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# Milk Production

An Up-to-Date Overview of Animal  
Nutrition, Management and Health

*Edited by Narongsak Chaiyabutr*





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**MILK PRODUCTION –  
AN UP-TO-DATE OVERVIEW  
OF ANIMAL NUTRITION,  
MANAGEMENT  
AND HEALTH**

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## Milk Production - An Up-to-Date Overview of Animal Nutrition, Management and Health

<http://dx.doi.org/10.5772/1525>

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First published in Croatia, 2012 by INTECH d.o.o.

eBook (PDF) Published by IN TECH d.o.o.

Place and year of publication of eBook (PDF): Rijeka, 2019.

IntechOpen is the global imprint of IN TECH d.o.o.

Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from [orders@intechopen.com](mailto:orders@intechopen.com)

Milk Production - An Up-to-Date Overview of Animal Nutrition, Management and Health

Edited by Narongsak Chaiyabutr

p. cm.

ISBN 978-953-51-0765-1

eBook (PDF) ISBN 978-953-51-5322-1

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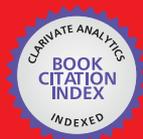
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# Meet the editor



Narongsak Chaiyabutr is Professor of Animal Physiology at Faculty of Veterinary Science, Chulalongkorn University, Thailand. He received his D.V.M. degree in 1969 from Faculty of Veterinary Science. From 1971-1973, he held FAO fellowship at The Royal Veterinary and Agricultural University, Denmark, for postgraduate training in Animal Physiology and Biochemistry. In 1980, he received the Ph.D degree at Glasgow University, UK under the supervision of Professor Dr. Malcolm Peaker at the Hannah Research Institute. He has received the Distinguished Researcher Awards (Animal Production) at 6th Asian-Australasian Association of Animal Production Society (AAAP) Congress. He has received the National outstanding researcher award (Agriculture and Biology) by National Research Council of Thailand and Distinguished Veterinarian Award (Academic discipline) by The Veterinary Medical Association of Thailand.



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## Preface

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When I was invited by InTech Open Access Publisher, to author/edit the books on Milk Production, I immediately realized both the importance of such books that are concerned with milk production. The difficulty of assembling such books rests on the fact that lactation is a complex process and the vast amount of research, fundamental and applied has been carried out. Milk production is not concerned only with the cow. The sense of milk production is concerned worldwide with the milk of cows, sheep, goats and buffaloes. The efficient milk production both quantity and quality need to improve in many areas. Involvement of many disciplines is essential to understand for more efficient production. Knowledge of animal nutrition, physiology, biochemistry, animal management and husbandry, animal breeding and genetics and animal health, must be considered together. Thus, it would be very difficult for one scientist alone to adequately collect so much data. I concluded that this book could best achieve its aim if presented from different perspective as recognized by eminent authorities in the field. These books permits the reader's exposure to the expert's experience and scientific style of interpreting and integrating available data into his own views. Because of space limitations, the subject is presented in two books: "Milk Production - An Up-to-Date Overview of Animal Nutrition, Management and Health", and "Milk Production - Advanced Genetic Traits, Cellular Mechanism, Animal Nutrition and Management".

The chapters in "Milk Production - An Up-to Date Overview of Animal Nutrition, Management and Health", are organized into three main sections and are concerned with the animal nutrition, animal management and, breeding and animal health. This book is intended for students, researchers, teaching staff, practicing professionals and those connected with dairy science, animal science, food science, nutrition, physiology, biochemistry, veterinary medicine and other related fields. It is also hoped that Milk Production - An Up-to Date Overview of Animal Nutrition, Management and Health, will help stimulate interest in improved milk production. Each chapter has an extensive bibliography which can future aid the reader in keeping abreast of the developments in this field.

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# Milk Production and Animal Nutrition

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# The Effect of Fatty Acids in Goat Milk on Health

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Dragomir Kompan and Andreja Komprej

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50769>

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## 1. Introduction

It has been recognized that components of foods can be contributing factors in human health and disease prevention. Based on the potential benefits to long-term human health there is interest in developing sustainable nutritional strategies for reducing saturated and increasing specific unsaturated fatty acids in ruminant milk. Despite the lower scale of milk production from goats compared with cows in Europe, there is an increasing interest in goat milk due to inherent species-specific biochemical properties that contribute to nutritional quality. Goat milk has been identified as a viable alternative for consumers that are sensitive or develop allergic reactions to bovine milk.

### 1.1. Synthesis and composition of goat milk fat

Fat composition in goat milk is one of the most important components of the technological, nutritional or dietetic quality of goat milk. Milk fat content in goat milk is high after parturition and then decreases during the major part of lactation. This is related to at least two phenomena: a dilution effect due to the increase in milk volume until the lactation peak, and a decrease in fat mobilization that decreases the availability of plasma non-esterified fatty acids, especially C18:0 and C18:1, for mammary lipid synthesis (Chilliard et al., 2003). Even that, total solids, fat, crude protein, lactose, and ash contents of goat milk are almost similar to cow milk, there are important differences in the individual fatty acids and casein fractions and fat globule sizes. Fat globules of goat milk are smaller in size and do not coalesce upon cooling because of lack of agglutinin, which is responsible for the aggregation of fat globules in cow milk.

Goat milk fat is composed primarily of triglycerids (or triacylglycerides) (in 98%) and in a small part from phospholipids and sterols. Triglycerids are synthesized on the outer surface of the smooth endoplasmic reticulum of the milk alveolar cells from precursor substances: fatty acids and glycerol. They are forming larger globules, which are travelling to the margin of cell. At the beginning, they attach to the membrane and they pass through. Then,

they are eliminated from the cell as fat globules of the milk. The synthesis is endogenous in a large extent, where the presence of the conjugated linoleic acid plays an important role (Hurley, 2009).

Fatty acids in goat milk are synthesized in epithelial cells of the mammary gland de novo or they are passing over from the blood (Chilliard et al., 2003). Two coenzymes have a major role in the synthesis of fatty acids in goat milk: acetyl-coenzyme A-carboxylase, which participates in the synthesis of fatty acids de novo and fatty acid synthase, which is a complex of enzymatic active substances and is responsible for the extension (elongation) of the fatty acid chain (Hurley, 2009). Fatty acids of exogenous origin are presented via the circulation to mammary epithelial cells either in the form of non-esterified fatty acids or esterified as the acyl groups of the triacylglycerol component of lipoprotein particles. In the mammary gland of ruminant animals, short and medium chain saturated fatty acids are the major products of de novo lipogenesis whereas plasma lipids contribute longer chain and mono unsaturated species. The acetate is the precursor of fatty acids synthesis in ruminants, while in monogastric animals, the precursor is glucose (Clegg et al., 2001).

## 1.2. Fatty acid composition in goat milk fat

Average goat milk fat differs in contents of its fatty acids significantly from average cow milk fat, being much higher in butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), linoleic (C18:2), but lower in stearic (C18:0), and oleic acid (C18:1) (Table 1). Three of the medium chain fatty acids (caproic, caprylic, and capric) have actually been named after goats, due to their predominance in goat milk. They contribute to 15% of the total fatty acid content in goat milk in comparison to 5% in cow milk (Haenlein, 1993). The presence of relatively high levels of medium chain fatty acids (C6:0 to C10:0) in goat milk fat could be responsible for its inferior flavour (Skjvedal, 1979).

Fatty acid	Goat milk <sup>1</sup>	Goat milk (from highland flock) <sup>2</sup>	Goat milk (from mountain flock) <sup>2</sup>	Cow milk <sup>1</sup>
C <sub>4:0</sub> butyric	130	-	-	110
C <sub>6:0</sub> caproic	90	-	-	60
C <sub>8:0</sub> caprylic	100	106	85	40
C <sub>10:0</sub> capric	260	433	321	80
C <sub>12:0</sub> lauric	120	228	149	90
C <sub>14:0</sub> myristic	320	441	392	340
C <sub>16:0</sub> palmitic	910	984	990	880
C <sub>16:1</sub> palmitoleic	80	-	-	80
C <sub>18:0</sub> stearic	440	333	300	400
C <sub>18:1</sub> oleic	980	-	-	840
C <sub>18:2</sub> linoleic	110	103	76	80
C <sub>18:3</sub> linolenic	40	32	26	50

**Table 1.** Fatty acid composition (mg FA 100 g<sup>-1</sup> milk) in goat milk fat in comparison to cow milk (1<sup>Posati & Orr, 1976</sup>; 2<sup>Zan et al., 2005</sup>)

### 1.3. The effect of nutrition on goat milk fat and fatty acids composition

Nutrition (forage-to-concentrate ratio, type of forages, etc.) is the main environmental factor regulating milk fat synthesis and fatty acid composition in ruminants (Nudda et al., 2003; Bernard et al., 2009). Forage in the diet is known to affect milk fat composition responses to plant oils, including trans-18:1 and conjugated linoleic acid isomer concentrations. Inclusion of fat in the diet enhances milk fat secretion in the goat in the absence of systematic changes in milk yield and protein content (Bernard et al., 2009; Chilliard et al., 2003, 2007). Bernard et al. (2009) found out that, changes in goat milk fatty acid composition were dependent on forage type and plant oil composition, with evidence of an interaction between these nutritional factors. Responses to lipid supplements were characterised as a reduction in fatty acids synthesised de novo (C10:0–C16:0) and an increase in C18:0, cis-C18:1, conjugated linoleic acid and polyunsaturated fatty acid concentrations, indicating that plant oils can be used to effect potentially beneficial changes in milk fat composition without inducing detrimental effects on animal performance. Moreover, goats fed a high level of pasture forage had higher milk fat contents of C4:0, C6:0, C18:0, C18:1, C18:3, C20:0, iso-, ante-iso-, and odd fatty acids, but lower values of C10:0, C12:0, C14:0, C16:0, and C18:2, than those fed the low levels of forage. However, high levels of alfalfa forage also produced the lowest contents of the less desirable trans-C18:1 fatty acids (LeDoux et al., 2002). The conclusion was that decreasing the fibre content and increasing the grain part in the goat daily ration would lead to higher contents of the undesirable trans-C18:1 fatty acids in milk. The composition of goat milk fatty acids differed also in goats grazing one flock on highland (615–630 m altitude) and one flock on mountain (1060–1075 m altitude) pasture by Žan et al. (2005). The most abundant fatty acids in milk of both flocks were C16:0, C18:1, n-9, C14:0 and C10:0 (Table 1). The average content of saturated fatty acids was 74.52 and 73.05% in milk from the highland and mountain flocks, respectively. Three saturated fatty acids (caprylic (C8:0), capric (C10:0) and lauric acid (C12:0)), were present at significantly higher amounts in milk from the highland flock than in milk from the mountain flock. Monounsaturated fatty acids represented 20.49 and 22.32% and polyunsaturated fatty acids 3.73 and 3.24% of the milk from the highland and mountain flocks, respectively. Among the monounsaturated fatty acids, palmitoleic + palmitelaidic acid (C16:1, n-7) showed a significantly higher concentration in milk from mountain flock than in milk from the highland flock. The content of linolelaidic acid (C18:2, n-6) was significantly higher in comparison to milk from the highland flock. The average quantity (32 mg 100 g<sup>-1</sup> milk) of essential  $\alpha$ -linolenic acid (C18:3, n-3) was slightly higher in milk of the highland flock than in milk from the mountain flock (26 mg 100 g<sup>-1</sup> milk). Hou et al. (2011) stated that the supplementation of fish oil can significantly increase the production of cis-9, trans-11 conjugated linoleic acid, and trans-11 C18:1, while lowering the amount of trans-10 C18:1 and trans-10, cis-12 conjugated linoleic acid in the ruminal fluid of goats. Increased cis-9, trans-11 conjugated linoleic acid, and trans-11 C18:1 can lead to a higher output of cis-9, trans-11 conjugated linoleic acid in milk product, and the decrease in trans-10 C18:1 and trans-10, cis-12 conjugated linoleic acid supports the role of fish oil in the alleviation of milk fat depression.

#### 1.4. Conjugated linoleic acid

Conjugated linoleic acid consists of a series of positional and geometric dienoic isomers of linoleic acid that occurs naturally in foods. It is a product of biohydrogenation in the rumen of ruminants and has a great influence on synthesis of fatty acids in milk in low concentrations (Bessa et al. 2000; Chouinard et al. 1999; Griinari & Bauman, 1999; Griinari et al. 2000; Khanal & Dhiman, 2004). Actually, the conjugated linoleic acid found in goat milk fat originate from two sources (Griinari & Bauman, 1999). One source is conjugated linoleic acid formed during ruminal biohydrogenation of linoleic acid (C18:2 n-6) that leads first to vaccenic (trans-11 C18:1) and finally to stearic acid (C18:0) (Nudda et al., 2003). The second source is conjugated linoleic acid synthesized by the animal's tissues from trans-11 C18:1, another intermediate in the rumen biohydrogenation of unsaturated FA. Thus, the uniqueness of conjugated linoleic acid in food products derived from ruminants relates to the incomplete biohydrogenation of dietary unsaturated fatty acids in the rumen. Ruminal biohydrogenation combined with mammary lipogenic and  $\Delta$ -9 desaturation pathways considerably modifies the profile of dietary fatty acids and thus milk composition (Chilliard et al., 2007).

Dietary sources from ruminants such as milk, cheese and meats contain more conjugated linoleic acid than foods of non-ruminant origin (Bessa et al. 2000; Khanal & Dhiman, 2004). The increase of linoleic acid intake is one of the feeding strategies for conjugated linoleic acid enrichment in ruminant fat since linoleic acid is the main precursor of conjugated linoleic acid (Bessa et al., 2000). The main available sources of linoleic acid in animal feeds are cereal and oilseed grains or oils obtained from these. Goat milk conjugated linoleic acid content increases sharply after either vegetable oil supplementation (Bernard et al., 2009) or fresh grass feeding containing unsaturated fatty acids, but does not change markedly when goats receive whole untreated oilseeds (Chilliard et al., 2003). Mir et al. (1999) found that it is possible to increase conjugated linoleic acid content of goat milk by manipulation of dietary regimen such as supplementation with canola oil. The pasture has major effects by decreasing saturated fatty acids and increasing fatty acids considered as favourable for human health (C9-18:1, C18:3n-3 and C9t11-CLA), compared to winter diets, especially those based on maize silage and concentrates (Chilliard et al., 2007). Investigations have shown that milk fat conjugated linoleic acid content can be also enhanced by manipulation of the rumen fermentation (Bessa et al., 2000; Griinari et al., 1999) or by direct addition of a dietary supplement of conjugated linoleic acid (Lock et al., 2008).

#### 1.5. Effect of fatty acids on health

Milk, apart from its nutritional traits, contains substances which have beneficial effects on human health and is, therefore, considered essential to a correct nutrition. In particular, in milk are present vitamin A, vitamin E,  $\beta$ -carotene, sphingomyelins, butyric acid, and conjugated linoleic acid, all with a strong antitumor effect (Parodi, 1999). Different FA (short and medium chain, saturated, branched, mono and polyunsaturated, *cis* and *trans*, conjugated) in the lipid fraction of milk are potentially involved as positive or negative

predisposing factors for human health (Parodi, 1999; Williams, 2000). In this respect, conjugated linoleic acid is the most characteristic one. One of the goat milk significance in human nutrition is treating people afflicted with cow milk allergies and gastro-intestinal disorders, which is a significant segment in many populations of developed countries. Fat in goat milk is more digestible than bovine milk fat which may be related to the lower mean milk fat globule size, higher C8:0–C10:0 concentrations and a larger proportion of short- and medium-chain fatty acids (Chilliard et al., 2006 as cited in Bernard et al., 2009). Because of predominance of smaller fat globules in goat milk, it is easier to digest than cow milk and this may be attributed to faster lipase activity on smaller fat globules due to a greater surface area (Chandan et al., 1992). Goat milk is therefore recommended for infants, old, and convalescent people.

The physiological and biochemical facts of the unique qualities of goat milk are just barely known and little exploited, especially not the high levels in goat milk of short and medium chain fatty acids, which have recognized medical values for many disorders and diseases of people (Haenlein, 2004). Goat milk exceeds cow and sheep milk in monounsaturated, polyunsaturated fatty acids, and medium chain triglycerides, which all are known to be beneficial for human health, especially for cardiovascular conditions. Capric, caprylic acids and medium chain triglycerides have become established medical treatments for an array of clinical disorders, including malabsorption syndromes, chyluria, steatorrhea, hyperlipoproteinemia, intestinal resection, premature infant feeding, non-thriftiness of children, infant malnutrition, epilepsy, cystic fibrosis, coronary by-pass, and gallstones, because of their unique metabolic ability to provide direct energy instead of being deposited in adipose tissues, and because of their actions of lowering serum cholesterol, inhibiting and limiting cholesterol deposition (Alferez et al., 2001; Greenberger & Skillman, 1969; Kalser, 1971; Schwabe et al., 1964; Tantibhedhyanangkul & Hashim, 1978).

Conjugated linoleic acid was recognized as having antioxidative and anticarcinogenic properties in animal model studies (Ip et al., 1991; Jiang et al., 1996; Parodi, 1997). Several *in vitro* and *in vivo* studies showed also antiatherogenic, anti-obesity, anti-diabetes and immune-stimulating properties of conjugated linoleic acid (McGuire & McGuire, 1999). By Parodi (1997), conjugated linoleic acid inhibited proliferation of human malignant melanoma, colorectal, breast and lung cancer cell lines. Anticarcinogenic effects of conjugated linoleic acid appear to be dose dependent, from 0.1 to 1% in the diet (Ip et al., 1991). Conjugated linoleic acid reduced the incidence of chemically induced mouse epidermal tumors, mouse forestomach neoplasia and aberrant crypt foci in the rat colon. They have been also shown to stimulate immune response and protect against arteriosclerosis (Cook et al., 1993; Lee et al., 1994). When rabbits were fed conjugated linoleic acid, LDL cholesterol to HDL cholesterol ratio and total cholesterol to HDL cholesterol ratio were significantly reduced. Examination of the aortas of conjugated linoleic acid fed rabbits showed less atherosclerosis (Lee et al., 1994).

Somatic cells in milk are the total sum of white blood cells present in milk and udder epithelial cells, which may be an indicator of the udder health status (Das & Singh, 2000; Manlongat et al., 1998; Zeng & Escobar, 1996; Wilson et al., 1995). They are present in milk

all the time. In cows, a somatic cell count above the regulatory standard is generally considered as an indication of mastitis. An increased number of somatic cell count is either the consequence of an inflammatory process due to the presence of an intramammary infection or under non-pathological conditions due to physiological processes such as oestrus or advanced stage of lactation. For this reason, the somatic cell count of milk represents a sensitive marker of the health of the udder and is considered a useful parameter to evaluate the relationship between intramammary infection and changes in milk characteristics. The standard for the permissible number of somatic cell count for cow milk exists, while it is still under study for goat milk due to considerable fluctuations. When the udder is tired during late lactation, the number of somatic cells in normal conditions can considerably enlarge, and approximately 80% of the cells may be polymorphonuclear leukocytes (Manlongat et al., 1998). The same authors found that normal nonmastitic late-lactation-stage goat milk is significantly higher in polymorphonuclear leukocytes chemotactic activity than early-lactation-stage goat milk. The chemotactic factor(s) present in the milk of normal late-lactation-stage goats is nonpathological and may play a physiologic regulatory role in mammary gland involution. On the other hand, the increase of leucocytes is a response to the inflammatory process in the mammary gland or somewhere in the body. The number of leucocytes increases due to bacterial infections, but it could also be increased due to the stage of lactation, age of the animal, stress, season of the year, nutrition and udder injuries. The variability of somatic cell count in goat milk is very high, which exists among the animals and within the time span of individual animals (Das & Singh, 2000). Therefore, it is important to determine how nutrition can influence the reduction of somatic cell count in goat milk. Gantner & Kompan (2009) found that a five-day supplementation of  $\alpha$ -linoleic acid in Alpine goat diet had a significant effect on lower somatic cell count in milk. Based on this experiment, it was concluded that  $\alpha$ -linoleic acid supplementation had no effect on milk yield; it had low effect on milk components and significant effect on somatic cell count. A decrease in somatic cell count was determined in the 1<sup>st</sup> day of the treatment period and continued until 30<sup>th</sup> day after the treatment period. The supplementation of the goat diet with  $\alpha$ -linoleic acid could be used as a method of choice for reduction of somatic cell count in goat milk.

The aim of our study was therefore to ascertain the changes in goat milk yield and its contents of fat, protein, lactose, dry matter, somatic cell count, and total number of microorganisms when goats are supplemented with the following fatty acids:  $\alpha$ -linoleic acid, eicosapentanoic acid, and docosahexanoic acid and how these three fatty acids influence on the content of particular fatty acids during and after the supplementation.

## 2. Material and methods

### 2.1. Material

The research was performed on the farm with 90 Slovenian Alpine and Slovenian Saanen goats. Goats were machine milked. During the experiment, goats were in different stages of lactation. The average body weight of the goats was  $51 \pm 6$  kg. All kids were weaned. Goats

were arranged into three pens according to their stage of lactation, namely, after kidding from the fourth to the tenth week of lactation (pen A), from the 11<sup>th</sup> to the 20<sup>th</sup> week of lactation (pen B), and after the 20<sup>th</sup> week of lactation (pen C). Goats were milked twice a day, at 6 a.m. ( $\pm$  30 min) and at 6 p.m. ( $\pm$  30 min). Diet was composed from hay (2 kg/animal/day) which was given to goats twice a day. Goats were supplemented with feed mixture at milking parlor during the milking time. Supplemental feed mixture contained 50% of grounded maize grains, 30% of dried beet pulp, and 20% of wheat bran. Goats from pen A were supplemented with 500 g, goats from pen B with 350 g, and goats from pen C with 250 g of feed mixture. Vitamin-mineral supplement and water were offered to goats *ad libitum*. After the tenth day preparing period, 62 goats from pens A and B were selected and randomly arranged into four experimental groups. At the beginning of the experiment (September 17<sup>th</sup>, 2000), goats were 28 to 105 days after kidding. The experiment lasted 63 days. During this time, experimental goats were added fats or oils.

## 2.2. Methods

### 2.2.1. Measuring performance and milk sampling

The whole experiment was performed in three periods:

**1<sup>st</sup> period:** Preparatory period - measuring before adding fats or oils. The preparatory period lasted 10 days. During this period, milk yield in goats was measured, milk samples were collected, and animals were adapting to the working group. Goats were adapted to the work and people after a week, so they were not under the stress any more. Milk yield was measured every day at morning and evening milking, when 70 ml of milk sample was taken for the analysis of milk content, somatic cells, and bacteriological analysis, and 2 ml for fatty acid content analysis.

**2<sup>nd</sup> period:** Experimental period – adding fatty acids. After the tenth day preparing period, 62 goats from pens A and B were randomly selected into four experimental groups, named EPA, ALFA, DHA, and KONT. There were 15 goats in groups EPA, ALFA, and DHA and 17 goats in the group KONT. Supplementation of the fats was performed 5 days (from the 11<sup>th</sup> to the 15<sup>th</sup> day), after morning milking in groups EPA, ALFA, and DHA. Each goat was caught and individually administered the appropriate quantity of fatty acids into its mouth with a special sound. Group EPA was receiving a preparation rich in eicosapentaenoic acid (EPA; 20 g/day), group ALFA was receiving a linseed oil rich in  $\alpha$ -linoleic acid (ALA; 20 g/day), and group DHA was receiving a preparation rich in docosahexaenoic acid (DHA; 20 g/day). Group KONT was a control group, which was receiving no preparation. Measuring of the milk yield and collecting milk samples was followed the same procedure as in the first period.

**3<sup>rd</sup> period:** This period lasted from the 16<sup>th</sup> day, after the end of administering fatty acids to goats. Milk yield measuring and milk samples collection was continuing until the 20<sup>th</sup> day. From the 21<sup>st</sup> day of the experiment, milk yield measuring and milk samples collection was performing every five days, at the morning and evening milking, until the end of the

experiment (63<sup>rd</sup> day). All together, 30 morning and 30 evening records were collected by each goat.

### 2.2.2. Milk yield measuring

There were 90 goats all together in the flock, which were milked on the milking parlor with 24 places for milking goats connected to milk pipeline. Goats were milked every morning between 5:40 and 7:20 a.m. and every evening between 6:20 and 8:00 p.m. A measuring gauged flask was connected to milking unit to measure milk yield. Milk yield was written down for every goat. A milk sample was also taken for the analysis. During the experiment, 30 daily records were collected for every goat, which means 60 records for each goat and 60 milk samples by 70 ml for milk analysis (sample A) and 60 samples by 2 ml (sample B) for fatty acid analysis. The preservative azidiol on the basis of  $\text{NaN}_3$  in concentration 0.02% with the addition of chloramphenicol for the stabilization of microorganisms was added to the sample A. For every 50 to 70 ml of the milk sample, 0.2 ml of the preservative was added. Milk samples A were then delivered to the Laboratory for dairying, while milk samples B were delivered to the Chemical laboratory at Biotechnical Faculty in Ljubljana.

### 2.2.3. Analyses of milk samples

**Chemical composition, somatic cell count, and total number of microorganisms:** Fat, protein, lactose, and dry matter content, somatic cell count and total number of microorganisms were determined in the collected milk samples A in the Laboratory of dairying at Biotechnical Faculty in Ljubljana. Furthermore, fatty acid composition of milk lipids was determined. Chemical composition of goat milk was determined by the instrument MilkoScan 133 B, which operates on the principle of infrared spectrometry. Somatic cell count was determined using apparatus Fossomatic 5000, which operates on the basis of automatic epifluorescent technique, by the principle of flow cytometry. The total number of microorganisms was determined using the apparatus Bactoscan 8000, type 27000.

**Fatty acid composition of milk lipids:** Milk samples B were stored in liquid nitrogen immediately after milk recording. They were stored then in freezer chamber at  $-70^\circ\text{C}$  until the analysis. Before the analysis, milk samples were warmed to  $38-40^\circ\text{C}$  in water bath and mixed up. After that, 500 mg of the milk sample were weighed out into tubes, where 300  $\mu\text{l}$  of methylenchloride and 3 ml of fresh prepared 0.5M of sodium hydroxide in methanol were added. To determine the fatty acid composition of milk lipids, the analysis of methyl esters of fatty acids was done. This analysis was performed on gas chromatograph Hewlett Packard HP AGILENT 6890 SERIES GC SYSTEM, USA. Processing of chromatographic data was conducted using ChemStation Plus software. Furthermore, factor of the responsiveness of the flame ionization detector was determined. Total lipids in the sample are composed of both fatty acids and glycerol from triglycerids, phosphate from phospholipids, and sterol. For the calculation of the fatty acid value in the sample in mg, special factors are used, which express the proportion of acids in total fat.

#### 2.2.4. Statistical analysis of the data

The statistical package SAS (SAS/STAT, 2000) and partly the statistical package S-PLUS (1966) were used to analyse the data. The statistical analysis did not include records collected during the first six days of the preparation period. In the meantime, the situation in the stable was stabilizing and the team who participated in the experiment was introducing in the everyday milk measuring and collecting samples.

Due to the large fluctuations in individual values of the somatic cell count and number of microorganisms among animals and among observations within animals, we analyzed each animal individually as its time series, and for the most variable ones the logarithm of the values was found ( $X = \log_{10}Y$ ).

The time series were first standardized (S) in the way that last four days (from the 7<sup>th</sup> to 10<sup>th</sup> day) of the preparatory period (before supplementing with fatty acids) were taken as a starting point. Mean value of this period was calculated by the median (Me), the measure of variability was the average absolute deviation (AD). In this way we reduced the impact of outliers. Although, it is usual to standardize by the average and standard deviation, we decided for median and absolute deviation. In this way, the standardized time series for the animal was calculated using the following equation:

$$S = ((X - \text{Me}) / \text{AD}) \dots \quad (1)$$

In this way, the standardized time series (S) are comparable for animals with different values. Then, we calculated the median for the three periods on the standardized time series:

- median for the period from the seventh to tenth day of the experiment (preparatory period), which was in all cases zero (=0);
- median for the period from 11<sup>th</sup> to the 15<sup>th</sup> day of the experiment (the period of supplementing with fatty acids);
- median for the period from the 16<sup>th</sup> to the 63<sup>rd</sup> day of the experiment (the post supplementation period of the fatty acids).

For each animal, the corresponding median has become an input data for the statistical analysis. In this way, we analyzed milk yield (ml), the content of milk proteins (g/100 ml), milk fat (g/100 ml), milk lactose (g/100 ml), dry matter (g/100 ml), non-fat dry matter (g/100 ml), total number of microorganisms ( $n \cdot 10^3/\text{ml}$ ), and somatic cell count ( $n \cdot 10^3/\text{ml}$ ) in milk.

In this way, a comparison of groups with a simple analysis of variance was made where the zero assumption was checked for that the averages by groups were the same. If a statistically significant difference test was found (5% level of significance was considered), then the groups were compared also by the Duncan test or by the contrast analysis, where each group was compared with the control group.

All other traits were analyzed by the GLM procedure (General Linear Model) with statistical package SAS, which included the impacts of the group (4) and period (3). Differences

among groups were estimated by the linear contrasts, while connections between the properties were calculated by the Pearson correlation coefficient. The limit of statistical significance was taken at  $P < 0.05$  and highly statistically significance was taken at  $P < 0.001$ .

### 3. Results and discussion

#### 3.1. Milk yield and the chemical composition of milk

The average milk yield and its content of fat, proteins, lactose, dry matter, non-fat dry matter, total number of microorganisms, and somatic cell count in different periods of the experiment by groups is shown in Table 2. In the preparatory period, only somatic cell count statistically significantly differed among groups. Statistically significant differences among groups in the experimental period appeared in dry matter, somatic cell count, and logarithm of the somatic cell count. In the third period of the experiment, statistically significant differences among groups appeared in the majority of observed traits.

It seems that the short time fatty acid supplementation into goat's diet does not negatively affect their milk yield. Milk yield did not vary statistically significant during the observed period (Table2). As found by Sampelayo et al. (2002), the supplemented fatty acids into the diet of Granadina goats did not affect their milk yield and the content of fat, proteins, lactose, and dry matter in milk.

Group	EPA	ALFA	DHA	KONT	EPA	ALFA	DHA	KONT	EPA	ALFA	DHA	KONT
Trait/Period	1	1	1	1	2	2	2	2	3	3	3	3
Milk (ml)	780 <sup>a</sup>	748 <sup>a</sup>	869 <sup>a</sup>	766 <sup>a</sup>	790 <sup>a</sup>	708 <sup>a</sup>	888 <sup>a</sup>	824 <sup>a</sup>	765 <sup>a</sup>	719 <sup>a</sup>	884 <sup>a</sup>	789 <sup>a</sup>
Fat (g/100 ml)	3.05 <sup>a</sup>	3.15 <sup>a</sup>	3.00 <sup>a</sup>	2.99 <sup>a</sup>	2.65 <sup>a</sup>	3.40 <sup>b</sup>	2.52 <sup>a</sup>	2.91 <sup>a</sup>	2.84 <sup>a</sup>	3.30 <sup>b</sup>	2.77 <sup>a</sup>	3.06 <sup>a</sup>
Proteins (g/100 ml)	2.93 <sup>a</sup>	3.12 <sup>a</sup>	2.98 <sup>a</sup>	3.01 <sup>a</sup>	3.01 <sup>a</sup>	3.21 <sup>b</sup>	3.01 <sup>a</sup>	3.06 <sup>a</sup>	3.15 <sup>a</sup>	3.28 <sup>a</sup>	3.29 <sup>a</sup>	3.07 <sup>b</sup>
Lactose (g/100 ml)	4.59 <sup>a</sup>	4.55 <sup>a</sup>	4.49 <sup>a</sup>	4.53 <sup>a</sup>	4.58 <sup>a</sup>	4.54 <sup>a</sup>	4.48 <sup>a</sup>	4.44 <sup>a</sup>	4.50 <sup>a</sup>	4.54 <sup>a</sup>	4.44 <sup>a</sup>	4.46 <sup>a</sup>
NFDM (g/100 ml)	8.32 <sup>a</sup>	8.48 <sup>a</sup>	8.26 <sup>a</sup>	8.33 <sup>a</sup>	8.39 <sup>a</sup>	8.55 <sup>a</sup>	8.29 <sup>a</sup>	8.30 <sup>a</sup>	8.45 <sup>a</sup>	8.62 <sup>b</sup>	8.53 <sup>a</sup>	8.33 <sup>a</sup>
DM (g/100 ml)	11.37 <sup>a</sup>	11.62 <sup>a</sup>	11.26 <sup>a</sup>	11.33 <sup>a</sup>	11.04 <sup>a</sup>	11.76 <sup>b</sup>	10.81 <sup>a</sup>	11.21 <sup>a</sup>	11.29 <sup>a</sup>	11.92 <sup>b</sup>	11.30 <sup>a</sup>	11.39 <sup>a</sup>
MO (n*10 <sup>3</sup> /ml)	653 <sup>a</sup>	609 <sup>a</sup>	498 <sup>a</sup>	505 <sup>a</sup>	315 <sup>a</sup>	334 <sup>a</sup>	350 <sup>a</sup>	494 <sup>a</sup>	266 <sup>a</sup>	267 <sup>a</sup>	267 <sup>a</sup>	347 <sup>a</sup>
SCC (n*10 <sup>3</sup> /ml)	1316 <sup>a</sup>	1095 <sup>a</sup>	585 <sup>b</sup>	526 <sup>b</sup>	1631 <sup>a</sup>	975 <sup>b</sup>	1166 <sup>a</sup>	1258 <sup>a</sup>	1531 <sup>a</sup>	915 <sup>b</sup>	1884 <sup>a</sup>	1364 <sup>a</sup>
log <sub>10</sub> _MO (n*10 <sup>3</sup> /ml)	2.64 <sup>a</sup>	2.62 <sup>a</sup>	2.47 <sup>a</sup>	2.58 <sup>a</sup>	2.44 <sup>a</sup>	2.44 <sup>a</sup>	2.48 <sup>a</sup>	2.62 <sup>b</sup>	2.35 <sup>a</sup>	2.34 <sup>a</sup>	2.34 <sup>a</sup>	2.48 <sup>a</sup>
log <sub>10</sub> _SCC (n*10 <sup>3</sup> /ml)	2.69 <sup>a</sup>	2.68 <sup>a</sup>	2.62 <sup>a</sup>	2.54 <sup>a</sup>	2.82 <sup>a</sup>	2.72 <sup>a</sup>	2.80 <sup>a</sup>	2.99 <sup>a</sup>	2.83 <sup>a</sup>	2.77 <sup>b</sup>	2.88 <sup>a</sup>	2.99 <sup>a</sup>

<sup>a,b</sup> – values which are not marked with the same letter are statistically significantly different at least  $P < 0.05$   
 NFDM – non-fat dry matter; DM – dry matter; SCC – somatic cell count; MO – microorganisms;

**Table 2.** Average values of the observed traits in different periods of the experiment by groups

Milk fat yield statistically significantly increased in ALFA group from 3.15 to 3.40 g/100 ml on average when goats were supplemented with linseed oil rich in  $\alpha$ -linoleic acid (Table 2) and it slightly decreased to 3.30 g/100 ml until the third period of the experiment. In groups EPA and DHA, milk fat yield firstly decreased, while it increased slightly after the end of supplementation with fatty acids.

There were no statistical significant differences among the groups of goats in milk protein yield before the supplementation with fatty acids (Table 2). During the supplementation of goats with fatty acids, milk protein yield increased and it was increasing also after the end of supplementation. Group ALFA had the highest protein yield in milk in the whole time of the experiment.

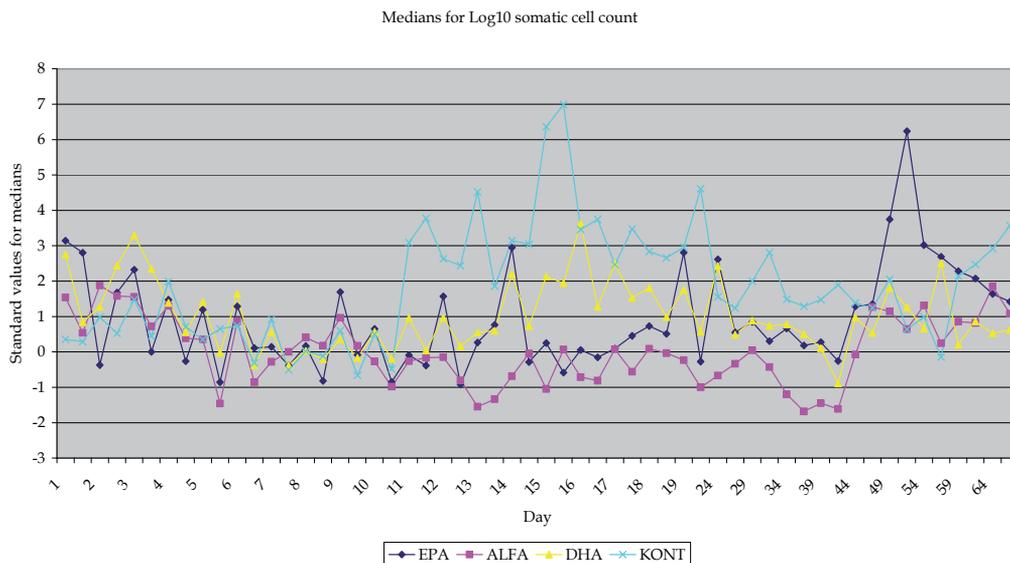
In general, lactose in milk varies little, what was confirmed also in our research. There were no statistical significant differences in lactose yield among the observed groups, neither during the supplementation with fatty acids nor after that (Table 2).

Non-fat dry matter increased during the experiment in all observed groups which were supplemented with fatty acids, but not in the control group KONT (Table 2). Differences among groups were not statistically significant. Total dry matter decreased after supplementing with fatty acids in groups EPA, DHA, and KONT, while it increased in ALFA group. After the end of supplementing with fatty acids, total dry matter increased in all groups. Group ALFA statistically significantly differed in milk dry matter from other observed groups in the second and third period of the experiment.

The number of microorganisms in milk mostly depends on milking hygiene, which includes staff, animals, facilities, equipment, hygiene maintenance, and cleaning of the equipment. It also depends on the health of the udder and the presence of mastitis. Soon after the beginning of the experiment, the hygiene and cleaning improved, and the number of microorganisms in milk decreased (Table 2). There was no mastitis detected in the whole time of the experiment. No statistically significant differences were noticed among groups in the number of microorganisms in milk.

Somatic cell count was one of the most variable traits in our experiment, since we found that values ranged from 13.000 to 24,312.000 of somatic cells in ml of milk. Despite the great variability, transformation of somatic cell count to the logarithmic value enabled to find the possible impacts of supplementation with fatty acids on somatic cell count (Figure 1). Preliminary report by Košmelj et al. (2001) showed the impact of supplementing alpha-linolenic fatty acid to goats, which was reflected in a reduction of the number of somatic cells during the supplementation and four weeks after.

The average values for medians during the supplementation with fatty acids ( $Me_1$ ) and for medians five days after the supplementation with fatty acids ( $Me_2$ ) are shown in Table 3. Results showed statistical significant differences among groups of goats for medians during the supplementation with fatty acids and also for medians five days after the supplementation with fatty acids. The average of medians ( $Me_1$  and  $Me_2$ ) in group ALFA is negative, so it could be affirmed, that the supplementation of linseed oil rich in  $\alpha$ -linoleic acid decreases the number of somatic cell count in milk.



**Figure 1.** Standardization and log<sub>10</sub> value median for number of somatic cells by groups

Period / Group	EPA	ALFA	DHA	KONT
Average for Me <sub>1</sub>	1.01 <sup>b</sup>	-3.11 <sup>a</sup>	1.68 <sup>b</sup>	3.52 <sup>b</sup>
Average for Me <sub>2</sub>	1.75 <sup>b</sup>	-2.47 <sup>a</sup>	1.78 <sup>b</sup>	3.20 <sup>b</sup>

<sup>a, b</sup> Groups with different letter are statistically significantly different (P<0.05)

**Table 3.** Average value of Me<sub>1</sub> in Me<sub>2</sub> in different group

On average, somatic cells in goat milk are present in a greater number than in cow milk. Zeng et al. (1997) reported that 17% of goat milk samples recorded on goat farms which are members of the Association of goat farmers in the U.S. exceeded the standard 1.0x10<sup>6</sup> of somatic cells ml<sup>-1</sup> when the experiment of daily monitoring of somatic cells in milk was carried out.

Das & Singh (2000) studied somatic cells in goat milk and electrical conductivity of milk. In the blood samples total leucocytes and differential leucocytes (lymphocytes, monocytes, neutrophils, eosinophil, and basophils) were also determined. Somatic cell count in goat milk was high during early lactation and decreased subsequently as the lactation advanced. There were found individual variations (P<0.01) in somatic cell counts between different lactation periods as well as among and within animals. For example, one goat had very high somatic cell count in comparison to other goats from the beginning to the end of the experiment. The goat was then tested for mastitis using California mastitis test and it was found to have normal milk. Similar results were found in our experiment. Total leucocyte count in blood also decreased as the lactation progressed and remained fluctuated during late lactation in the study by Das & Singh (2000). Lymphocytes and neutrophils were low during early lactation and with establishment of lactation stabilized to normal levels. Protein content of milk did not vary during different periods of lactation. However, lactose

decreased and fat percent increased with advanced lactation. It is interesting that the connection between somatic cell count and milk yield and between somatic cell count and milk composition was not found in any stage of lactation.

Mastitis is typically associated with a large number of somatic cells in small ruminants. In our experiment, the number of somatic cells significantly reduced only in the ALFA group and lasted statistically significant 39 days after the supplementation with fatty acids. For  $\alpha$ -linolenic fatty acid is known, that it could incorporate into phospholipids five hours after ingestion (Adam et al., 1986). The other two, eicosapentaenoic acid and docosahexaenoic acid can incorporate into phospholipids only after a few days supplementation. The statistically significant effect of the  $\alpha$ -linolenic fatty acid only on somatic cell count could be explained by the rapidness of incorporation into membrane phospholipids of this fatty acid.

The fluctuations of the somatic cell count in goat milk are subjected to many influences. Researchers have not explored other reasons for the number of somatic cells in goat milk except the hygiene measures. Ruminants are in the last 20 years fed adding n-3 fatty acids to improve the fatty acid composition of milk and meat, but the impact on the number of somatic cells have not been monitored. Our experiment clearly shows that the supplementation of the  $\alpha$ -linolenic fatty acid had a relatively long time impact on reducing somatic cell count or to a low level of somatic cells in milk. The interpretation may be possible, that we achieved a more appropriate relationship between n-3 and n-6 long chain fatty acids with the supplementation of  $\alpha$ -linolenic fatty acid which was not provided by the diet.

### 3.2. Composition of fatty acids in goat milk

Chemical analysis of goat milk fat was done for fatty acids from 10:00 to 24:6, n-9. The fat composition of goat milk was studied by each milking during the experiment time. Therefore, values listed below (Table 3) represent the percentage of the all analyzed fatty acids rather than total fat in goat milk.

During our experiment, there was from 9.0 to 14.0 wt % of the **capric** acid (10:0) in the goat milk fat. Some authors (Hurley, 2009; Jandal, 1996; Sanz Sampelayo et al., 2002) indicated values from 8.4 to 11.1%. EPA group had the lowest level of capric acid before supplementing with fatty acids, while its level exceeded groups ALFA and KONT during the supplementation and declined to the lowest level among groups in the last period of the experiment. DHA group had the highest level of the capric acid during the supplementation with fatty acids as well as all the time after the supplementation. It is known that goat milk has more short-chain fatty acids ( $C_{4:0}$  to  $C_{10:0}$ ) than cow's milk, which are easier to digest than long-chain fatty acids.

We found that the **lauric** acid (12:0) in goat milk fat presented between 3.8 and 7.7 wt %. During the supplementation with fatty acids, the lauric acid increased for 2% in DHA group and for 1% in EPA group. The increase in EPA group lasted two days after the end of the supplementation, and four days in DHA group. Hurley (2009) found that there is 3.3% of the lauric acid in goat milk fat, Jandal (1996) reported about 6.0%, while Sanz Sampelayo et al. (2002) found from 4.69 to 5.11% of the lauric acid in goat milk fat.

Experimental period	1	1	1	1	2	2	2	2	3	3	3	3
FA / GROUP	EPA	ALFA	DHA	KONT	EPA	ALFA	DHA	KONT	EPA	ALFA	DHA	KONT
10:0	9.55 <sup>a</sup>	11.63 <sup>a</sup>	10.84 <sup>a</sup>	11.16 <sup>a</sup>	11.64 <sup>a</sup>	11.20 <sup>a</sup>	13.13 <sup>b</sup>	11.60 <sup>a</sup>	9.84 <sup>a</sup>	10.64 <sup>a</sup>	12.09 <sup>b</sup>	10.08 <sup>a</sup>
12:0	4.43 <sup>a</sup>	6.06 <sup>a</sup>	4.97 <sup>a</sup>	5.38 <sup>a</sup>	5.41 <sup>a</sup>	5.65 <sup>a</sup>	6.56 <sup>a</sup>	6.06 <sup>a</sup>	4.93 <sup>a</sup>	5.56 <sup>a</sup>	6.21 <sup>a</sup>	4.96 <sup>a</sup>
14:0	10.79 <sup>a</sup>	12.31 <sup>a</sup>	11.44 <sup>a</sup>	11.88 <sup>a</sup>	11.88 <sup>a</sup>	10.90 <sup>b</sup>	11.62 <sup>a</sup>	12.49 <sup>a</sup>	11.25 <sup>a</sup>	11.25 <sup>a</sup>	11.39 <sup>a</sup>	11.14 <sup>a</sup>
16:0	25.62 <sup>a</sup>	24.92 <sup>a</sup>	27.61 <sup>a</sup>	26.01 <sup>a</sup>	23.98 <sup>a</sup>	22.79 <sup>a</sup>	25.24 <sup>a</sup>	25.04 <sup>a</sup>	24.75 <sup>a</sup>	23.29 <sup>a</sup>	24.12 <sup>a</sup>	23.81 <sup>a</sup>
16:1, n-7	1.25 <sup>a</sup>	1.19 <sup>a</sup>	1.32 <sup>a</sup>	1.18 <sup>a</sup>	1.24 <sup>a</sup>	1.18 <sup>a</sup>	1.51 <sup>b</sup>	1.16 <sup>a</sup>	1.22 <sup>a</sup>	1.25 <sup>a</sup>	1.57 <sup>b</sup>	1.25 <sup>a</sup>
18:0	11.09 <sup>a</sup>	9.98 <sup>a</sup>	10.65 <sup>a</sup>	10.24 <sup>a</sup>	7.26 <sup>b</sup>	10.32 <sup>a</sup>	4.23 <sup>b</sup>	9.54 <sup>a</sup>	11.14 <sup>a</sup>	11.08 <sup>a</sup>	7.31 <sup>b</sup>	11.04 <sup>a</sup>
18:1, n-9c, 18:1, n-9t, 18:1, n-12t, 18:1, n-7c	26.19 <sup>a</sup>	23.08 <sup>a</sup>	22.29 <sup>a</sup>	23.68 <sup>a</sup>	21.89 <sup>a</sup>	22.80 <sup>a</sup>	19.94 <sup>b</sup>	22.62 <sup>a</sup>	24.25 <sup>a</sup>	24.58 <sup>a</sup>	22.50 <sup>a</sup>	26.00 <sup>a</sup>
CLA (1)	0.82 <sup>a</sup>	0.81 <sup>a</sup>	0.74 <sup>a</sup>	0.79 <sup>a</sup>	1.73 <sup>b</sup>	1.35 <sup>b</sup>	2.89 <sup>b</sup>	0.92 <sup>a</sup>	1.19 <sup>b</sup>	1.06 <sup>a</sup>	2.50 <sup>b</sup>	0.93 <sup>a</sup>
18:2, n-6c	2.31 <sup>a</sup>	2.14 <sup>a</sup>	2.22 <sup>a</sup>	2.17 <sup>a</sup>	2.81 <sup>b</sup>	3.10 <sup>b</sup>	2.40 <sup>a</sup>	2.19 <sup>a</sup>	2.22 <sup>a</sup>	2.48 <sup>a</sup>	2.39 <sup>a</sup>	2.55 <sup>a</sup>
18:3, n-3	0.70 <sup>a</sup>	0.57 <sup>a</sup>	0.53 <sup>a</sup>	0.52 <sup>a</sup>	0.97 <sup>a</sup>	2.98 <sup>b</sup>	0.60 <sup>a</sup>	0.80 <sup>a</sup>	0.87 <sup>a</sup>	0.95 <sup>b</sup>	0.66 <sup>a</sup>	0.78 <sup>a</sup>
18:3, n-6	0.05 <sup>a</sup>	0.04 <sup>a</sup>	0.03 <sup>a</sup>	0.00 <sup>a</sup>	0.14 <sup>a</sup>	0.29 <sup>b</sup>	0.14 <sup>a</sup>	0.17 <sup>a</sup>	0.08 <sup>a</sup>	0.12 <sup>a</sup>	0.09 <sup>a</sup>	0.04 <sup>a</sup>
20:3, n-3	0.03 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.32 <sup>b</sup>	0.03 <sup>a</sup>	0.04 <sup>a</sup>	0.03 <sup>a</sup>	0.12 <sup>b</sup>	0.04 <sup>a</sup>	0.02 <sup>a</sup>	0.03 <sup>a</sup>
20:3, n-6	0.02 <sup>a</sup>	0.03 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.06 <sup>b</sup>	0.03 <sup>a</sup>	0.04 <sup>a</sup>	0.02 <sup>a</sup>	0.03 <sup>a</sup>	0.02 <sup>a</sup>	0.04 <sup>a</sup>	0.03 <sup>a</sup>
20:4, n-6	0.25 <sup>a</sup>	0.21 <sup>a</sup>	0.22 <sup>a</sup>	0.21 <sup>a</sup>	0.39 <sup>b</sup>	0.21 <sup>a</sup>	0.47 <sup>b</sup>	0.20 <sup>a</sup>	0.32 <sup>b</sup>	0.22 <sup>a</sup>	0.30 <sup>b</sup>	0.23 <sup>a</sup>
20:5, n-3	0.08 <sup>a</sup>	0.07 <sup>a</sup>	0.07 <sup>a</sup>	0.06 <sup>a</sup>	2.41 <sup>b</sup>	0.14 <sup>a</sup>	0.48 <sup>a</sup>	0.13 <sup>a</sup>	0.50 <sup>b</sup>	0.15 <sup>a</sup>	0.30 <sup>a</sup>	0.09 <sup>a</sup>
22:3, n3	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.04 <sup>b</sup>	0.00 <sup>a</sup>	0.07 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.07 <sup>b</sup>	0.02 <sup>a</sup>
22:4, n6	0.07 <sup>a</sup>	0.08 <sup>a</sup>	0.07 <sup>a</sup>	0.07 <sup>a</sup>	0.10 <sup>a</sup>	0.07 <sup>a</sup>	0.10 <sup>a</sup>	0.09 <sup>a</sup>	0.10 <sup>a</sup>	0.09 <sup>a</sup>	0.10 <sup>a</sup>	0.10 <sup>a</sup>
22:5, n-3	0.18 <sup>a</sup>	0.15 <sup>a</sup>	0.14 <sup>a</sup>	0.13 <sup>a</sup>	0.64 <sup>b</sup>	0.19 <sup>a</sup>	0.49 <sup>b</sup>	0.15 <sup>a</sup>	0.60 <sup>b</sup>	0.24 <sup>a</sup>	0.33 <sup>b</sup>	0.18 <sup>a</sup>
22:6, n-3	0.07 <sup>a</sup>	0.06 <sup>a</sup>	0.05 <sup>a</sup>	0.06 <sup>a</sup>	0.13 <sup>a</sup>	0.16 <sup>a</sup>	2.27 <sup>b</sup>	0.13 <sup>a</sup>	0.15 <sup>a</sup>	0.11 <sup>a</sup>	0.79 <sup>b</sup>	0.10 <sup>a</sup>
n-3	0.99 a	0.81 a	0.76 a	0.73 a	4.13 b	3.34 b	1.68 a	1.11 a	1.99 b	1.38 a	1.38 a	1.10 a
n-6	2.70 a	2.50 a	2.56 a	2.47 a	3.47 b	3.70 b	3.17 b	2.67 a	2.69 b	2.93 a	2.92 a	2.95 a
n-3/n-6	0.37 a	0.32 a	0.30 a	0.30 a	1.19 a	0.90 b	0.53 b	0.42 a	0.74 b	0.47 a	0.47 a	0.37 a
n-3 : n-6 (1:X)	2.73 a	3.09 a	3.37 a	3.38 a	0.84 b	1.11 b	1.89 a	2.41 a	1.35 b	2.12 a	2.12 a	2.68 a
LC PUFA n-3	0.36 a	0.30 a	0.28 a	0.27 a	3.29 b	0.52 a	3.35 b	0.44 a	1.27 b	0.54 a	1.51 b	0.42 a
LC PUFA n-6	0.34 a	0.32 a	0.31 a	0.30 a	0.52 b	0.31 a	0.63 b	0.31 a	0.39 a	0.33 a	0.44 a	0.36 a
LC PUFA n-3/ LC PUFA n-6	1.06 a	0.94 a	0.90 a	0.90 a	6.33 b	1.68 a	5.32 b	1.42 a	3.26 b	1.64 a	3.43 b	1.17 a
LC n-3 : n-6 (1:X)	3.11 a	2.93 a	2.91 a	3.00 a	12.17 b	5.41 a	8.44 b	4.58 a	8.35 b	4.96 a	7.80 b	3.24 a

<sup>a,b</sup> – values which are not marked with the same letter, are statistically different at least P<0.05

FA – fatty acid; CLA – conjugated linoleic acid; LC PUFA – long chain polyunsaturated fatty acid

**Table 4.** Average values of fatty acids, secreted in milk in different periods of the experiment by groups (wt %)

**Myristic acid (14:0)** in goat milk fat represented from 10.0 to 13.5 wt % of fatty acids. The content was similar than in Sanz Sampelayo's et al. (2002) research. Throughout supplementing the fatty acids, a statistically significant reduction of myristic acid level in milk fat was noticed only in the ALFA group ( $p < 0.05$ ). Other variations were not statistically significant and the level of myristic acid was similar among groups. Myristic content in goat milk fat was very stable during the experiment.

**Miristoleic acid (14:1)** in goat milk fat was detected in the content from 0.12 to 0.40 wt %, while Sanz Sampelayo et al. (2002) listed the values between 0.41 and 0.64%. We have not observed differences among groups and even daily fluctuations of miristoleic acid in goat

milk fat were very small. Miristoleic acid values were fluctuating at least in DHA group. Differences among groups were not found in any period of the experiment.

There was between 20 and 29 wt % of the **palmitic** acid (16:0) in the goat milk fat. Sanz Sampelayo et al. (2002) indicated values of the palmitic acid between 24.6 and 27.7%. There were no statistically significant differences observed among groups before the supplementation of the fatty acids to the goat diet. There was a trend of decreasing values during and immediately after the supplementation of fatty acids, especially in groups DHA and ALFA as well as in the EPA group.

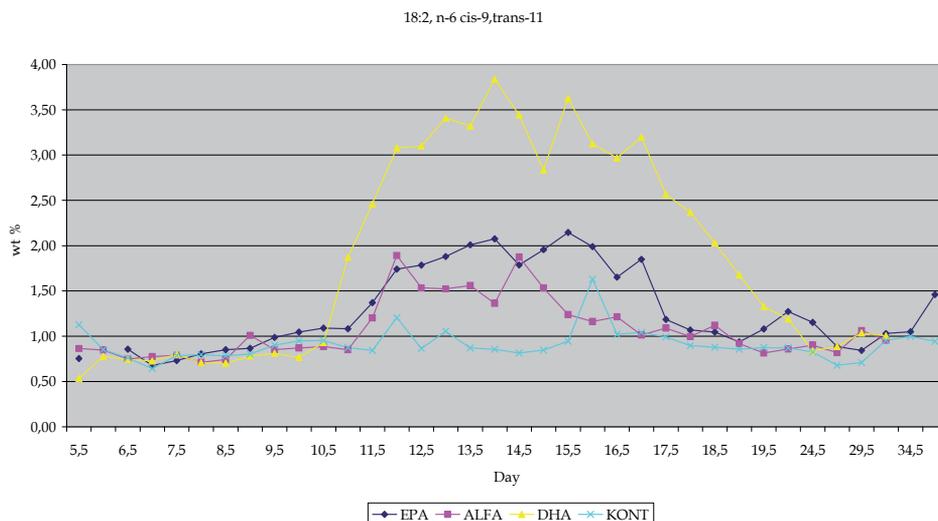
In goat milk fat, between 1.06 and 1.73 wt % of the **palmitoleic** acid (16:1, n-7) was determined. There were no differences in the level of this fatty acid among groups before the supplementation with fatty acids. Among groups EPA, ALFA, and KONT, no statistically significant differences in the content of the palmitoleic acid in milk fat were observed neither during the supplementation with fatty acids nor after that. The content of palmitoleic acid in DHA group increased statistically significant (from 1.30% to 1.70%) from the second day of the supplementation with fatty acids. The high level of this fatty acid lasted till the ninth day after the supplementation ( $p < 0.001$ ). The supplementation with unprotected n-3 fatty acids in cows reduced the content of palmitoleic acid in milk fat (Chilliard et al., 2001), what is contrary to our results.

**Stearic** fatty acid (18:0) in the goat milk fat was presented in the level from 2 to 14 wt %. There were no differences in the stearic acid content among groups before supplementation with fatty acids. Differences appeared during the supplementation with fatty acids, which were expressed the most in DHA group, where the percentage of stearic fatty acid fell from about 10 to less than 3% ( $p < 0.001$ ). The fall of stearic acid during the supplementation with fatty acids was detected also in EPA group ( $p < 0.05$ ), which was somewhat less pronounced, and the level of stearic acid re-established to the previous level within two days after the end of the supplementation with fatty acids. The previous level of stearic acid in DHA group was re-established five days after the end of supplementation with fatty acids. In ALFA and KONT group, there were no statistically significant differences in the levels of stearic fatty acid throughout the experiment. This information is a further indication, that the biodegradation of long-chain fatty acids (DHA) does not expire until the stearic acid, but there are several isomers of conjugated cis- and trans- C 18:2 fatty acids (Gulati et al., 1997; Gulati et al., 2000).

The content of **oleic** fatty acid (18:1, n-9) was in our experiment determined in the concentration from 19.0 to 28.0 wt %. During the supplementation with fatty acids, the content of oleic acid statistically significantly declined in EPA and DHA groups. An increase of the content of oleic acid in milk was observed in groups KONT and ALFA, as during as well as after the supplementation with fatty acids, but differences in these two groups before and after the supplementation were not statistically significant. Sanz Sampelayo et al. (2002) noted the content of oleic acid in goat milk around 22 to 24% and stated that despite the addition of various concentrations of protected polyunsaturated fatty acids the content of oleic fatty acid in goat milk remained fairly constant.

**Conjugated linoleic** acids (CLA) are a family of at least 28 isomers of linoleic acid found mainly in the meat and dairy products derived from ruminants. Several names could be

found for conjugated linoleic acid, most often conjugated linoleic acid, then rumenic or ruminal acid or cis-9, trans-11 octadecadienoic acid. It is one of those found only in ruminants and is a product of incomplete hydrogenation of fatty acids in the rumen (Clegg et al., 2001; Chouinard et al., 1999). In goats fed with fish oil (Gulati et al., 2000) mainly vaccenic fatty acid is formed due to the altered pattern of the biohydrogenation. In our experiment (Figure 2), goat milk of all observed groups contained less than 1.0% of the conjugated linoleic acid before the supplementation with fatty acids. During the second period, groups EPA, ALFA, and DHA statistically significantly differed ( $p < 0.05$ ) from KONT group. The largest increase of the conjugated linoleic acid content during the supplementation appeared in DHA group, to over 3.0%. The content of conjugated linoleic acid in EPA group increased to 2.0%, and in ALFA group to 1.5%. The effect of the conjugated linoleic acid in DHA group was detected ten days after the supplementation with fatty acids. In nature, the most of the conjugated linoleic acids have their origin in alpha linolenic acid (Gulati et al., 2000), while in our experiment, the conjugated linoleic acid increased the most after feeding goats with docosahexaenoic acid (group DHA). Chilliard et al. (2001) fed cows with 200 to 300 g of the fish oil daily where the content of the conjugated linoleic acid increased from 0.2 to 0.6% to 1.5 to 2.7%. Authors mentioned that mainly rumenic acid increased which is presented also in our results, whereas the vaccenic acid occurred only in trace amounts and only a short time so that the findings published by Gulati et al. (2000) we could not confirm.



**Figure 2.** Average value of rumenic acid in goat milk

Conjugated linoleic acid is an intermediate product of the biohydrogenation, therefore its high concentration in DHA group was logical, since the degradation of the docosahexaenoic acid in the rumen is the slowest. The concentration of the conjugated linoleic acid in goat milk fat was relatively high also in ALFA group, knowing that the biohydrogenation of the  $\alpha$ -linolenic acid is the fastest (Gulati et al., 1999), what we also observed in an increased concentration of C 18:1 in ALFA group. The conjugated linoleic acid is synthesised in the

mammary gland of lactating animals and in the muscles of young animals. In our experiment, the conjugated linoleic acid probably did not originate only from the supplemented fatty acids, what was found also by Griinari et al. (2000).

Before the supplementation with fatty acids, there was from 2.00 to 2.66 wt % of the **linoleic** acid (18:2, n-6) determined in goat milk fat in all groups. During the supplementation, the percentage increased in EPA group to 2.92% and in ALFA group to 3.4% ( $p < 0.001$ ). Three days after the end of supplementation, the percentage dropped back to the previous value. There were no changes in the content of linoleic acid during the whole experiment in DHA and KONT groups.

**$\alpha$ -linolenic** (18:3, n-3 or octadecatrienoic) acid in goat milk was found in 0.50 to 1.00 wt %. During the supplementation with fatty acids, the percentage of  $\alpha$ -linolenic acid increased only in the ALFA group to 3.20% and it dropped back to the previous level 0.50% ( $p < 0.001$ ) four days after ending the supplementation. Thus, goats can successfully build linolenic fatty acid into milk fat when they are supplemented with this fatty acid.

There was less than 0.06 wt % of the  **$\gamma$ -linolenic** or *cis*-6,9,12-octadecatrienoic acid (18:3, n-6) in goat milk fat in all observed groups at the beginning of the experiment. After the addition of fatty acids into the goat diet, the content of the  $\gamma$ -linolenic acid increased in EPA group to 0.18%, in DHA group to 0.20% ( $p < 0.05$ ), while the maximum increase to 0.33% appeared in ALFA group ( $p < 0.001$ ). The increased content reflected three days after the end of supplementation with fatty acids and then decreased to the started value. Thus,  $\gamma$ -linolenic fatty acid is also successfully transferred into the milk fat, the fastest from  $\alpha$ -linolenic fatty acid.

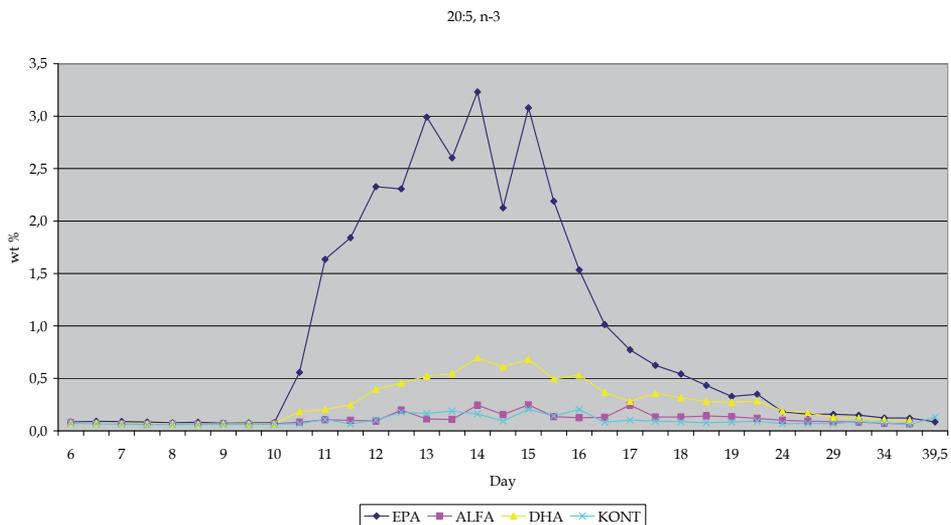
The content of ***cis*-11,14,17-eicosatrienoic** acid (20:3, n-3) in goat milk fat at the beginning of the experiment was 0.02 to 0.04 wt %. During the supplementation with fatty acids, the content increased only in the EPA group to 0.43% ( $p < 0.001$ ). The content did not statistically significantly change in the other three groups. It is obviously, that eicosapentaenoic fatty acid was formed as a product of biohydrogenation, which occurred as an intermediate product only in milk fat of the EPA group.

At the beginning of the experiment, the content of ***cis*-8,11,14-eicosatrienoic** acid (20:3, n-6) was 0.02 to 0.03 wt %. During the supplementation with fatty acids, a slight increase of the content of *cis*-8,11,14-eicosatrienoic acid in DHA group to 0.04 to 0.05% and in EPA group to 0.08% was detected. Statistically significant increase of the *cis*-8,11,14-eicosatrienoic acid in goat milk fat occurred only in EPA group, from the third to the fifth day of the supplementation ( $p < 0.05$ ). Immediately after ending the supplementation, the percentage of the *cis*-8,11,14-eicosatrienoic acid decreased in all observed groups to the value before the supplementation.

**Arachidonic** acid (20:4, n-6) was found in goat milk fat at the beginning of the experiment on average 0.20 wt %. During the supplementation with fatty acids, the percentage increased to 0.40% in EPA group and even to 0.60% in DHA group. Three days after ending the supplementation, the content of arachidonic acid in EPA group decreased to its starting level, while in DHA group, the content of arachidonic acid decreased after five days after the

end of the supplementation. The statistically significant increase in arachidonic acid content during the supplementation with fatty acids occurred in EPA and DHA groups ( $p < 0.05$ ).

**Eicosapentaenoic acid** (20:5, n-3 or EPA) was determined in the goat milk fat at the beginning of the experiment in the content from 0.10 to 0.25 wt %. During the supplementation with fatty acids, the percentage changed in DHA group to 0.50 to 0.69%, while in EPA group the percentage rose to 2.00 to over 3.23%, as shown in Figure 3. Results showed that the level of eicosapentaenoic acid increased more than 30-times in milk, when animals consumed the eicosapentaenoic acid in the diet ( $p \leq 0.001$ ). Statistically significantly higher content of eicosapentaenoic acid was observed in goat milk fat also five days after the end of supplementation, but only in EPA group.



**Figure 3.** Average value of cis-5,8,11,14,17-eicosapentaenoic acid in goat milk

The maximum concentration of **eicosapentaenoic acid** was found on the fourth day of the supplementation with fatty acids, while Kitessa et al. (2001) noted the maximum on the sixth day, but they added only 160 mg of eicosapentaenoic acid per day as unprotected supplement, which was 125-times lower than in our case. Chilliard et al. (2001) stated the efficiency of transfer of the unsaturated fatty acids into cow's milk. The transfer was 2.6% for the eicosapentaenoic acid into cow's milk. In goats fed unprotected fatty acids, the transfer was 3.5% and 7.6% in goats fed protected fatty acids (Kitessa et al., 2001). The transfer of the eicosapentaenoic acid in our experiment was 7.1%, what had probably several reasons. The first reason can be relatively large dose of the supplemented eicosapentaenoic acid, the second short-term administration, whereas the ruminal microflora could not adapt for biohydrogenation of the eicosapentaenoic acid in this short time, and third, that according to the method of administering fatty acids the eicosapentaenoic acid partially passed through the rumen over esophageal gutter directly into the stomach.

According to the fact that the transfer of eicosapentaenoic acid through diet into the milk can be so effective, it is important how to produce milk enriched with n-3 and n-6 fatty

acids. Consumers are increasingly use milk with lower fat content. Thus, milk enriched with n-3 and n-6 fatty acids would significantly help to more correct and balanced diet, especially in children and elderly people.

Before supplementation with fatty acids, the content of **docosatrenoic** fatty acid in goat milk fat (22:3, n-3) was in all groups below the detection limit. During the supplementation, the increased content of docosatrenoic fatty acid was detected in EPA group, 0.03 to 0.06 wt %, and in DHA group, 0.6 to 0.11 wt % ( $p < 0.001$ ). The increased value of the docosatrenoic fatty acid lasted until the 18<sup>th</sup> day of the experiment, and then it fell again below the detection limit. The value of the KONT group and ALFA group was below the detection limit through the whole time of experiment.

There was from 0.046 to 0.136 wt % of the **docosatetraenoic** fatty acid (22:4, n-6) in goat milk fat. During the supplementation, a slight increase of the docosatetraenoic fatty acid in EPA and DHA groups was noticed, but differences between groups in different periods of the experiment were not statistically significant.

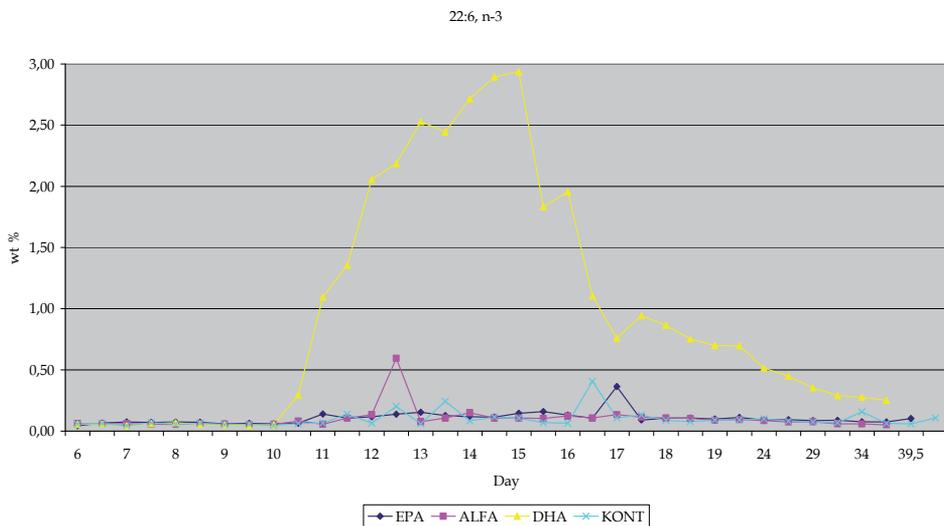
**Docosapentaenoic** fatty acid (22:5, n-3) in goat milk fat was found in the concentration from 0.15 to 0.22 wt %. During the supplementation with fatty acids, the percentage of docosapentaenoic fatty acid increased in DHA group to 0.59% and in EPA group to 0.85% ( $p < 0.001$ ). In both groups, an increased concentration of docosapentaenoic fatty acid reflected still 15 to 20 days after the end of the supplementation. The concentration was statistically highly significantly greater than the ALFA and KONT groups. It looks like docosapentaenoic fatty acid passes into the udder directly by blood, as it is not produced in the mammary gland de novo.

Only 0.05 to 0.1 wt % was the concentration of **docosahexaenoic** (22:6, n-3 or DHA) fatty acid in goat milk fat at the beginning of the experiment. During the supplementation with fatty acids, the percentage increased only in DHA group to 2.80%, and after the end of supplementation, it gradually declined. Even nine days after the end of supplementation with fatty acids, milk fat contained more than 0.50% of docosahexaenoic fatty acid (Figure 4). There was 3 to 4-times higher content of docosahexaenoic fatty acid in DHA group than in other groups ( $p < 0.001$ ) even 20 days after the supplementation. The maximum concentration of docosahexaenoic fatty acid in goat milk fat in our experiment was found on the fifth day, while Kitessa et al. (2001) found the maximum concentration on the sixth day, but they added only 580 mg of docosahexaenoic fatty acid per day as an unprotected supplement, which is 34.5 times less than in our experiment.

The effectiveness of transfer the docosahexaenoic fatty acid into milk was observed in cows by Chilliard et al. (2001), which amounted 4.1%. In goats, it amounted 3.5% for unprotected fatty acids and 7.6% for protected fatty acids (Kitessa et al., 2001). The estimated transfer of docosahexaenoic fatty acid in our experiment was 7.84.

There was 53 to 57 wt % of the **medium chain** fatty acids in goat milk fat before the supplementation with fatty acids. After the supplementation, a decrease of the medium chain fatty acids was noticed in EPA, DHA, and ALFA group to 46 to 50%. The level of

medium chain fatty acids re-established to the starting level in three days after ending the supplementation in EPA and ALFA groups and in ten days in DHA group ( $p < 0.05$ ).



**Figure 4.** Average value of cis-4,7,10,13,16,19- docosahexaenoic fatty acid in goat milk

As reported Kitessa et al. (2001), a significant decrease appeared in C10 to C16 fatty acids after adding fish oil into the diet for goats, but when Chilliard et al. (2001) fed cows with fish oil only, they noticed a slight decrease in C4 to C14 fatty acids, or even 1.3% increase of these fatty acids when adding fish oil in the duodenum. In the experiment by Kitessa et al. (2001), a group of animals were supplemented a protected fish oil from 19<sup>th</sup> to 26<sup>th</sup> day and then unprotected fish oil from the 37<sup>th</sup> to 42<sup>nd</sup> day. Due to the significantly reduced feed intake and milk production in sheep the unprotected fish oil was administered a short time. Between one and another type of feeding was only eight days, which is questionable. It is possible that there was an influence of the previous supplementation, because our data showed that the effect of supplementation with some types of fatty acids can take more than 10 days on changes in the fermentation of medium chain fatty acids. Even Sanz Sampelayo et al. (2002) in goats found that the percentage of total unsaturated fatty acids reduced after the supplementation with protected polyunsaturated fatty acids.

The content of **monounsaturated** fatty acids in goat milk fat in our experiment ranged from 23 to 28 wt %, which reduced during the supplementation with fatty acids to 22% in EPA group and to 21% in DHA group. The decrease was statistically significant ( $p < 0.05$ ) during the supplementation in EPA and DHA groups, while the reduction of monounsaturated fatty acids did not occur in ALFA and KONT groups. As reported Sanz Sampelayo et al. (2002), the supplementation of 9% polyunsaturated fatty acids only slightly increased the content of monounsaturated fatty acids, while the supplementation of 12% polyunsaturated fatty acids significantly increased the content of monounsaturated fatty acids.

Before the supplementation with fatty acids, **polyunsaturated** fatty acids were found in goat milk fat in the concentration from 4 to 6 wt %. The same level of polyunsaturated fatty acids

stayed in ALFA group throughout the whole time of experiment. A statistically significant ( $p=0.001$ ) increase of the polyunsaturated fatty acids concentration appeared during the supplementation with fatty acids in EPA group (to 11%), ALFA group (9 to 10%), and in DHA group (11 to 11.9%). The peak in concentration of polyunsaturated fatty acids was achieved in EPA and DHA group on the fourth and fifth day of the supplementation and in ALFA group on the third day of the supplementation. The increased percentage of polyunsaturated fatty acids in goat milk fat persisted from 10 to 14 days in EPA, ALFA, and DHA groups.

The passage of the supplemented polyunsaturated fatty acids from the gastrointestinal tract into milk was estimated on the basis of the differences between the content of fatty acids before supplementation and the difference between KONT group and other groups during the supplementation and thereafter, taking into account the amount of milked milk during the supplementation and 14 days thereafter. The results are shown in Table 3, where it is clear that the passage of the conjugated linoleic acid into milk was 12.79%, 14.03% of the eicosapentaenoic acid, and 21.13% of the docosahexaenoic acid. The differences were statistically significant ( $p < 0.05$ ).

GROUP	EPA	ALFA	DHA	KONT
Supplement	EPA	CLA	DHA	no
Supplemented PUFA during experiment (g)	95	72	75	0
PUFA appeared in milk from the 6 <sup>th</sup> to the 34 <sup>th</sup> day (g)	50.78	46.66	53.3	37.45
Difference or estimated passage (%)	14.03	12.79	21.13	0

EPA – eicosapentaenoic acid; CLA – conjugated linoleic acid; DHA - docosahexaenoic acid; PUFA –polyunsaturated fatty acids

**Table 5.** Estimated passage of the supplemented polyunsaturated fatty acids from food into milk

The **ratio between n-3:n-6** fatty acids before the supplementation with fatty acids was the same in all groups (1:3.50) and remained unchanged throughout the experiment only in KONT group. In all other groups, the ratio reduced during the supplementation with fatty acids to 1:1 and even to 1:0.67. It was gradually establishing back more than 20 days after the end of the supplementation. The differences before and after supplementation were statistically highly significant ( $p < 0.001$ ).

### 3.3. Correlations between somatic cell count and some fatty acids

Correlations between somatic cell count and some fatty acids during the experiment were calculated by the Pearson correlation coefficient. The same correlations were calculated also for the second and third period of the experiment (from the 11<sup>th</sup> to the 65<sup>th</sup> day) and for the period from the 21<sup>st</sup> to the 65<sup>th</sup> day of the experiment. Statistically significant correlations between somatic cell count and C10 throughout the whole experiment were found in EPA group ( $r=0.24$ ), ALFA ( $r=-0.18$ ), and ( $r=-0.17$ ) KONT group. The correlations between somatic cell count and C12 and between somatic cell count and C14 were statistically significant

throughout the whole experiment only in EPA group ( $r=0.25$  and  $r=0.24$ , respectively;  $p<0.01$ ). From the 11<sup>th</sup> to the 65<sup>th</sup> day of the experiment, there were only correlations between somatic cell count and C10 in DHA group ( $r=-0.30$ ), between somatic cell count and C12 in DHA group ( $r=-0.37$ ), and between somatic cell count and C14 in ALFA ( $r=0.26$ ) and DHA ( $r=-0.29$ ) groups found statistically significant ( $p<0.05$ ). From the 21<sup>st</sup> to the 65<sup>th</sup> day of the experiment, correlations between somatic cell count and C10 in EPA ( $r=-0.45$ ) and DHA ( $r=-0.46$ ) groups, between somatic cell count and C12 in EPA ( $r=-0.43$ ), DHA ( $r=-0.53$ ), and KONT ( $r=0.39$ ) groups, and between somatic cell count and C14 in ALFA ( $r=-0.59$ ), DHA ( $r=-0.57$ ), and KONT ( $r=0.44$ ) groups were statistically significant ( $p<0.05$ ).

Correlation between somatic cell count and C18:1 was statistically significant only in EPA group ( $r=-0.24$ ) throughout the whole experiment, in DHA group ( $r=0.47$ ) from the 11<sup>th</sup> to the 65<sup>th</sup> day of the experiment, and in EPA ( $r=0.42$ ), ALFA ( $r=-0.49$ ), and DHA ( $r=0.67$ ) groups from the 21<sup>st</sup> to the 65<sup>th</sup> day of the experiment. Between somatic cell count and C18:3, the correlation was statistically significant only in ALFA ( $r=-0.43$ ) group from the 11<sup>th</sup> to the 65<sup>th</sup> day of the experiment. No correlations between somatic cell count and C20:4 throughout the whole experiment were statistically significant. There were only correlations between somatic cell count and C20:4 in EPA group from the 11<sup>th</sup> to the 65<sup>th</sup> day of the experiment ( $r=0.36$ ) and from the 21<sup>st</sup> to the 65<sup>th</sup> day of the experiment ( $r=0.66$ ) statistically significant.

Statistically significant correlation between somatic cell count and monounsaturated fatty acids throughout the whole experiment was found only in ALFA group ( $r=-0.22$ ) and from the 11<sup>th</sup> to the 65<sup>th</sup> day of the experiment in DHA group ( $r=0.50$ ). From the 21<sup>st</sup> to the 65<sup>th</sup> day of the experiment, this correlation was statistically significant in EPA ( $r=0.43$ ), ALFA ( $r=-0.50$ ), and DHA ( $r=0.68$ ) groups. Between somatic cell count and polyunsaturated fatty acids, only the correlation in ALFA group from the 21<sup>st</sup> to the 65<sup>th</sup> day of the experiment was found statistically significant ( $r=-0.49$ ).

#### 4. Conclusions

Our research proved that the supplementation of fatty acids into the diet had no effect on daily milk yield of goats. In ALFA group, a statistically significant impact on the increase of the protein content in milk ( $p<0.01$ ) during the supplementation and thereafter was observed. Fat content was increasing during the supplementation and thereafter in ALFA group, while in EPA and DHA groups, fat content significantly reduced during the supplementation with fatty acids ( $p<0.001$ ) and a few days thereafter. This finding indicates that the supplementation with fatty acids (eicosapentanoic and docosahexanoic fatty acid) had a negative impact on the milk fat production. Lactose content did not change significantly during the supplementation and no differences were found among groups. Non-fat dry matter content was the highest in ALFA group, its increased value reflected even after the end of the supplementation with fatty acids.

The supplementation of  $\alpha$ -linoleic fatty acid decreased somatic cell count in milk, even 30 days after the end of the supplementation. Statistically significant decrease of somatic cell

count, compared to the period prior to the supplementation, was appeared till the 29<sup>th</sup> day after the end of the supplementation ( $p < 0.05$ ). The number of micro organisms in milk is the result of hygienic conditions at milking, hygiene of milking personnel, equipment, environment and hygiene of the animals. In the case of our study, it has been established that the lower number of micro organisms was the consequence of better hygiene during the experiment due to the experimentalists' presence.

The supplementation of  $\alpha$ -linoleic, eicosapentanoic and docosahexanoic fatty acids had different effects on the composition of fatty acids in milk fat. Eicosapentanoic fatty acid supplemented into the diet of EPA group increased the following fatty acids: capric, lauric, myristic, conjugated linoleic, linoleic,  $\gamma$ -linolenic, cis-11,14,17-eicosatrienoic, cis-8,11,14-eicosatrienoic, arachidonic, eicosapentaenoic, docosatrienoic, docosatetraenoic, and docosapentaenoic acid. The supplementation of eicosapentanoic fatty acid decreased palmitic, stearic, and oleic fatty acid.  $\alpha$ -linoleic fatty acid supplemented to ALFA group increased the following fatty acids: lauric, miristoleic, oleic, conjugated linoleic, linoleic,  $\alpha$ -linoleic,  $\gamma$ -linolenic acid. This means that there was no elongation from  $\alpha$ -linoleic acid into fatty acids with longer chain. A decrease was observed in myristic, palmitic, and docosatetraenoic acid. DHA group was supplemented with docosahexanoic fatty acid where the increase of the following fatty acids was recorded: capric, lauric, myristic, palmitoleic, conjugated linoleic, linoleic,  $\gamma$ -linolenic, cis-8,11,14-eicosatrienoic, arachidonic, eicosapentaenoic, docosatrienoic, docosatetraenoic, docosapentaenoic, docosahexanoic acid, while a decrease was noticed in the following fatty acids: miristoleic, palmitic, stearic, and oleic acid. In the control group, only slight variations in some fatty acid levels were recorded, which were not statistically significant.

Research showed that the supplementation of  $\alpha$ -linoleic acid had a positive impact on reduction of the somatic cell count in goat milk. However, the surprising result was found, that the supplementation of eicosapentanoic and docosahexanoic acid did not affect the reduction of somatic cell count in the same extent. There is a question, whether this is the result of the supplement or of the n-3:n-6 ratio which changed after the supplementation. Since the ratio n-3:n-6 changed to the similar value when other fatty acids were supplemented and the effect was not the same, it seems that the n-3:n-6 ratio was not the cause of this effect. It is suggested that  $\alpha$ -linoleic acid could be rapidly incorporated into cell membranes, which displace arachidonic acid. This is resulted in more flexible cell membranes and better anti-inflammatory effect. Perhaps this mechanism was the one which contributed to the reduction of somatic cell count. For further research, it would be necessary to also include this kind of analysis. The results also showed that the transition of long chained polyunsaturated fatty acids into goat milk appeared relatively in large extent, therefore, polyunsaturated fatty acids occur in milk fat very quickly after their consumption.

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# **Nutrition and Health of Dairy Animals**

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50804>

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## **1. Introduction**

Modern breeds of dairy animals are able to produce huge amount of milk. In attempt to consume, digest and metabolize enough nutrients to satisfy lactation needs, those animals are exposed to serious stress conditions that can affect their health. Health problems which arise from those conditions are mainly related to impaired ability to metabolize enough nutrients to compensate for those lost in milk. They are known as metabolic or production diseases and may be of great economic importance in milk production systems.

Although metabolic diseases have become a common problem on dairy farms, they still require a serious attention to be controlled. The incidences of these disorders can be reduced by proper nutrition of animals. Also, some of the specific strategies in feeding practice offer additional advantages in prevention of nutrition-related metabolic diseases.

## **2. Nutrition-related metabolic disorders, their etiology and consequences**

The most important metabolic diseases in dairy cows, ewes and goats can be discussed as energy and/or fiber related, lipid related or vitamin and mineral related disorders. However, they are not easily categorized according to their cause, thus the pathogenesis and hallmarks of each disease should be considered for their categorization. Energy related disorders (related to energy density of the diet or feed intake) include fatty liver and ketosis, rumen acidosis, laminitis, displaced abomasums and milk fat depression. As energy density and fiber content of the diets are often inversely related, most of these diseases can be considered as fiber related too. Fatty liver and ketosis also can be categorized as lipid related disorders due to changes in lipid metabolism in affected animals (Grummer, 1993). The most important mineral/vitamin related disorders are hypocalcaemia, hypomagnesaemia, udder edema, retained placenta and metritis. Not all of them are ultimately caused by mineral composition of the diet, but may be prevented by manipulation of minerals or vitamins in the diet. Broken homeostatic mechanisms often become major etiological factors in the

development of metabolic disease, and consequences varies from reduced production and impaired reproductive performances to increased risk to develop other diseases.

## 2.1. Fatty liver

Fatty liver (hepatic lipidosis, fat cow syndrome) is a metabolic disorder characterized by a high content of lipids and triglycerides in the liver (Ingvarstsen, 2006). The disease occurs in periparturient period, primarily in the first 4 weeks after calving (Grummer, 1993), and as a secondary disease of other production diseases that depress appetite or increase body fat mobilization. The clinical symptoms comprise depression, lack of appetite and weight loss, and the cows are weak and apathetic (Radostits et al., 2000). Most cows suffer from non-specific clinical signs including reduced rumen motility and decreased milk yield. However, this disease occurs especially in its subclinical form and can be a problem for up to 50% of cows in early lactation (Ingvarstsen, 2006).

Risk factors for fatty liver in dairy cows may be nutritional, managerial, and genetic. Prepartum risk factors include obesity, severe feed restriction, feeding excess energy, and long calving interval. Postpartum risk factors are different diseases and infection, fasting and feed restriction, as well as ketogenic diets and sudden feed changes (Bobe et al., 2004). Among the highest risk factors of fatty liver is a high rate of mobilization of body lipids around calving in overconditioned cows which have been overfed in late lactation and the dry period and had low feed intake around calving (Ingvarstsen, 2006). Increased prepartum body weight gain or body condition increase liver fat content and indicate increased risk of fatty liver and related disorders. Therefore, the primary nutritional risk factor for fatty liver is obesity. In obese cows, lipolysis of adipose tissue is increased more during periparturient period than in cows with normal body condition. Obese cows have a greater decrease in feed intake around calving and, therefore, have a more severe negative energy balance (Bobe et al., 2004). During the negative energy balance body fat is mobilized into the bloodstream in the form of non-esterified fatty acids (NEFA). NEFA are taken by the liver in proportion to their supply, but the liver does not have capacity sufficient to oxidize and use all amount of NEFA for energy. Therefore, cows are predisposed to accumulate NEFA as triglycerides within the liver when large amounts of NEFA are released from adipose tissue (Overton & Waldron, 2004). Rate of triglyceride synthesis is proportional to plasma NEFA concentration, thus fatty liver is likely to develop when plasma NEFA are elevated (Grummer, 1993).

Severe negative energy balance caused by low feed intake around calving leads to increase in body fat mobilization and plasma NEFA concentration. Thus, the severity of fatty liver may be reduced if drop in feed intake before calving is prevented (Bertics et al., 1992).

Diets deficient in cobalt (Co) have been shown to cause fatty liver in sheep. It has been termed as ovine white liver disease. Hepatic lipidosis in goats due to feeding low levels of Co in their diet has also been reported (Johnson et al., 2004).

Fatty liver is associated with decreased health status and reproductive performance. In severe cases, milk production and feed intake are also decreased (Bobe et al., 2004).

Although fatty liver is often a reversible condition, it predisposes cows to reduced liver function and to a number of other production diseases (Ingvartsen, 2006). The incidence of fatty liver is strongly associated with the incidence of especially ketosis and displaced abomasum because these disorders are related to severe negative energy balance too (Bobe et al., 2004). Inhibition of gluconeogenesis may also occur when triglycerides accumulate in liver (Overton & Waldron, 2004) and additionally increase the risk for ketosis. Different aspects of the immune response are also suppressed in cows with fatty liver (Bobe et al., 2004; Overton & Waldron, 2004) and those animals are more prone to infectious diseases. However, the exact costs of fatty liver are difficult to estimate because condition can be diagnosed only by liver biopsy (Bobe et al., 2004).

## 2.2. Ketosis

Ketosis (acetonemia) is a metabolic disorder characterized by elevated concentrations of the ketone bodies (acetoacetate, beta-hydroxybutyrate and acetone), and a low to normal concentration of glucose in the blood. This disorder occurs both subclinically and clinically. Prevalence of subclinical ketosis is the highest in the first 2 weeks of lactation ranging from 8,9% to 34% in various studies (Ingvartsen, 2006; Rushen et al., 2008). Blood glucose in clinically affected cows fall below the level required to support nerve and brain function and cows often exhibit signs of central nervous system dysfunction. Ketotic cows also suffer inappetence which further exacerbates the negative energy balance. Milk production falls seriously which helps the cow to cope with the negative energy balance (Goff, 2006a).

Four main types of ketosis are: primary ketosis, secondary ketosis, butyric acid ketosis and underfeeding ketosis (Ingvartsen, 2006). Classical or primary ketosis (also called spontaneous or production ketosis) generally occurs in cows during the first 2 to 4 weeks of lactation (Goff, 2006a). It develops when the glucose demand exceeds the gluconeogenesis capacity of the liver resulting in increased ketogenesis, and thus in high concentrations of ketone bodies in blood, milk, and urine. The disorder is mainly seen in obese cows. Secondary ketosis results from another disease that depresses feed intake and increases body fat mobilization (Ingvartsen, 2006).

Butyric acid ketosis is caused by large amounts of butyrate in the feed and probably by related depressed feed intake. Silage with high butyrate concentrations results in an increased concentration of beta-hydroxybutyrate in the blood, and such silage is consumed in lower amount than normal one. Underfeeding ketosis occurs especially in cows that are fed insufficiently. Underfed animals are deficient in glucogenic precursors and this condition then leads to increased ketogenesis (Ingvartsen, 2006).

Another form of ketosis often seen in US dairies usually occurs in cows less than 10 days in lactation. It can often be difficult to treat as is generally accompanied by some degree of fatty liver (ketosis/fatty liver complex). Ketosis, like fatty liver, also occurs during periods of elevated plasma NEFA. Clinical symptoms are similar to classical ketosis, but ketotic state was actually preceded by an increase in fat accumulation in the liver (Goff, 2006a).

Major factors directly or indirectly increasing the risk of fatty liver and ketosis are: overconditioning at calving, excessive mobilization of body fat, low nutrient intake, some nutrient or diet specific factors and management and environmental stress (Ingvarsen, 2006). Ketosis is a metabolic condition that occurs primarily when a cow is in negative energy balance immediately after calving. To support the energy demands, the body mobilizes fat reserves. Excessive release of NEFA from fat depots may overwhelm the capacity of the liver to use the fatty acids as a fuel. They are instead converted to ketone bodies (Rushen et al., 2008). Feed intake is naturally depressed by about 20% around the time of calving. Simultaneous with the feed intake depression, plasma NEFA and liver triglyceride concentrations exhibited their greatest increase (Goff, 2006a). All factors exacerbating negative energy balance and depression of feed intake around calving increase the risk of ketosis, especially in overconditioned cows.

Negative energy balance, high blood levels of NEFA or beta-hydroxybutyrate and ketosis reduce the response capacity of white blood cells so that invading bacteria can out compete the immune system of the cow (Zadoks, 2006). Hypoglycemia alone is not likely to exacerbate periparturient immunosuppression, but hyperketonemia appears to have multiple negative effects on immune functions (Overton & Waldron, 2004). Impaired immune functions around calving make cow more prone to infectious diseases. Cows that have suffered ketosis have a higher risk of ketosis in the following lactation (Ingvarsen, 2006). Affected animals produce 0,7-3,3 kg/day less milk in the rest of lactation (Fourichon et al., 1999) or about 200 kg annually (Guard, 1996). In addition, prolonged and pronounced negative energy balance in early lactation adversely affects reproductive performance of cows and increases the risk of other metabolic diseases (Rushen et al., 2008).

Ewes experience ketosis typically during the last month of pregnancy and is known as pregnancy toxemia (Schlumbohm & Harmeyer, 2003). Ovine pregnancy toxemia (twin lamb disease, pregnancy disease) occurs primarily in ewes carrying more than one fetus (Andrews et al., 1996; Schlumbohm & Harmeyer, 2003). It is usually seen when the plane of nutrition has been static or falling over the last month of gestation when the requirements of the ewe are increasing to allow the growth of the fetuses (Andrews et al., 1996). In goats, two clinical form of ketosis have been described: pregnancy toxemia during the last month of pregnancy and primary ketosis during the first month of lactation (Stelletta et al., 2008).

Limitation of the availability of glucose has been found to be a crucial factor for the development of pregnancy toxemia. Hyperketonemia lowered plasma glucose concentration and depressed endogenous glucose production in sheep by approximately 30%, and this facilitates the onset of pregnancy toxemia (Schlumbohm & Harmeyer, 2003).

Mortality is high if pregnancy toxemia is not treated, with only about 20% of affected ewes recovering without treatment. Encephalopathy can results from depressed glucose metabolism in the brain (Andrews et al., 1996).

### **2.3. Rumen acidosis**

Ruminal acidosis is a nutritional disorder of ruminants generally resulting from ingestion of large amounts of feeds rich in readily fermentable carbohydrates, particularly when animals

have not been gradually acclimated to those feeds (Bramley et al., 2008). The disorder usually occurs in high-producing dairy cows. Subacute or subclinical ruminal acidosis (SARA) is considered to be one of the major threats to the welfare of lactating dairy cows and may affect up to 20% of cattle in early to mid lactation (Rushen et al., 2008). Subclinical rumen acidosis is defined as a condition where rumen fluid pH is below 6,0 while acute rumen acidosis is when rumen pH is below 5,5 associated with rumen motility that is weak or ceased (Ingvarstsen, 2006).

Rumen acidosis classically occurs when an animal consumes the excess of grain (Stone, 2004). Fermentation of the high grain diet reduces rumen pH which can cause undesirable changes in microbial populations within the rumen (Goff, 2006a). Rumen pH is lowered due to large quantities of volatile fatty acids and lactic acid produced during grain fermentation (Bramley et al., 2008). Lactic acid production is not the hallmark of rumen acidosis in dairy cows, as observed in beef feedlot cattle. Instead, it is the total organic acid load induced by the high grain diet combined with the inability of the cow to buffer the acids with salivary secretions (Goff, 2006a; Stone, 2004). However, lactate accumulation may occur in cows after calving if the shift in fermentable carbohydrates between the diets fed before and after calving is too dramatic (Stone, 2004).

Low rumen pH can cause rumenitis, metabolic acidosis, lameness, hepatic abscesses formation, pneumonia, and even death (Bramley et al., 2008). Reduced ruminal efficiency, liver and lung abscesses, and laminitis all can be related to SARA (Stone, 2004).

## 2.4. Laminitis

Laminitis and laminitis-related hoof problems (sole ulcer, white line abscess, solar hemorrhage, etc.) are one of the leading causes of lameness in cows. Laminitis has been associated with nutrition, and specifically with ruminal acidosis either in its acute or subacute form. Exact relationship between laminitis and SARA is not known. One of the theories states that damage of the ruminal epithelium induced by acidosis allows absorption of histamine and endotoxins into the blood. These and possibly other compounds affect circulation within the hoof and cause inflammation leading to the condition known as laminitis (Stone, 2004). Cows fed the higher level of crude protein may have increased incidence and duration of lameness. It is considered that products of degradation of protein excess in the rumen may be the causative agents for lameness.

It is widely accepted that nutrition helps maintain claw health through the production of good quality horn (Baird & Muelling, 2009). For example, heifers fed on hay before calving might have better foot health than those fed silage, even if both groups are being fed the same diets postpartum. Forage type and level of moisture in the diet could influence lameness and this effect is exerted even before first calving (Logue & Offer, 2001).

It has also been shown that nutritional supplements such as biotin and zinc (Zn) can help reduce lameness through improving claw horn quality (Baird & Muelling, 2009; Goff, 2006a). Biotin is essential for two major metabolic pathways in keratinisation, keratin protein synthesis and lipogenesis. Other vitamins also may play important roles in

maintaining claw integrity, including vitamin A and vitamin E. Zinc, as a component of many enzyme systems, has a role in major functions during keratinisation including the formation of structural proteins (Baird & Muelling, 2009). This element improves claw integrity by speeding wound healing and epithelial tissue repair. Other trace minerals that impact claw condition include iodine (I), selenium (Se), copper (Cu), manganese (Mn) and Co. Calcium and phosphorus (P) are also needed for normal claw growth and integrity.

Laminitis reduces profitability of the dairy herd. It is estimated that 15% of cows culled for slaughter are culled due to laminitis. In clinically lame cows, milk yield was reduced from 4 months before and for the 5 months after treatment. The total mean estimated reduction in milk yield per lactation was approximately 360 kg (Green et al., 2002).

## 2.5. Displaced abomasum

Displaced abomasum is a multifactorial disease where the abomasum is dilated as a result of gas accumulation and dislocated to the left (left-displaced abomasum) or to the right (right-displaced abomasum) in the stomach in relation to the normal placing (Ingvarstsen, 2006). The passage of feed to the intestines is partly or totally blocked. Approximately 80-90% of the incidences are left-displaced abomasums. The disease is most frequent in high producing cows in early lactation and 80–90% of the cases are seen in the first 4 weeks postpartum (Shaver, 1997). It occurs in approximately 3,5% of dairy cows each year (Goff, 2006a).

Nutrition has been implicated as a major risk factor in the etiology of displaced abomasums, but the precise cause is still unclear. Low feed intake and increased negative energy balance prepartum have been found to increase the risk of displaced abomasums in cows (Goff, 2006a; Ingvarstsen, 2006). Cows fed high concentrate diets in early lactation or diets with inadequate particle size are also at increased risk of displaced abomasums (Goff, 2006a). Sudden changes in the diet and rapid increase in concentrate allowance in early lactation are also the risk factors. Some feeds increase the risk of the disease compared to others. Thus, a higher risk of displaced abomasum has been found for cows fed silage compared to those fed hay, probably because silage is often finely chopped. Risk can be almost eliminated if cow eats a kilogram of straw daily (Ingvarstsen, 2006).

Displaced abomasum develops if three major conditions are met. The first one is reduced contractility and atony of abomasum which is gas-dilated. The next condition is that the mesentery shall stretch for the abomasum to be able to dislocate, and a third condition is the space in the abdominal cavity (Ingvarstsen, 2006). The conditions leading to atony and reduced motility are still unclear, but hypocalcaemia around calving is a possible factor. Reduction in blood Ca concentration around calving results in a reduction of abomasal contractility, which can lead to its atony and dilatation (Shaver, 1997). Hypocalcemic cows after calving have 3,4 to 4,8 times increased risk of development of abomasal displacement (Massey et al., 1993).

Volatile fatty acids (VFA) in the abomasum also have been reported to reduce abomasal motility. Effects of VFA on motility may be exacerbated by low ruminal absorption of VFA

during the transition period (Shaver, 1997). Hypocalcaemia also may play a role in this process. Due to reduced feed consumption, slow contractions and inadequate filling of the rumen which therefore does not reach the ventral abdominal wall, empty space appears for movement of abomasum. Then usually more fatty acids escape absorption in the rumen and reach the abomasum. Those VFA, along with hypocalcaemia and accumulated gases, contribute to reducing abomasum contractility and development of atony (Goff & Horst, 1997c). Thus, inadequate feed consumption and insufficient rumen fill with reduced motility and strength of abomasal contractions together contribute to the onset of this disorder (Goff & Horst, 1997c).

Low intake of concentrates during the prepartum period also may increase the risk of left displaced abomasum because absorptive capacity of the ruminal papillae is not increased sufficiently and microbial population of the rumen is not adapted prior to intake of high energy postpartum diets (Shaver, 1997). Too rapid increase of concentrates after calving may reduce roughage intake and potentially increase the risk of displaced abomasum (Ingvarsen, 2006). Uncomplicated ketosis, fatty liver, retained placenta, and metritis are also risk factors for left displaced abomasums (Bobe et al., 2004; Shaver, 1997).

Treatment of displaced abomasum often requires surgical treatment, and deaths are not uncommon so that potential losses due to disease are very high. In addition, cows that recover from abomasal displacement produce about 350 kg less milk during the next month (Shaver, 1997) or 0,8-2,5 kg/day in the rest of lactation (Fourichon et al., 1999). According to Guard (1996) the average losses amounted to about 380 kg of milk annually.

## **2.6. Milk fat depression**

Nutrition influences both the quantity and composition of milk fat. In modern dairy production cows are fed diets with high level of concentrates to maximize milk production but such diets often causes drop in milk fat. Condition is known as milk fat depression (MFD) or low-fat milk syndrome. Although milk volume and yield of other milk constituents may not be affected or may be even increased, depression of fat in milk can be a serious economic problem for dairy producers. This syndrome is not a disease, but rather metabolic consequence of attempts to reach the higher milk production in animals.

Milk fat depression refers to a marked reduction in milk fat yield with no change in milk yield or yield of other milk components (Harvatine et al., 2009). It represents a level of milk fat production below the genetic potential of the cow, usually as below 3,2% fat in milk for Holstein, or below 4,2% fat in the milk for a Jersey herd. When problem with MFD exists, the ratio of milk fat/milk protein will be less than 1,0 for Holstein herds. In severe cases the ratio will be less than 0,8. Milk fat yield can be reduced up to 50% and the effect is specific for milk fat (Bauman et al., 2008). The problem has been observed in many feeding situations and dietary conditions, including high level of concentrates and low level of dietary fiber, and diets supplemented with unsaturated oils. The fat content of milk can also be affected by the physical characteristics of the roughage, including grinding and pelleting (Perfield & Bauman, 2005). In diet-induced MFD, the yield of all individual fatty acids is

reduced, but the decline is greatest for short- and medium-chain fatty acids that are synthesized *de novo* in mammary gland (Bauman et al., 2008).

It is generally accepted that induction of MFD requires both an altered rumen environment and the presence of unsaturated fat in the rumen which biohydrogenation pathways are altered (Perfield & Bauman, 2005). Changes in ruminal microbial processes are an essential component for the development of MFD and are often associated with a decrease in rumen pH and a shift in the acetate/propionate ratio.

Historically, there have been several theories proposed to explain the basis for diet-induced MFD. The biohydrogenation theory was proposed by Bauman & Griinari (2001) focusing on the latest research in MFD problem. The authors suggested that "under certain dietary conditions the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates which are potent inhibitors of milk fat synthesis". The theory was born after identification of *trans*-10,*cis*-12 conjugated linoleic acid (CLA) as a highly potent inhibitor of milk fat synthesis (Bauman & Griinari, 2001). This isomer is a rumen precursor of *trans*-10 18:1 in biohydrogenation pathway that may arise under certain dietary conditions, including high concentrate/low fiber diets. The biohydrogenation of linoleic acid in rumen involves the formation of *trans*-11 18:1 and *cis*-9,*trans*-11 CLA as the main intermediates. However, under certain dietary situations the other CLA pathways of biohydrogenation can happen and some of the intermediates produced during the process may be potent inhibitors of milk fat synthesis (Bauman & Griinari, 2001). The most extensively studied is *trans*-10,*cis*-12 CLA. Minimal quantities of this isomer markedly inhibited milk fat synthesis and a curvilinear reduction in milk fat yield occurred with increasing *trans*-10,*cis*-12 CLA in the rumen content (Baumgard et al., 2001).

Isomer *trans*-10,*cis*-12 CLA alone does not completely explain the extent of the decrease in milk fat. Additional biohydrogenation intermediates produced in the rumen probably inhibit milk fat synthesis and two of them (*trans*-9,*cis*-11 and *cis*-10,*trans*-12 CLA) have already been identified (Bauman et al., 2008).

Consequences of MFD are almost exclusively related to losses in yield of milk fat and lower price of milk. As problem is normally reversible, health consequences are usually not a matter of concern. However, MFD inducing diet is usually risky for rumen homeostasis due to high level of carbohydrates and low level of effective fiber which may reduce ruminal pH and cause acidosis (Bergen, 2009). If acidosis is not compensated and drop in ruminal pH is not prevented, the condition may become an important animal health issue.

## 2.7. Parturient hypocalcaemia

The lack of Ca in the diet does not lead to any changes in health or production for a long time because it is compensated by mobilization of Ca reserves in the skeleton. The change of Ca content in the blood may not occur until the cows show symptoms of osteoporosis and bone fractures. A unique example, however, is an acute hypocalcaemia in dairy cows that occurs in the periparturient period as a result of sudden loss of great amounts of Ca in

colostrum along with temporary dysfunction of the mechanisms of Ca mobilization from bones. From a relative inactivity in the dry period, these mechanisms often fail to achieve full activity quickly enough to maintain normocalcaemia around calving (Horst et al., 1994). More pronounced hypocalcaemia causes progressive neuromuscular dysfunction which is manifested as a specific clinical syndrome known as paresis puerperalis (milk fever, parturient paresis, calving paralysis). More often, however, and in a larger number of cows, hypocalcaemia exists in a subclinical form with very few or no symptoms (subclinical hypocalcaemia).

Hypocalcaemia was defined as the content of total Ca in blood below 2 mmol/L with or without clinical signs of paresis (Oetzel et al., 1988; Radostits et al., 2000), which is roughly equivalent to 1 mmol/L of ionized Ca (Massey et al., 1993; Oetzel, 1996).

Milk fever (MF) is non-febrile disease of adult dairy cows accompanied by general weakness, circulatory collapse and depression of sensation (Oetzel, 1988). It is one of the most common metabolic disorders in intensive dairy production (Horst, 1986) and a typical nutritional disorder (Ender et al., 1971). The disease attacks 5-10% of adult dairy cows in the U.S. and Europe annually, primarily those with high productivity. The classic syndrome of MF occurs immediately before or after parturition with about 87% of cases in the first 48 hours after calving and only about 9% cases before or during the parturition (Oetzel, 1988). The disease is characterized by pronounced hypocalcaemia which is accompanied by hypophosphataemia in most cases, and by more or less serious hypermagnesaemia (Phillippo et al., 1994).

Phenomena like paresis may rarely occur in beef cows, ewes (before or after lambing) and more frequently in dairy goats (Oetzel, 1988). In mares, the disease can occur several weeks after birth and is known as milk tetany or eclampsia (Radostits et al., 2000).

Milk fever has been recognized as a nutritional disorder with various endocrine abnormalities which occur more or less as a secondary phenomenon (Ender et al., 1971). The characteristic of the disease is an acute inability to mobilize Ca from bone (Horst, 1986) despite apparently normal endocrine dynamics in most affected cows (Radostits et al., 2000). The problem seems to lie in the reduced sensitivity of target tissues (bone, kidneys and intestines) on calcitropic hormones due to reduced number of receptors or because of their dysfunction (Horst et al., 1994).

Widely accepted theory for a long time was that MF was caused by a high content of Ca in the diet consumed before calving, as well as by unfavorable ratio of Ca/P. However, further studies emphasized importance of the high content of monovalent cations in the diet, mainly potassium (Block, 1984; Ender et al., 1971; Goff & Horst, 1997b; Horst & Goff, 1997). As a relative excess of cations in the diet affects blood acid-base balance toward alkalosis, the disruption of integrity of tissue receptors is related to metabolic alkalosis that normally exists in cows at the time of calving (Goff, 2000). Thus, the primary cause of MF is considered to be the temporary inability of cow tissues to adequately respond to stimuli generated by calcitropic hormones, mainly parathyroid hormone (PTH) (Goff et al., 1991; Goff, 2000).

In addition to monovalent ions, risk factors also include dietary magnesium (Sansom et al., 1983), dietary P (Curtis et al., 1984), and possibly vitamin D (Goff, 2000; Horst, 1986). Animal factors as the risks for hypocalcaemia include age (Curtis et al., 1984; Dishington, 1974) and breed (Goff, 2000; Oetzel, 1988). Adult cows are at increased risk for the disease. Occurrences are more frequent from the third lactation onwards, reaching the plateau of 12-15% in cows 6-10 years of age (Curtis et al., 1984; Dishington, 1974). The most predisposed breeds for milk fever are Jersey and Guernsey, followed by Holstein and Brown Swiss. In the case of Jerseys possible reasons are considered to be the higher milk production per unit of body weight (Oetzel, 1988) and a higher content of Ca in the colostrum (Goff, 2000). In addition, the number of receptors in the intestine is about 15% lower in Jerseys than in Holsteins (Goff, 2000; Horst et al., 1997).

Depending on the age, breed, feeding regimen and housing conditions, as many as 50-80% of cows can suffer MF annually in some herds (Oetzel, 1988). Concerning that well-timed treatment of MF easily solves the problem and is relatively inexpensive, the disease was not considered as a factor of economic importance in dairy production for a long time (Radostits et al., 2000). However, later findings confirmed its close relationship with other health disorders in the puerperium (Curtis et al., 1985; Massey et al., 1993). Many of these disorders are now considered as complications of hypocalcaemia. They generally occur as a complex, rarely as isolated diseases (Curtis et al., 1985) and include most of metabolic and reproductive disorders in periparturient period: dystocia, retained placenta, metritis, uterine prolapse, ketosis, mastitis and displaced abomasum (Horst et al., 1997). Risk of occurrence of each of them is much higher in hypocalcemic cows (Curtis et al., 1985). A well known physiological effect of hypocalcaemia is atony of smooth and skeletal muscles because their contractility is a function of Ca concentration in extracellular fluid (Ramberg et al., 1984). Most of the possible complications are related specifically to reduced contractility and to atony of smooth muscles of digestive and reproductive tract (Beede, 1995).

Spontaneous recovery of MF is usually not possible and approximately 75% of affected cows die if treatment fails (Oetzel, 1988). Even under normal conditions about 8% of treated cows still die due to various complications, 12% of them are culled (Guard, 1996) and about 25% require another treatment. Due to increased susceptibility to other health disorders and possible complications, the production life of cows that experienced MF was reduced by an average of 3-4 years (Horst et al., 1997).

Subclinical hypocalcaemia influences in the same manner as the clinic one, but to a lesser extent. As is more common in the herd, adverse effects of subclinical hypocalcaemia on herd economy can be equal or even greater than the effects of MF due to its broader influence on feed intake, secondary disease conditions, and milk production during early lactation (Horst et al., 1994).

## **2.8. Hypomagnesaemia**

Magnesium is an essential mineral with many functions in the body but its homeostasis is not hormonally regulated. The concentration of Mg in the blood is dependent exclusively on

its absorption from the diet. If Mg secretion in milk and its endogenous losses exceeds absorption from the forestomachs hypomagnesaemia occurs because of the lack of hormonal control. Hypomagnesaemia is common problem in ruminants and may be one of the major health problems of cattle, sheep and goats in large scale production systems especially in temperate climates (Mayland, 1988; Stelletta et al., 2008).

Clinical hypomagnesaemia is also called hypomagnesemic tetany, grass tetany, spring tetany, or lactational tetany. Clinical signs usually occur when the animal is both hypomagnesaemic and hypocalcaemic (Robinson et al., 1989). In its subclinical form, however, hypomagnesaemia may be risk factor for other diseases. Around calving it may be the important risk factor for MF (Sansom et al., 1983; Schonewille et al., 2008).

Hypomagnesaemia may develop due to Mg deficiency in the diet or due to its low utilization in the gut. It may also occur because of a need for increased amounts of Mg during parturition and early lactation (Mayland, 1988). Clinical hypomagnesaemia in cows with plasma Mg concentrations below 0,4 mmol/L is manifested as grass tetany (Schonewille et al., 2008). Grass tetany has been investigated extensively, but its complex etiology is not well understood. Large number of factors influences the development of the disease (Robinson et al., 1989). It appears within 2-4 weeks after cattle or sheep have been turned out to rapidly growing pasture. Ewes with twins are more susceptible to grass tetany than are ewes with single lamb (Mayland, 1988). Specifically, older lactating animals consuming lush, intensively fertilized, cool-season grasses during the early spring are the most frequently affected (Robinson et al., 1989). Older animals also have reduced ability to mobilize body reserves of Mg. Lower average environmental temperatures (<14°C) facilitate the onset of the disorder (Mayland, 1988). However, periods of rapid plant growth during any season, resulting in forage of low Mg and high moisture, high nitrogen (N) and K, present dietary conditions that increase the potential for the development of tetany (Robinson et al., 1989). Grass tetany also may develop if animals graze forage that is high in digestible protein, but low in digestible energy (Mayland, 1988).

Grass tetany generally occurs when the dietary intake of total Mg is not particularly low, but factors which increase the animal's requirement for Mg or reduce the availability of dietary Mg are present (Mayland, 1988). The Mg concentrations in forage and subsequently in the blood of cattle are influenced strongly by high amounts of fertilizer K and, to some extent, fertilizer N. Absorption of Mg by plants is reduced by high levels of K in the soil (Mayland, 1988; Robinson et al., 1989). Mg absorption by ruminants is also reduced by a high intake of K (Schonewille et al., 2008), and by high content of Ca and P in the diet (Sansom et al., 1983). High concentrations of N in the forage may additionally decrease the availability of Mg (Mayland, 1988).

Absorption of Mg is dependent on the concentration of Mg in the rumen fluid and the functionality of the Mg active transport process across the rumen wall. The active transport mechanism is critical for the animal when dietary Mg concentration is less than 0,25%. Several factors, such as dietary K, can inhibit Mg absorption by this pathway. At high concentration of Mg in the rumen it will be absorbed by passive transport, and this

mechanism is not affected by high K but only by the solubility of Mg in the rumen fluid. This mechanism requires dietary Mg to be not less than 0,35% (Goff, 2006b).

Mg deficiency results in reduced appetite which decreases total nutrient intake (Robinson et al., 1989), thus chronic hypomagnesaemia results in reduced feed intake and milk production. It may progress quickly into acute hypomagnesaemia which terminates in convulsions, coma and death. One-third of animals with clinical symptoms die (Mayland, 1988). Even mild form of hypomagnesaemia (Mg <0,85 mmol/L) significantly reduces the mobilization of Ca from the skeleton around calving (Goff, 2000; Sansom et al., 1983). That is why hypomagnesaemia may be a risk factor for parturient hypocalcaemia and milk fever. According to Sansom et al. (1983) possible reasons for inhibition of Ca mobilization are considered to be:

- a. influence on PTH secretion; there are indications that serious Mg deficiency may impair or completely interrupt secretion of PTH,
- b. interference with the action of PTH on target tissues; the integrity of interaction between PTH and its receptors is disrupted and activity of initiated enzymes is disabled,
- c. interference with the metabolism of vitamin D; the first phase of vitamin D hydroxylation in the liver requires Mg<sup>2+</sup> ions, while the second phase in the kidney takes place in the presence of PTH.

## 2.9. Udder edema

Udder edema occurs sporadically in cows and heifers near parturition, peaking in severity during the immediate prepartum period. Unless complicated, recovery of edema is spontaneous within a few days after calving. However, if edema is sufficiently severe it can interfere with suckling by the calf, milking, and may cause other complications including udder inflammation. Edema is caused by excessive accumulation of fluid underneath the skin and at least some mammary edema is associated with pregnancy and parturition, especially in primigravid heifers (Malven et al., 1983).

Investigations of the causes of udder edema and the development of strategies to reduce its prevalence have progressed in very limited degree during last several decades (Goff, 2006a). Some authors (Schmidt & Schultz, 1959) proposed that severity of udder edema is based on an inherent physiological phenomenon, because a cow may tend to have the same amount of edema each year regardless of the feeding. Feeding program had little if any effect on udder edema (Randall et al., 1974). "Steaming up" method of feeding dry pregnant cows and high level of concentrates in their diet was initially blamed for high incidences of udder edema. However, investigation found that the amount of edema at calving was not related to grain feeding during the dry period or after calving (Schmidt & Schultz, 1959). Edema also is not correlated with the body condition of the cows (Schmidt & Schultz, 1959). However, addition of Na or K to the diet before calving can increase the incidence and severity of udder edema in dairy cows and first calf heifers (Nestor et al., 1988). In practical terms, high level of NaCl in the feed during the dry period may be one of the major factors in the development of udder edema around calving. Conflicting results from some earlier studies on effect of grain on

udder edema could be explained with salt content of the diets. However, KCl as a replacement for NaCl results in edema of about the same severity (Randalu et al., 1974).

Cows with severe edema that requires veterinary treatment are more likely to have additional health problems. Authors found higher culling rates for cows with more severe edema. Thus, there would be a substantial economic benefit to minimizing edema in dairy herds (Dentine & McDaniel, 1984).

## 2.10. Retained placenta

Inadequate antioxidant status or “oxidative stress” of the cow contributes to a poorly functioning immune system and increases the risk of mastitis as well as retained placenta (retained fetal membranes, placental retention). Fetal membranes are retained if not expelled longer than 12 hours after parturition. Se and vitamin E are important dietary antioxidants and their low levels in the diet are associated with a high incidence of mastitis and retained fetal membranes (Goff, 2006a). Subsequently, addition of vitamin E or Se may improve antioxidant status and decrease the incidence of those diseases. The similar effects can be obtained by addition of beta-carotene in the diet. Blood lymphocyte proliferation was higher in cows supplemented with beta-carotene, and phagocytic activity of blood neutrophils was enhanced as well as intracellular killing by blood neutrophils. Therefore, dietary beta-carotene can elevate blood beta-carotene and enhance peripartum host defense mechanisms by enhancing lymphocyte and phagocyte function (Michal et al., 1994).

Hypocalcaemia, among others, may be an important risk factor in the development of retained placenta. Muscle weakening or absence of uterine contractions in hypocalcemic animals does not contribute to the expulsion of fetal membranes (Goff & Horst, 1997c; Oetzel, 1988). In cows with hypocalcaemia placental retention is 3,2 to 4 times more frequent than in normocalcemic cows (Curtis et al., 1985). Hypocalcaemia also delay the physiological involution of uterus and increases the incidence of metritis (Beede, 1995). Moreover, hypocalcaemia is considered as one of the main causes of uterine prolapse, also due to loss of uterine muscles tone.

Cows with retained placenta were 3 times more likely to develop mastitis than animals without retained placenta (Overton & Waldron, 2004). It has been reported that placental retention, metritis and mastitis predisposes dairy cows to foot problems. Moreover, uncomplicated ketosis, retained placenta, metritis, and hypocalcaemia at parturition are also risk factors for left displaced abomasums (Shaver, 1997). Cows with retained placenta or metritis produce 0,3-2,3 kg/day less milk during subsequent lactation (Fourichon et al., 1999). In the case of retention alone, Guard (1996) stated that losses are on average 350 kg of milk annually.

## 2.11. Metritis

Metritis and endometritis are inflammatory uterine diseases. They frequently occur soon after calving and may severely compromise reproductive performances. Metritis and

endometritis refer to the inflammation of the uterus and of its endometrial lining - both conditions are referred to subsequently as metritis (Urton et al., 2005). Younger cows were more likely to have dystocia or assisted deliveries, while older cows were most likely to have retained placenta and metritis (Lewis, 1997).

In some herds, up to 40% of the postpartum cows may be diagnosed with, and treated for uterine infections. However, the exact causes of uterine infections are unknown but are associated with several factors. Cows with dystocia, retained placenta, or stillbirths, and other metabolic disorders are more likely to develop metritis than healthy cows. Impaired immune functions before and after calving seem to predispose cows to severe uterine infections. It has been suggested that the function of neutrophils is impaired in cows that develop uterine infections. Thus, methods for regulating immune function in periparturient cows may have potential for preventing uterine infections. However, prevention of uterine infections is still difficult because the primary causes cannot be defined clearly (Lewis, 1997). Malnutrition influences the ability of the immune system to function, which affects the incidence of diseases such as mastitis and metritis (Goff, 2006a).

Cows suffering metritis exhibit reduced milk yield and reproductive performances (Urton et al., 2005). Few cows die from uterine infections, but affected animals are more likely to be culled for poor reproductive performances. The estimated cost to producers for each cow with metritis was 106 U.S. dollars (Lewis, 1997). Many of the financial costs of metritis are indirect, such as increased days open or predisposition to other diseases, and are thus difficult to measure. As mentioned previously, metritis is one of the diseases that predispose dairy cows to foot problems and left displaced abomasums (Shaver, 1997).

In contrast, the effects of metritis on milk production can be measured immediately, and losses during first four months after calving can be almost 270 kg. Also, besides reduced milk production in sick animals, some pharmaceuticals for treatment of uterine infections contaminate milk with residues, and the milk must be discarded. This is how uterine infections may have an indirect effect on milk production (Lewis, 1997).

### **3. Use of blood and milk analyses to evaluate nutritional and disease status**

If recognition of subclinical diseases is difficult, the condition may be confirmed by analyzing blood, milk or sometimes urine, although some of them are still difficult to diagnose in practice, including rumen acidosis and fatty liver (Ingvarstsen, 2006). Blood and milk analyses as tools to evaluate nutritional and disease status of individual animal or whole herd are aimed to help making decisions for improvement of nutritional strategies and production management. However, many problems are associated with interpretation of results of laboratory analyses, including Compton metabolic profile test, as well as other tools for assessment of metabolic status of dairy animals.

### 3.1. Milk analyses

Producers can use changes in milk production or in milk composition to monitor the health of their animals, but these tools are not always completely reliable because milk production corresponds poorly with mild or subclinical infectious disease. Nevertheless, measuring energy balance from changes in milk composition, most likely changes in milk fat and protein contents, could provide a cheap and reliable estimator of energy balance. A study clearly showed that there was a strong relationship between energy balance and milk composition under stable feeding conditions (Friggens et al., 2007). Milk composition varies with energy status and was proposed for measuring energy balance on-farm. Ratio milk fat/protein below 1,4 indicates the optimal or positive energy balance, and above it balance is negative (Pehrson, 1996; Zadoks, 2006). During peak lactation many cows with negative energy balance have this ratio in milk even above 2,1 (Pehrson, 1996).

The biological basis for the relationship between fat/protein ratio and energy status of the animal can be found in two physiological features: a) mechanism that maintains milk energy output by increasing milk fat content when yield is compromised due to a deficit in energy supply, and b) decreased milk protein content under negative energy balance (Friggens et al., 2007). Negative energy balance also may be reflected in elevated ketone bodies which are excreted in milk and urine. Milk has about half the ketone level of blood, and is recommended to check milk ketone levels for detection of ketosis.

Milk fat depression is an example of disorder that can be diagnosed exclusively by milk analysis. Specific blood test for MFD diagnosis does not exist, but milk analyses are usually enough to evaluate risk for or presence of MFD in a herd. Every drop in milk fat percentages with no changes in the content of other milk constituents and milk yield can be considered as MFD. In serious cases, diet-induced MFD can result in reduction of milk fat yield of up to 50% or even more (Bauman & Griinari, 2001; Bauman et al., 2008) and represents one of the greatest risks for production economy.

### 3.2. Blood analyses

Analyses of blood constituents have long been a matter of concern as a possible tool for assessment of diet adequacy or metabolic and health status of animals. Compton metabolic profile test (CMPT) originally involved the analysis of a set of blood variables from three groups of seven cows, one near peak lactation, and others in midlactation and in late dry period (Kronfeld et al., 1982). The samples are collected at least three times yearly: summer, autumn and winter, or when nutritional imbalance is expected, using the same procedures and timings (Radostits et al., 2000). Means of variables are calculated for each lactational group in each herd, and this set of means constitutes the metabolic profile. Each group mean is compared to reference ranges determined from corresponding means plus or minus two standard deviations that are based on group-means for all herds (Kronfeld et al., 1982). The test was based on the concept that laboratory measurement of certain components of the blood will reflect the nutritional status of an animal with or without presence of clinical abnormalities. However, the results of research indicate that CMPT may be useful only as an

aid in the diagnosis of the nutritional imbalances and production diseases. It must be carefully planned and is still expensive. Laboratories with automated analytical equipment should be available and this is often a major limiting factor (Radostits et al., 2000).

Compton metabolic profile test includes following analyses: blood glucose, packed cell volume, hemoglobin, blood urea nitrogen, serum inorganic phosphate, serum Ca, Mg, K and Na, total serum protein, albumin and globulins, serum Cu and iron (Fe), and plasma NEFA. Obtained results are interpreted with other relevant information taken on the day of sampling related to individual animals or to the herd: age, milk yield, days in milk, concentrates and forage intake, etc (Radostits et al., 2000).

Workers tested the CMPT as a guide to the nutritional status of dairy cows, and found that blood variables were not reliable predictors of energy and nutrients consumption relative to the requirements. Then they supposed that, for prediction of nutritional status, selection of blood variables should be probably different. Those workers have suggested, however, that protein intake may be reflected in blood concentrations of urea, albumin, and hemoglobin. Similarly, blood glucose concentration has attracted attention as an index of energy intake, although results from study to study have been inconsistent (Kronfeld et al., 1982).

Despite limitations and unconformity of metabolic profile tests, blood analyses still can help in diagnostics of some nutritional imbalances. Negative energy balance in postpartum cows can be detected by change in concentration of some blood metabolites (Rushen et al., 2008). As condition that affect immune functions and predispose animals to many metabolic disorders, it is important that negative energy balance is reflected in a higher levels of NEFA and beta-hydroxybutyric acid (BHBA) in the blood. To screen a herd for negative energy balance, Zadoks (2006) recommends having at least 12 animals tested. Testing of NEFA is done 2-14 days before calving and BHBA testing at 2-21 days after calving. If more than 10-15% of animals have NEFA levels above 0,40 mEq/L or BHBA levels above 1,36 mmol/L, the herd is considered to be suffering from excessive negative energy balance. Ketosis and fatty liver are diseases typically associated with negative energy balance for long periods of time. Increased ketone bodies and NEFA in the blood, and decreased blood glucose level are common findings in cows with ketosis and fatty liver. Values of glucose below 2,2 mmol/L are considered subnormal. However, fatty liver in cows currently can be diagnosed only by liver biopsy (Bobe et al., 2004).

The concentration of BHBA in the blood may be taken as a guide for correction of ration supplement for ewes during the final weeks of pregnancy to prevent pregnancy toxemia. It has been recommended to check 10% of the flock and feed is increased if blood concentration of BHBA exceeds 0,8 mmol/L (Morgante, 2004). Besides hypoglycemia and hyperketonaemia, plasma cortisol levels also may be elevated in sheep with pregnancy toxemia (Andrews et al., 1996). Early in the disease, both does and ewes will show a positive test for ketone bodies in the urine (Morgante, 2004).

There are no specific blood variables for detecting metabolic diseases like laminitis, displaced abomasums, udder edema, retained placenta and metritis. However, if these diseases are herd problems, blood metabolic profile should be checked for more than one

variable, and carefully used as an aid for development of preventive strategies. When laminitis is a problem, a metabolic profile on both dry and lactating cows as well as springing heifers should include both red and white blood cells count, packed cell volume, Se, Zn, Cu, Fe, blood urea nitrogen, vitamins A and E, and beta-carotene. When retained placenta is a herd problem, blood profile should include serum minerals, Se, vitamin E, and beta-carotene. In individual cases, blood urea nitrogen and packed cell volume should be included. Metritis requires testing metabolic profile on dry and fresh cows which should include white blood cells count, Se, Zn, Cu, Fe, Mg, blood urea nitrogen, vitamins A and E, and beta-carotene.

Use of blood analyses to evaluate degree of hypocalcaemia is sufficient diagnostic test for this disorder. Concentration of Ca may be determined in whole blood, but more often determination is performed in plasma or serum. When there are no disturbances in acid-base status and protein metabolism, ionized and total Ca are strongly correlated and therefore total Ca concentrations in plasma may serve as an acceptable diagnostic value (Oetzel, 1988).

Hypocalcaemia means that the content of total Ca in the blood is below 2 mmol/L, or below 1 mmol/L of ionized Ca (Massey et al., 1993; Oetzel, 1996). Signs of paresis can occur at total Ca level of 1,9 mmol/L, but most of the cows remain on their feet down to 1,0-1,25 mmol/L. A reduction in the plasma Ca concentration at parturition is usually accompanied by hypophosphataemia and hypermagnesaemia (Phillippo et al., 1994). However, this is not always the case, especially if hypocalcaemia is not severe and is not clinically manifested (Crnkic et al., 2010; Joyce et al., 1997; Oetzel et al., 1988).

Blood analysis is also sufficient diagnostic test for hypomagnesaemia. Based on blood serum or plasma Mg levels, animals may be normally magnesemic (0,74-1,23 mmol/L), chronically hypomagnesemic (0,20-0,74 mmol/L) or acutely hypomagnesemic (<0,20 mmol/L) (Mayland, 1988). Sampling and analysis of the blood of several cows within 12 hours after calving is a good indicator of Mg status of the periparturient period. If serum Mg concentrations are less than 0,82 mmol/L in 90% of cows it suggests insufficient dietary Mg absorption (Goff, 2006a).

#### **4. Nutritional strategies to reduce metabolic diseases incidences**

Although metabolic disorders are not easily categorized as to their cause, nutritional strategies have been developed to help prevent many of these disorders (Goff, 2006a). Scientific justification of those measures can be found in many research articles published in scientific journals during the last decades. However, problems in application of the strategies in the field still exist, as well as their limitations and possible negative consequences on milk production economy in certain circumstances. Strategies are based on major factors directly or indirectly increasing the risk of diseases such as overconditioning at calving, excessive mobilization of body fat, low nutrient intake, nutrient or diet specific factors and management and environmental stress (Ingvarstsen, 2006). Strategies are expected to be able to reduce morbidity and at the same time improve reproduction and production.

To prevent metabolic disorders in the periparturient period nutritional strategies must start prior to calving. In a survey conducted by Curtis et al. (1985) consumption of nutrients before calving was directly related to the occurrence of metabolic disorders, and directly or indirectly to the occurrence of reproductive disorders after parturition. In most cases, these disorders occur as a complex and many of them are interrelated in their occurrence (Beede, 1995; Curtis et al., 1985). Consequently, strategies to reduce one disease can help preventing others. For example, strategies to reduce liver triglyceride accumulation at calving may decrease incidence of ketosis, etc.

Goff & Horst (1997c) recommended that three basic physiological functions must be maintained during the periparturient period if disease is to be avoided: adaptation of the rumen to lactation diets that are high in energy density, maintenance of a strong immune system, and maintenance of normocalcaemia. Whenever one or more of these functions are impaired, the incidence of both metabolic and infectious diseases is increased. However, measures to maintain these physiological functions must be conducted carefully avoiding other risk factors such as overconditioning and excessive mobilization of body fat around calving.

Metabolic disorders that may appear in the rest of lactation also require adequate prevention strategy to be controlled, including rumen acidosis, laminitis, hypomagnesaemia and milk fat depression.

#### **4.1. Adaptation of the rumen**

Fully adapting the rumen flora to a high starch diet that will be fed after parturition requires about 3 to 4 weeks, and full development of rumen papillae requires about 5 weeks of concentrate feeding (Goff & Horst, 1997c). It is, therefore, important to start increasing concentrates in the diet 3-4 weeks before calving and continue during the first 1-2 weeks after parturition to fully adapt rumen to lactation diet. If fresh cow is abruptly switched to a high starch lactation diet, the risk of developing rumen acidosis exists because the lactate production and accumulation. During the rest of lactation, the most reliable means of preventing rumen acidosis is to apply feeding methods that ensure a more even distribution of feed intake over the day. Feeding TMR only once or twice a day may result in that cows eat an excessive amount of feed during a short period of time, because cows are strongly attracted by the arrival of fresh food (Rushen et al., 2008). Buffering agents such as Na-bicarbonate or alkalizing agents such as Mg-oxide are added to high concentrate ration to reduce the risk of acidosis (Goff, 2006a). Na-bicarbonate should be supplemented particularly to corn silage-based diets at the rate of approximately 0,8 to 1% of DM (Stone, 2004). Too rapid increase in concentrate allowance during early lactation may reduce roughage intake and increase the risk of not only rumen acidosis, but also displaced abomasums (Ingvarsen, 2006).

A highly significant relationship between forage neutral detergent fiber (NDF) content in the diet and ruminal pH has been found. NRC (2001) recommended 19% of forage NDF as absolute minimum when formulating rations in the field. A system of “physically effective”

NDF (peNDF) relates the ability of a feedstuff to stimulate chewing relative to a hypothetical long grass hay containing 100% NDF. The peNDF of a feed is the product of its physically effective fiber (pef) and NDF content. The diet should contain about 22% peNDF to maintain ruminal pH of 6,0 (Stone, 2004).

Gradual adjustment of the rumen to a high concentrate diet can help avoiding serious drop in feed intake around calving and minimize body fat mobilization in early lactation. The plasma NEFA concentration is negatively correlated with DMI and depression in feed intake around the time of calving was largely responsible for fatty liver development (Goff, 2006a). Therefore, all nutritional measures that prevent drop in DMI before parturition may be useful in prevention of fatty liver. However, it has been assumed that increased energy and nutrient density of the diet may assure maintenance of the same intake of nutrients and energy despite lower DMI around calving, and decrease rate of lipid mobilization. Increase in nutrient density during the last 2–3 weeks prepartum by increasing concentrates in the ration has been referred to as “steaming-up” or “close-up” diet (Ingvarlsen, 2006). Use these diets should not last for too long because of risk of overfeeding energy and development of obesity. NRC (2001) recommends increasing the energy content of the precalving diet from 5,2 MJ/kg of DM during the “far-off” dry period, to 6,8 MJ/kg of DM for the 3 weeks before calving. This strategy is thought to prepare the cow for the metabolic demands of early lactation and thereby minimize the need for body tissue mobilization (Urton et al., 2005).

As fatty liver and ketosis in cows often occur as a complex, all measures to reduce fatty liver incidence may decrease incidence of ketosis (Grummer, 1993). Moreover, positive effects on ketosis incidence and lipid transport also have been seen when niacin or rumen-protected choline is fed to dry and fresh cows (Goff, 2006a). The treatment of pregnancy toxemia in ewes is usually unsuccessful, therefore the prevention is of key importance to reduce occurrence of the disease. Grain is a high source of available energy. Feeding 0,5-1 kg of grain daily along with high quality hay during the last four to six weeks of pregnancy will help prevent pregnancy toxemia.

Manipulation of the nutritional program of dairy cows affects rumen health, which influences hoof health (Stone, 2004). It is very evident that feeding diets that cause drop in rumen pH will result in increase in laminitis cases. To minimize the drop in rumen pH it is necessary to limit amount of concentrate fed per meal to no more than 3,6 kg and provide fresh feed at the bunk throughout the day. Diet should contain at least 25% total NDF or 19% acid detergent fiber (ADF), with non-fiber carbohydrate (NFC) levels between 35 and 40% or non-structural carbohydrate (NSC) levels between 30 to 35%, and minimize abrupt changes of ration. Adding buffers to diets may help maintain claw integrity as buffers, such as Na-bicarbonate, minimize the drop in rumen pH. Supplements that contain combinations of complexed trace minerals Zn, Mn, Cu and Co positively influence claw health, and it has been advised to feed them when lameness is problem in a herd.

Increased energy content of the diet fed during the prepartum period was also associated with decreased incidence of displaced abomasum (Curtis et al., 1985). Risk of displaced abomasum for cows fed silage can be almost eliminated if every cow eats a kilogram of straw daily (Ingvarlsen, 2006).

## 4.2. Maintenance of immunity

Maintaining of a strong immune system may help to reduce incidence of infectious diseases after parturition including those related to nutritional factors: retained placenta, metritis, mastitis, laminitis, etc. Inadequate nutrition may contribute depression of the immune system that occurs around calving time (Rushen et al., 2008) when stress of parturition and metabolic challenges experienced by the dairy cow at the onset of milk production impair immune cell function (Goff, 2006a).

Dietary factor that may influence immune functions include vitamin E and Se as the most important vitamin and mineral related to immunity. To a lesser extent vitamin A and beta-carotene, Cu and Zn may play a role (Zadoks, 2006). Metabolic conditions related to parturition also may exacerbate immunosuppression, including negative energy balance (Zadoks, 2006), hypocalcaemia (Ganj Khanlou et al., 2010), and hyperketonemia (Overton & Waldron, 2004). Therefore, strategies to maintain strong immune system must include feeding adequate or increased levels of all dietary components that influence immunity, as well as prevention of metabolic disorders that may exacerbate immunosuppression.

Most measures explained in the preceding section related to adaptation of the rumen to lactation diets may help maintain immune functions throughout reduction in negative energy balance and hyperketonemia. Measures to reduce hypocalcaemia around calving are presented in the following section. Feeding vitamin E and Se have long been associated with reducing incidences of retained placentas and uterine infections. Increased protein content of the diet fed during the prepartum period was also associated with decreased incidences of retained placenta (Curtis et al., 1985).

Although feeding is generally not related to the incidence and severity of udder edema, some dietary measures can help prevention of the problem. Restriction of the salt NaCl in the diet reduces the severity of udder edema (Randall et al., 1974). Restriction or exclusion of other Na or K sources from the diet is also recommended until the levels of dietary Na and K are not in line with dietary recommendations. NRC (2001) recommends at least 0.1% Na in dietary DM for close-up dry cow diet and at least 1% K. As membrane permeability may be a primary cause of udder edema, vitamin E supplementation precalving may be a supportive preventive measure (Thomas et al., 1990). More recently, feeding anionic diets or diets with additional antioxidants have shown some promise in reducing udder edema (Goff, 2006a). Prevention of udder edema is important as condition has temporary effects of pain and stress for the cow, and may increase risk of udder inflammation and additional health problems.

## 4.3. Prevention of parturient hypocalcaemia

Concerning effects of hypocalcaemia on many physiological functions it is obvious that health, production and reproduction can be compromised in hypocalcemic cows even in the absence of clinical signs of paresis (Oetzel, 1996). Therefore, measures to prevent decline of blood Ca in cows after calving can improve milk production in herds that apparently have

no problem with this disease. Prevention aims to increase the mobilization of Ca from the skeleton, or its absorption in the gastrointestinal tract, or both. The most important nutritional measures for prevention of hypocalcaemia include manipulation of minerals in the diet which is nutritionally balanced in accordance with requirements of the cows several weeks before calving. This method of prevention is based on limiting the consumption of Ca or increasing the content of anions in the diet.

#### 4.3.1. Anionic diet

An anionic, or acidic, diet is one supplemented with anionic salts to provide more anions ( $\text{Cl}^-$  and  $\text{S}^{2-}$ ) relative to the cations ( $\text{Na}^+$  and  $\text{K}^+$ ). It has a negative dietary cation-anion difference (DCAD), calculated as  $\text{mEq/kg of DM} = (\text{Na} + \text{K}) - (\text{Cl} + \text{S})$ . It is well known that anionic diets help maintain blood Ca at parturition and prevent milk fever when fed to cows during the last several weeks of pregnancy (Block, 1984). Feeding an anionic diet before parturition has been advised if the incidence of milk fever in a herd exceeds 10%, and also when it is desired to improve the health status and production in herds in which MF is not a serious problem (Horst et al., 1994; Oetzel, 1993).

Diet with excess of anions, mainly  $\text{Cl}^-$  and  $\text{S}^{2-}$ , relative to the cations  $\text{Na}^+$  and  $\text{K}^+$  acidifies the body and is therefore considered as acidogenic (Goff & Horst, 1997b). The two PTH-dependent functions, including bone resorption and production of  $1,25\text{-(OH)}_2\text{D}$ , were enhanced after feeding acidogenic diet which contributes to lower incidence of hypocalcaemia after calving (Goff, 2000). However, the diets of dry cows in the field are always more or less alkalogenic and have a positive DCAD. When feeding diets with positive DCAD the acid-base balance tends to metabolic alkalosis, and vice versa. Formulation of acidogenic diet is only possible by supplementation with chloride and sulfate in a quantity that provides a relative excess of  $\text{Cl}^-$  and  $\text{S}^{2-}$  in the diet. Manipulation of dietary cations and anions, however, is limited by metabolic requirements and by tolerance levels of minerals in the diet.

The literature offered a dozen different formulas to calculate DCAD, which include some or all dietary minerals with or without the use of coefficients related to their utilization from the diet or the degree of influence on acid-base status:

A. $(\text{Na}+\text{K}) - (\text{Cl}+\text{S})$	Ender et al. (1971)
B. $(\text{Na}+\text{K}) - (\text{Cl}+\text{S}+\text{P})$	Lomba et al. (1978)
C. $(\text{Na}+\text{K}) - \text{Cl}$	Gaynor et al. (1989)
D. $(\text{Na}+\text{K}+\text{Ca}+0,3\text{P}) - (\text{Cl}+\text{S})$	Goff et al. (1991)
E. $(\text{Na}+\text{K}+0,38\text{Ca}+0,3\text{Mg}) - (\text{Cl}+0,6\text{S}+0,5\text{P})$	Goff (1992)
F. $(\text{Na}+\text{K}+0,38\text{Ca}+0,25\text{P}+0,3\text{Mg}) - (\text{Cl}-0,6\text{S})$	Goff (1992)
G. $(\text{Na}+\text{K}+0,38\text{Ca}+0,3\text{Mg}) - (\text{Cl}-0,6\text{S})$	Horst et al. (1997)
H. $(\text{Na}+\text{K}+0,15\text{Ca}+0,15\text{Mg}) - (\text{Cl}+0,2\text{S}+0,3\text{P})$	Oetzel (2000)
I. $(\text{Na}+\text{K}+0,15\text{Ca}+0,15\text{Mg}) - (\text{Cl}+0,25\text{S}+0,5\text{P})$	Goff (2000)
J. $(\text{Na}+\text{K}) - (\text{Cl}+0,6\text{S})$	Goff et al. (2004)

Equations A, B, and C imply the complete absorption of each dietary element that can be considered accurate only in the case of equation C. Equations E and G take into account the coefficients of utilization of those elements that are not completely utilized. Equations H and I could be considered biologically and functionally most accurate because they include the degree of influence of individual ions on acid-base status, and are physiologically the most relevant (Horst & Goff, 1997; Goff, 2000).

Equation F can be used to assess the risk of developing MF. All the elements that increase the risk of MF are on the left side, while those who decrease the risk are on the right side of equation. Equation D can serve the same purpose (Goff et al., 1991). The last equation J,  $(Na + K) - (Cl + 0,6S)$ , was the most recently proposed by Goff et al. (2004).

Oetzel (1991) collected data from 75 published experiments and determined the risk factors of diet in the development of MF using meta-analysis technique. Comparing the three different equations to calculate DCAD he found that the equation  $(Na + K) - (Cl + S)$  was strongly correlated with the appearance of MF. The author in the same study well justified the inclusion of S in the calculation of DCAD. Charbonneau et al. (2006) used the same technique but found that the equation  $(Na + K) - (Cl + 0,6S)$  was the most highly associated with clinical milk fever ( $R^2 = 0,44$ ) and urinary pH ( $R^2 = 0,85$ ).

Element	Ar	Valence	Equivalent weight (g/Eq)	Conversion factor (from % in mEq/kg)
Na	22,99	1	22,99	434,98
K	39,09	1	39,09	255,77
Ca	40,08	2	20,04	499,00
Mg	24,31	2	12,16	822,88
Cl	35,45	1	35,45	282,06
S	32,06	2	16,03	623,83
P	30,97	3	10,32	968,56

**Table 1.** Mineral elements in the diet used for calculation of DCAD

Mineral content in feed is usually expressed as a percentage so it is necessary to convert it in mEq/kg for calculation of DCAD. Equivalent weight (g/Eq) of each element is calculated by dividing the relative atomic weight (Ar) with its valence. For example, Ar of S is 32,06. Dividing 32,06 by 2 we get the equivalent weight of S which is 16,03 g/Eq. Factor for conversion of percentage in mEq/kg is calculated by dividing the 10.000 with equivalent weight of the element. For S it is  $10.000/16,03 = 623,83$  (Table 1). Multiplying this factor with percentage of the element in dietary DM its content is expressed in mEq/kg DM. When this is calculated for each element in the equation, then the sum of cations is subtracted from the sum of anions and DCAD value is obtained. The example of calculating DCAD of alfalfa hay and rapeseed meal using equation  $(Na + K) - (Cl + S)$  is given in Table 2.

Alfalfa hay	K	Na	Cl	S
Content in DM, %	1,71	0,12	0,38	0,28
Factor for multiplication (F)	255,77	434,98	282,06	623,83
Content in mEq/kg DM (% × F)	437,37	52,20	107,18	174,67
DCAD mEq/kg DM [(Na+K) – (Cl+S)]	489,57 – 281,85 = + 207,72			
Rapeseed meal				
Content in DM, %	1,36	0,10	0,11	1,25
Factor for multiplication (F)	255,77	434,98	282,06	623,83
Content in mEq/kg DM (% × F)	347,85	43,50	31,03	779,79
DCAD mEq/kg DM [(Na+K) – (Cl+S)]	391,35 – 810,82 = - 419,47			

**Table 2.** Example of calculating DCAD in alfalfa hay and rapeseed meal

The example in Table 2 clearly shows the difference in the alkali-acid production potential of the two feeds depending on their mineral content. A positive DCAD of alfalfa hay is result of the relatively high K content. Extremely negative DCAD of rapeseed meal is result of high S content.

Most diets commonly used for dry cows have DCAD value of +100 to +250 mEq/kg DM (Oetzel, 2000) and even up to +500 mEq/kg DM (Pehrson et al., 1999). By choosing appropriate feeds DCAD can be reduced, but not sufficiently to influence Ca metabolism. It is therefore necessary to enrich the diet with Cl and S by adding anionic salts in a quantity that provides a negative DCAD. That is, indeed, a standard diet that contains all essential nutrients required by dry cow and was further enriched with sources of Cl and S in amount that causes a mild metabolic acidosis.

Acidogenic, or anionic mineral salts are chlorides and sulfates with a high content of Cl and S, not containing Na and K. Alkalogenic salts are, on the other hand, Na and K salts that contain organic anion which is metabolized and are also called cationic salts ( $\text{Na}_2\text{CO}_3$ ,  $\text{NaHCO}_3$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{KHCO}_3$ , etc). They have the effect opposite to anionic salts and are undesirable in the diet of dry cows.

To formulate an anionic diet three chlorides and three sulfates are commonly used (Table 3). Two or more of them are usually added depending on mineral content of the diet. Ca and Mg salts also serve to meet requirements in these two minerals whose content in anionic diet should be higher than in standard diet (NRC, 2001). Ammonium salts, besides Cl and S, are also a source of non-protein nitrogen.

Acid production potential or acidifying activity of certain salt depends on preferential absorption of anions in relation to cations which the salt is consisted of (Goff et al., 1991). Phosphates, for example, have a weak acidifying activity as they are absorbed only slightly better than the corresponding cations (Horst et al., 1997). NaCl is neutral salt as both elements are absorbed completely and none is metabolized, so it does not affect neither DCAD nor acid-base status.

Salt	Mr	Val	g/Eq	DM, %	% of DM					DCAD mEq/kg DM
					Ca	Mg	N	Cl	S	
MgSO <sub>4</sub> ·7H <sub>2</sub> O <sup>1</sup>	246,51	2	123,26	48,83		20,20			26,63	-16.613
MgCl <sub>2</sub> ·6H <sub>2</sub> O <sup>2</sup>	203,33	2	101,67	46,83		25,53		74,46		-21.002
CaSO <sub>4</sub> ·2H <sub>2</sub> O <sup>3</sup>	172,18	2	86,09	79,07	29,44				23,55	-14.691
CaCl <sub>2</sub> ·2H <sub>2</sub> O <sup>4</sup>	147,02	2	73,51	75,49	36,11			63,88		-18.018
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> <sup>5</sup>	132,14	2	66,07	100,0			21,20		24,26	-15.134
NH <sub>4</sub> Cl <sup>6</sup>	53,49	1	53,49	100,0			26,19	66,27		-18.692

<sup>1</sup>magnesium sulfate heptahydrate (Epsom salt), <sup>2</sup>magnesium chloride hexahydrate, <sup>3</sup>calcium sulfate dihydrate (gypsum), <sup>4</sup>calcium chloride dihydrate, <sup>5</sup>ammonium sulfate, <sup>6</sup>ammonium chloride, Mr – relative molecular weight of a substance (sum of atomic weights of all atoms in the molecule), Val – valence, g/Eq – equivalent weight (Mr/valence of cation), DCAD = (Na+K) – (Cl+S).

**Table 3.** Characteristics and chemical composition of anionic salts

Anionic salts are not harmless substances and require caution for the use and handling them. The main limiting factors are bitter and salty taste and potential toxicity of higher doses. NH<sub>4</sub>Cl is considered the most toxic, followed by CaCl<sub>2</sub>. The combination of salts is the best solution because it reduces the possibility of harmful effects (Oetzel, 2000). The most acceptable method of administration is mixing salts in the feed.

An alternative to the anionic salts is adding mineral acids directly into a diet. Very potent in causing systemic acidosis is hydrochloric acid (HCl), while sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) is less efficient (Goff & Horst, 1997a). An additional advantage of mineral acids is that cows prefer diets with acidic taste rather than bitter-salty taste of anionic salts. Goff & Horst (1998) found that efficient prevention of MF can be achieved with only 1,5 Eq of HCl in the diet, and that with no adverse consequences up to 2,5 Eq of the acid can be fed daily. Moreover, the addition of HCl in the diet had, unlike anionic salts, a beneficial effect on the consumption of feed. For safety, it is not advisable to use pure acid or keep it on farm, but rather as commercial products in the form of acidified feeds where the acid is mixed with some feed as a carrier. Byproducts of fermentation are usually used for this purpose, and also soybean meal or sugar beet pulp (Goff & Horst, 1997a, 1998).

Feeding anionic diet few weeks before calving significantly reduces the incidences of MF and subclinical hypocalcaemia in the herd, and improves milk production and reproductive performances of cows in the subsequent lactation (Beede et al., 1991; Block, 1984; DeGroot et al., 2010). Metabolic acidosis caused by anionic diet is subclinical, mild and compensated, and with no significant impact on animal health, but may influence some physiological functions if lasts long enough. Increased sensitivity of PTH receptors in renal tissue contributes to increased production of 1,25-(OH)<sub>2</sub>D before parturition (Gaynor et al., 1989; Goff et al., 1991). Cows fed anionic diet have a higher content of 1,25-(OH)<sub>2</sub>D in plasma, although changes in secretion of PTH does not occur (Phillippo et al., 1994). The active form of vitamin D takes part in osteolysis with PTH (Horst et al., 1994; Horst, 1986) and stimulates Ca reabsorption in renal tubules (Goff, 1992). Since 1,25-(OH)<sub>2</sub>D is important for the functioning of both Ca compensation mechanisms, change in its secretion is considered as an important effect of anionic diet.

Oetzel (1993) recommended the application of anionic salts in the herds with high incidence of MF (>10%) if not possible to formulate low Ca diet, or to improve production and reproduction in the herd which apparently does not have high incidence of MF. Adding anions in the diet is, therefore, to be considered not only for preventing MF, but also for prevention of subclinical hypocalcaemia which is responsible for the frequent occurrence of other metabolic and reproductive disorders in puerperium (Horst et al., 1994). Beede (1992) states that even in well-kept herds, where cows are in proper condition with no major problems with metabolic disorders, an additional 250-500 kg of milk can be achieved by using anionic salts in diets before parturition probably because lower incidence of subclinical hypocalcaemia.

Based on results of earlier studies, Horst et al. (1997) found that the most effective diets for prevention of MF are those with DCAD from -50 to -100 mEq/kg DM, while others state that it is between -50 and -150 (Beede, 1995), or -200 (Horst et al., 1994). However, Beede et al. (1991) achieved a good preventive effect with DCAD -250 mEq/kg, and Goff et al. (1991) with -230 mEq/kg. Too low DCAD, irrespective of the amount of added salts, can reduce feed consumption because too strong metabolic acidosis which depresses the appetite in animals (Goff & Horst, 1997b). Preventive effect of anionic diet on MF incidence occurs only if DCAD is below -40 mEq/kg DM in most cases, regardless of the type and quantity of added salts.

A risk for many health and reproductive disorders in puerperium is increased in cows with hypocalcaemia (Curtis et al., 1985; Massey et al., 1993). The prevention of hypocalcaemia can, in addition to better health status of cows in puerperium, improve milk production and increase reproduction performances in the herd (Beede et al., 1991; Block, 1984; DeGroot et al., 2010). Comparing the milk production in healthy cows with those who suffered MF, Block (1984) found greater milk production by about 14% in healthy animals. The same author found that cows fed anionic diet in the dry period produced on average 486 kg (7,3%) more milk than those fed cationic diet. Beede et al. (1991) conducted a field experiment with 510 cows and showed three positive effects of anionic diet: enhanced Ca metabolism in periparturient period, increased milk production in subsequent lactation, and better reproductive performances. Cows fed anionic diet before parturition produced 327 kg more milk (about 3,6%) than control cows.

Because of unpleasant taste, anionic salts can have depressing effect on feed intake (Joyce et al., 1997; Vagnoni & Oetzel, 1998), but this is minimal if salts are properly used (Block, 1984; Moore et al., 2000; Oetzel et al., 1988). Up to 300 mEq of anions/kg DM (3-3,5 Eq of salts per day per cow) can be added to the diet without depressing effect on feed intake (Horst et al., 1994; Oetzel, 2000). Intake also could be influenced by the type of salt because more acidic salts have less favorable taste. Feed intake depression was at least expressed with  $MgSO_4$ , and much more with other salts, mainly  $CaCl_2$  and  $NH_4Cl$  (Oetzel & Barmore, 1993). Last two also have much higher acidifying activity in relation to  $MgSO_4$ .

The best and easiest method of application of anionic salts is mixing in TMR which effectively masks their taste. In the conventional feeding system application of anionic salts is difficult, but still possible. The salts should be manually mixed in silage or haylage, or in concentrates (Oetzel & Barmore, 1993). In the latter case, salts should be mixed in sufficient amount of concentrates containing palatable feeds, at least 2,3-2,6 kg (Oetzel & Barmore, 1993; Pehrson et al., 1999).

Oetzel (2000) finds that preventive effect of anionic salts can be achieved if cows consume anionic diet at least 5 days before calving, while others state that at least 10 days is necessary. Since the expected and actual date of calving are matter of discrepancies in practice, it is advisable to start feeding salts 3-4 weeks before expected parturition, at least 2-3 weeks, to ensure that most of the cows consume the diet at least 10 days (Beede 1992, 1995).

NRC (2001) gives fairly broad recommendations for the content of Ca in anionic diet for dry cows, from 0,6 to 1,5% DM, which is not difficult to formulate when anionic Ca salts are used. Anionic salts should not be fed if Ca intake is below 50 g/day (Oetzel, 1993). As hypomagnesaemia negatively affects Ca metabolism at the time of calving, many authors emphasize the importance of adequate content of Mg in dry cow diet (Sansom et al., 1983; Thilsing-Hansen et al., 2002). Recommendation has increased from earlier 0,20-0,25% to the current 0,35-0,40% DM either in standard or in anionic diet (NRC, 2001). Increasing dietary Mg up to 0,40% DM has no negative consequences so, even if not prove useful, poses no risk or any practical problem in the formulation of a diet when anionic Mg salts are used (Wang & Beede, 1992). On the other hand, the Mg level of 0,4% DM has been also set as the maximum tolerable (NRC, 2001).

If we assume that DCAD in efficient anionic diet should be around -100 mEq/kg DM, it means that the initial DCAD should not be greater than +200 to +250 (Horst et al., 1997). Therefore, the diet must be formulated using feeds with lower content of K, such as corn silage, grass hay, etc. If necessary, part of the forage with high content of K can be replaced with concentrates rich in fiber, such as brewers' grains, sugar beet pulp, malt sprouts, etc. The most common problem is grass silage and alfalfa silage because they can contain more than 3% K. Most cereal grains have almost neutral DCAD, while it is more negative only in rapeseed meal and brewers grains primarily due to high content of S (NRC, 2001).

In field conditions, a marked decrease in feed intake may occur when anionic salts are included in the diet for the first time, therefore, adjusting cows should last at least three days (Oetzel, 2000)

The most accurate biological indicator of the degree of acidification of the body is pH of urine (Vagnoni & Oetzel, 1998). Urinary pH in cows was decreased linearly with decreasing DCAD (Charbonneau et al., 2006; Ganjkanlou et al., 2010). The optimum pH of urine to prevent puerperal hypocalcaemia in Holsteins fed anionic diet is 6 - 6,5 (Horst & Goff, 1997) or wider, 6 - 7 (Moore et al., 2000). In Jersey cows, however, the pH of urine is necessary to reduce to 5,8 - 6,2. If the pH is lower than the specified, amount of anionic salts should be reduced and vice versa.

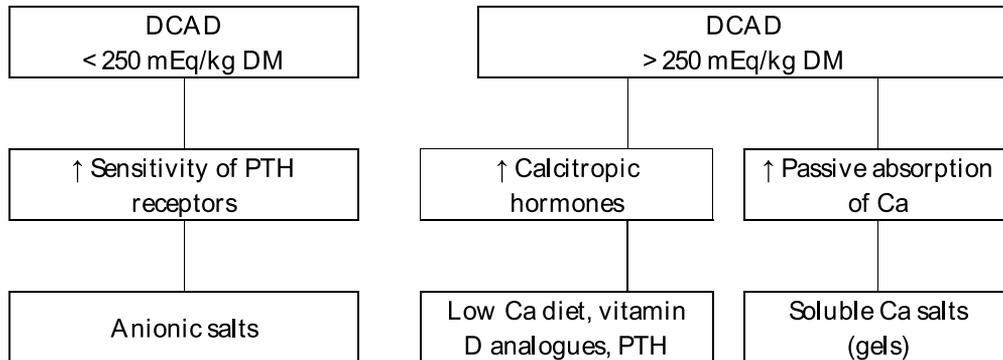
#### 4.3.2. *Low calcium diet*

Maintenance requirements of the cow and requirements of fetus during high pregnancy are usually satisfied with 35-45 g Ca/day (NRC, 2001). The content of Ca in the diet is often beyond that, and the requirements in this case are met mainly by passive absorption, while mechanisms of mobilization of body reserves and active absorption of Ca in the intestine are suppressed. Inactivity of these mechanisms in the dry period makes cows difficult to adapt to the sudden loss of Ca in colostrum at the moment of mammary gland activation. A few days required for starting these mechanisms causes temporary crisis in Ca homeostasis that results in decline of Ca in the blood (Goff, 1992).

When Ca content in the diet is below the minimum requirement, the animal is dependent on Ca mobilization from bone with simultaneous increase of active Ca absorption in the gut (Horst, 1986). These mechanisms can be activated before parturition and maintained active by constant stimulation up to the critical moment of parturition. Restriction of Ca intake to below 20 g/day caused negative balance of Ca and stimulates the secretion of PTH which increases tubular Ca reabsorption, bone resorption and production of 1,25-(OH)<sub>2</sub>D (Goff, 1992; Horst et al., 1997). This allows the cow to use Ca more efficiently from the diet and from body reserves immediately after calving (Goff, 1992). Introduction of low Ca diet leads to a slight decrease of Ca and P concentrations in plasma which returns to the base level in 3-4 days due to simultaneous increase in secretion of PTH that remained increased up to calving time (Goings et al., 1974). Prolonged exposure of the tissues to elevated PTH levels after feeding low Ca diet can overcome tissue resistance to PTH that might be induced by high dietary K (Goff, 2006a). This is the way to reduce or avoid period of adaptation to sudden losses of Ca which usually takes several days (Goff, 1992). Rations formulated to contain less than 20 grams of Ca proved to be very effective in preventing MF and significantly reduce the occurrence of hypocalcaemia too (Goings et al., 1971, 1974; Wiggers et al., 1975). For the best effect, restrictions of Ca consumption in the dry period should last at last 7-10 days (Goings et al., 1974; Wiggers et al., 1975) and be followed by a high Ca diet immediately after calving (Horst et al., 1994; Oetzel, 2000). Cows fed in this way at the moment of calving mobilize about 10 g of Ca from the skeleton daily that is enough to prevent the occurrence of MF (Wiggers et al., 1975). Increasing Ca in the diet above daily needs and manipulation of its content within these limits does not affect the blood Ca status if other dietary minerals are maintained unchanged (Goff & Horst, 1997b).

Feeding low Ca diet to dry cows is considered the traditional way of preventing MF. For this reason, the use of feeds rich in Ca is usually avoided and the diet is formulated with corn silage and grass hay. Consumption of Ca can be reduced to 50-60 g/day in this way what often gives good results in the field (Goff, 1992; Horst et al., 1997). However, significant stimulation of parathyroid gland and complete preventive effect can be achieved only if Ca consumption is restricted to below 20 g/day. The formulation of such diet is, unfortunately, unrealistic in practice and that is the reason for its limited application (Goff, 1992). This type of diet has no adverse effects on production in the subsequent lactation (Goings et al., 1974), but the problem is, in addition to impracticality, that it may not last longer than 2-3 weeks because negative balance of Ca can be too exhausting for body reserves (Van Saun & Sniffen, 1996).

In the strategy for prevention of MF, Horst et al. (1997) recommended measures depending on the baseline cation-anion difference of the diet (Fig. 1). If it is less than 250 mEq/kg DM, the use of chloride and sulfate is justified without danger of low feed intake. If this value is above 250 mEq/kg DM it is necessary to consider other preventive measures, such as low Ca diet, short-term administration of oral Ca salts or some pharmacologic therapy (vitamin D analogs and active metabolites, PTH injections, etc.).



**Figure 1.** Strategy for preventing milk fever (adapted from Horst et al., 1997)

Thilising-Hansen et al. (2002) reviewed research conducted over the past 50 years and concluded that any measures for preventing MF, even if used under ideal conditions, rarely reach preventive effect of 100%. The same authors calculated that efficiency of low Ca diets is best, reaching 80-100%, and anionic diets efficiency was 65-80%.

#### 4.4. Prevention of hypomagnesaemia

Commonly used parameter to characterize the grass tetany potential of forage is the ratio  $[K/(Ca+Mg)]$  (Mayland, 1988). Forages containing less than 0,2% Mg and a "tetany ratio"  $[K/(Ca+Mg)]$  greater than 2,2 have increased risk of inducing grass tetany (Crawford et al., 1998). The fertilizers containing N and K are the most important factors increasing  $[K/(Ca+Mg)]$  ratio in forages. Key step in nutritional prevention of hypomagnesaemia is finding a forage with lower ratio  $[K/(Ca+Mg)]$ , or modify this ratio in the diet by adding more Ca and Mg supplements. Commercial grade  $MgO$ ,  $MgCl_2$ ,  $MgCO_3$  and  $MgSO_4$  are good sources. As hypomagnesaemia may induce hypocalcaemia, including Ca in supplements to prevent Mg tetany may have a beneficial effect (Robinson et al., 1989).

NRC (2001) recommends Mg level of 0,35-0,40% DM in the diet for dry cows, although studies showed no benefit from increasing dietary Mg above 0,2% DM when diets contain less than 3% K (Wang & Beede, 1992). Van Saun & Sniffen (1996) recommended increasing dietary Mg above 0,20% when the content of K exceeds 1,2 to 1,5% DM. However, Goff (2006a) recommends that Mg content of the close-up dry cow ration and the early lactation ration should be between 0,35 and 0,40% as insurance against the possibility that the active transport processes for Mg absorption are impaired. In pregnant cows high Ca intake decreased utilization of dietary Mg and increases its excretion in urine (Sansom et al., 1983)

that can be even worsened in metabolic acidosis (Wang & Beede, 1992). So it seems logical to increase Mg content in anionic close-up diet because it usually contains more Ca.

#### 4.5. Prevention of milk fat depression

As milk fat depression has been observed over a wide range of feeding situations, this problem on dairy farms remains one of the more challenging tasks within overall nutritional management of dairy cows. Diets high in concentrates and low in fiber, and diets supplemented with plant or fish oil are the most often associated with MFD, but many other dietary factors also can affect milk fat synthesis including those which are able to alter rumen environment and those related to supply of polyunsaturated fatty acids (PUFA). Factors that can alter rumen environment include low level of physically effective NDF, feed particle size, total fiber in the diet, starch and non-structural carbohydrates, feeding pattern etc. On the other side, factors related to supply of PUFA are variation in fat content and fatty acid composition of feed ingredients, amount and availability of PUFA, and also feeding pattern.

Factor that alter rumen environment are first to consider in nutritional strategy to prevent or solve the problem of MFD, and those who affect rumen pH are the most important. Lower than normal ruminal pH, even without signs of acidosis, causes the change in bacterial population favoring those that have alternative pathways of biohydrogenation of dietary fatty acids. Inclusion of adequate level of so-called "effective fiber" and appropriate buffers in lactation diets can prevent drop in ruminal pH and markedly decrease MFD (Bergen, 2009). Forages, including long stemmed hay, are the main sources of effective fiber, thus the best method to maintain an adequate fat percentage in milk is to feed a balanced ration with adequate forage. The low levels of effective fiber may result from overfeeding of concentrates or the lack of forage, from consumption of large amounts of lush pasture and from silage or haylage that is too finely chopped (Perfield & Bauman, 2005).

Additional management practices to maintain a stable milk fat percentage in dairy herds include regular feeding of diet without abrupt changes, as well as feeding buffers such as Na-bicarbonate and/or Mg-oxide. Buffers are particularly useful when more than 5,5 kg of concentrate is fed per feeding and when frequent changes in diet are made.

Adding different sources of fat in dairy cow rations is a practice which has been favored when it is necessary to increase the energy consumption. However, higher amounts of fat can inhibit the activity of rumen bacteria and reduce the efficiency of fiber digestion, thus leading to a reduction in milk fat content (Schroeder et al., 2004). Although unsaturated vegetable oils can have a number of positive effects on fatty acid composition of milk fat, a negative effect on fat content must not be neglected due to the fact that the fat in milk is one of the main determinants of the price of milk and profits that farmers make. High levels of unsaturated oil in the diet can reduce fat content in milk along with possibly other adverse effects on production performances, such as drop in milk yield, decreased protein content in milk, low feed intake, etc. (Shingfield et al., 2006). Looor et al. (2002) found that the upper limit for dietary supplementation of unsaturated vegetable oils is 3,5% DM without serious negative consequences on production. If fish oil is the supplement, it is at most 1% DM (Donovan et al., 2000).

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# Vitamin-Like Supplementation in Dairy Ruminants: The Case of Choline

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50770>

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## 1. Introduction

Recent developments in nutrition have established that choline is an essential nutrient for mammals when a sufficient supply in methionine and folates are not available in the diet. Vitamin B12 is also involved in this process. The dynamic interactions between these components introduced the concept of choline as vitamin-like compound. Two types of choline functions are known: as choline *per se*, for which the choline moiety is required, and functions as a methyl donor. Choline *per se* plays a major role in lipid metabolism, particularly in lipid transport, as lipotropic agent. Choline is also an important source of labile methyl groups for the biosynthesis of other methylated compounds. Based on this second function choline and methionine are interchangeable, as sources of methyl groups. Accordingly choline occupies a key position between energy and protein metabolism in mammals.

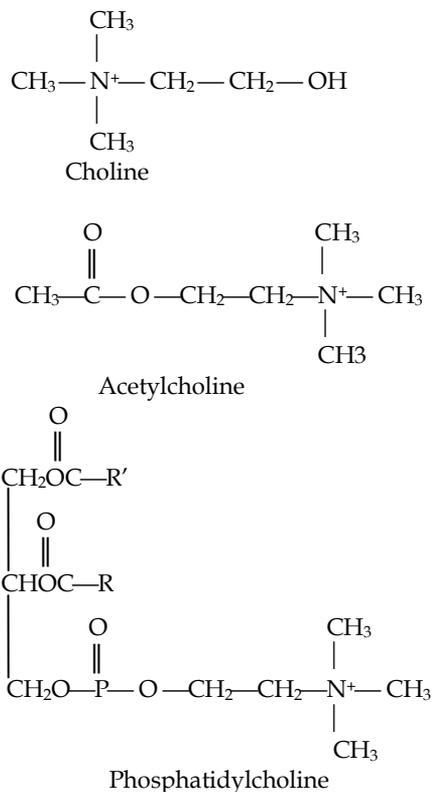
Choline and methyl group metabolism in ruminants however are different. In adult ruminants, choline is extensively degraded in the rumen; for this reason dietary choline contributes insignificantly to the choline body pool and methyl group metabolism is generally conservative with a relatively low rate of methyl catabolism and an elevated rate of *de novo* synthesis of methyl groups via the tetrahydrofolate (THF) system. This can be exacerbated in lactating dairy ruminant, in which the dietary availability of choline is nearly non-existent, but the output of methylated compounds in milk is high, while methionine as well as other sources of methyl groups are likely to be in short supply, especially at the onset of lactation. In light of this, the hypothesis that choline can be a limiting nutrient for milk production has been formulated and tested in several studies. Accordingly this chapter will focus on the effects of rumen-protected choline (RPC) supplementation to transition and early lactating dairy ruminants (cows and goats) on milk production and on metabolic health. Experimental data will be discussed in a systematic analysis, in order to define possible recommendations for high yielding dairy ruminants.

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## 2. What is choline?

In 1849 Adolph Strecker, a German chemist, isolated a compound from pig bile, to which he subsequently (in 1862) applied the name choline (from the Greeke, *chole*, bile). In 1867, Bayer, determined the chemical structure of choline (McDowell, 1989).

Choline is a beta-hydroxyethyltrimethylammonium hydroxide (figure 1), which is widely distributed in nature as free choline, acetylcholine, and more complex phospholipids and their metabolic intermediates. Its role in the body is complex. It is needed for neurotransmitter synthesis (acetylcholine), cell-membrane signaling (phospholipids), lipid transport (lipoproteins), and methyl-group metabolism (homocysteine reduction) (Zeisel & Da Costa, 2009).



**Figure 1.** Structural formula for free choline, acetylcholine, and phosphatidylcholine (lecithin). R and R' refers to any fatty acids (adapted from McDowell, 1989).

### 2.1. Vitamin or not?

In mammals choline has been classified as one of the B-complex vitamins but it does not satisfy the standard definition of a vitamin (Pinotti et al., 2002): it is synthesised endogenously and there is no evidence that it is an enzyme co-factor; furthermore, unlike other water-soluble vitamins, it is difficult to identify a deficiency syndrome for choline in

healthy mammals because of its interrelation with methionine, folic acid, and vitamin B12 (Scott, 1999; Zeisel, 1988). Finally, choline is a vital component of tissues and is required in the diet of non-ruminant species at much higher levels than the water-soluble vitamins (g vs. mg) (Whitehead & Portsmouth, 1989). However, the presence of an endogenous synthetic pathway does not render choline dispensable and deficiency results in several dysfunctions when other nutrients are limiting (Zeisel et al., 1991). For this reason it has been suggested that choline may be an essential nutrient (or vitamin like compound) for mammals when excess methionine and folic acid are not available in the diet (Zeisel & Da Costa, 2009). Consequently choline was officially recognized as an essential nutrient for humans by the Institute of Medicine in 1998 (Food and Nutrition Board, 1998).

## 2.2. How requirement for choline can be met

The metabolic need for choline can be met in two ways: either by dietary choline and via *de novo* biosynthesis through the methylation of phosphatidylethanolamine to phosphatidylcholine. From the point of view of animal nutrition, relatively rich sources of choline are soybean, soybean meal, rapeseed meal, fish meal and dried yeast, even though its bioavailability is considered "moderate" (Pinotti et al., 2002; Whitehead & Portsmouth, 1989). Dietary choline from a variety of choline-containing feed and food is absorbed by the intestine and uptake is mediated by choline transporters nutrient (McDowell, 1989). *De novo* synthesis of choline occurs by the sequential methylation of phosphatidylethanolamine, the methyl groups being supplied by S-adenosyl-L-methionine (SAM) (Mato et al., 1994). However, *de novo* synthesis of choline alone is not sufficient or rapid enough to satisfy all the animal's need. Methyl groups needed in this pathway may be derived from exogenous sources such as methionine, and betaine but can arise *de novo* in the body from the tetrahydrofolate (THF) system (Zeisel, 1988; Zeisel, 1992); vitamin B12 is involved in this process (Kennedy et al., 1995). Thus, dietary factors such as methionine, betaine, myo-inositol, folic acid, and vitamin B12 or combination of different levels of fat, carbohydrates, and protein in the diet, as well as the physiological state, all have influence on the "requirements" of choline (McDowell, 1989; Zeisel & Da Costa, 2009).

## 2.3. Functions of choline

Choline is considered a vitamin-like compound with two main functions: as choline *per se*, for which the choline moiety is required, and as methyl donor, although the two roles overlap.

Choline *per se* is an essential constituent of all cell membranes, where it is required to make the phospholipids phosphatidylcholine, lysophosphatidylcholine, choline plasmalogen, and sphingomyelin. Phosphatidylcholine, one of the most abundant phospholipids in higher plants and animals, is the predominant phospholipid (>50%) in most mammalian cell membranes (Kuksis & Mookerjee, 1978; Ruiz et al., 1983; Zeisel, 1988; Zeisel, 1992). Choline *per se* plays a major role in lipid metabolism, particularly in lipid transport, as lipotropic agent because of its ability to prevent or correct excess fat deposition in the liver generally arising as a result of its dietary deficiency (Kuksis & Mookerjee, 1978; Zeisel, 1988). Impaired triglyceride secretion to very low density lipoproteins (VLDL) is considered a major cause of fatty liver in dietary choline deficiency (Zeisel, 1988). In this context, it is noteworthy that

bovine VLDL phospholipids are mainly phosphatidylcholine, with smaller proportions of sphingomyelin and phosphatidylethanolamine (Moore & Christie, 1981). Thus, when massive mobilisation of fatty acids – as at the onset of lactation in dairy ruminants – is associated with lipotropic factor deficiency (e.g. choline) triglycerides accumulate in the liver and may lead to the development of fatty liver (Gruffat et al., 1996). Choline *per se* is required to prevent hemorrhagic kidney lesions in rats (Kuksis & Mookerjea, 1978); and together with other nutrients particularly manganese salts, is required to prevent perosis, a bone disease of poultry (Ruiz et al., 1983). In the prevention of perosis, choline is needed as constituent of phospholipids required for normal maturation of the cartilage matrix of the bone, whereas in the prevention of hemorrhagic kidney lesions choline seems involved in the renal phospholipids turnover (Kuksis & Mookerjea, 1978).

Choline *per se* is also essential for the synthesis of the neurotransmitter acetylcholine (Kuksis & Mookerjea, 1978; Zeisel, 1988). It plays important roles in brain and memory development in the fetus and appears to decrease the risk of the development of neural tube defects (Zeisel & Da Costa, 2009).

As methyl donor, choline, like methionine, is an important source of labile methyl groups for biosynthesis. Actually, the two principal methyl donors in animal metabolism are betaine, a choline metabolite, and S-adenosyl-L-methionine (SAM) a metabolite of methionine (Pinotti et al., 2002 for references). At least 50 SAM-dependent reactions have been identified in mammals, and it is likely that the number is much higher. Such methylation reactions play major roles in biosynthesis of lipids, the regulation of several metabolic pathways, and detoxification in the body (Zeisel & Da Costa, 2009). Accordingly it has been suggested that choline, and methionine are closely interrelated metabolically (Mato et al., 1994); it is also clear that choline has only little capacity to reduce the requirement for methionine, even though choline seems important to spare methionine as a methyl donor.

Although the choline-methionine interrelationship has been studied extensively (see Pinotti et al, 2002 for reference), less emphasis has been placed on the link between choline and its oxidation product, betaine. Betaine serves as an osmoregulator and is a substrate in the betaine–homocysteine (HCy) methyltransferase reaction, which links choline and betaine to the folate-dependent one-carbon metabolism (Kempson & Montrose, 2004; Ueland, 2011). The availability of choline and its metabolite betaine appear to influence the conversion or recycling of the HCy moiety of methionine, through betaine-HCy methyltransferase activity (Stipanuk, 1986). However, betaine fails to prevent fatty livers and haemorrhagic kidney (McDowell, 1989), indicating that the requirement for choline *per se* must be met as choline, and that betaine can substitute only the methyl donor function of choline, probably because betaine cannot be reduced to choline (Zeisel, 1988). Thus, choline *per se* must provide at least 50% of the total choline requirement, while the remaining portion of the choline requirement can be replaced by betaine (Dilger et al., 2007).

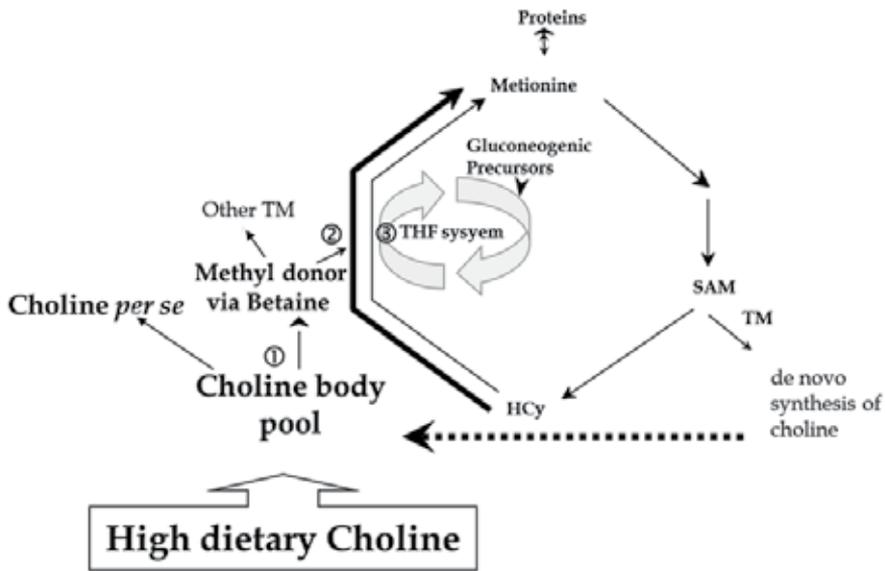
### 3. Choline in ruminants

Ruminants differ from most other mammals in regard to their choline and methyl group metabolism. In adult ruminants, choline is extensively degraded in the rumen. Quantitative

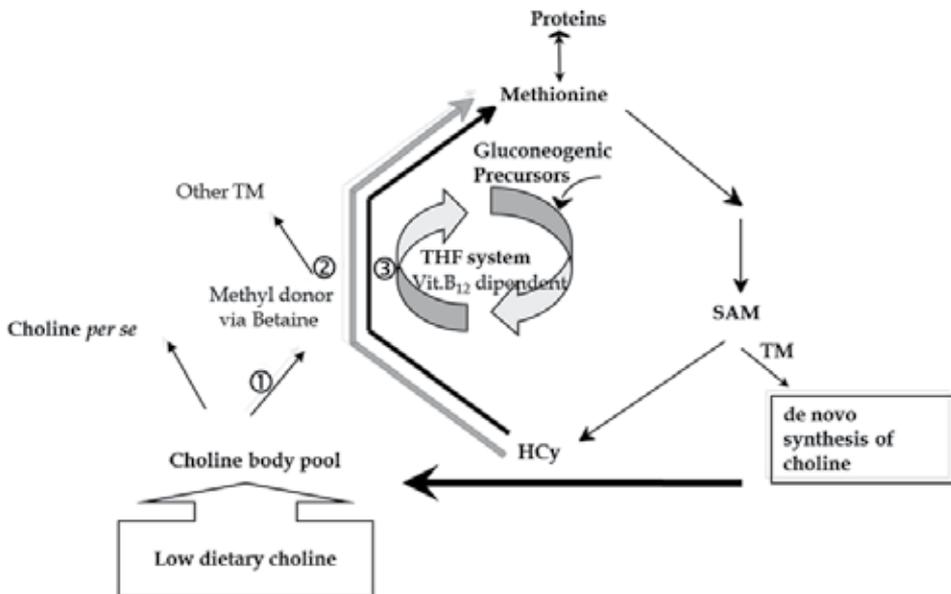
studies in sheep showed that 76% of [<sup>14</sup>C]-choline injected into the rumen was expired as methane over 6h, whereas approximately 15% accumulated as trimethylamine. Under such conditions, less than 10% of choline escapes degradation by incorporation, as phosphatidylcholine, into the structural membranes of ciliate protozoa (Neill et al., 1979). Nevertheless, the concentration of phosphatidylcholine in ruminal digesta was higher than in abomasal digesta, suggesting that protozoa are selectively retained in the rumen (Neill et al., 1979). In sheep with a defaunated rumen, the concentration of phosphatidylcholine in the abomasal digesta was higher than in the rumen (Dawson et al., 1981), leading to the suggestion that some of this abomasal Phosphatidylcholine was derived from non-dietary sources (regurgitation of bile from the lower digestive tract) (Robinson et al., 1984). In fact, intravenous injection of labelled choline in sheep indicated that the small amount of phosphatidylcholine present in abomasal digesta is largely (69%) of non-dietary or ruminal origin (Dawson et al., 1981).

The physiological state however, is important (Table 1). In the pre-ruminant lamb the activities of liver Cho-oxidase and betaine-HCy methyltransferase increase markedly after birth, but subsequently decrease as the animals reach the ruminant state (Xue & Snoswell, 1986a). The increasing activities of liver choline oxidase and betaine-HCy methyltransferase in pre-ruminant lambs are probably related to the abundance of choline-containing compounds in the milk (Xue & Snoswell, 1986a- Figure 2). By contrast, in adult ruminants only small quantities of methyl group nutrients are available from the diet and methionine synthase assumes a much more important role (Dawson et al., 1981; Neill et al., 1978; Neill et al., 1979; Kennedy et al., 1995). Methionine synthase is responsible for the de novo synthesis of methionine methyl groups from one-carbon units furnished by tetrahydrofolate (THF) (figure 1). The enzyme utilises methyl-THF as methyl donor and methylcobalamin as tightly-bound coenzyme. De novo synthesis of methyl groups via this system appears to be minimal when labile methyl group intake is sufficient or excessive (Stipanuk, 1986).

Dietary choline therefore contributes insignificantly to the choline body pool in adult ruminants (Table 1; Figure 3). Methyl group metabolism is generally conservative with a relatively low rate of methyl catabolism and an elevated rate of de novo synthesis of methyl groups via the tetrahydrofolate (THF) system (Girard et al., 2010; Henderson et al., 1983; Robinson et al., 1984; Snoswell & Xue, 1987; Xue & Snoswell, 1986a; Xue & Snoswell, 1986b). In dairy ruminants the situation can be even worse (Table 1): the dietary availability of choline is still low while the output of methylated compounds to milk is particularly high, and methionine, as well as other methyl group sources - one-carbon units for methylneogenesis via the THF system-, is likely to be in short supply, especially at the onset of lactation (Pinotti et al., 2002). In dairy ruminants producing large quantities of milk in fact, methionine is also the first limiting amino acid. This means that the elevated requirement for methionine for transmethylation reactions and milk protein synthesis may lead to altered methyl group metabolism (Girard et al., 2010; LaCount et al., 1995; Lobley et al., 1996). The effects of lactation on methyl group metabolism have been examined in sheep (Xue & Snoswell, 1985); it was found that the activities of hepatic phospholipid methyltransferase (for phosphatidylcholine synthesis) and methionine synthase were significantly higher (+ 33% and +34%, respectively) in lactating than non-lactating ewes (Xue & Snoswell, 1985).



**Figure 2.** Choline and methyl groups metabolism in pre-ruminants. SAM, S-adenosyl-L-methionine. HCys, Homocysteine. TM, Transmethylation pathway. THF Tetrahydrofolate system. ①Choline oxidase (EC 1.1.3.17). ② Betaine-homocysteine methyltransferase (EC 2.1.1.5). ③ Methionine synthase (EC 2.1.1.13). (adapted from Pinotti et al., 2002).



**Figure 3.** Choline and methyl groups metabolism in adult ruminants in positive energy balance (adapted from Pinotti et al., 2002). SAM, S-adenosyl-L-methionine. HCys, Homocysteine. TM, Transmethylation pathway. THF Tetrahydrofolate system. ①Choline oxidase (EC 1.1.3.17). ② Betaine-homocysteine methyltransferase (EC 2.1.1.5). ③ Methionine synthase (EC 2.1.1.13). (adapted from Pinotti et al., 2002).

Physiological Stage	Availability	Enzymes Activities
Pre-Ruminants	↑ choline ↑ betaine	↑ Cho-oxidase ↑ Betaine-HCy-methyltransferase
Adult Ruminants	↓ choline ↓ betaine ↑ methyl syntheses via THF	↑ Methionine synthetase
Lactating Ruminants	↓ choline ↓ betaine ↓ methionine ↓ gluconeogenetic precursors ↑ methyl syntheses via THF*	↑ Methionine synthetase

**Table 1.** Evolution of metabolism in methyl groups in ruminants in three physiological stages[\*at the onset of lactation, the substrates of this path often become limiting](adapted from Pinotti et al., 2002).

#### 4. Choline in dairy cows nutrition

The earliest investigations were interested in determining the effects of added dietary choline on milk production. For this purpose supplementation experiments using unprotected choline (Erman et al., 1984; Atkins et al., 1988; Sharma & Erdman, 1988b), were performed. In these studies using unprotected choline, neither milk production nor milk composition were affected, due, it was suggested, to rapid choline degradation in the rumen (Atkins et al., 1988; Sharma & Erdman, 1988b). In fact the work of Atkins et al. (1988) indicated that choline chloride was more degradable than naturally occurring choline in feed, while increasing choline chloride intake from 23 to 326 g/d only raised duodenal choline flow from 1.2 to 2.5 g/d, so that recovery was low indeed (Sharma & Erdman, 1988b). The mean estimates of rumen degradable choline (measured *in vitro*) actually were 80-98% for common feedstuffs and supplements (Table 2), indicating that use of choline-rich feedstuffs or additives in diets can only marginally increase the post ruminal flow of choline in ruminants (Sharma and Erdman, 1989a).

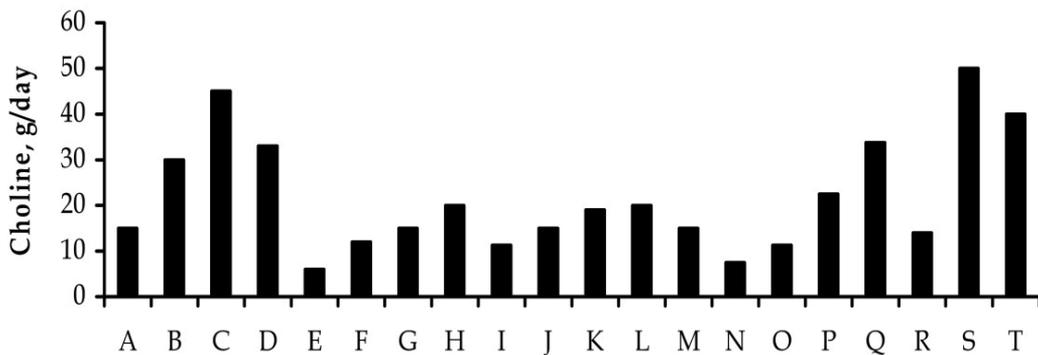
However, others studies (Sharma & Erdman, 1988a; Sharma & Erdman, 1989b) on the effects of abomasally infused choline, suggested a possible requirement for supplementing choline in lactating dairy cows. For example, Sharma & Erdman (1988a) investigated abomasal infusion of choline or methionine in dairy cows, either alone or with the choline synthesis inhibitor 2-amino-2-methyl-1-propanol (2AMP). They found that abomasal infusion of 30 g/d of choline was more effective than abomasal infusion of 45.6 g/d methionine (the molar equivalent of 30 g choline) in increasing milk yield and milk fat content. Additionally, milk production, milk fat percentage, milk fat yield, milk protein percentage, and milk protein yield of cows infused with 2AMP plus methionine were all lower than in cows infused with 2AMP plus choline (Sharma & Erdman, 1988a). These data suggest not only that supplemental choline is required to achieve maximal performance, but also that choline formed from methionine (Emmanuel & Kennelly, 1984) may be partially responsible for these stimulatory effects on milk production. From a technical/feeding point of view these studies also evidenced that choline must be rumen-protected to be effective in dairy cow nutrition.

Feedstuffs	Effective degradation, %
Barley	79.4
Cottonseed	84.7
Soybean meal	82.9
Fish-meal	83.8
Supplements	
Choline stearate	98.0
Choline chloride	98.6

**Table 2.** Rumen stability: In vitro choline degradation (Sharma & Erdman, 1989a). Data obtained during four individual fermentation runs, samples were incubated in vitro with an inoculum mixture containing rumen fluid obtained from a rumen-fistulated dairy cow fed 17.5% corn silage and 28.7% grass silage and 53.8% concentrate diet.

#### 4.1. Recent advance in choline supplementation in dairy cow

Although the choline requirement of dairy cows is still unknown (National Research Council, 2001), higher choline availability (by feeding rumen-protected choline, RPC) can have a favorable effect on milk production (Erdman and Sharma 1991; Erdman, 1994; Hartwell et al. 2000; Piepenbrink and Overton 2003; Pinotti et al. 2003; Scheer et al. 2002). In dairy ruminants, in fact choline has been proposed as limiting nutrient, especially at the onset of lactation (Erdman and Sharma 1991; Pinotti et al., 2002). Based on those considerations, the effects of rumen-protected choline (RPC) supplementation to transition cows have been investigated in several studies (figure 4).



**Figure 4.** Levels of choline chloride supplementation [provided in a rumen-protected (RP) form] to transition dairy cows in published studies. Citations keys are reported on the x axis: (A)–(C) Erdman & Sharma, 1991; (D) Erdman, 1994; (E)–(F) Hartwell et al., 2000; (G) Scheer et al., 2002; (H) Pinotti et al., 2003; (I)–(K) Piepenbrink & Overton, 2003; (L) Pinotti et al., 2004; (M) Janovick Guretzky et al., 2006; (N)–(Q) Xu et al., 2006; (R) Zahra et al., 2006; (S) Elek et al., 2008; (T) Davidson et al., 2008. Choline chloride supplementation is shown on the y axis.

Findings in transition and early lactating dairy cows suggest that greater choline availability can improve not only milk production (Erdman and Sharma, 1991; Hartwell et al., 2000; Pinotti et al., 2003), but also lipid (Piepenbrink Overton, 2003; Pinotti et al., 2003), methyl group metabolism (Baldi & Pinotti, 2006) and choline secretion in milk (Deuchler et al., 1998; Elek et al., 2008; Pinotti et al., 2003; Pinotti et al., 2004). Furthermore, in the Pinotti et al. (2003) study, although plasma concentrations of vitamin E declined after parturition in choline-supplemented animals, the reduction was less than in controls, suggesting improved vitamin E status. However, the mechanisms of this effect have not been elucidated with certainty, even though an improved fat absorption and transport induced by choline supplementation has been proposed (Pinotti et al., 2003). Janovick Guretzky et al. (2006) found that choline supplementation did not affect milk production in either Holstein or Jersey transition cows, but in discussing their results they noted that calculated methionine balance was negative postpartum, so that supplemental choline might not have spared enough methionine to produce a physiological benefit. This conclusion is in line with another study (Brüsemester & Südekum, 2006) that, in reviewing several published works on choline supplementation in dairy cows, concluded that negative methionine balance – and therefore a basal diet of adequate quality and composition (Baldi & Pinotti, 2006) – is essential for obtaining a response to rumen-protected choline. A further study of Zahra et al. (2006), reported that choline supplementation improves milk production (1.2 kg/day) in the first 60 d of lactation, although supplementation was only from 21 d prepartum to 28 days in milk. However, choline's effect on milk yield was attributable mainly to increased milk production (4.4 kg/day) in animals with body condition score  $\geq 4$  three weeks before calving, that were also consuming more feed. Supplementation of rumen-protected choline would therefore seem to be essential for optimising high-quality milk production in high-yielding dairy cows, as also reported in subsequent studies (Davidson et al., 2008; Elek et al., 2008; Xu et al., 2006), particularly in animals fed basal diets that limit post-ruminal methionine supply. The magnitude of the production response is likely to be affected by basal diet composition, the dose and mode of administration of the rumen-protected choline, and the stage of lactation, as discussed elsewhere (Baldi & Pinotti, 2006; National Research Council, 2001).

With regard to metabolic health, few studies in dairy cow also investigated the effects of choline supplementation on lipid metabolism (Piepenbrink & Overton, 2003; Pinotti et al. 2003; Pinotti et al. 2004 ), and hepatic lipidosis (Grummer, 2006; van Vuuren et al., 2010). In dairy ruminants it is known that the necessity to increase the lipolysis of adipose fat stores is most critical during the first stage of lactation (Grummer, 1993; McCarthy et al., 1968). During this stage, fat mobilisation leads to increased blood levels of non-esterified fatty acids (NEFA) which are taken up by the liver and oxidised to ketone bodies or carbon dioxide or esterified to triglycerides. The liver normally packages the triglycerides in VLDLs and secretes them, but sudden increases in plasma NEFA may not be adequately processed by the ruminant liver (Gruffat et al., 1996; Pullen et al., 1990). Choline serves as methyl donor in the synthesis of carnitine (Griffith, 1987) which is essential for fatty acid oxidation.

The esterification of NEFA to triglycerides, and their secretion to VLDL also involves choline (Gruffat et al., 1996). In this context the NEFA/cholesterol ratio can be considered a measure of the fat retained in or metabolized by the liver and its lowered value observed in RPC cows suggested a lower risk of fatty liver in these animals (Holtenius, 1989). Thus the lowered plasma NEFA levels and the NEFA/cholesterol ratio in RPC-supplemented cows at parturition suggest more efficient liver function and improved lipid metabolism in general (Pinotti et al. 2003).

A subsequent study (Pinotti et al. 2004) provided similar results, in that plasma NEFA was reduced by 18% on day 20 of lactation in supplemented animals. Likewise, Chung et al. (2009) found that RPC have a dose dependent effect on reducing plasma NEFA concentration by 11% and 23% in cows receiving 25g and 50 g of rumen-protected choline. These findings are also consistent with the evidence that RPC supplementation to dairy cows throughout the periparturient period decreased liver accumulation of lipids (stored as intracellular triglycerides- van Vuuren et al., 2010) and increased liver glycogen content (Piepenbrink & Overton, 2003). The effects of choline at liver level have been recently confirmed in transition dairy cows (van Vuuren et al., 2010), in which RPC supplementation resulted in lower TAG concentration, by 36% and 32% compare to untreated animals, in week 1 and 3 post-partum, respectively. Results obtained in these studies indicated that not only hepatic fatty acid metabolism and cow performance are responsive to increasing the supply of choline (Piepenbrink & Overton, 2003), but also that choline supplementation can reduce cellular lipid accumulation in the liver especially during the transition period.

A further aspect investigated in some of these studies was the effect of choline on methyl group status of lactating cows. Studies carried out by Pinotti et al. (2004) showed that the administration of 50 g of (RPC, providing 20 g of choline chloride) to transition dairy cows increased plasma levels of choline-containing phospholipids and folates by the end of the transition period (20 days in milk). These findings indicate that that RPC supplementation is effective both in increasing choline availability and in optimizing methyl group metabolism: the increase in plasma folates may be attributable to a sparing effect of choline on methyl group metabolism. As noted, choline is an important source of labile methyl groups, but when it is in short supply, methyl groups (e.g. for methionine resynthesis from homocysteine) must be synthesized *de novo* by the THF system or derived from other sources. The THF system consumes gluconeogenic precursors, so in periods of glucose imbalance (e.g. early lactation) such precursors can also be limiting (Armentano 1994; Rukkwamsuk et al. 1999). An unexpected finding of studies in this area is that plasma vitamin B12 concentrations in RPC-supplemented cows were lower than in controls (Baldi & Pinotti 2006). Vitamin B12 is considered a marker of methyl group status, and the lower concentrations in treated animals appear inconsistent with the observed increases in plasma folates and choline-containing phospholipids. However, vitamin B12 is required as a coenzyme in the biosynthesis of methionine from homocysteine and in gluconeogenesis from propionate. Increased gluconeogenesis may therefore deplete vitamin B12, as indicated

by the finding of a tendency toward higher concentrations of plasma glucose in RPC-supplemented cows. Nevertheless, these results were obtained from a limited number of animals, and therefore they should be considered with caution since our knowledge of the interaction of choline with vitamins and other essential nutrients are incomplete.

#### 4.2. Systematic analysis of choline supplementation in dairy cows

In the recent past different models (linear and logistic) were developed to permit the calculation of changes in milk yield and milk composition from amounts of rumen-protected choline in dairy cow diets. For example, Pinotti et al. (2010) investigated the effects of RPC administration on milk production by a regression model based on the results of 11 different studies (42 experimental groups) published between 1991 and 2008 (Davidson et al., 2008; Deuchler et al., 1998; Elek et al., 2008; Erdman et al., 1991; Hartwell et al., 2000; Janovick Guretzky et al., 2006; Pipenbrink and Overton, 2003; Pinotti et al., 2003; Pinotti et al., 2004; Xu et al., 2006; Zahra et al., 2006). The analysis included RPC supplementation (control/RPC) as fixed effect, the variability among experiments as random effect and their interaction. Treatments schedule, dry matter intake and dietary composition, accounted for most of the variability among experiments and were highly correlated in a preliminary data analysis; therefore, these variables were considered as experimental effects and treated as random components in the mixed model, assuming that the results of different experiments are affected by different experimental conditions. For each study, data were also weighted by the reciprocal of the squared standard error of the mean value, in order to consider the unequal variance among studies.

Choline amount administered	Milk yield estimate (kg/d)	RPC effect
Control (0 g/d)	32.35	-
RPC low level (<10 g/d)	33.40	0.15
RPC high level (> 10 g/d)	35.18	<0.001

**Table 3.** Results from mixed model when the different amounts of RPC administered were grouped into three supplementation levels (adapted from Pinotti et al., 2010).

The data reviewed in this analysis were consistent with the fact that choline supplementation significantly increased milk yield in dairy cows, even though, as expected, the dose of choline administered is also relevant (table 3). Considering three supplementation classes, i.e. control (no choline: 0 g/d), low level (<10 g/d of choline chloride in a rumen protected form) and high level (>10 g/d of choline chloride in a rumen protected form), the meta-analysis indicated that a low level (less than 10 g per day) of RPC supplementation tended to increase milk yield and a high level of supplementation of RPC significantly increased milk yield. This results were in line with another meta-analysis,

performed using a nonlinear model (Sales et al., 2010), on the effects of rumen-protected choline in dairy cows, in which choline supplementation has a positive effect on milk yield and milk protein content, but not on milk fat content.

## 5. Other dairy ruminant

In the case of small ruminants the effects of choline infusion on transmethylation reactions have been studied in sheep by Lobley et al. (1996), while Emmanuel and Kennely (1984) investigated methionine and choline incorporation into plasma and milk of lactating goats. More recently Banskalieva et al. (2005) assessed the efficacy of rumen-protected choline supplementation in meat goats. However, studies on choline supplementation and its effects on milk production, and lipid and methyl group status in periparturient dairy goats have been published only recently (Pinotti et al., 2008; Savoini et al., 2010). In a production trial (Pinotti et al., 2008) a supplementation of 4g/d of choline in rumen protected form was given to periparturient dairy goats, starting 4 weeks prior to expected kidding and continuing for 5 weeks after parturition. The quantity of choline given was based on experiments in dairy cows (Pinotti et al., 2005) and metabolic Body weight ( $BW^{0.75}$ ) of the goats at the beginning of the experiment. During the first 6 weeks of lactation, milk yield and four percent fat-corrected milk (FCM) yield were increased by 7 and 12%, respectively, in RPC supplemented than non-supplemented goats. Milk fat concentration, fat and protein yield were also increased by RPC treatment. The link between choline supplementation and milk response has been mainly attributed to the metabolic interchangeability of choline and methionine, in the sense that both can furnish labile methyl groups. This interchangeability has been investigated by Emmanuel and Kennely (1984) in goats, who estimated that 6% of the choline pool was derived from methionine and that approximately 28% of methionine is used for choline synthesis via the pathway for the *de novo* biosynthesis of the choline moiety involving sequential methylation of phosphatidylethanolamine. Reduction of methyl group demands from the methionine pool may therefore offer production benefits to dairy ruminants and might be achieved by nutritional manipulation other than alteration of the methionine supply (Lobley et al., 1996). This was probably the situation in the study by Pinotti and coworkers (2008), in which during the early stages of lactation of dairy goats dietary methionine was probably limiting (goats received about 80% of the optimal level of methionine). These considerations imply that feed composition, mainly protein supply and the availability of methionine (National Research Council, 2001) influence the effects of choline supplementation not only in dairy cows (Baldi & Pinotti, 2006; Brüsemeister & Südekum, 2006), but also in goats.

The milk production response to choline supplementation in dairy goats, was obtained without any detrimental effect on methyl groups status. In fact, folate availability at kidding was higher in choline-supplemented than in non-supplemented goats, while during lactation lack of a drop in plasma folate and vitamin B12 in the choline-supplemented animals, suggests that good methyl group status was maintained in choline-treated goats

(Pinotti et al., 2008; Savoini et al., 2010). This results was obtained, even though RPC receiving goats were producing more milk than the choline un-supplemented ones.

In a parallel study (Baldi et al. 2011) the effect of rumen-protected choline administration on metabolic profile, selected liver constituents, and on mRNA expressions of selected enzymes, transcription factors, and nuclear receptors involved in mammary lipid metabolism in dairy goats, have been investigated. It was evident from this study that at comparable milk yield during the first month of lactation, choline supplemented goats showed a lower level of plasma  $\beta$ -hydroxybutyrate, whereas no other differences were observed for other metabolites. The reasons for this difference are uncertain: authors conjecture that it might be due to the role of choline in lipid metabolism as already reported in dairy cows (Baldi & Pinotti, 2006; Chung et al., 2009). With regard to liver variables, it has been reported that total liver lipid/ DNA content was significantly lower (-35%) in RPC than CTR goats. As already reported above, dairy cows receiving choline have also been reported to have reduced hepatocellular lipid accumulation (Cooke et al., 2007; Grummer, 2006).

Within the mammary tissue many selected enzymes [e.g. lipoprotein lipase (LPL)], transcription factors and nuclear receptors that can be affected by choline availability are involved in lipid uptake and milk fat secretion. Furthermore, choline is actively secreted into mammalian milk. The major choline-containing compounds in bovine milk are unesterified choline, phosphatidylcholine, and sphingomyelin (Pinotti et al., 2003). Kinsella (1973) reported that a bovine mammary gland yielding 25 litres milk secretes  $10 \pm 3$ g phospholipids per day, corresponding on average to 5% of the phospholipids of the mammary tissue. The phospholipids of the membrane of the milk fat globule constitute the major choline-containing component of bovine milk (McPherson & Kitchen, 1983). The above data suggest that choline is an important metabolite in lactating mammary tissue, that it is used avidly when available (Kinsella, 1973), mainly in the lipid metabolism. Thus, it has been proposed that choline supplementation can have positive effects on lipid trafficking particularly lipid transport to and within extra-hepatic tissues (Baldi & Pinotti, 2006; Cooke et al., 2007; Piepenbrink & Overton, 2003) including the mammary gland. However, at udder level in lactating goats, choline did not affect the expression of several mammary gland transcripts involved in lipid metabolism (Baldi et al. 2011). The effects of nutrition on the expression of mammary lipogenic genes in dairy ruminants (cows, ewes, goats) have been investigated mainly by feeding milk-fat depressing diets, characterized by high levels of concentrates, or conjugated linoleic acids (CLA), or other lipids (fish oil, vegetable oils, full fat seeds, etc.) (Bauman, 2008; Bernard et al., 2006; Harvatine and Bauman 2006; Lock et al., 2008). Absence of significant effects on gene expression observed in goats could be due to the dose or to the type of supplementation. Thus, while in Baldi et al. (2011) were supplied 4 g/d choline (about 0.2% of DMI), other studies used higher levels of supplementation (for fats 3.6-11.2% of DMI) or more potent nutritional factors (e.g. CLAs are potent inhibitors of mammary gland fat synthesis) (see Bernard et al., 2006 for review). Nevertheless, these results were obtained from a limited number of animals, and choline effects and function in lactating mammary tissue deserve further investigation.

## 6. Conclusions

It can be concluded from this outline that dietary supply of choline may not always be sufficient to maximize milk production in both dairy cows and goat; and although the requirement for choline can in theory be satisfied by other nutrients, it is unlikely that this happens in practice especially at the onset of lactation. The magnitude of the production response is likely to be affected by basal diet composition, the dose of the rumen-protected choline, and the stage of lactation (Baldi & Pinotti, 2006; National Research Council 2001). In particular with respect to lactation cycle several studies provided convincing evidence that protected choline positively affects lactation performance especially when feeding is initiated prior to calving and is continued during early lactation (Grummer, 2011). In fact, assuming that choline is limiting in high yielding dairy ruminants, the greatest demand for choline should occur at lactation onset. It is also at this stage that the availability of other choline-related nutrients (i.e. methionine, folates, and vitamin B12) is often low (Baldi & Pinotti, 2006; Girard et al., 2010). From a metabolic and hepatic point of view, since choline is a lipotropic factor, it may be particularly beneficial at this time in view of the adipose and liver metabolism changes that occur during the transition from late pregnancy to early lactation (Piepenbrink and Overton 2003): choline may optimize the balance between fat retained and fat metabolized by the liver, thereby improving lipid metabolism in general. These roles of choline however, do not exclude that RPC supplementation is also effective in optimizing methyl group metabolism, which might be an added benefit of adequate choline supplementation. With regard to dose, even though an estimate of the minimum choline needed in dairy cows for maintenance functions (based on metabolic body size), is approximately 4 to 6 g/day (Donkin, 2011), production response were abstained when higher doses were fed (Pinotti et al., 2010). Consequently, although insufficient data are available to establish the choline requirement, it can be suggested that supplementation doses in this range are likely to be adequate for transition dairy ruminants.

However, it is also evident that our knowledge of the interaction of choline with vitamins and other essential nutrients is incomplete in the dairy ruminants. For example there are indications that both methyl group precursors (including choline) and appropriate co factors (folate and vitamin B12) are important for the optimal metabolic support of milk production (Pinotti et al., 2008), even though methionine may not always be involved in this scenario (Preynat et al, 2009). These aspects not only reflect the inadequacy of a nutritional approach based on the supply and utilisation of individual nutrients, but also suggest that the requirements of B-complex vitamins in dairy ruminants should be reconsidered.

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## Acknowledgment

This chapter has been developed in the frame of the COST Action FA0802 Feed for Health. The author wish also to thanks Dr. Gerwin Meijer from the Animal Science Group, Wageningen University, for his advisor in the early literature study on this topic, Prof. Antonella Baldi and Prof. Vittorio Dell'Orto, as well as all the member of the Animal Nutrition Research Group from the Veterinary Medicine Faculty of the Università di Milano (Italy), for their collaboration and support in several experiments carried out in this area.

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# **Effect of Rumen-Inert Fat Enriched with High Levels of Poly-Unsaturated Fatty Acids on the Productive and Reproductive Response in Ruminants**

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50879>

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## **1. Introduction**

The chapter will discuss the effect of rumen-inert fat enriched with high levels of poly-unsaturated fatty acids (PUFA) on the productive and reproductive responses in ruminants. The discussion will include characteristics of the milk and its derivatives such as goat cheese. The expertise presented in the chapter will cover the authors' experience about the interrelationship between nutrition and reproduction in ruminants from the College of Veterinary Sciences at the Central University of Venezuela.

The success of milk and meat production systems with ruminants depends largely on an efficient reproductive performance of the herd. Thus, numerous researchers have tried to identify the different factors affecting the productive and reproductive behavior of these herds, being the nutritional component one of the factors that has the highest impact on tropical livestock. Marked shortcomings of forage quality and quantity generate undernourished animals, originating low production parameters in our livestock, far below the desired levels. In countries located in the tropical region of the world the reproductive efficiency in beef and dairy cattle is low, no more than 40-45% (Diaz, 2009). In addition, the pregnancy rate in first calf cows is around 30%, post-partum anestrous period for cattle ranges from 150 to 210 days (Montaño and Ruiz, 2005) and early embryonic mortality in lactating dairy cows, is around 30% (Bach, 2003).

Furthermore, during the early postpartum period the lactating cow (dairy, beef and dual purpose cows), as well as the dairy goats, show a gap between the consumption and the amount of energy needed, especially during the time of maximum requirements, when the cow and the goat do not meet their nutritional requirements, which leads to the massive

mobilization of its body reserves, particularly energy, generating a state known as the Negative Energy Balance (NEB), which can cause deleterious effects on milk production, health and reproductive performance.

A practical way to control this NEB is through the increase of the food consumption by animals and/or the increase of the energy density of the diet. Thus, various food technologies have been developed for this purpose: energy banks, the use of strategic crops such as sugar cane, liquid diets with high levels of energy, multi-nutritional blocks and energy supplementation with rumen-inert fat. Increasing the fat content of the ration is a way to decrease the NEB, because of fats have double the energy than sugars. However, there are limitations on their use, particularly if the fat is not a rumen-inert fat.

If rumen-inert fat is used to feed ruminants, some undesirable effects of active fats at ruminal level, would be avoided, and higher amount of fat could be used.

On the other hand, if the fat is rich in polyunsaturated fatty acids (PUFA; linoleic, linolenic, docosahexanoic [DHA], or eicosapentaenoic acid [EPA]), the rumen-inert fat enriched not only supplies energy, but allows the incorporation of the beneficial effects that these fatty acids have on the animal body and in the productive and reproductive response, therefore having a nutraceutical effect, i.e., producing meat and milk with high levels of these fatty acids, which may have a beneficial effect on human health.

## 2. The fat and the feeding of ruminants

Fats are important in ruminant nutrition because of its high energy content. In this regard, the complete combustion of one gram of fat produces around 9.45 Kcal of net energy, while a typical carbohydrate generates around 4.4 Kcal. So, lipids, in general, provide 2.25 times more energy than traditional sources. Also, it is important to consider the intake of fat-soluble vitamins and essential fatty acids (Mayes, 1988).

Fats are part of a group of organic molecules called lipids, which accomplish many functions in the animal body: structural functions (part of cell membranes), hormonal functions (some hormones are of lipidic nature: estradiol, progesterone, testosterone, among others) and immunological functions. Some vitamins (A, D, E and K) are of lipid nature.

Fatty acids (FA) are fundamental molecules in the structure of a lipid, with hydrogenated carbon chains which contain an acidic or carboxyl group in one end and a methyl group in the other end (Jenkins, 2004). The length of the chains of the FA goes from 2 to 24 or more carbon atoms. It is common to name them according to the number of atoms and the number of double bonds, which serves to classify them as saturated or unsaturated fatty acids.

Animal tissues do not synthesized linoleic and linolenic acids (Omega 6 and Omega 3, respectively); they should therefore be incorporated within the diet (Jenkins, 2004). Both FA are known as essential in animal nutrition because they are required for many metabolic processes. Omega fatty acids belong to one of the three Omega families ( $\omega$ ):  $\omega$ -6,  $\omega$ -3 and  $\omega$ -9 (Table 1). Each family has a parental fatty acid, which can be converted into other biologically active acids within the same  $\omega$  family (Jenkins, 2004).

$\omega$ -Family	Parental fatty acid	Mayor Metabolite
$\omega - 9$	C 18:1 $\omega$ -9; Oleic acid	C 20:3 $\omega$ -9; Eicosatrienoic acid
$\omega - 6$	C 18: 2 $\omega$ -6; Linoleic acid	C 20:4 $\omega$ -6, Arachidonic acid
$\omega - 3$	C 18:3 $\omega$ -3; Linolenic acid	C 20:5 $\omega$ -3, Eicosapentaenoic acid C 22:6 $\omega$ -3, Docosahexaenoic acid

(Adapted from Jenkins, 2004.)

**Table 1.** Parental fatty acids and its main metabolites within each Omega family.

The sources of lipids in ruminant feeding systems are forages, cereals, oleaginous seeds, by-products of the industry, such as the tallow, yellow fats, mixtures of vegetable and animal fats, hydrogenated fat, oil palm and calcium soaps. Tropical forages are relatively low in their content of lipids; the concentration of fatty acids in forages rarely exceeds 1.5% of the dry matter of the diet.

Both oils and fats, belong to the group of lipids, but differ in that the first are liquids at ambient temperature, while fats are solid. Another difference is that fats are usually from animal origin (tallow) while most of the oils are from vegetables (excluding fish oil). Fats have high levels of saturated fatty acids, while the oils contain more PUFA.

Fats and oils have limitations to be incorporated in the feeding of ruminants. It has been reported that levels >5% of dry matter produced a decrease in food consumption of the animal. In this regards, Jenkins (1993) and Palmquist (1996) mention some of the possible ways of how fats can affect the microorganism action in the rumen:

- Rumen microorganisms cannot use the fiber because the formation of a film on the surface of the fiber, thus preventing the enzymatic and bacterial attack and disturbing the fermentation process.
- Reduction of microbial activity by adsorption of fat to the surface of the bacterial membrane.
- Possible formation of calcium and magnesium soaps in the rumen, which reduce the availability of essential minerals for the fermentative activity in the rumen.
- Elimination of a fraction of the microbial population due to possible toxic effects of some PUFA, especially on cellulolytic bacteria.

The above generates a reduction in the ruminal microbial growth, which translates into a change in the production of volatile fatty acids in the rumen, with consequences to the acetic:propionic ratio and a decrease in the amount of acetic acid available for the production of fat in the mammary gland. In the particular case of unsaturated fatty acids, once they are free in the rumen, they suffer a massive hydrogenation process known as biohydrogenation (Jenkins, 1993), which consists in the addition of hydrogen atoms on the double bonds, thus transforming the unsaturated fatty acids into saturated fatty acids. Thus, PUFA as oleic (C18:1), linoleic (C18,  $\omega$ -6) and linolenic acid (C18,  $\omega$ -3), are transformed into

the stearic FA (C18:0). Eicosapentaenoic acid (C20:5,  $\omega$ -3) and docosahexaenoic acid (C22:6,  $\omega$ -3) undergo very little hydrogenation in the rumen (Mattos *et al.*, 2000). These two fatty acids are commonly found in oil and fishmeal.

When we use a source of fat, not protected or not a rumen-inert fat, with high levels of PUFA, most is lost due to the biohydrogenation, which is particularly important for PUFA  $\omega$ -6 and  $\omega$ -3, which are considered as essential in the diet and which have an important role on reproductive, immunological, metabolic and hormonal functions. Therefore, this type of susceptible fat to interact in the rumen, are known as active fat and its use is limited in ruminants.

New technologies have generated chemically modified fats that allow its use at higher levels and with a lower level ruminal interaction, which reduces the deleterious effects of lipids on the ruminal bacteria activity. This kind of fats is known as inert fat, by-pass fat, or protected fat. In this regard, Jenkins (2004) defines the inert greases as those fats that have been specifically designed to have very little, or no negative effect on the digestibility of foods in ruminants. Rumen-inert fats are often carboxylate calcium salts (calcium soaps), saturated fatty acids or hydrogenated fats.

The use of calcium soaps allows the incorporation of a higher level of unsaturated fatty acids in the diet of ruminants. This is particularly important in the case of essential fatty acids ( $\omega$ -6 and  $\omega$ -3) which not only provide an energy effect *per se*, but may have specific effects on the metabolism of tissues and organs (Staples *et al.*, 1998). The melting point of rumen-inert fats, is usually above 100°C and solubility occurs at pH levels below 5.5. These values of temperature and pH are present in the rumen, which allows the fat to by-pass the rumen. However, at the level of the abomasum and first portion of the duodenum pH levels are much lower, allowing the dissociation of the carboxylate salt, leaving the fatty acids available for absorption. Therefore, it can be concluded that supplementation of rumen-inert fats to ruminants generates an increase in the availability of unsaturated fatty acids at the intestinal level, and therefore may increase the absorption and their incorporation into tissues (Table 2).

Source of fat	Fat source fed (Kg/day)	Linoleic acid fed (g/day)	Linoleic acid appearing in small intestine (g/day)
Whole cottonseeds	2.8	300	30 to 120
Whole soybeans	2.8	300	30 to 120
Yellow grease	0.45	77	8 to 31
Tallow	0.45	23	2 to 9
Calcium soaps (8,5% $\omega$ -6)	0.45	38	25 to 34

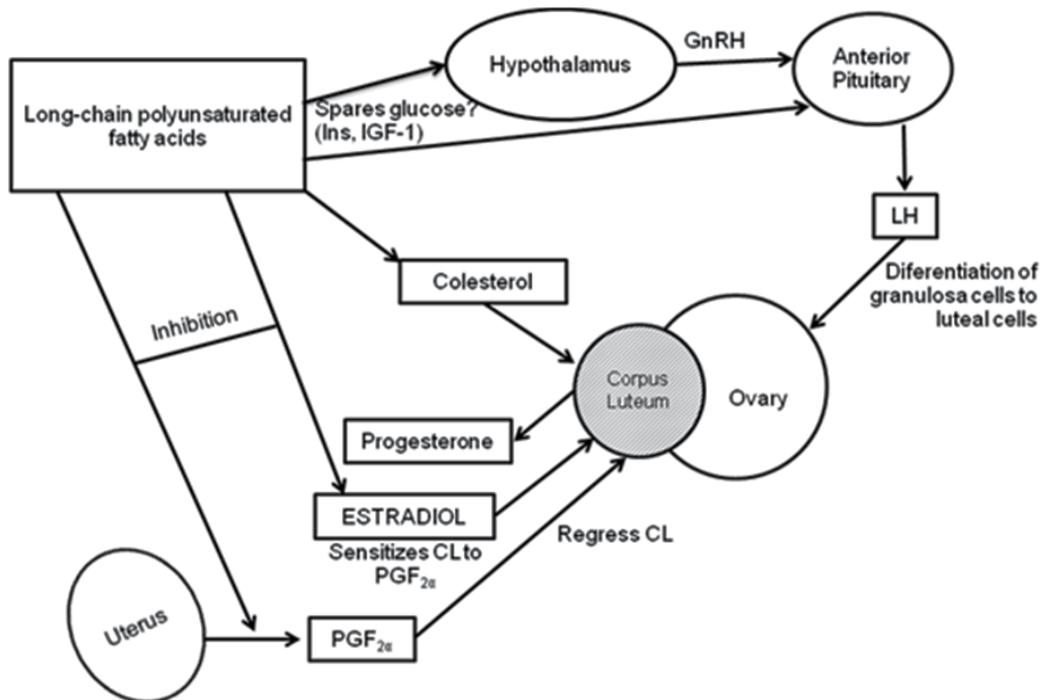
(Adapted from Staples *et al.*, 1998)

**Table 2.** Estimated amount of linoleic acid that reaches the small intestine in cows, depending on the source of fat.

### 3. Rumen-inert fat and the productive and reproductive responses in ruminants

When the rumen-inert fat is included into the diet it incorporates more PUFA, which is not only an energy source, but also generates non-energetic benefits related to the impact of these FA on the metabolism, hormonal and immune responses. The energetic effect is related to the greater amount of energy that lipids have, which helps to reduce the NEB during the postpartum period, which translates into increased production of luteinizing (LH) and follicle-stimulating hormone (FSH) by the pituitary gland, generating more growth and follicular development and promoting the ovulation (Diaz *et al.*, 2009).

Non-energetic effects are associated with the type of FA presents in the supplied fat, these effects are related with the increase in cholesterol (particularly the HDL fraction or "good cholesterol") levels, direct effects on ovaries and uterus, increase the levels of progesterone (P<sub>4</sub>) and the modulation of the prostaglandins production (especially PGF<sub>2α</sub>). In addition, rumen-inert fat has direct effects on hormones and growth factors involved with the reproductive and productive activity (insulin, IGF-I, among others). Most of these non-energy effects are favored when PUFA (ω-6 and ω-3) are included in the diet. Figure 1 shows various of the non-energetic proposed mechanisms, that can generate this type of FA.



**Figure 1.** Proposed mechanisms of action by which PUFA supplementation can affect reproductive function. (Adapted from Staples *et al.*, 1998).

The impact of this type of fat is associated with the energy balance of the animal, particularly in the cow during early lactation, decreasing the deleterious effects of the NEB and improving the reproductive activity during the early postpartum. On this regard, Staples *et al.* (1998) mentioned that 11 out of 18 studies reviewing the effect of fat in cow's reproduction, reported an increase in reproductive performance, either because it improves the conception rate at first service or by increasing in the overall conception or pregnancy rates.

In addition, the supplementation with high levels of PUFA can generate a favorable response in milk production and composition, and an improvement in the fatty acid profile of the milk, particularly the levels of conjugated linoleic acid (CLA). Angulo *et al.* (2005) have described that some anticancer and antidiabetogenic properties of the CLA as well as the prevention of atheroma formation, the potentiation of the immune response and the improvement of bone mineralization. It may also increase levels of essential fatty acids ( $\omega$ -6 and  $\omega$ -3) in milk, which can cause the milk produced by cows or goats fed with this type of fat, would be a nutraceutical food.

#### 4. Experiences in Venezuela

Experiences in Venezuela with the use of rumen-inert fat enriched with high levels of PUFA are promissory, either dairy and beef cattle, and small ruminants such as goats. Since 2007 the research group in the area of Nutrition - Reproduction interaction, in conjunction with researchers from the area of Animal Production at the College of Veterinary Sciences, Universidad Central de Venezuela, have been working on the effect of the rumen-inert fat enriched with high levels of polyunsaturated fatty acids on the productive and reproductive response in ruminants.

The research has been carried out in different ecological areas, with different types of production, levels of intensification, both in experimental stations and commercial farms, using the supplementation with rumen-inert fat given in different ways: multinutritional blocks (MNB); top-dress supplementation (fat placed on the concentrate supplement), in addition to the use of calcium soaps and mixed with mineral or concentrated supplement.

Regarding the effect of rumen-inert fat enriched with high levels of PUFA on the production of milk in a commercial trial, conducted with multiparous Carora breed cows, we evaluated the milk production during six months, obtaining a higher production per day and per lactation (20% more) in cows supplemented with 400 g of rumen-inert fat enriched with high levels of PUFA (Energras®) compared to cows in the control group. Results are shown in Table 3.

In crossbred Canary goats under tropical conditions, Salvador *et al.* (2009) evaluated the effect of by-pass fat supplementation (80 g Energras® / day) on milk production and composition. They reported a beneficial effect on the duration of lactation (+44 d), the milk production (+29.4%) and the content of milk components (41% more fat, and 31.2% more protein), without altering the physical characteristic (WMT, acidity, pH, cryoscopy) of the milk the supplemented goats (Table 4).

Parameters	Treatment group (400 g Energras® /day)	Control group
Nº animals	8	8
Liters/day	13.8	11.7
Total milk yield (Liters)	16,852	14,055
Diference (Liters/day)	+ 2.1 (17.9%)	
Diference in total milk yield (Liters)	+2,797 (19.9%)	

Otero (2007; unpublished data)

**Table 3.** Effect of supplementation with rumen-inert fat on milk production of Carora cows.

Parameter	Treatment group (80 g Energras®/day)	Control group	Probability
Milk yield (kg)	274.4 ± 18.5	193.6 ± 17.9	**
Days of milk	272.6 ± 15.9	228.8 ± 15.4	NS
Fat (kg/lactation)	13.83 ± 1.02	8.16 ± 1.05	**
Protein (kg/lactation)	11.29 ± 0.67	7.76 ± 0.69	**
Casein (kg/lactation)	7.59 ± 0.44	5.19 ± 0.46	**
Ashes (kg/lactation)	2.12 ± 0.14	1.49 ± 0.14	**
Lactose (kg/lactation)	12.44 ± 0.80	8.99 ± 0.83	**
Non fatty Solid (kg/lactation)	25.86 ± ±1.59	18.25 ± 1.65	**
Total solid (kg/lactation)	39.70 ± 2.56	26.41 ± 2.65	**

\*\* P < 0.01; NS= not significant

(Adapted from Salvador *et al.*, 2009)

**Table 4.** Effect of rumen-inert fat on milk production, days on lactation and composition of crossbred Canary goats

On the other hand, the milk lipid profile can be manipulated when supplementation with rumen-inert fat is given. Milk tends to have low levels of unsaturated fatty acids, being oleic acid (C18:1), a monounsaturated fatty acid, the most abundant (20% approx.). The proportion of unsaturated fatty acids is less than the saturated ones. The formation of the milk fat may come from the *novo* synthesis fatty acids in the mammary gland or from the incorporation of fatty acids from the diet or body reserves. Regarding the long chain fatty acids (including ω-6 and ω-3 fatty acids) which are incorporated in the milk, about 40-45% come from the diet (Palmquist, 1996). Therefore, manipulating the diet by the incorporation of higher levels of fatty acids from rumen-inert fat, could be an strategy to change the milk fat composition in ruminants.

In recent years, the manipulation of milk fat content has taken great importance, with the aim of increasing the concentration of CLA in dairy products, which may contribute significantly to generate milk with nutraceutical properties of essential fatty acids that would contribute to the human health. In this regard, Zamora (2010), supplementing crossbred Canary goats with rumen-inert fat enriched with high levels of fatty acids (45 g/day ENERGRAS®), found significant differences (P<0.01) on oleic and linolenic acids

concentrations between cheeses from treated and control goats: ( $331.34 \pm 63.25$  vs.  $28.02 \pm 67.29$  mg/g and  $9.78 \pm 1.22$  vs.  $5.41 \pm 1.30$  mg/g respectively), when compared to the cheeses made from milk of goats supplemented (treatment vs. control) respectively. In addition, there was a trend in linoleic acid concentrations to be higher in these from treated goats: ( $30.26 \pm 9.74$  vs.  $19.26 \pm 10.37$  mg/g), not affecting the quality and organoleptic properties of fresh cheeses (Table 5).

VARIABLE	Treatment group (80 g Energras®/day)	Control group	Probability
<b>Performance</b>			
Performance to 0 H	$3.9 \pm 0.1$	$4.0 \pm 0.1$	NS
Performance to 48 H	$4.8 \pm 0.1$	$5.2 \pm 0.1$	NS
<b>Characteristic:</b>			
Acidity	$13.1 \pm 1.7$	$13.5 \pm 1.7$	NS
Clorures	$2.9 \pm 0.3$	$2.9 \pm 0.3$	NS
pH	$5.6 \pm 0.1$	$5.4 \pm 0.1$	NS
<b>Components:</b>			
fat (%)	$24.5 \pm 1.2$	$21.9 \pm 1.2$	NS
Protein (%)	$10.2 \pm 0.4$	$10.7 \pm 0.4$	NS
Humidity (%)	$48.1 \pm 0.9$	$48.4 \pm 0.9$	NS
Ash (%)	$4.3 \pm 0.2$	$4.1 \pm 0.2$	NS
Solids (%)	$51.8 \pm 0.9$	$51.5 \pm 0.9$	NS
Solids no fat (%)	$27.3 \pm 1.3$	$29.6 \pm 1.3$	NS
Lactose (%)	$12.7 \pm 1.0$	$14.6 \pm 1.0$	NS
Oleic C18:1 (mg/g)	$331.3 \pm 63.2$	$28.0 \pm 67.2$	**
Linoleic C18:2 ( $\omega$ -3) (mg/g)	$30.2 \pm 9.7$	$19.2 \pm 10.3$	NS
Linolenic C18:3 ( $\omega$ -6) (mg/g)	$9.7 \pm 1.2$	$5.4 \pm 1.3$	*

NS: No significant; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$  (Adapted from Zamora, 2010).

**Table 5.** Effect of rumen-inert fat on performance, characteristics and composition of fresh pasteurized goat's cheese.

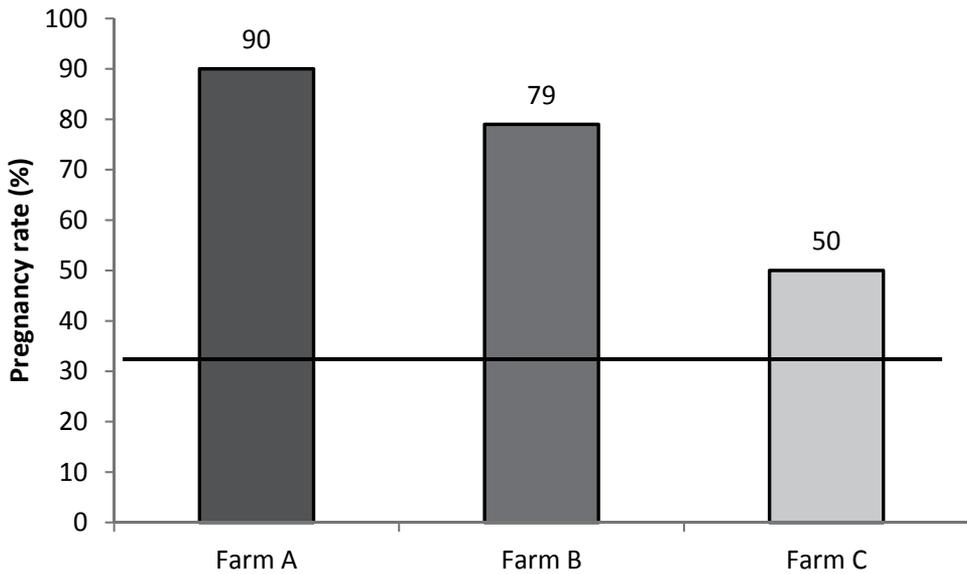
In addition, the performance of milk in terms of kilograms of cheese showed no significant differences ( $P > 0.05$ ) between cheese made from milk of goats supplemented with rumen-inert fat and cheese made from milk of goats without fat supplementation, (at 0 h and 48 h), after the removal from the mould. Nevertheless, the cheeses made from milk of the treatment group needed less milk to produce 1 kg of fresh cheese (140g and 400g less at 0 and 48 hours after the removal from the mould, respectively). At the moment of curd preparation there was not difference in terms of content of water retained (48.1% and 48.4% for treatment and control groups respectively). This could be due to, the protein content of

the serum that could affect the production of cheese (cheese making performance and the drainage of whey, especially when milk from treated goats was heated; Raynal-Ljutovac *et al.*, 2008). However, it is important to mention there were not statistical differences in terms of kg of cheese obtained from treated and control goats, the performance improved for processed cheese made from milk of goats that consumed rumen-inert fat, although they had a higher milk production, there were 47.95 kg of fresh cheese from the treated group and 25.37 kg of fresh cheese in the control group, with a difference of 47.09% for the treatment group.

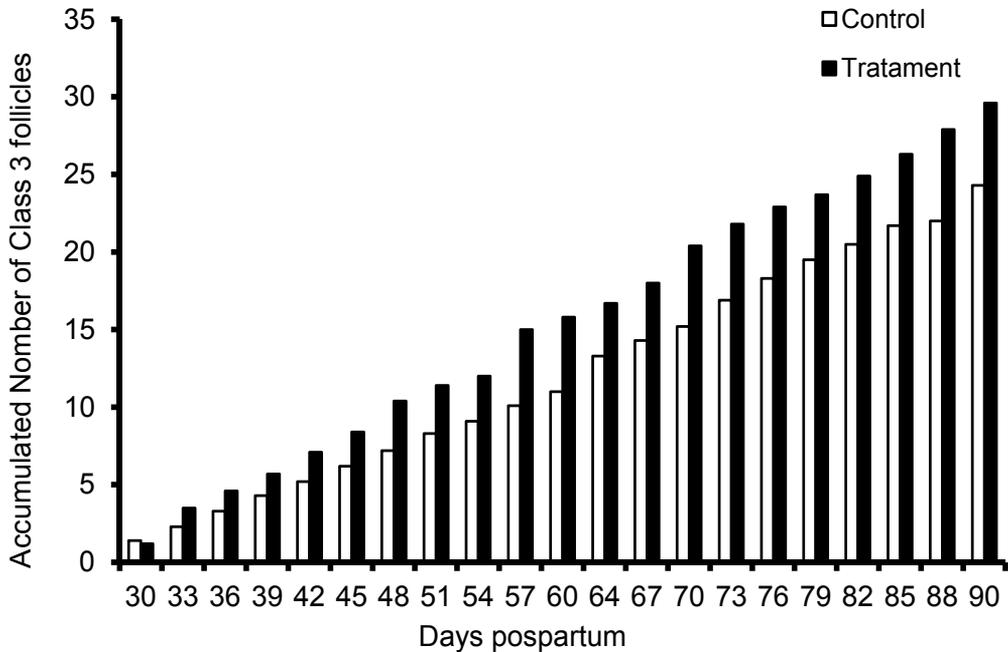
It is important to mention that the rumen-inert fat did not affect the characteristics of the cheese, nor the chemical composition neither of its components, except FA, concentrations and minerals in the treatment group, which had a trend to be higher. Some additional results showed that the effect of the consumption of rumen-inert fat on the proportion of *cis* and *trans* FA found in fresh goat cheese, was not significant ( $P > 0.05$ ). However, the proportion was higher in milk from treated goats than in milk from control goats ( $95.97 \pm 4.31$  and  $85.73 \pm 4.31$  respectively) which aggregates a value to the cheese, having a product with unsaturated *cis* FA, which has benefits to human health, because *trans* FA from of partially hydrogenated oils have been linked to deaths due to cardiovascular diseases (Giacopini, 2008).

Regarding to reproductive responses, the use of rumen-inert fat enriched with high levels of PUFA has been evaluated with promising results, particularly in first-calf Brahman cows in areas of well drained savannas in Cojedes States (Farms A and B) and Guárico States (Farm C) with acidic soils, in conditions of limited range and forage quality. In these studies Diaz *et al.* (2008; 2009) used multinutritional blocks that were formulated with the aim of providing non protein nitrogen, rumen-inert protein, minerals (macro and microelements) and rumen-inert fat (with 17% of linoleic acid [ $\omega$ -6]). In the case of Farm A, the breeding season was during the rainy season, while for the two other farms (B and C) breeding seasons were during the dry period. Data of pregnancy rate are shown in Figure 2. Results showed higher pregnancy rates than those reported for the same type of animals under tropical conditions (30%). Pregnancy rate for first calf Brahman cows in Farm A during the previous year was 46% much lower than the present results.

Diaz *et al.* (2009) and Hernandez (2010) also reported a beneficial effect of the supplementation with rumen-inert fat enriched with high levels of PUFA, during the postpartum period on ovarian activity of first-calf Brahman cows. Their results showed that supplementation with 150 g of fat/cow/d increased the accumulated number of Class 3 follicles ( $\geq 10$  mm) during the first 90 d post-partum ( $P < 0.06$ ; Figure 3). In this regard Diaz (2009) suggested that a greater accumulated number of Class 3 follicles could be an indicator of a greater likelihood of having preovulatory follicles, so cows would have a greater chance of ovulation during the first 90 d postpartum. On the other hand, it is an indirect indicator of the onset of LH secretion after parturition, as this hormone is responsible for the final maturation of preovulatory follicles and subsequent ovulation.



**Figure 2.** Pregnancy rate for first calf Brahman cows supplemented with multinutritional blocks enriched with rumen-inert fat. The line represents the pregnancy rate for first calf Brahman cows in Venezuela (Adapted from Diaz *et al.*, 2008; 2009).



**Figure 3.** Accumulated number of Class 3 follicles ( $\geq 10$  mm) between 30 and 90 d postpartum in first-calf Brahman cows supplemented or non with-rumen-inert fat.

Also, Hernandez (2010) reported that independent of significant difference in reproductive efficiency parameters between cows supplemented with rumen-inert fat and cows in the control group, there were numeric differences of 14 percentage points in conception rate, in favor of the supplemented cows (91% vs. 77%) and 12 d shorter interval from calving to conception (days open: 90 vs. 102 d in treatment and control group respectively). Those numbers would translate into an increase in reproductive efficiency and therefore the profitability herd.

In another experiment (cows received 250 g of rumen-inert fat/cow/d) we found a 20% reduction in the interval between calving and first estrus (control cows: 98 d vs. 78 d in supplemented cows). In this case the fat used as a supplement contained 5%  $\omega$ -3 and 17%  $\omega$ -6, (Unpublished data). It is important to note that the cows supplemented with  $\omega$ -6 presented problems of consumption at the beginning of the trial.

Diaz *et al.* (2009) considered that the  $\omega$ -6 fatty acids should be provided to stimulate cyclic ovarian activity early in the post-partum, while the  $\omega$ -3 fatty acid (linolenic acid, DHA and EPA) should be supplied during early pregnancy. In this regard, several authors (Petit *et al.*, 2002;) Mattos *et al.*, 2003) indicate that  $\omega$ -6 fatty acids stimulate synthesis of PGF<sub>2 $\alpha$</sub> , as well as  $\omega$ -3 stimulates the synthesis of series 3 prostaglandins, which can block the synthesis of the former, specifically the PGF<sub>2 $\alpha$</sub>  by competitive inhibition of enzymes that regulate their synthesis.

However, there are contrasting evidences regarding the role of the  $\omega$ -6 fatty acids and the synthesis of PGF<sub>2 $\alpha$</sub> . To this respect, Staples *et al.* (2002), indicate that linoleic acid has inhibitory effects both *in vitro* and *in vivo*, probably dose related, as the excess of this fatty acid might reduce the synthesis of prostaglandins in series 1 and 2 (i.e., PGF<sub>2 $\alpha$</sub> ). These same authors point out, that the inhibition may be due to a competitive effect with arachidonic acid by the prostaglandin endoperoxide synthase enzyme (PGSH). Therefore, a rich source of  $\omega$ -6 fatty acids could reduce the synthesis of PGF<sub>2 $\alpha$</sub> , prolonging the lifespan of the corpus luteum, allowing the implantation of the embryo and decreasing the early embryonic mortality.

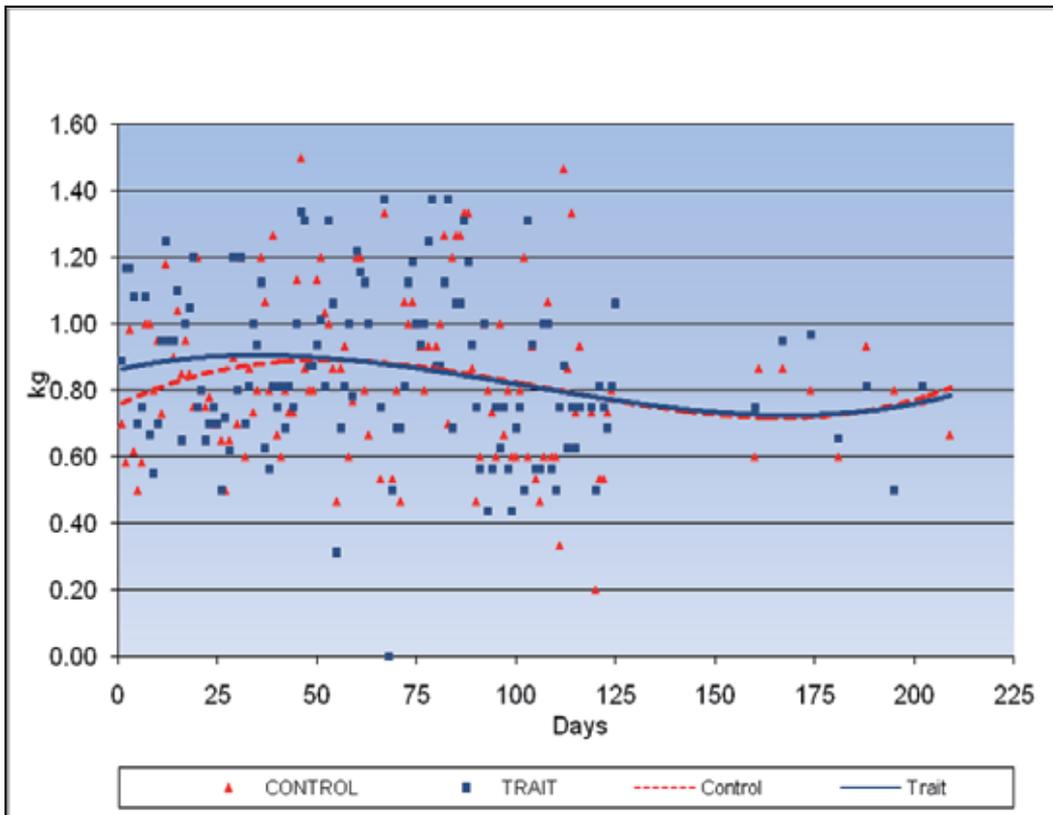
On the other hand, Diaz *et al.* (2009) argue that both the maintenance of pregnancy, and the onset of postpartum ovarian activity may occur with the single supply of linoleic acid. Therefore, the use of rumen-inert fat enriched with high levels of  $\omega$ -6 and/or  $\omega$ -3 could contribute positively on uterine involution, the resumption of postpartum ovarian activity and reduction of days open in beef and dairy cows.

## **5. Effect of rumen-inert fat supplementation on the voluntary consumption**

Salvador *et al.* (2009) reported that there were not significant differences between groups in terms of voluntary consumption of food. Both groups consumed the whole dose of concentrate (200 g with 18% of crude protein) across the lactation period. Also, there were

no differences ( $P > 0.05$ ) in terms of consumption of hay of Bermuda grass (*Cynodon dactylon*) offered ad libitum between groups ( $0.85 \pm 0.02$  kg and  $0.83 \pm 0.02$  kg for treatment and control group respectively). These results are consistent with those reported by Teh *et al.* (1994), González and Bas (2002) and Sanz Sampelayo *et al.* (2002a).

Figure 4 shows that there was a significant effect ( $P < 0.05$ ) of days in milk on the consumption of hay, showing that hay consumption increased immediately after the peak of lactation and decreased as the milk production reduces, coinciding with results reported by Jimeno *et al.* (2003) who points out that the lowest level of consumption is just one week before calving. On the other hand, the voluntary consumption increased after calving up to the maximum consumption between 6 to 10 weeks; However, it was not linear increase. Then, subsequent to the peak of milk the consumption declined linearly as the milk production decreased.



**Figure 4.** Effect of rumen-inert fat on the voluntary consumption of hay in crossbreed Canarian goats during lactation.

Regarding to the consumption of the of rumen-inert fat (80 g) by goats in the treated group, there was no rejection of the fat, consuming all the by-pass offered.

Results showed that the addition of this type of rumen-inert fat has no a substitute effect of the main component of the diet of ruminants (grass) but works as a supplement, thereby improving the quality of the diet, reducing the NEB, and improving the productive and reproductive parameters.

## 6. Final considerations

Supplementation with rumen-inert fat enriched with high levels of PUFA, should start enough time before parturition, to promote energy reserves of the animal (to improve body condition score) and to maintain body condition during the early postpartum period, in order to reduce the negative effects that has NEB on the production and reproduction in ruminants. Diaz *et al.* (2009) recommend that this type of fat supplementation, should begin between 21-40 days before calving, with doses between 100 and 400 g/cow/day, according to the type of cow (dairy or dual purpose cows). The time that the supplementation will be given varies according to the farm, the basal diet, the level of production, the type of animal, etc., but in general it is recommended to keep it during 90 days postpartum, period in which requirements of the cows are higher because they reach the peak of milk production.

Salvador *et al.* (2009) y Zamora (2010) recommend in dairy goats, that supplementation with this type of fat, would be given during the same way and time but with lower levels (45 and 80 g/goat/d) depending on the weight and the level of production of the goat.

Although the rumen-inert fat is not the solution to all problems of our farms, it is a food resource of great potential, that contains high levels of polyunsaturated fatty acids that allows the incorporation of beneficial effects that essential, fatty acids ( $\omega$ -6 and  $\omega$ -3) may have on the reproductive and productive activity of our herds and at the same time generating meat, milk and other derivatives with better nutritional quality.

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# **Fatty Acid Profile and Conjugated Linoleic Acid Content of Milk from Confined Holstein Cows During the Summer and Winter Seasons**

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50774>

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## **1. Introduction**

In recent decades, research in dairy cattle has been focused on the evaluation of factors that may cause changes in the lipid composition of milk, due to the fact that unsaturated fatty acids, conjugated linoleic acids (CLA) and high monounsaturated fatty acids/saturated fatty acids (MUFA/SFA) and polyunsaturated fatty acids/saturated fatty acids (PUFA/MUFA) ratios in milk have shown beneficial properties, including antiatherogenic and anticarcinogenic effects in humans. The principal factors that determine these effects are the breed (Lawless et al., 1999; Wood et al., 1980) and feeding regime (Cooper et al., 2004) in addition to less-studied factors, such as the parity, days in milk (DIM) and extreme temperatures. CLA are a mixture of linoleic acid isomers that contain conjugated double bonds. Studies in experimental animals have demonstrated that CLA has properties that may be beneficial for humans, providing anticarcinogenic, antidiabetic, antiobesity, antiatherogenic and immune stimulatory effects (Huth et al., 2006; Nirvair et al., 2007; Pariza et al., 2001; Parodi., 1999). Milk and dairy products are the primary sources of CLA, and approximately 75 to 90% of the total CLA content in milk fat is represented by *cis*-9, *trans*-11-CLA (Chin et al., 1992; Kay et al., 2004). CLA (9-*cis*, 11-*trans*-CLA) in milk fat is produced in the mammary glands via an endogenous synthesis pathway in which  $\Delta^9$ -desaturase converts vaccenic acid (*trans*-11 C18:1) to CLA (Bauman et al., 2001; Kay et al., 2005). Under conditions fostered by a certain combination of feed production system, additives, diet, breed, stage of lactation, and season, vaccenic acid is increased in the rumen, which results in a concomitant increase in the 9-*cis*, 11-*trans*-CLA content in milk fat (Grinari et al., 2000; Lock & Bauman, 2004). However, the factors mentioned above have been studied under

controlled conditions. In contrast, our study focused on the evaluation of some of these factors on the fatty acid profile and 9-*cis*, 11-*trans*-CLA content in milk under commercial conditions where extreme temperatures occur. To this end, we evaluated the fatty acid profile and 9-*cis*, 11-*trans*-CLA content in Holstein cow milk during the winter (14 °C) and summer (40 °C) on a commercial dairy farm in Northwestern Mexico (Sonora) by studying the effects of feed composition, parity, stage of lactation (DIM), and milk yield.

## 2. Material and methods

### 2.1. Location and sampling

This study was carried out on a commercial dairy farm in Sonora, México, which was located at 29.07 ° longitude and 110.90 ° latitude. A total of 240 Holstein dairy cows were included in the study, and the parity of the cows ranged from 1 to 6. The cows were exposed to all of the environmental elements that can affect the behaviour and milk yield of dairy cattle, including solar radiation, rain, and wind, as shown in Figure 1. The cows were divided into parity groups, including the following: primiparous (**P**, 1 parity, n = 84); earlier multiparous (**EM**, from 2 to 3 parities, n = 96); and late multiparous (**LM**, from 4 to 6 parities; n = 60). The milk was sampled in proportion to the parity of the cows; exclusion criteria included the presence of mastitis and more than 350 DIM.



**Figure 1.** Physical and environmental conditions of the dairy farm.

The diets were formulated using the Cornell-Penn-Miner Dairy model (Moate et al., 2004), and the ingredients and chemical compositions of summer and winter feed are shown in Table 1. Milking was carried out twice per day (0400 h and 1600 h), and the two milk samples (50 mL each) were combined. Milk samples were collected during the summer (from June to August 2006) and the winter (December 2006 to February 2007). The milk yield was measured using a Waikato MKV milk meter (Waikato MKV, Milking Systems, NZ) and evaluated on the last day of each month, which was standard practice on this dairy farm. The samples were transported on ice to the laboratory and stored at -20 °C for later analysis. Feed samples were collected on each sampling date. The DIM were also recorded.

The meteorological station “El Perico”, which was located 5 km from the farm, recorded the daily weather data, and this information was used to calculate the temperature-humidity index (**THI**) for the winter and summer seasons. The THI was calculated as follows:  $THI = td - (0.55 - 0.55RH) \times (td - 58)$ , where  $td$  = the dry bulb thermometer in °F and  $RH$  = the relative humidity expressed as a decimal (NOAA, 1976). A  $THI < 72$  was taken as an indicator of zero stress for the dairy cows. THI values from 72 to 79 indicated that the dairy cows were under mild stress, whereas THI values from 79 to 88 indicated that cows were under high stress (Armstrong, 1994).

## 2.2. Feed analysis

### 2.2.1. Feed chemical composition

The chemical composition of the diets was analysed by the AgroLab México laboratory (Gómez Palacio, Dgo). The components of the feeds that were evaluated in winter and summer were the crude protein, neutral detergent fibre, acid detergent fibre, ether extract, and net absorbable energy.

### 2.2.2. Feed fatty acid analysis

Total lipids were extracted with a chloroform and methanol mixture (2:1, vol:vol), as described by Folch et al., (1957). Fatty acid methyl esters (**FAMES**) were prepared by the standard method (AOAC, 1995). FAMES were identified and quantified by gas chromatography using a Varian Star 3400 CX Gas Chromatograph (VARIAN Inc, Walnut Creek, CA) with a DB-23 (J & W Scientific, Folsom CA) capillary column (30 m x 0.25 mm). Helium was used as the carrier gas with a flow rate of 1 mL/min; the airflow was 300 mL/min, and the hydrogen flow was 30 mL/min. The column temperature was initially set at 50 °C and held for 1 min. It was then increased at 10 °C/min to 166 °C, and then at 1 °C/min to 174 °C and held for 30 s; next, it was increased at 2 °C/min to 194 °C and held for 30s. As a final step, the column temperature was increased at a rate of 3.5 °C/min to 215 °C and held for 5 min. The total running time was 42.6 min. Identification of the fatty acid profile was performed with an external standard FAME mix (C4-C24, Sigma, USA).

## 2.3. Milk quality

The milk composition (fat, protein, lactose, and total solids) was determined by near infrared spectrometry (Milkoscan Minor FT120).

### 2.3.1. Fatty acid analysis of the milk

#### 2.3.1.1. Extraction

Milk fat extraction was carried out according Luna et al., (2005). In brief, 30 mL of milk was centrifuged at  $17,800 \times g$  for 30 min at 8 °C (Beckman Coulter centrifuge, Mod. Allegra 64R).

Approximately 350 mg of fat was subsequently removed for lipid extraction with 18 mL of a hexane:isopropanol mixture (3:2 v:v) per g of fat; next, 12 mL of sodium sulphate per g of fat was added as described by Hara & Radin (1978).

### 2.3.1.2. Methylation

The milk fatty acids were transesterified with methoxide sodium according the method of Christie (1982) as modified by Chouinard et al., (1999). Briefly, the fatty acids were mixed with 2 mL of hexane per 40 mg of fatty acid, and 40  $\mu$ L of methyl acetate was added. We then vortexed the mixture and added 40  $\mu$ L of methylation reagent (1.75 mL of methanol mixed with 0.4 mL of 5.4 M sodium methoxide). The mixture was again vortexed and allowed to react for 10 min. We then added 60  $\mu$ L of termination reagent (1 g of oxalic acid in 30 mL of diethyl ether). Next, the sample was centrifuged for 5 min at  $2400 \times g$  at  $5^\circ\text{C}$ , and the hexane layer was removed and transferred to a new tube for evaporation with nitrogen. When the resulting methylated fatty acids had dried, 50 mg was transferred to an amber vial, and 200  $\mu$ L of hexane was added. At that point, the sample was ready for quantification of fatty acids and 9-*cis*, 11-*trans*-CLA.

### 2.3.1.3. Identification and quantification of FAMES by gas chromatography

The FAMES were identified and quantified by gas chromatography using a Varian Star 3400 CX Gas Chromatograph (VARIAN Inc., Walnut Creek, CA), with a DB-23 (J & W Scientific, Folsom, CA) capillary column (30 m  $\times$  0.25 mm). The procedure was identical to that described above for the *Feed Analysis of Fatty Acids* with the same total running time of 42.6 min. Identification of the fatty acid profile was performed with an external standard fatty acid methyl ester mix (C4-C24, Sigma, USA). Conjugated linoleic acid isomers were identified using 98% pure standards (Matreya Inc., PA).

### 2.3.2. Desaturase index

The desaturase index was calculated as reported by Kelsey et al. (2003), using four pairs of fatty acids where each pair represented one product and one substrate of  $\Delta^9$ -desaturase. The fatty acid pairs (product and substrate) were as follows: *cis*-9 14:1 and 14:0; *cis*-9 16:1 and 16:0; *cis*-9 18:1 and 18:0; and *cis*-9 *trans*-11 CLA and *trans*-11 18:1. Kelsey et al. (2003) have defined the desaturase index as follows: [product of  $\Delta^9$ -desaturase]/[product of  $\Delta^9$ -desaturase + substrate of  $\Delta^9$ -desaturase]. For example, the desaturase index for *cis*-9 16:1 would be (*cis*-9 16:1)/(*cis*-9 16:1 + 16:0).

## 2.4. Statistical analysis

The PROC MIXED procedure provided in the SAS software (SAS Inst. Inc., Cary, NC, 2001) was used to adjust a model for analysing the fatty acid data. The model included the fixed effects of season and month within the season and parity; the random effects included milk yield and DIM (with linear and quadratic effects) and the residual term of animal per parity. The data were considered significant when  $P < 0.05$ . The response variable was reported as the least square mean  $\pm$  SEM. The adjusted model was evaluated as follows:

$$Y_{ijklmn} = \mu + A_i + B_j(A_i) + C_k + D_l + E_m + F_n + \varepsilon_{ijklmn},$$

where  $Y_{ijklm}$  is the response variable (fatty acid content),  $A_i$  is the season,  $B_j$  is the month in the season,  $C_k$  is the parity,  $D_l$  is the days in milk,  $E_m$  is the quadratic of days in milk,  $F_n$  is the milk yield, and  $\varepsilon_{ijklmn}$  is the residual error term.

When significant effects of the factors studied were found, mean comparisons of the response variables were performed by the LSMEANS procedure of SAS.

### 3. Results and discussion

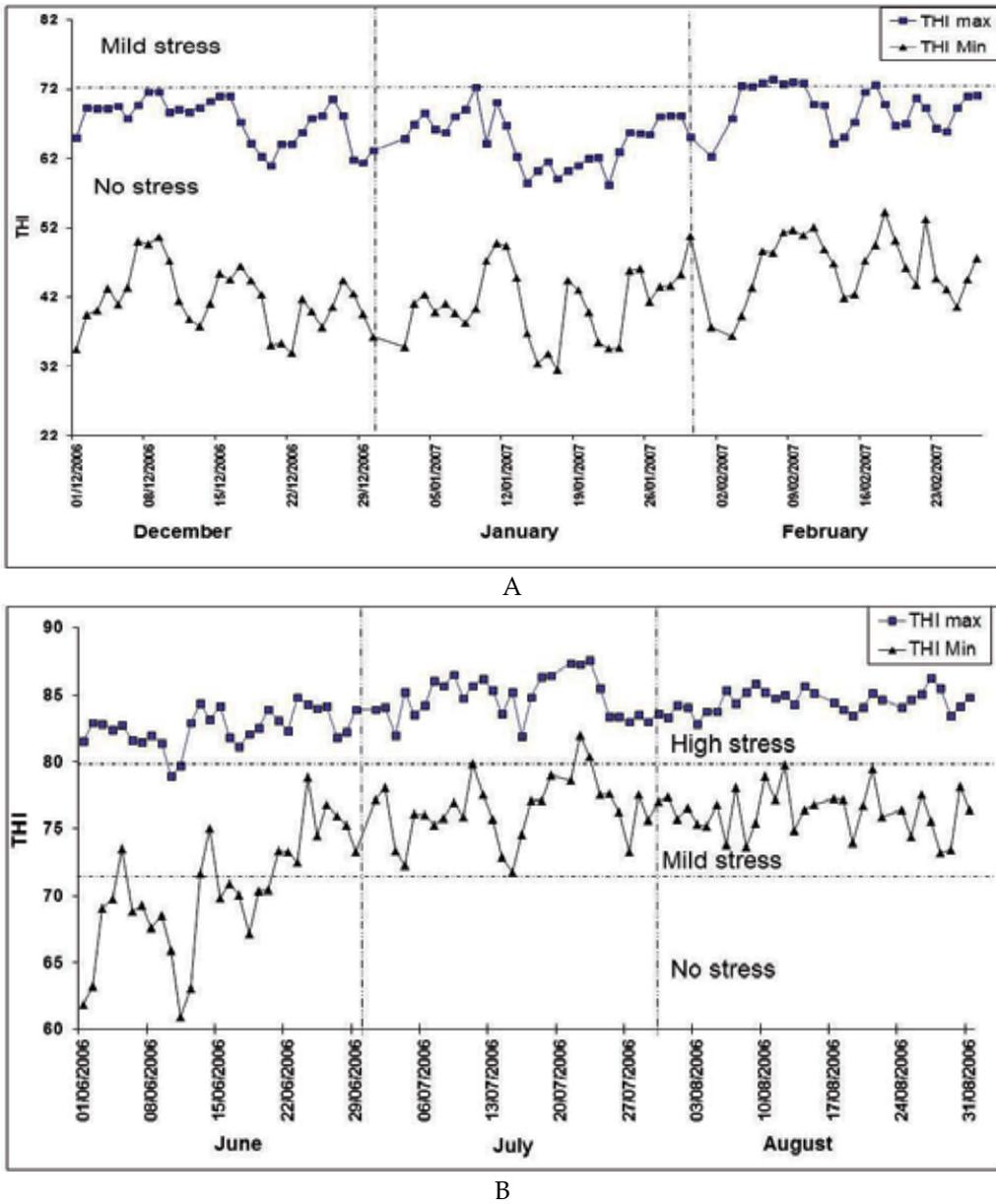
#### 3.1. Temperature-humidity index

In desert climates, the ambient temperatures are extreme and vary widely between winter and summer. To our knowledge, the previous studies that have evaluated variations in the milk 9-*cis*, 11-*trans*-CLA content in different seasons had been performed in countries with moderate ambient temperatures. Therefore, our objective was to evaluate the 9-*cis*, 11-*trans*-CLA content in Holstein cow milk in the winter and summer in a hot environment in the Northwestern region of México. We used the THI as an indicator of the heat stress of the dairy cattle in both seasons (Figure 2). As anticipated, during the winter, with an average maximum temperature of 24 °C and 20% RH, the dairy cows were not under heat stress according to the maximum THI measured (average  $57 \pm 8.7$ , range from 33 to 74). In contrast, with an average maximum temperature of 43 °C and 50% RH, the dairy cows were under heat stress during the summer (average THI maximum =  $80 \pm 5.1$ , range from 63 to 88).

#### 3.2. Feed composition

According to the components and chemical analysis of the feed (Table 1), the summer and winter diets were similar in caloric, nitrogen, and fibre content. Nevertheless, changes in the ingredients were made according to the standard practice on the dairy farm. These changes included the use of whole cottonseeds, maize silage and less alfalfa content in the feed during the winter. The final forage-to-concentrate ratios of the diets were 58:42 and 59:41 for winter and summer, respectively. Accordingly, the fatty acid composition of the diets showed variations between the two seasons (Table 1). Because these compounds are precursors of CLA in both ruminal and mammary gland biosynthesis, the discussion is focused on the concentrations of *cis*-9 18:1, C18:2 and C18:3 in the feed. Despite a lower 18:2 concentration in the summer diet (37.21% of FAMES) compared to the winter diet (45.45% of FAMES), the 9-*cis*, 11-*trans*-CLA content in the milk during the summer was higher (9.36 mg/g fatty acids) compared to that observed during the winter (7.03 mg/g fatty acids). However, the concentration of the other CLA precursors *cis*-9 18:1 and C18:3 were higher ( $P < 0.05$ ) during the summer compared to the winter diets. This finding may explain, in part, the increased content of CLAs in the milk during the summer. Although the *cis*-9 18:1 isomer is not a well-recognised CLA precursor, Mosley et al. (2002) have demonstrated the ability of mixed ruminal microbes to convert *cis*-9 18:1 to several *trans*

positional isomers, including *trans*-11 18:1, which is a precursor of CLA (Griinari et al., 2000; Lock & Bauman, 2004). These data suggest that biosynthesis in the mammary gland may explain the increased milk 9-*cis*, 11-*trans*-CLA content observed during the summer (Soyeurt et al., 2008).



**Figure 2.** Maximal and minimal THI values during the winter (A) and summer (B) and the relation of THI to heat stress in dairy cows.

	Winter	Summer
Ingredients <sup>1</sup>		
Concentrate	42.21	41.13
Maize ground	21.09	25.49
Whole Cottonseed	6.48	0
Soya 47% Protein	3.53	5.59
Soybean 70	4.27	2.91
Vitamins and minerals	2.28	2.08
Fat mixture <sup>2</sup>	1.42	1.85
Cane Molasses	3.15	3.07
Alfalfa	30.19	58.87
Maize silage	27.60	0
F:C <sup>3</sup>	58:42	59:41
Chemical analysis <sup>1</sup>		
CP	15.7	15.4
NDF	24.2	25.2
ADF	33.6	34.4
EE	5.56	4.86
ENI	1.55	1.54
Fatty acids of the diet <sup>4</sup>		
C12:0	0.00 <sup>b</sup>	0.78 <sup>a</sup>
C14:0	0.00 <sup>b</sup>	0.77 <sup>a</sup>
C16:0	20.54 <sup>a</sup>	20.04 <sup>a</sup>
C18:0	6.71 <sup>a</sup>	5.70 <sup>a</sup>
C18:1 c9	21.56 <sup>b</sup>	26.15 <sup>a</sup>
C18:2	45.45 <sup>a</sup>	37.21 <sup>b</sup>
C18:3	5.74 <sup>b</sup>	9.35 <sup>a</sup>

<sup>1</sup>Values were determined using dry matter (% of DM); <sup>2</sup>Tallow and soybean oil; <sup>3</sup>Forage:concentrate ratio; <sup>4</sup>Fatty acid values were calculated as the percentage of methylated fatty acids; Means within a row with different superscripts were significantly different.

**Table 1.** Ingredients and chemical composition of the feed in the winter and summer.

### 3.3. Milk yield and composition

The different climate conditions also had effects on the milk yield and composition (fat, protein, lactose, and total solids) as shown in Table 2. The milk yield was significantly lower ( $P < 0.05$ ) in the summer ( $15.8 \pm 0.5$  kg/d) compared to the winter ( $18.9 \pm 0.4$  kg/d). This reduced yield may have been due, at least in part, to the reduced energy intake and the heat stress observed during the summer season, similar to the results reported by others (Fuquay, 1981; Granzin, 2006; Rhone et al., 2008; West, 2003). In addition, the reduction in

milk yield agreed with the findings of West (2003), who has reported a reduction in the milk yield of 0.2 kg per unit increase in THI when the THI was above 72. Accordingly, in our study, the milk yield was 3.1 kg/d lower in the summer than in the winter; this reduction corresponded to the 16 THI units above 72 that were recorded during the summer (average  $80 \pm 5.1$ , range from 63 to 88). The percentages of fat, protein, lactose, and total solids in the milk were also lower during the summer season. This result may also have been attributable to the reduced feed intake induced by heat stress (Collier et al., 2006).

	Winter	Summer
Milk yield, kg/d	$18.9^a \pm 0.40$	$15.8^b \pm 0.50$
Milk fat, %	$2.69^a \pm 0.10$	$1.91^b \pm 0.06$
Milk protein, %	$3.45^a \pm 0.03$	$3.34^b \pm 0.03$
Lactose, %	$4.41^a \pm 0.02$	$4.30^b \pm 0.02$
Total solids, %	$11.5^a \pm 0.11$	$10.4^b \pm 0.09$

<sup>a,b</sup> Means within a row with different superscripts were significantly different ( $P < 0.05$ )

**Table 2.** Cow milk yield and composition during the summer and winter.

### 3.4. Fatty acid profile in milk

Table 3 shows the significance, determined by the analysis of variance, of the effects of the season, month in the season, parity, DIM, and milk yield on the content of CLA, individual fatty acids, SFA, MUFAs, PUFAs, and the desaturase index. Most of the individual fatty acids in milk and the sum of the saturated fatty acids, the sum of the monounsaturated fatty acids and the sum of the polyunsaturated fatty acids, the MUFA/SFA and PUFA/SFA ratios and desaturase index were affected ( $P < 0.05$ ) by the season. In contrast, only the C14:0, C16:0, and unsaturated fatty acids, such as C16:1, C18:1 t11, C18:2 and 9-*cis*, 11-*trans*-CLA, and the sum of PUFA and both ratios MUFA/SFA and PUFA/SFA were affected by parity. Only the season, month in the season, parity, and DIM had significant effects ( $P < 0.001$ ) on the milk CLA content. In addition, the season, month in the season, and milk yield had significant effects ( $P < 0.05$ ) on the desaturase activity, as evaluated indirectly by the desaturase index for *cis*-9 14:1.

Table 4 shows the fatty acid profiles of the milk during both seasons. A low content of saturated fatty acids (C14:0, C16:0, and C18:0) and a low sum of saturated fatty acids were observed during the summer. In contrast, the sum of monounsaturated fatty acids, the sum of polyunsaturated fatty acids, 9-*cis*, 11-*trans*-CLA, and the MUFA/SFA and PUFA/SFA ratios were higher in the summer than in the winter. The milk fat samples obtained during the summer had higher contents of 9-*cis*, 11-*trans*-CLA ( $P < 0.001$ ) than those obtained during the winter ( $9.36 \pm 0.28$  mg/g fatty acids vs.  $7.03 \pm 0.29$  mg/g fatty acids, respectively). As mentioned above, this difference in the 9-*cis*, 11-*trans*-CLA content was attributable to the summer feed content of 18:2 and mammary gland precursors of CLA (*cis*-9 18:1 and 18:3).

Fatty acid	Model adjusted term					
	Season	Month (season)	Parity	DIM	DIM <sup>2, 1</sup>	Milk yield
C4:0	*	**	NS	NS	NS	NS
C6:0	**	**	NS	NS	NS	NS
C8:0	**	**	*	*	*	NS
C10:0	**	**	**	*	*	NS
C12:0	*	**	*	NS	NS	NS
C14:0	*	**	*	NS	NS	NS
C14:1	**	NS	NS	NS	NS	*
C15:0	NS	**	*	NS	NS	NS
C16:0	**	*	*	NS	NS	NS
C16:1	NS	0.064	*	*	*	NS
C17:0	NS	**	NS	NS	NS	NS
C18:0	*	*	NS	NS	NS	NS
C18:1, t11	*	**	*	NS	NS	NS
C18:1, c9	**	**	NS	NS	NS	NS
C18:2	**	**	*	NS	NS	*
C18:3	**	**	NS	NS	NS	NS
CLA <sup>2</sup>	**	**	*	<b>0.051</b>	<b>0.070</b>	NS
C20:0	*	**	NS	NS	NS	NS
Unknown	NS	NS	*	NS	NS	NS
Σ SFA <sup>3</sup>	**	**	NS	NS	NS	NS
Σ MUFA <sup>4</sup>	**	**	NS	NS	NS	NS
Σ PUFA <sup>5</sup>	**	**	*	NS	NS	*
MUFA/SFA ratio	**	**	**	NS	NS	NS
PUFA/SFA ratio	**	**	*	NS	NS	NS
Desaturase index						
<i>cis</i> -9 14:1	**	*	NS	NS	NS	*
<i>cis</i> -9 16:1	*	*	NS	*	*	NS
<i>cis</i> -9 18:1	**	**	NS	*	*	NS
<i>cis</i> -9, <i>trans</i> -11 CLA	**	**	NS	*	*	NS

<sup>1</sup>Quadratic term of days in milk; <sup>2</sup>9-*cis*, 11-*trans*-CLA; <sup>3</sup>Sum of saturated fatty acids; <sup>4</sup>Sum of monounsaturated fatty acids; <sup>5</sup>Sum of polyunsaturated fatty acids

\*P < 0.05; \*\*P < 0.001; NS = Not significant

**Table 3.** Significance of factors of the adjusted model on individual milk fatty acids.

Fatty acid	Winter		Summer		P
	Mean ± SE	CI (95%)	Mean ± SE	CI (95%)	
C4:0	1.80 ± 0.18	1.44 – 2.14	0.90 ± 0.12	0.67 – 1.13	*
C6:0	9.69 ± 0.25	9.19 – 10.18	6.45 ± 0.30	5.86 – 7.03	**
C8:0	10.36 ± 0.14	10.07 – 10.64	8.28 ± 0.18	7.90 – 8.64	**
C10:0	26.10 ± 0.40	25.30 – 26.90	21.21 ± 0.41	20.40 – 22.02	**
C12:0	31.70 ± 0.48	30.74 – 32.65	27.74 ± 0.45	26.85 – 28.63	*
C14:0	109.05 ± 0.97	107.13 – 110.96	100.53 ± 1.01	98.51 – 102.54	*
C14:1	8.71 ± 0.21	8.29 – 9.13	11.83 ± 0.28	11.27 – 12.39	**
C15:0	11.53 ± 0.13	11.28 – 11.78	11.69 ± 0.19	11.30 – 12.08	NS
C16:0	332.31 ± 2.37	327.62 – 337.00	299.90 ± 2.73	294.54 – 305.37	**
C16:1	14.04 ± 0.30	13.45 – 14.63	15.97 ± 0.38	15.22 – 16.72	NS
C17:0	6.09 ± 0.10	5.88 – 6.30	5.54 ± 0.11	5.32 – 5.75	NS
C18:0	125.18 ± 1.63	121.90 – 128.42	105.86 ± 1.68	102.50 – 109.21	*
C18:1, t11	9.64 ± 0.23	9.17 – 10.11	8.18 ± 0.28	7.62 – 8.73	*
C18:1, <i>cis</i> -9	237.75 ± 2.50	232.80 – 242.70	288.23 ± 2.77	282.70 – 293.70	**
C18:2 <sup>1</sup>	22.79 ± 0.29	22.21 – 23.37	39.59 ± 0.84	37.92 – 41.26	**
C18:3 <sup>2</sup>	2.82 ± 0.08	2.65 – 2.98	4.37 ± 0.09	4.18 – 4.56	**
<b>CLA<sup>3</sup></b>	<b>7.03 ± 0.29</b>	<b>6.46 – 7.59</b>	<b>9.36 ± 0.28</b>	<b>8.81 – 9.91</b>	<b>**</b>
C20:0	1.25 ± 0.07	1.10 – 1.39	0.68 ± 0.08	0.51 – 0.84	*
Others	32.62 ± 0.30	32.01 – 33.22	33.12 ± 0.63	31.86 – 34.38	NS
Σ SFA <sup>4</sup>	665 ± 28.8	659.8 – 670.3	588.8 ± 41.4	581.3 – 596.3	**
Σ MUFA <sup>5</sup>	270.1 ± 25.3	265.6 – 274.7	324.2 ± 31.2	318.5 – 329.9	**
Σ PUFA <sup>6</sup>	32.3 ± 4.0	31.5 – 33.0	53.8 ± 11.2	51.7 – 55.8	**
MUFA/SFA ratio	0.408 ± 0.007	0.398 – 0.419	0.557 ± 0.007	0.539 – 0.574	**
PUFA/SFA ratio	0.048 ± 0.001	0.047 – 0.05	0.092 ± 0.001	0.088 – 0.097	**

<sup>1</sup>Linoleic acid; <sup>2</sup>Linolenic acid; <sup>3</sup>9-*cis*, 11-*trans*-CLA; <sup>4</sup>Sum of saturated fatty acids; <sup>5</sup>Sum of monounsaturated fatty acids; <sup>6</sup>Sum of polyunsaturated fatty acids; \*P < 0.05; \*\*P < 0.001; NS = Not significant; N = 240 cows (120 in winter and 120 in summer); ± Standard error

**Table 4.** Cow milk fat composition (mg/g fatty acids) by season.

These results show that the attributes of the milk were modified by the season, but this modification of the fatty profile and 9-*cis*, 11-*trans*-CLA content was mainly attributable to the diet because the summer diet (28% more of alfalfa) was richer in PUFA and CLA

precursors than the winter diet, promoting an increase in the *Butirivibrio fibrisolvens* isomerase activity and resulting in an increase in 9-*cis*, 11-*trans*-CLA production and MUFAs and PUFAs during the summer. The importance of manipulating the fatty acid composition in the milk, thus favouring a high content of unsaturated fatty that includes 9-*cis*, 11-*trans*-CLA, is due to the fact that their consumption can help to prevent chronic diseases in humans. A decrease in the SFA levels and/or a concomitant increase in the MUFA and PUFA contents of ruminant milk may confer benefits for human health and may provide a basis for marketing claims. The PUFA/SFA ratio in humans is an important risk factor for cardiovascular diseases (Sacks & Katana, 2002; Simopoulos, 1999), and thus the proportions of total unsaturated and PUFAs in regard to the total SFAs are relevant from a human health perspective. Indeed, the PUFA/SFA ratio has been used to calculate the risk factor of foods. According to Wood et al., (2008), the recommended value is at least 0.4, although, others have suggested that the minimum PUFA/SFA ratio is 0.12 (Hoffman, Muller, & Cloete, 2003). However, in our study, the PUFA/SFA ratios for the milk in both seasons (0.048 for winter and 0.092 for summer season) was lower than 0.12. It is important to note that there was no manipulation on the original formulation of the diets to modify the PUFA/SFA ratios obtained.

In contrast, it is well known that most CLA are produced in the mammary gland via  $\Delta^9$  desaturase. As shown in Table 5, the activity of this enzyme was higher in summer than in winter for the four substrates (C14:0, C16:0, C18:1, and *trans*-C18:1) of the desaturated products, C14:1, C16:1, *cis* 9-C18:1, and CLA, respectively. Because the C14:0 content in milk fat is produced via de novo synthesis in the mammary gland, desaturation is the only source of C14:1; therefore, the ratio of C14:1 to C14:0 is the best indicator of  $\Delta^9$ desaturase activity (Lock & Garnsworthy, 2003). Our results showed that the desaturase index was lower for C14:1 and C16:1 compared to 18:1 and CLA. Kelsey et al. (2003) have reported a similar pattern in the desaturase index (0.06, 0.04, and 0.67 for C14:1, C16:1, and 18:1, respectively).

Desaturase index <sup>1</sup>	Winter		Summer		P
	Mean ± SE	CI (95%)	Mean ± SE <sup>2</sup>	CI (95%)	
cis-9 14:1	0.074 ± 0.001	0.071 – 0.077	0.104 ± 0.002	0.100 – 0.109	**
cis-9 16:1	0.040 ± 0.001	0.038 – 0.042	0.05 ± 0.001	0.048 – 0.052	**
cis-9 18:1	0.65 ± 0.002	0.65 – 0.66	0.73 ± 0.003	0.72 – 0.73	**
c-9, t-11 CLA	0.41 ± 0.007	0.39 – 0.42	0.55 ± 0.008	0.53 – 0.57	**

<sup>1</sup>Calculated as the  $\Delta^9$ -desaturase product divided by the sum of the  $\Delta^9$ -desaturase product and substrate. For example, the desaturase index for *cis* 9 16:1 would be (*cis*-9 16:1)/(*cis*-9 16:1 + 16:0). \*\*P < 0.001; <sup>2</sup>Standard error

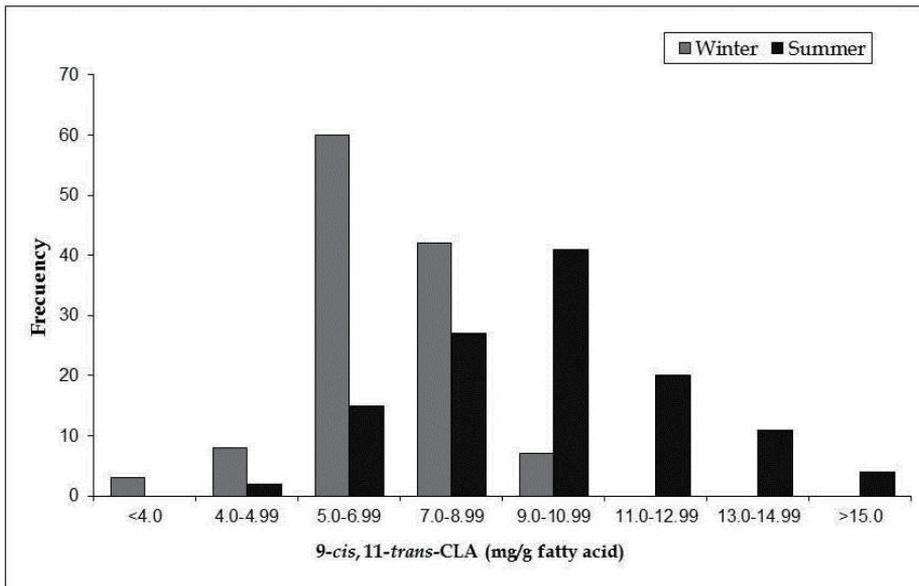
**Table 5.** Estimated  $\Delta^9$ -desaturase activity in milk fatty acids during the winter and summer.

Figure 3 shows the frequency distributions of the 9-*cis*, 11-*trans*-CLA content in milk obtained during the winter and summer. In the winter, the CLA content ranged from 3.49 to

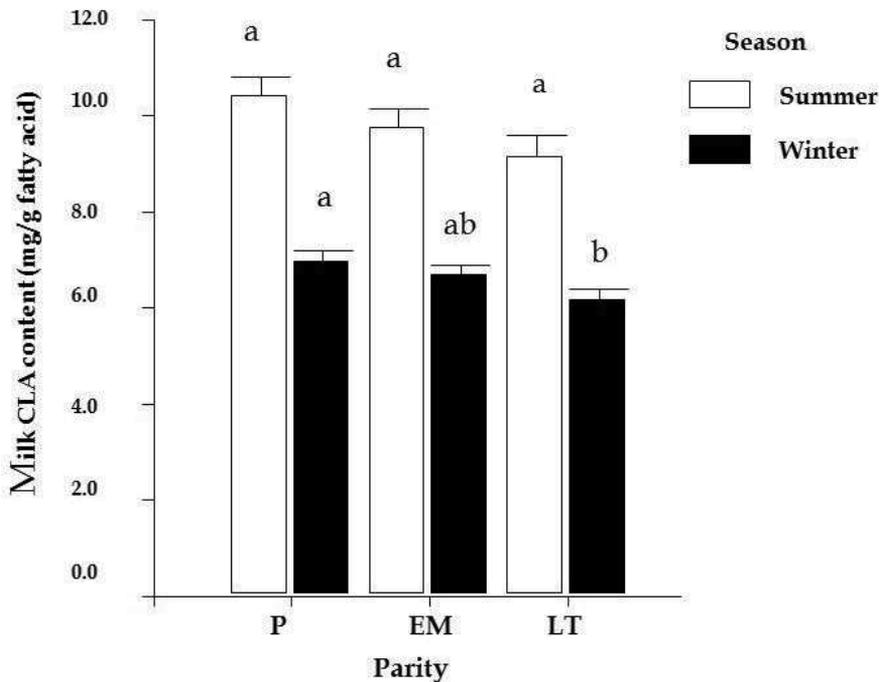
10.45 mg/g fatty acids (with a mean value of  $6.65 \pm 1.4$  mg/g fatty acids), with a peak frequency (59/120) within the range of 5.0 to 6.99 mg/g fatty acids. In the summer, the CLA content ranged from 4.03 to 17.86 mg/g fatty acids (with a mean value of  $9.83 \pm 2.58$  mg/g fatty acids), with a peak frequency (40/120) within the range of 9.0 to 10.99 mg/g fatty acids. Additionally, in the summer, 34 out of 120 cows had CLA values above 10.99 mg/g fatty acids. There was a broad variation in the content of 9-*cis*, 11-*trans* CLA in milk within a season and among animals despite the same diet and environmental conditions. Similar results have been reported by other authors (Bell et al., 2006; Lawless et al., 1999; Stanton et al., 1997; Staszak, 2005;).

In the present study, parity affected the CLA content in milk fat as shown in Figure 4. For this analysis, dairy cows were grouped by parity status by season. In the summer, there were no differences in the 9-*cis*, 11-*trans*-CLA content between parity levels, and the range of CLA was from 9 to 10 mg/g fatty acids. In contrast, we found differences between the parity groups and the 9-*cis*, 11-*trans*-CLA content in milk during the winter where primiparous cows had a higher CLA content ( $6.96 \pm 0.2$  mg/g fatty acids,  $P < 0.05$ ) compared to the milk from LM cows ( $6.17 \pm 0.2$  mg/g fatty acids), but the difference was not significantly greater than with the milk from EM cows ( $6.69 \pm 0.25$  mg/g fatty acids,  $P > 0.05$ ). To date, studies have been scarce on the effect of parity on the CLA content in milk fat. Peng et al. (2008) have reported that the milk from primiparous yak cows had a lower CLA content than multiparous yak cows. These authors have also suggested that the differences found may be related to the stage of growth of the mammary glands, which are one of the sites of the synthesis of fatty acids in milk, or that the differences may be related to forage, as there were differences on forage intake in the Yaks of different parities. In contrast, Kelsey et al., (2003) have studied the effect of parity and did not find differences in the milk CLA content between primiparous and multiparous dairy cows. This discrepancy with respect to our results for the winter season led us to hypothesise that the differences in CLA may be due, at least in part, to the hierarchy of the cows within the paddock area. Multiparous cows are generally older, bigger, and heavier than primiparous cows, therefore, they are the first to attain feed (Phillips & Rind, 2002). This hierarchy may have resulted in the consumption by the multiparous cows of high amounts of concentrate, which provoked a decrease in the ruminal pH, which, in turn, induced an adverse environment for the *Butirivibrio fibrisolvens* bacteria in the rumen that are responsible for CLA production.

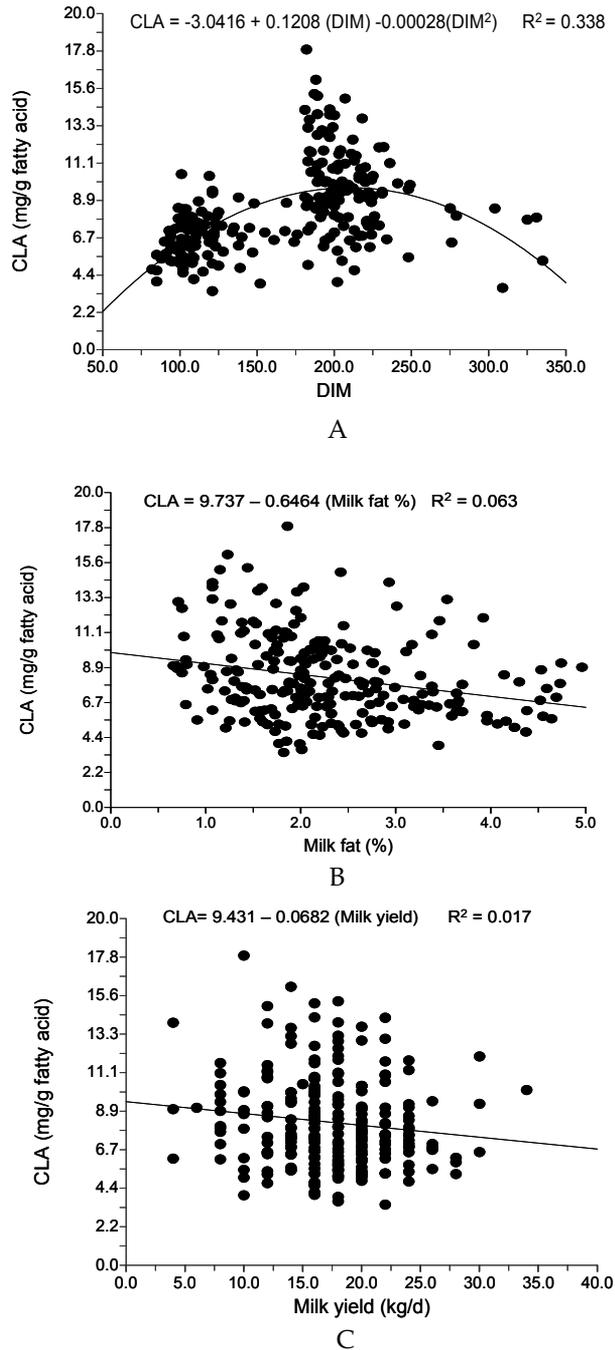
The relationships between the milk CLA content and DIM, milk fat, and milk yield are shown in Figure 5 (Panels A, B, and C, respectively). The DIM had an effect on the milk CLAs with an  $R^2 = 0.338$ ,  $P < 0.05$ . In our study, the DIM explained a third (33.8%) of the variation in the CLA content. This result contrasted with previous reports that have shown that this factor explained less than 10% of the variation (Lock et al., 2005; Kelsey et al., 2003). The milk fat and milk yield did not have effects on the milk CLA content with  $R^2 = 0.063$  and  $R^2 = 0.017$ , respectively; this result is in agreement with Kelsey et al., (2003).



**Figure 3.** Frequency distribution for the 9-cis, 11-trans-CLA content in cow's milk collected during the winter and summer seasons.



**Figure 4.** Milk CLA (9-cis, 11-trans) relative to parity by season. Bars with different superscripts (a, b, and ab) within the season indicate significant differences ( $P < 0.05$ ). Primiparous (P): one parity; early multiparous (EM): dairy cows with two and three parities; late multiparous (LM): dairy cows with four to six parities.



**Figure 5.** Relationships between the milk CLA content and the (A) DIM, (B) milk fat, and (C) milk yield. Data points represent milk samples collected during both the winter and summer seasons from 240 dairy cows

## 4. Conclusions

We found that the practical management of the adjustment of the ingredients in the diet to maintain energy intake and avoid decreased milk yield during the summer (when temperatures may exceed 40 °C) was beneficial because this adjustment of the diet allowed an improvement in the unsaturated fatty acid profile and CLA content in the milk, despite the animals' experiencing heat stress. The alterations in the fatty acid profile and CLA content in milk suggest that this milk could be healthier for the consumer in the summer, due to the increased MUFA/SFA and PUFA/SFA ratios.

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## Acknowledgement

The authors thank Dr. Arturo Madrid Lopez, Nutritionist, for his advice and assistance. We also thank Ing. Roy Swanson, the manager of the dairy farm where the present study was conducted. We also thank Amparo Nieblas Almada, Ingrid Rebeca Esquerra Brauer and Francisco A. Vazquez Ortiz for their excellent technical assistance.

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# Factors That Affect Energy Efficiency and Indirect Response of Selection for Efficiency on Related Traits

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50776>

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## 1. Introduction

The breeding goal of most livestock operations is maximizing profitability, which is a function of input and output ( Veerkamp & Emmans, 1995; Archer et al., 1999; Crews, 2005). In most livestock populations, selection programs have primarily focused on progressively improving means for output traits such as live weight gain, fertility, meat and milk yield (Archer et al., 1999; Sainz & Paulino, 2004; Crews, 2005). However, there has recently been a renewed interest in another component of profitability, namely the reduction of inputs or the increase in efficiency (Crews, 2005). Feed is one of the most important input components, and it represents more than one-half of the total costs in most livestock operations (Kennedy et al., 1993). In the dairy industry, feed cost represents about 40 to 50% of the total milk production cost and has increased substantially over the last few years (Garcia, 2009). Gibb & Macallister (1999) reported that the economic effect of a 5% improvement in feed efficiency is four times greater than a 5% improvement in average daily gain. Therefore, reducing production costs or increasing feed efficiency are the two most important ways to improve production efficiency and profitability. They also decrease environmental pollution and the carbon footprint (Capper et al., 2010). In order to gain the maximum benefits of genetic selection for energy efficiency, factors that influence energy efficiency and its indirect effects on other traits should be known. The most common measures of energy efficiency and their properties are reviewed in this chapter. It also deals with factors that practically affect energy efficiency. Furthermore, as there are very few reports on direct selection for energy efficiency in dairy cattle (Linn, 2006), the authors reviewed the indirect effect of selection for energy efficiency on other traits in beef cattle as well as in other species in addition to dairy cattle.

## 2. Energy efficiency traits

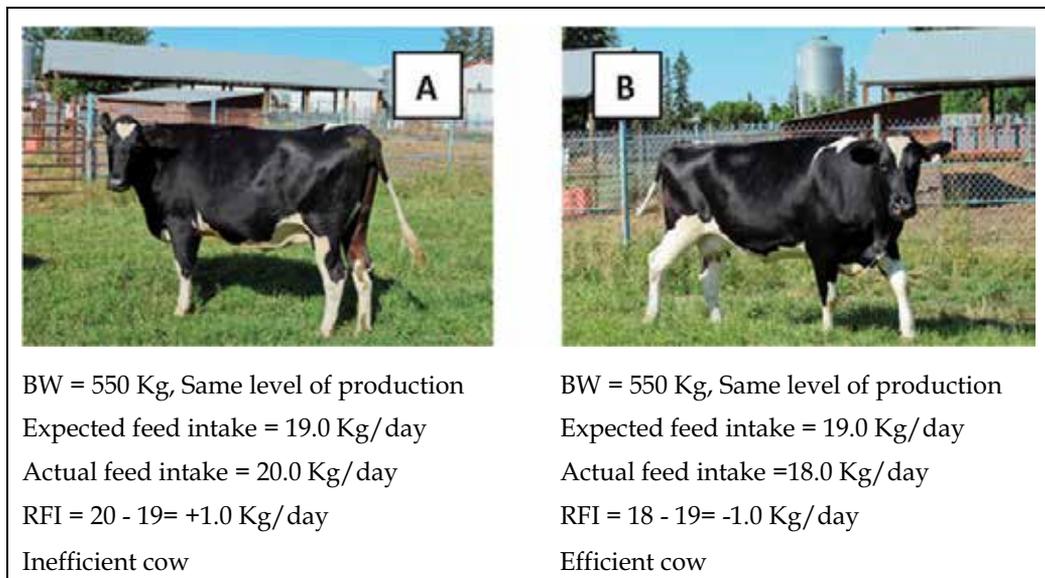
Researchers have proposed many measures of energy efficiency such as feed conversion ratio (FCR), gross energy efficiency (GEE), residual feed intake (RFI) (Koch et al., 1963; Archer et al., 1999; Crews, 2005) and life time efficiency (LTE) (Vandehaar, 1998; Vandehaar & St-Pierre, 2006). Their definition, applications and benefits are different.

FCR and GEE are the most common measures of efficiency in the literature. FCR is the ratio of input (e.g. feed) to output (e.g. weight gain or milk production) (Crews, 2005). In the dairy cow, the GEE is defined as the energy in the milk divided by the total energy intake (Veerkamp & Emmans, 1995). These approaches lead to only limited insight into efficiency of the entire production system (Crews, 2005). The problems of GEE and FCR have been discussed in numerous studies (Korver et al., 1991; Veerkamp & Emmans, 1995; Crews, 2005) and are mainly categorized in three groups. First, the energy intake has different partial efficiencies for maintenance, lactation, pregnancy and body tissue gain or loss, but the GEE and FCR do not distinguish between them (Veerkamp & Emmans, 1995). Secondly, FCR and GEE are well known to be phenotypically and genetically correlated with measures of growth, production and mature size. Therefore, selection of animals based on these measures may increase the maintenance requirements. Finally, changes in GEE and FCR can be the result of changes in either intake (numerator), yield (denominator) or both (Gunsett, 1984; Veerkamp & Emmans, 1995) and selection direction cannot be predicted very well. Then, selection for improvement of FCR (i.e. decreased FCR) and GEE would result in increased growth rate, mature size, and consequently mature maintenance requirements (Korver et al., 1991). It can be concluded that improving FCR and GEE by selection for increased growth rate do not necessarily improve net feed efficiency, because of drawbacks associated with increased maintenance requirements (Van der Werf, 2004; Crews, 2005).

Lifetime efficiency (LTE), another measure of energy efficiency, is defined as “the capture of feed energy in milk, conceptus, and body tissue divided by gross energy intake during the life of cow, starting at birth” (Vandehaar, 1998; Vandehaar & St-Pierre, 2006). This index attempts to summarize an animal’s entire life efficiency and is a good criterion to set up a long term vision. In order to compare the LTE in dairy cows, total milk production should be standardized for all factors such as housing, feeding, age at first calving and calving interval. The LTE mostly depends on the precalving interval and intercalving intervals. The Precalving Interval is defined as the period from birth to first parturition and Intercalving Intervals are the intervals between successive calvings (King, 2006). The main concerns related to LTE are: lots of information is required to calculate the LTE, it is applicable for the entire life, and it is influenced more by precalving and intercalving intervals.

To overcome the problems associated with FCR, GEE, LTE and other measures of energy efficiency, an alternative measure can be expressed as residual feed intake (RFI). RFI is a measure of feed utilization corrected for live weight and production, and it is often referred

to as net feed or energy efficiency ( Koch et al., 1963; Korver et al., 1991; Luiting et al.,1992). The concept of RFI can be described as “the difference between the actual feed intake and that predicted on the basis of mean requirements for body weight maintenance and levels of production” (Koch et al., 1963); it is explained schematically in figure 1. RFI relies simply on partitioning feed energy intake into portions required for body maintenance, stage and levels of production, and a residual portion. This residual portion is related to the true metabolic efficiency of an animal and would be comparable across individuals (Crews, 2005). Variation in RFI probably reflects underlying biological efficiency after adjustment for energy deposition (Crews, 2005; Herd & Arthur, 2009 ). In a population, the mean RFI index over all individuals is zero and approximately half of all individuals have RFI values below or above the mean. The efficient animals have low RFI values; it implies they consume less feed without compromising their production (Crews, 2005). Indeed, RFI is a net feed efficiency measurement and it can be calculated at any time of an animal’s life.



**Figure 1.** Schematic concept of residual feed intake (RFI). Two animals which have the same BW and ADG, are expected to consume the same amount of feed but in reality cow A consumes more than expected while cow B consumes less, so cow B is more efficient than A.

### 3. Factors affecting energy efficiency in dairy cattle

Several factors influence energy efficacy in dairy cattle. It is practically influenced by dry matter intake (DMI), production level, body tissue changes, age at first calving (AFC), and environmental factors (Vandehaar, 1998; Linn, 2006). Their approaches to affect the energy efficiency are different.

### 3.1. Dry matter intake and production

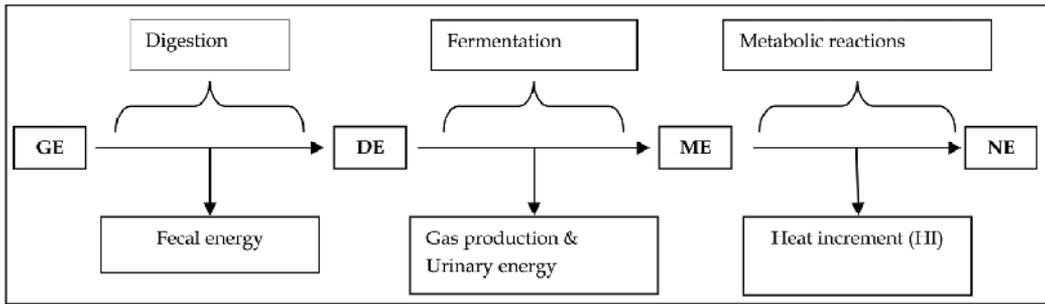
Dry matter intake (DMI) and production are the direct components of most energy efficiency traits. Dry matter intake (DMI) affects energy efficiency through energy transformation mechanisms from gross to net energy. The basic rule of most common efficiency measures, FCR and GEE, is increased production means increased efficiency, but the relationship between the marginal benefit of increased production and efficiency is not always linear.

DMI establishes the amount of nutrients available to an animal for maintenance and production. Inadequate intake of nutrients negatively affects production, efficient nutrient utilization and health status of the animal. Supplying nutrients in excess also increases feed costs and can result in excretion of nutrients into the environment (NRC, 2001; Collier et al., 2006). In dairy cows, the average DMI is 22.7 kg/d, and it ranges between 19.8 to 26 kg/d (Ordway et al., 2009; Vallimont et al., 2010). Heritability of DMI was reported from 0.16 to 0.48, and its genetic correlation with energy intake ranged from 0.8 to 0.9 (Veerkamp, 1998; Vallimont et al., 2010). Therefore, DMI and energy intake are genetically almost the same trait. However, the energy transformation mechanisms of DMI, which affect energy efficiency, involve digestion, fermentation and metabolic processes. Gross energy (GE) is the amount of released energy in heat combustion. Net energy (NE) is the energy which is directly used to support maintenance functions, including conversion to milk, conceptus growth and body tissue gain (NRC, 2001). Feedstuffs have energy in the gross form and it is converted to net energy in several steps (figure 2). Some amounts of the GE are indigestible and ultimately appear in feces; the remaining part is called digestible energy (DE). Some part of DE is lost due to gas production (mainly methane) and urinary energy (mainly urea) during the fermentation process. The remaining DE after deduction for gas and urinary production is called metabolizable energy (ME). Finally, converting the ME to net energy (NE) requires metabolic reactions, which produce heat that is termed the heat increment (NRC, 2001; Vandehaar & St-Pierre, 2006). Therefore, the accessible amount of NE from GE depends on the amount of losses in digestion, fermentation and metabolic processes. Practically, it depends on many factors, such as DMI levels, passage rate, and dietary fibre (especially, effective neutral detergent fiber, eNDF) level (NRC, 2001). Some studies have been conducted to determine the relationship between these factors and amount of nutrient losses in the different steps of the transformation mechanisms (Moe, 1981; Van Soest et al., 1992); they concluded there is an optimum point between them. For example, Vandehaar (1998) reviewed the literature and showed that the relationship between level of DMI and DE is not linear. When a dairy cow consumes DMI for its maintenance requirements, almost 80% of GE captured is in the form of DE. Furthermore, there is a reduction in digestibility as DMI increases (Vandehaar, 1998). Overall, NRC (2001) suggested that digestibility is depressed linearly at 4% per multiple of maintenance intake. It assumes that most of the cows consume 3 times their maintenance requirement, which is an optimum point of GEE. In addition, it has been shown that there is an optimum point of NDF level in terms of converting GE to DE, and it is reported to be between 25 to 30%. Amounts of NDF in the diet beyond this range will decrease energy intake. Higher levels of NDF fill the rumen,

whereas lower levels may cause some health problems (Eastridge, 2006; Vandehaar & St-Pierre, 2006). Finally, the portion of losses in the different steps of energy transformation shifts and it is dependent on DMI levels. At higher levels of DMI the losses into feces increase while the amounts of losses as heat increment are greater at lower levels of intake (Vandehaar & St-Pierre, 2006).

Although production is a fundamental component that determines efficiency, the relationship between marginal benefit of increased production and efficiency is not linear for all the time. During the past 18 years, the average milk production of Canadian Holstein cows has increased about 115 kg/cow/year and currently the average milk yield of a primarily Holstein herd is 9793 kg/cow/yr. The average rate of increase was 1.35% between 1991 and 2009 (DHI, 2009), and it likely will continue to increase. In addition, milk yield heritability is reported as 0.3 (Lee et al., 1992; VanRaden et al., 2009) and ranges between 0.16 to 0.5 (Veerkamp, 1998). This means that still there is still room to increase milk production by exploiting genetic selection. The genetic correlation between GEE and milk production in dairy cattle ranged from 0.88 to 0.95 (Pitchford, 2004). It confirms that selecting dairy cows for milk yield automatically improved GEE (Veerkamp & Emmans, 1995). Consequently, FCR (4% FCM/DM) has increased from 0.91 in 1991 to 1.2 in 2006, and a common goal is 1.5 (Eastridge, 2006). Korver (1991) concluded that the improved GEE and FCR mostly reflects the dilution of maintenance. Dilution of maintenance means that as cows consume more, a relatively small fraction of energy is used for maintenance and a larger portion is captured in milk. Although there is no evidence to suggest that the maintenance requirements depend on milk production and breed, cows with similar body weight and breed may vary for maintenance requirements by about 8 to 10% (NRC, 2001). These assumptions need further investigation. To set a vision for the future, Vandehaar (1998) modelled the optimum point of milk yield. He proposed that above 15000 kg/yr, the marginal increase in efficiency approaches zero. Therefore, the positive correlation between milk production and efficiency that has existed in the past may change in the future, when average milk production surpasses 15000 kg/yr/cow (Vandehaar, 1998).

DMI and milk yield are tightly linked as their genetic correlation is reported to be 0.5 (Vallimont et al., 2010) ranging from 0.46 to 0.84 (Veerkamp, 1998). Consequently, selection decisions which change milk yield and body weight (BW) also change DMI (Veerkamp & Emmans, 1995). Genetic selection mostly focuses on milk yield and it indirectly affects DMI. However, with increased milk production per animal, there is a limit to the increase in DMI because of rumen fill; therefore, the density of NE in dairy rations has been elevated as milk production increased in the last 30 years. For instance, the dietary NE density of dairy cattle rations has increased from 1.23 in 1980 to more than 1.6 Mcal/kg in 2006 (Eastridge, 2006). Thus, it can be inferred that some of the improved efficiency due to increased milk production is withdrawn by increasing the dietary energy concentration in terms of expenses. Furthermore, the linear relationship between milk production and efficiency may change in the future. Therefore, these concerns drive researchers to define net energy efficiency using concepts such as RFI, which is independent from production and maintenance in dairy cattle.



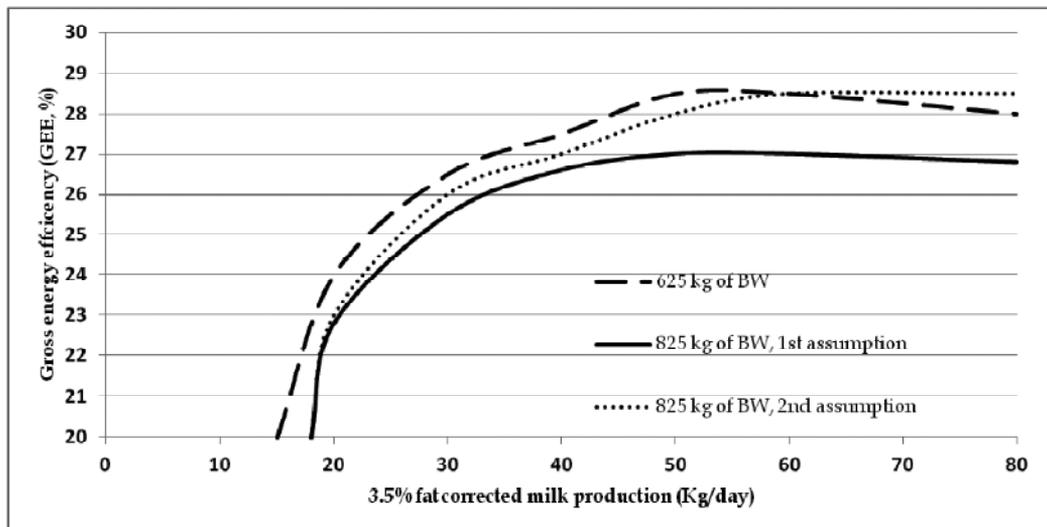
**Figure 2.** Energy transformation processes from gross energy (GE) to net energy (NE). The portion of lost energy in different steps is dependent on DMI level.

### 3.2. Body weight

Body weight influences energy efficiency through its relationship with milk production and digestive capacity. Heritability of body weight (BW) is reported to be in a range of 0.26 to 0.88 (Verrkamp, 1998). BW is genetically correlated with milk production. Although some researchers (Veerkamp, 1998; Vallimont et al., 2010) reported a negative correlation (range of -0.01 to -0.42), some other researchers reported positive correlations between BW and milk production (range of 0.05 to 0.45) (Veerkamp, 1998). This inconsistency in results could be due to mean differences of BW and milk production between populations under estimation. It can also be suggested that there is an optimum point of relationship between BW and milk production, and consequently energy efficiency. In order to illustrate this optimum relationship, Vandehaar (1998) modelled the relationship between body size, milk production and energy efficiency. He considered two possible relationships in which there were function of BW and digestive capacity (figure 3). In the first, he assumed that the digestive capacity of animals is not a function of BW and it is constant, the solid curve and dashed curve in figure 3. Therefore, increased BW increases the maintenance requirements and consequently decreases energy efficiency. In the second model, the digestive capacity was assumed to be a function of BW, so with increased BW digestive capacity will increase, and consequently a large cow would be efficient, dot-dashed curve and dashed curve in figure 3. In this case if a cow had a 200 kg greater BW (825 kg VS 625 kg), she should produce 60 kg/day more milk to become efficient. He concluded that the relationship between body size and efficiency depends on the relation between digestive capacities with body size (Vandehaar, 1998), and that there is an optimum point of relationship between BW and energy efficiency.

### 3.3. Body tissue changes

Body tissue changes increase energy efficiency by supporting milk production and tissue mobilization is a crucial factor in determining energy efficiency of dairy cattle. Although the conversion ratio of lost body reserves to milk production is less than that of regaining the



**Figure 3.** Adapted from Vandehaar (1998). He discussed the two possible relationships between digestive capacity and BW. In the first one, digestive capacity was not a function of BW while in the second, the digestive capacity was a function of BW.

reserves from feedstuffs, reasonable levels of losses still increase the energy efficiency. From an evolutionary point of view, mammals use their stored energy reserves to produce milk and support their young when their requirements exceed DMI consumed. In nature as the calf grows older, it gradually relies less on mothers' milk and the mother has an opportunity to regain energy resources for the next lactation (Bewley et al., 2008). Similarly, in the dairy industry, as the feed intake peak occurs later than the milk yield peak, dairy animals have a mechanism to use their body reserves to support milk production in early lactation and regain the body reserves in late lactation (Coffey et al., 2001; Bewley et al., 2008). In early lactation when energy intake is less than that used for milk, maintenance and activity, the cows are in a negative energy balance (NEB). Therefore, they sacrifice their body resources in this period to meet the requirements. Up to one-third of the total milk solids that are produced in early lactation, comes from body tissue reserves (Bewley et al., 2008). Practical measures of changes in energy resources include changes in BW and body condition score (BCS). BCS is a management technique used to appraise the body fat reserves in cattle (Coffey et al., 2001) and it is measured with either a 5 or a 9 point scale. The BCS represents 65, 55 and 66 percent of fat, protein and energy variation in dairy cattle, respectively (NRC, 2001). The ability to manage body reserves varies between animals, and they have a different pattern of BW and BCS changes during lactation and across lactations (Bewley et al., 2008). Heritability of changes in BW reportedly range from 0.1 to 0.27 (Verrkamp, 1998). Heritability estimates for BCS change depend on stage of lactation and range from 0.08 to

0.6 (Bewley et al., 2008). Negative and positive correlations have been reported between milk yield and BW changes (-0.41 to 0.45) (Verrkamp, 1998) and BCS changes (Bewley et al., 2008). One BCS unit (5 point scale) is equivalent to ~400 Mcal of ME and its conversion ratio to milk is estimated at 0.82. It is enough to produce an additional 8 kg milk/day in the first 60 days in milk (Vandehaar, 1998; Bewley et al., 2008). Therefore, losing one unit of BCS supports around 2000 kg of increased milk production over 305 days and it is expected to increase GEE from 25 to 26.5% in cows with a production of 8000 kg milk (Vandehaar, 1998). The lost energy reserves are replaced by cows in late lactation, and its replenishment conversion ratio is less (0.7) than that for loss (0.8) (Moe, 1981), but loss of BCS still increases efficiency (Vandehaar, 1998). Besides the increased efficiency, some researchers point out that side effects of losing energy reserves on other traits like reproduction and health should be considered (Vandehaar, 1998; Bewley et al., 2008). For example, cows restart reproduction activity after they pass the NEB period (Goff, 2006). Some of the metabolic diseases such as ketosis/fatty liver complex are highly correlated with NEB (Collier et al., 2006). Researchers proposed that there is a curvilinear relationship between BCS at calving and milk production; furthermore, maximum milk production is associated with 3.25 to 3.5 BCS at calving (Roche et al., 2007; Bewley et al., 2008). Indeed, during early lactation, a controlled loss of body condition of 0.5 to 1.0 units is associated with optimal milk production, health, and reproductive performance. Moreover, excessive BCS losses at calving predispose the animal to metabolic disorders such as ketosis and fatty liver (Spain, 1996; Bewley et al., 2008).

### **3.4. Age at first calving (AFC)**

Age at first calving (AFC) is the period between birth and first calving. It represents a period when animals cost the farmer due to yardage expenses. Yardage expenses include costs related to housing, feeding and veterinary care, which represent 15 to 20% of animal expenditures toward the cost of milk production (Mayer et al., 2004). Breeding programs aim to have AFC at 22 to 24 months of age, and reducing the AFC can increase animal life time efficiency (LTE) (Mayer et al., 2004, Vandehaar & St-Pierre, 2006). Reduced AFC should not compromise weight at calving. The data suggest that the optimum weight for Holstein cows right after calving, is 570 kg. The results also showed that milk yield will be reduced about 70 kg for every 10 kg body weight below the optimum (Vandehaar & St-Pierre, 2006). Therefore, AFC can be reduced by a combination of increasing average daily gain and decreasing age at breeding (Mayer et al., 2004). Decreased AFC, and consequently yard cost, is associated with increased feed cost to support a rapid growth rate. Furthermore, if the optimum breeding weight is not achieved, there will be a negative effect on subsequent milk production (Vandehaar & St-Pierre, 2006). Indeed, the economic benefit of a decreased AFC is not well understood and there is a need for further investigation.

### **3.5. Environmental factors**

Changes in environmental conditions (temperature and humidity) and photoperiod are called seasonal changes. Seasonal changes affect energy efficiency by altering hormone

signals and the target cell's responsiveness to hormonal stimulation (Collier et al., 2006). The thermoneutral zone is a range in which animals do not spend energy to maintain their normal body temperature. The upper critical range for dairy cattle is 25 to 26 °C and lower critical range depends on DIM and production level. The lower critical range is 2, -4 and -10 °C for an animal at maintenance or producing 10 kg or 20 kg of milk, respectively. Dairy cows in cold stress do not need to change their energy requirements due to high heat production but it has an effect on feed digestibility. Research has shown that there is a 2% reduction in digestibility for every 10 degree reduction in ambient temperature; this can possibly be attributed to the increase in passage rate of digesta (NRC, 2001). Therefore, cold stress does not affect energy efficiency significantly in dairy cattle; while, mild to severe heat stress increases the maintenance requirements from 0.7 to 2.4 %, respectively, and decreases DMI. Heat stress affects animal behaviour, metabolism and efficiency (NRC, 2001).

Photoperiod, another environmental factor, affects lactation, reproduction, production, growth and immune function. Most studies are done using short or long day photoperiod concept. Results demonstrated that the physiological basis of attainment of puberty is controlled by photoperiod rather than ambient temperature. Long photoperiod causes early puberty that is associated with rapid growth in calves, and greater mammary parenchyma (Collier et al., 2006). Long day photoperiod can affect energy efficiency by lowering AFC, and increases milk production, but it does not affect feeding behaviour. In addition, other temporary environmental factors such as milking frequency can also affect milk production and energy efficiency. Wall & McFadden (2007) concluded that milking 2 times more frequently than usual (4 vs 2 times/day) for a 3 week interval during early lactation significantly increases milk production.

#### **4. Indirect effects of selection for energy efficiency on some related traits**

To this point, factors that practically and directly affect energy efficiency in dairy cattle have been discussed; to maximize gain due to genetic selection for energy efficiency, its genetic base and indirect effects on other traits should also be known. Although reports on direct/indirect selection for efficiency in dairy cattle are scarce (Linn, 2006), many studies have been conducted to study its heritability and the direct/indirect effect that selecting animals based on efficiency traits has on other related traits in different species. The reviewed results showed that the weighted mean of 28 and 9 estimates of heritability in beef for FCR and GEE were reported as  $0.32 \pm 0.02$  and  $0.37 \pm 0.05$ , respectively (Koots et al., 1994). The weighted mean of 35 estimates of heritability for RFI in 7 species was reported  $0.25 \pm 0.02$  (Pitchford, 2004). In order to point out the potential effect of selection for efficiency on other related traits, authors discussed this effect on reproduction, activities, organs, body composition, metabolites and health in beef cattle as well as other species in addition to dairy cattle (table 1).

Species	Reproduction	Activities	Organs	Chemical composition	Metabolites	Health
Dairy	Decrease daughter fertility	Data not available.	Data not available.	Data not available.	Data not available.	Increases the incidence of metabolic diseases (Wassmuth et al., 2000)
Beef	Decreases age at puberty, does not affect pregnancy rate (Shaffer et al., 2010). Did not affect bull performance (Wang et al., 2012).	Less feeding duration and less head-down time, (Durunna et al., 2010; Nkrumah et al., 2006; Kelly et al., 2010).	Did not affect tissues of gastro intestinal organs and internal organs (Richardson et al., 2001).	Less body fat (Richardson et al., 2001) more empty body water (Basarb et al., 2003).	Low plasma protein, blood concentration of urea and aspartate amino transfer (Herd & Arthur, 2009) high insulin, glucose and NEFA (Kelly et al., 2010).	Data not available.
Pig	Decreased litter size (Estany et al., 2002)	Less feeding time, less visits per day, less total time in feeder (Von Felde et al., 1996)				
Mice	Decreased litter size, ovulation rate (Nielsen et al., 1997)	Less activities (Hastings et al., 1997; Rau et al., 2000)	Larger livers, caeca, stomachs but smaller hearts (Hughes & Pitchford, 2004)	Fatter (Hughes & Pitchford, 2004)		Data not available.
Chicken	Increased fertility, hatchability, decreased mortality (Morrison et al., 1997). No losses in egg production (Bordas et al., 1992)	Less activities (Luiting & Urf, 1991)		Controversial results, increase or decrease fat traits (Liting & Urf, 1991)		

**Table 1.** Summary of indirect response of selection for energy efficiency on related traits in different species

#### 4.1. Reproduction

Reproductive performance and milk production are two main entities in the profitability of dairy cattle industry (LeBlanc, 2010). Although milk production and energy efficiency have increased, the genetic trend of average daughter fertility in Canadian Holsteins has shown a 2% reduction over 14 years. It decreased from 101.9 in 1995 to 99.9 in 2009 (Van Doormaal, 2010). As a result, a selection objective to increase milk production seems to favour cows that genetically produce more milk, but consequently are prone to experience more negative

energy balance (NEB). It has been reported that the time of first estrus is closely related to NEB during the first 2 - 3 weeks after calving (Coffey et al., 2006) and “cows appear to resume reproductive activity only after the nadir of NEB has passed” (Veerkamp, 1998).

Some researchers studied the indirect effect of selection for energy efficiency on reproduction traits in beef, and other species. For example, Shaffer et al. (2010) allocated beef heifers into three groups based on their efficiency (low, medium and high RFI) and studied the indirect effects of selection for efficiency on reproduction performance. They reported a negative relationship between RFI and age at puberty. The efficient animals reached puberty later than inefficient animals but it did not affect pregnancy or conception rates. They also quantified this relationship and reported that each unit increase in RFI corresponds to a decrease of 7.5 days in age at puberty. Wang et al. (2012) studied the effect of RFI on bull's reproductive performance and fertility. They had 20 high RFI (inefficient) and 22 low RFI (efficient) beef bulls in a multi-sire breeding system on pasture and examined the association between RFI and semen quality traits (density, progressive motility and morphology), progeny per sire and some other related traits. They concluded that selection for RFI does not have a negative effect on reproductive performance and fertility in bulls bred in multi-sire groups on pasture.

In other species, Nielsen et al. (1997) divergently selected mice for energy efficiency, based on heat loss, over 15 generations. They had high efficient, low efficient and control groups, and each group had three replicates. Indirect effects of selection for energy efficiency on reproduction performance (litter size, ovulation rate, number of foetuses at 7 days of gestation and ovulation success) were measured. The results showed that the high efficient line (low heat loss) had 20% smaller litters at first parity in the 15<sup>th</sup> generation. The efficient line also had a 23% lower ovulation rate when measured at the second parity. However, the high efficiency line had a higher ovulation success rate (86%) than the low efficiency line (84%), but the differences were not significant (Nielsen et al., 1997). A report on pigs demonstrated that pigs with high litter size had a poorer efficiency compared to the control group (Estany et al., 2002). However, Morisson et al (1997) divergently selected hens for RFI over 18 generations and studied the effect of energy efficiency selection on reproduction and sperm characteristics. Contrary to mice and pigs, they found that a high efficient line of hens had only 6% unfertilised eggs compared with 30% in a low efficiency line. The early mortality rate in the inefficient line was twice that of the efficient line. Overall, the efficient line had a better hatchability performance (Morrisson et al., 1997). The better reproductive performance of efficient hens is supported by other researchers who selected hens for low RFI without losses in egg production (Bordas et al., 1992). It could be inferred that some species sacrifice litter size and maintain energy to better take care of the fetus. There is a need to study the associated effects of selection for energy efficiency and reproductive performance in dairy cattle.

## 4.2. Activity

Energy expenditure of feeding depends on feeding behaviour. In addition, results of studies in different species have shown that selection for efficiency had effects on animal's feeding

behaviour. Durunna et al (2010) conducted a 3 year study on 402 and 419 steers on two different diets (grower and finisher). They measured feed intake, feeding duration, head-down time and bunk visits using the Growsafe system. Their results showed that the efficient steers (Low RFI) exhibited less feeding duration, head down time, and bunk visits. In another study, efficient beef cattle (low RFI) had less feeding duration, but a higher feeding frequency (Nkrumah et al., 2006). These results are also supported by other researchers studying finishing heifers (Kelly et al., 2010) that showed efficient heifers had less feeding duration.

Some studies have been done on mice to determine the effect of selection for RFI on activity. Hastings et al (1997) found that high efficiency (low RFI) mice were 67% less active than the low efficiency mice. Furthermore, Rauw et al (2000) selected mice for high litter size at birth (S line) and showed that the S line had higher RFI (low efficiency). They reported that low efficiency mice, when compared with control group, had more locomotion activity, and they ran faster in two types of runaway tests. In hens, Luiting &Urff (1991) reported that high efficient layer hens were less active than the control group. However, efficient boars had a lower feeding rate, less feed intake per visit, fewer visits per day, and less total time in the feeder per day (Von Felde et al., 1996). Herd & Arthur (2009) concluded that the positive and high genetic correlation of feeding time per day and eating sessions per day with RFI indicates that there are some common genes controlling feeding behaviour and RFI.

### **4.3. Organs and body composition**

Liver, the largest visceral organ, accounts for 17 to 31% of total body energy expenditures (Eisemann & Nienaber, 1990; Ortigues and Visseiche 1995). All of the visceral organs account for up to 40 to 50% of body energy expenditures in sheep and cattle (Perry et al., 1997). It was concluded that selection for efficiency may result in lower proportions of liver and visceral tissues (Pitchford, 2004). In female mice, the results contradicted this conclusion and the efficient mice (low RFI) had larger livers, caeca, intestines, and stomachs but smaller hearts (Hughes & Pitchford, 2004). In cattle divergently selected for RFI, the weight of highly activate tissues of gastrointestinal organs and internal organs were not significantly different. It was concluded that variation in ME intake and energy efficiency was due to metabolic processes rather than changes in body composition (Richardson et al., 2001).

Results of divergently selecting steers for RFI showed that there is a correlation between chemical composition and variation in RFI. Animals with low RFI had more whole-body chemical protein and less whole-body chemical fat (Richardson et al., 2001). Basarab et al (2003) also found that efficient steers had more empty body water but less empty body fat than low efficient steers. The divergently selected steers had almost the same amount of empty body protein. In another study, Shaffer et al (2010) grouped beef heifers of British breeds into low, medium and high RFI groups and found that efficient heifers (low RFI) had less lean meat area (cm<sup>2</sup>) per 100 kg of BW than inefficient (high RFI) heifers. In mice, the results have shown that the high efficiency lines had slightly lower post-weaning weight (0-12%), little differences in mature weight (0-30%) and were fatter (5-60% depending on the

age at measurement) than low efficiency lines. Luiting & Urff (1991) summarized reports of phenotypic and genetic correlations between RFI and body fat traits in chickens and found that they ranged from -0.4 to 0.45. Herd & Arthur (2009) concluded that the amount and direction of association between body composition and variation in energy efficiency in cattle depends on age and stage of maturity.

#### 4.4. Metabolites and health

There are some reports on associations between efficiency and some metabolites, which are indicators of production, and health. For example, high concentrations of total plasma protein, blood concentrations of urea and aspartate amino transfer were reported in high RFI cattle (inefficient) compared to low RFI (efficient). These metabolites are an index of protein turnover and inefficient cattle had higher protein turnover rates compared to low efficient cattle (Herd & Arthur, 2009). In other research, Kelly et al (2010) divergently selected heifers based on RFI and found that inefficient animals had higher plasma urea, B-hydroxybutyrate, and leptin concentration and lower NEFA, plasma glucose and insulin than efficient animals. Higher levels of cortisol and red and white blood cells were reported in high RFI steers, which indicates that these animals (inefficient) may be more susceptible to stress (Richardson et al., 2004). In another report, a positive correlation between IGF-I, a growth metabolite, and RFI was reported in beef cattle (Moore et al., 2005). However, separation of RFI into post weaning and feedlot periods determined that there is a positive correlation of IGF-I with RFI during post weaning time while there is a negative correlation during the feedlot period (Herd & Arthur., 2009). Kelly et al (2010) concluded that some plasma analytes such as B-hydroxybutyrate may be potential indicators of net efficiency in beef cattle.

Overall, animals are efficient and profitable, if they are healthy. Rauw et al (1998) reviewed undesirable effects of selection for high efficiency in farm animals and concluded that selection had a negative correlation with health traits. Wassmuth et al (2000) used feed intake data of 7752 young dairy bulls (2203 Danish Red, 4527 Danish Friesian and 1022 Danish Jersey), and combined the feed intake data with recorded incidence of mastitis, retained placenta, metritis, sole of ulcer and ketosis data of 473,613 dairy cows in their early lactation to investigate the relationship between efficiency and diseases in dairy cattle. They defined efficiency as “the feed energy intake per kilogram live weight gain” in bulls. The size and direction of relationship depended on breed, but the overall energy efficiency was positively correlated with incidence of diseases. Currently, selection indices in dairy cattle favour animals with high milk production and consequently negative energy balance (NEB). NEB is generally related to poorer health status and fertility and it can have an indirect economic effect (Goff, 2006; Veerkamp, 1998).

Overall, the physiological basis of energy efficiency (RFI) has been reviewed by Herd & Arthur (2009). The results of Angus steers divergently selected for net feed efficiency (RFI) revealed that feeding pattern, metabolism including turn over and stress, body composition, digestibility, heat increment of fermentation, and activity accounted for 2, 37, 5, 10, 9 and 10

% of the variation in RFI, respectively, and the remaining variation was attributed to other unknown processes (Herd & Arthur, 2009).

## 5. Conclusion

It can be concluded that there is an optimum point for the factors (DMI, milk production, body weight, AFC and environment factors) that influence the energy efficiency, and their relationship with energy efficiency is not linear. Hence, increasing output traits does not necessarily increase net energy efficiency. Therefore, the measures of energy efficiency that represent net efficiency, like RFI, which is independent from maintenance and production, need to be considered to improve efficiency in dairy cattle. It is proven that RFI is a robust measure of the animals' energy efficiency because it is independent from animals' maintenance requirements and level of production. Genetic improvement on energy efficiency can be achieved through selection for RFI in the dairy industry since the heritability estimates for RFI are moderate for most species ( $h^2 = 0.25$ ). Also, the traits are correlated and there are inconsistent results between species for indirect response of selection for energy efficiency on other related traits especially reproduction and health traits. Care should be taken when animals are selected for energy efficiency. Further research is required to define RFI in dairy cattle and to determine the indirect effects that selecting for efficiency may exert on other related traits, especially those related to reproduction and health.

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# Efficiency of Lactation

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50772>

## 1. Introduction

Dairy cows are one of the most efficient animals. Regarding to energy and protein yield per unit of land used, milk production is one of the most efficient enterprises in animal productions (Table 1). Likewise, the efficiency of solar energy used for production of energy and protein as milk is more efficient than other livestock products (Table 2). Moreover, efficiency of nutrient utilization is a major factor affecting profitability in modern dairy farms, because feed costs represent approximately one-half of the total costs in most livestock operations and 80% of the variable cost of milk production. (Milk Marketing Board, 1990). Thus, improvement of feed efficiency has a noticeable importance to increase profitability of lactating dairy cows.

Yield	Crude protein (kg/ha/yr)	Gross energy (MJ/ha/yr)
Cow milk	145	12000
Beef	31	2400
Lamb	22	2500
Pork	76	11400
Broiler's meat	145	4300

**Table 1.** Energy and protein yielded by meat and milk productions per unit of land used (Spedding et al., 1981)

Yield	Protein (gr)	Energy (MJ)
Cow milk	0.0044	0.00036
Beef	0.0009	0.00007
Lamb	0.0007	0.00008
Pork	0.0023	0.00035
Egg	0.0034	0.00018

**Table 2.** Efficiency of solar energy used for production of energy and protein as different animal productions (Spedding et al., 1981)

\* As received radiation of  $33 \times 10^6$  MJ/ha/yr

Dairy cows excrete about 2-3 times more nitrogen (N) in manure than in milk, which contributes to increased milk production costs and environmental N pollution (Broderick, 2005). Mass N balance studies showed that on typical dairy farms only 12 to 36% of the N input is retained in salable products, whereas up to about 70% is lost mainly through volatilization and leaching into the off-farm environment (Ipharraguerre and Clark, 2005). These evidences reveal that improving a cow's biological efficiency for converting feed to milk should be an important goal for the dairy industry and in animal breeding programs.

## 2. Measures of efficiency

There are several criteria to measure feed efficiency in lactating dairy cows. The most important measures of feed efficiency could be mentioned as follow.

### 2.1. General efficiency

This category of feed efficiency measures refers to the simple ratio criteria such as gross feed efficiency and feed conversion ratio.

*Feed conversion ratio (FCR)*: The ratio of feed intake over milk yield is called feed conversion ratio (Equation 1). By this measure, a less feed conversion ratio means more efficiency.

$$FCR = \frac{DMI}{MY} \quad (1)$$

Where, DMI and MY are dry matter intake and milk yield, respectively.

*Gross feed efficiency (GFE)*: This measure can be described as the ratio of milk produced to feed consumed. Gross feed efficiency is inversed of feed conversion ratio. (Equation 2)

$$GFE = \frac{MY}{DMI} \quad (2)$$

In calculation of FCR and GFE, milk compositions are ignored. To overcome this problem, fat corrected milk yield or solid corrected milk yields could be used for calculation of FCR and GFE.

Another problem arising from feed conversion ratio and gross feed efficiency is that the nutrient composition of feed intake affects the efficiency of animal. For example, a concentrated diet will improve the efficiency (decreased FCR and increased GFE). To overcome this problem, the efficiency could be determined for main dietary components, including energy and protein.

### 2.2. Energetic efficiency

Energetic efficiency is a common measure of biological efficiency, because energy is the most limiting nutrient for dairy cow, and the intake of which is most closely related to the

level of milk production; furthermore, protein is a form of feed energy and accounted for in calculations of energetic efficiency as well (VandeHaar, 1998).

Several criteria for the measurement of energetic efficiency have been proposed. The most important measures of energetic efficiency are gross energy efficiency, net energy efficiency and residual feed intake.

*Gross energy efficiency (GEE):* Gross energy efficiency is the percentage of a given category of feed energy recovered in milk (Brody, 1945). GEE could be calculated by Equation 3.

$$GEE = \frac{ECM}{EI} \quad (3)$$

In this equation, ECM and EI are energy contents of milk and energy intake, respectively.

Gross energy efficiency does not consider the role of body reserves in energetic efficiency of lactating cows. For example, at the first stages of lactation, when the cow is in negative energy balance, a considerable amount of energy is taken from body tissues and the cow seems more efficient in at these stages of lactation. Vice versa, at the last stages of lactation, a considerable energy is stored in the body tissues and the cow sounds less gross energy efficiency. This problem could be eliminated by the next measure of efficiency, called net energy efficiency.

*Net energy efficiency (NEE):* Net energy efficiency is the ratio of energy contained in the milk over the available portion of energy intake used to produce it above maintenance requirements (Brody, 1945; Buttazzoni and Mao, 1989; Miraei-Ashtiani et al, 2005). NEE could be calculated using Equation 4.

$$NEE = \frac{ECM}{MEI - NE_m - NE_{preg} - ER} \quad (4)$$

Where, ECM is energy content of produced milk and MEI,  $NE_m$ ,  $NE_{preg}$  and ER are metabolizable energy intake, energy requirement for maintenance, energy requirement for pregnancy and net energy required for replenishment of reserves or net energy available if reserves are mobilized.

Feed conversion ratio, gross feed efficiency, and gross and net energy efficiencies are calculated as ratios. When feed efficiency is expressed as a ratio, there may be two disadvantages (Wang et al, 1992): the first is an increase in the error variance as a proportion of total variance in the statistical analysis (Lison, 1958) and the second is a strong positive phenotypic and genetic correlations between milk yield and feed efficiency which could be the result of an almost automatic correlation (Mason et al, 1957). To overcome the probable problems of ratio measures, an alternative linear calculating criterion could be more appropriate to measure energetic efficiency. The next measure, called residual energy intake, has a linear calculating method.

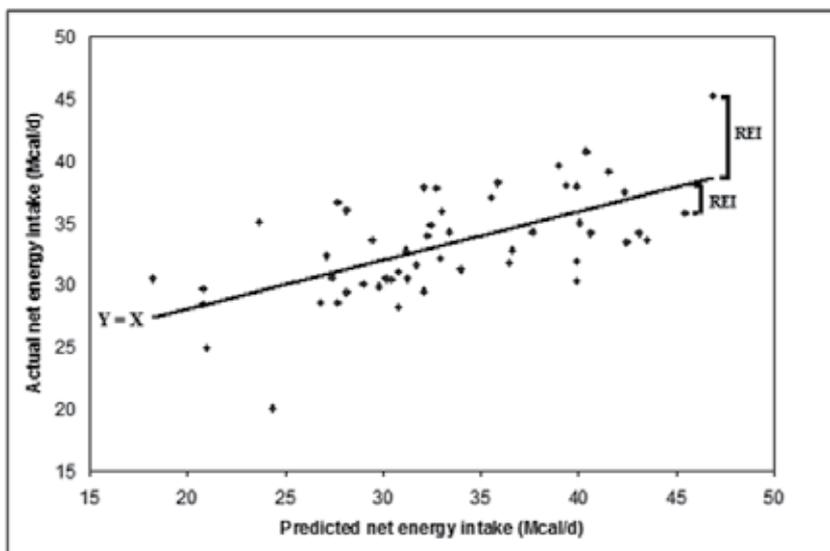
*Residual energy intake (REI)*: Residual energy intake or residual feed intake (RFI) can be defined as the difference between actual energy intake and the one predicted on the basis of requirements for maintenance, lactation, and body weight change of an animal (Equation 5). Residual energy intake seems to have been first proposed by Koch et al. (1963) and then studied by other researchers (e.g. Kennedy et al., 1993; Veerkamp et al., 1995; Zamani et al., 2008).

$$REI = AEI - PEI \quad (5)$$

In this equation, AEI and PIE are actual and predicted energy intakes, respectively.

Energy intake could be predicted using energy requirement prediction models, such as NRC (National Research Council, 2001) and so on. But the most proper and traditional method is prediction of energy intake regarding to the population studied. In this method, energy intake is predicted using a regression model, fitting actual energy intake on some independent variables such as milk yield, milk compositions, body weight and body weight change, thus residual effect of the fitted model will be residual energy intake.

In this method, the average of REI in the population is zero, whereas the animals with negative REI are more efficient and the animals with positive REI are less efficient in use of energy intake (Figure 1). In another word, more efficient animals use more proportion of the energy intake for production, thus their actual energy intake is less than the prediction, so have negative REI.



**Figure 1.** Residual energy intake (REI) as the difference between actual and predicted energy intakes. The animals over the regression line have positive REI (less efficient) and the animals under the line have negative REI (more efficient).

### 2.3. Protein efficiency

As it was mentioned before, dairy cows excrete about 2 to 3 times more N in manure than in milk, which contributes to increased costs of milk production and environmental N pollution (Broderick, 2005). Thus, recently, attention has focused on the relationship between nitrogen utilization in lactating cows and environmental pollution (Castillo et al, 2000).

Different criteria could be used to measure protein efficiency of lactation. For example, gross efficiency of crude protein (Huhtanen et al, 2008; Zamani et al, 2011a and 2011c), crude protein balance (Zamani et al, 2011a and 2011b) and residual protein intake (Zamani et al, 2011a) have been studied in literature.

*Gross efficiency of crude protein (GECP):* Gross efficiency of crude protein is the proportion of feed CP recovered in milk (Equation 6).

$$GECP = \frac{CPM}{CPI} \quad (6)$$

Where, CPM and CPI are CP produced in milk and CP intake, respectively.

*Crude protein balance (CPB):* Crude protein balance could be calculated as the difference between CP intake and protein excreted in milk (Equation 7), where the most efficient cattle present higher dietary protein recovery and consequently a lower CPB.

$$CPB = CPI - CPM \quad (7)$$

In this equation, CPI and CPM are CP intake and CP produced in milk, respectively.

*Residual protein intake (RPI):* Residual protein intake is similar to residual energy intake and is defined as the difference between actual and predicted CP intakes (Equation 8).

$$RPI = API - PPI \quad (8)$$

Where, API and PPE are actual and predicted protein intakes, respectively.

RPI is a measure of protein efficiency, because more efficient cows present lower protein intake for the same protein excretion in milk and body weight change and thus, have a lower RPI (Zamani et al, 2011a).

### 2.4. Economic efficiency

Economic efficiency is the most important goal for animal production enterprises. In dairy herds, several economic criteria such as economic selection index or economic merit (Veerkamp, 1998), the ratio of total costs to total incomes (Goddard, 1997) and proportion of total costs per unit of yield (Dickerson, 1970; Newman et al, 1985) are proposed in literature.

Generally, in dairy farms the profitability of a lactating cow is dependent on both outputs (incomes of milk, fat, protein, calving, etc.) and inputs (costs of feed, labour, facilities, etc).

Feed is the major cost input for all livestock farmers, including dairy producers (Harris and Newman, 1994) and has the highest correlation to profit (Balaine et al, 1981). Therefore, increase of main incomes (milk, fat and protein) and reduce of main variable cost (feed) potentially can be of considerable importance in profitability of dairy cows. *Income over feed cost (IOFC)* is defined as the income from milk, fat and protein yields minus the feed cost (Equation 9) and has been studied as an economic index for lactating dairy cows in several studies, such as Adkinson et al (1993), Baars (1998), Kelm et al (2000), Miraei-Ashtiani et al (2005) and Zamani et al (2004 and 2005).

$$IOFC = GI - FC \quad (9)$$

Where, IOFC, GI and FC are income over feed cost, gross income and feed cost, respectively.

### 3. Factors affecting efficiency of lactation

Efficiency of lactation is affected by different factors. Some of the practical factors affecting efficiency of energy and protein use are the level of milk production, type of diet, body size, changes in body tissue mass during lactation, environmental conditions, exercise, age at first calving, productive lifespan, genetics, metabolic modifiers, and growth rate as a heifer (VandeHaar, 1998). Moreover, other factors such as season, breed, parity number, days in milk and milking frequency are also proposed to have significant effects on the efficiency of lactation (Zamani, 2005).

Among different factors affecting efficiency of lactation, level of production is one of the most important factors, whereas higher milk production is associated with more efficient partitioning of feed nutrients (VandeHaar, 1998). In many studies, milk yield had a positive phenotypic and genetic correlation to efficiency (e.g. Korver, 1988; Buttazzoni and Mao, 1989; Britt et al, 2003; Miraei-Ashtiani et al, 2005; Zamani, 2005). However, it has been shown that the efficiency of protein use has a negative correlation to milk yield (Zamani et al, 2011a). The effect of nutrition and genetic on efficiency will be discussed in this section.

#### 3.1. Nutritional factors

Nutritional factors have noticeable effects on the efficiency. Too many nutritional factors could be mentioned to have an effect on the efficiency. But, some of the most important factors will be discussed here.

Source of energy is one of the most important factors, affecting energetic efficiency of feed (Smith, 1988). It is shown that the net efficiency of converting fiber to milk is less than that for starch and protein, and the net efficiency of converting dietary fat to milk is greater. This increased efficiency of fat is mostly due to a decrease in heat increment and thus an increase in partial efficiency of converting ME to NE<sub>L</sub> (VandeHaar, 1998). However, proper ratios of structural and non-structural carbohydrates, fat and protein must be included in diet to ensure proper function of ruminal microorganisms and whole digestive tract (Fox et al, 1992).

Dietary level of energy is also affecting the efficiency. Zamani et al (2011b) reported that a slight increase in dietary energy probably increases protein efficiency for lactation. This finding could be attributed to an increase of fermentable metabolizable energy (FME) in most of high NEL diets, because FME provides the energy needed to supply rumen microbes for capture of N. Moreover, the fate of absorbed peptides and amino acids once inside the microbial cell will depend on availability of energy. If energy is available, amino acids will be trans-aminated or used directly for microbial protein synthesis, otherwise, if energy is limiting, amino acids will be de-aminated and their carbon skeleton will be fermented (Bach et al., 2005).

In addition to dietary energy, dietary CP could also affect the efficiency. Producers often feed high CP diets to ensure a sufficient supply of the metabolizable protein required for maximal milk and protein production of dairy cows. Excess levels of dietary protein or high degradability of dietary crude protein increases ammonia production in the rumen and as a result, ammonia concentration in the plasma increases. More rate of ammonia conversion to urea in liver needs more energy consumption (Martin and Blaxter, 1965) and thus, reduced energetic efficiency. Negative correlation of dietary level of CP with gross energy efficiency has been reported by Zamani (2005).

Feeding excess protein has a high energetic cost. The energetic cost of feeding 1 kg of extra CP is equivalent to 0.72 Mcal of NE<sub>L</sub> (Oldham, 1984). If a cow producing 45 kg of milk/day and eating 25 kg of DM/day required 17% CP in its diet, then feeding an extra 2% CP (a diet with 19% CP) would cost 0.36 Mcal NE<sub>i</sub>/day, decrease milk yield by 0.5 kg/day, and gross efficiency by 0.3 percentage points (VandeHaar, 1998).

Some reports indicate that feeding diets with excessively high CP concentration and especially excess ruminally degradable CP, also decreases protein efficiency in lactating dairy cows (Olmos Colmenero and Broderick, 2006; Wang et al., 2007; Huhtanen et al., 2008; Zamani et al, 2011b and 2011c). Moreover, overfeeding CP reduces profit margins, because of the relatively high cost of protein supplements and poor efficiency of dairy cows fed high protein diets to utilize N (Broderick, 2003).

This has been reported that an increase in the ruminally undegradable protein (RUP)/ruminally degradable protein (RDP) ratio may improve protein efficiency (Zamani et al, 2011b and 2011c), because at a high RDP level, more N would be absorbed as ammonia or more amino acids deaminated, which might increase N excretion in urine (Castillo et al, 2001). The positive effect of RUP on protein efficiency is also reported by Flis and Wattiaux (2005) and Kalscheur et al (2006). Moreover, Castillo et al (2001) and Reynal and Broderick (2005) found that an increase in dietary CP degradability results in more urinary N excretion. However, post-ruminal digestibility of RUP and amino acids balance could be considered as an important factor for increasing metabolizable protein flow to the intestine (Noftsger and St-Pierre, 2003) and thus for optimizing dietary RUP to improve protein efficiency.

There are some reports about the effects of feed additives on the efficiency. For example, addition of buffers as sodium bicarbonate (NaHCO<sub>3</sub>) and magnesium oxide (MgO) reduced gross energy efficiency for milk production (Arambel et al, 1988).

These findings reveal that many nutritional factors may affect the efficiency of lactation. Generally, it is necessary to optimize different dietary components to maximize efficiency of lactation.

### 3.2. Genetic factors

Many evidences show genetic variation of the lactation efficiency. In several studies, the cows selected for high yield traits were more efficient in use of feed for milk production (Dunklee et al, 1994; Veerkamp et al, 1993, 1994, 1995).

There are many reports on estimates heritability ( $h^2$ ) for different measures of feed efficiency. Heritability estimates for gross energy efficiency had a wide range of 0.09 to 0.86 (e.g. Van Arendonk et al, 1991; Li et al, 1998; Zamani, 2005).

Buttazzoni and Mao (1989) estimated a heritability of 0.32 – 0.49 for net energy efficiency (NEE) of lactation. The estimate of heritability for NEE in another study was 0.34 (Miraei Ashtiani et al, 2005). In different studies NEE had moderate positive genetic correlations to milk yield. Genetic correlation of NEE to milk yield has reported from 0.51 by Miraei Ashtiani et al (2005) to 0.56 by Buttazzoni and Mao (1989).

Several studies have shown the genetic variation of residual energy intake (REI) in dairy cattle. Van Arendonk et al (1991) and Kennedy et al (1993) have reported heritability estimates of 0.19 and 0.14, respectively for REI. Veerkamp et al (1995) reported a heritability of 0.30 to 0.38 for REI, depending on the way of calculating the energy requirements from phenotypic regressions. In another study, the estimated heritability and repeatability for REI were 0.15 and 0.53 from univariate, and 0.21 and 0.60 from multivariate models, respectively (Zamani et al, 2008). On the other hand, Ngwerume and Mao (1992) and Svendsen et al (1993) found no evidence for any additive genetic variation in REI, where they have reported heritability estimates of 0.016 and 0.00 – 0.11 for REI, respectively. Unlike to other measures of efficiency, REI is, to some extent, independent of yield traits. In different studies REI had low and negative genetic correlations with milk yield (Madgwick et al, 1991; Van Arendonk et al, 1991; Kennedy et al, 1993; Veerkamp et al, 1995; Zamani et al, 2008).

Genetic variation of protein efficiency has been rarely studied in dairy cattle. Li et al. (1998) reported a value of 0.13 as estimate of heritability for crude protein efficiency of lactation. In another study, heritability estimates for crude protein efficiency in 90 and 305 days of lactation were 0.10 and 0.31, respectively (Ageeb, 1999). In a recent study Zamani et al (2011a) reported heritability estimates of 0.07, 0.40 and 0.03 for gross efficiency of crude protein, crude protein balance and residual protein intake, respectively. In their study, noticeable genetic correlations were observed between different measures of protein efficiency and some of yield traits. In this study this has been proposed that the protein efficiency could be improved by direct selection against crude protein balance, while other measures of protein efficiency, including gross efficiency of crude protein and residual protein intake did not seem to be suitable for direct selection. They proposed the selection

for milk fat and protein percentages as an effective way for indirect improvement of protein efficiency (Zamani et al, 2011a).

#### 4. Implications

Efficiency of nutrient utilization is a major factor affecting profitability in modern dairy farms. Several criteria are used to measure efficiency of lactation. Different measures of feed efficiency could be categorized as general, energetic, protein and economic criteria. Feed efficiency is controlled by different environmental and genetic factors. Feed efficiency could be improved by optimizing environmental effects, including nutritional factors, direct selection for feed efficiency or indirect selection for feed efficiency by the selection on yield traits.

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# Milk Production and Animal Management

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# Effects of Environmental Factors on Milk Yield, Lactation Length and Dry Period in Tunisian Holstein Cows

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50803>

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## 1. Introduction

Management, nutrition, lactation turn or the age, year and season in which lactation started are the leading environmental factors affecting lactation performance in cattle. Beside these factors, the persistency level of the highest milk production period reached on lactation is a significant factor [1]. Also, lactation performance in dairy cattle depends upon genetic and environmental factors. Genetic background, climate, diseases, feeding, year and season of calving have been reported to affect milk production, lactation length and dry period [2, 3]. Breed, age, stage of lactation, parity and milking frequency also influence performance production [2, 3]. Holstein cows were considered the most dairy cows in the World. As number of Holstein cows in Tunisia is more than other breeds, breeding of Holstein cows is very important. Milk yield and duration of lactation have marked effects on dairy economy [4, 5]. The persistency level of lactation milk production can be defined as the ability of keeping a high daily milk flow during lactation, in the milk productivity of lactation's first period's persistency level or the rest of the lactation as a measure of lactation curve diagram, the highest period productivity of the continuation level during lactation, continuation level of the highest productivity and after reaching the highest productivity the rate of decreasing seen on milk production in time. Milk yield is the most important single determinant of profit for the dairy cow. Moreover, effects of lactation number, age, and season and year of calving on milk yield and lactation length are well known. In addition, in breeding of dairy cows, the most important aims are to obtain a calf in a year and high milk yield from cows. To obtain a calf in a year from cows depends on some parameters of ideal limits (60 days dry period, 305 days lactation duration etc.). However, profitable breeding could be achieved by keeping lactation

duration, dry period and service period between optimal limits [6, 7]. The yields of farm animals are the result of the combined effects of genotype and environmental conditions. In order to increase the yield level, it is necessary to optimize the environmental conditions and to improve the genetic structure of the animals. In order to enhance productivity of a dairy animal, it is necessary to develop an understanding of the factors affecting its milk production. Environmental factors can be classified as factors with measurable effects (age, year, season, milking frequency, etc.) and factors with immeasurable effects (infectious diseases, parasitic infestations, etc.). The measurable effects can be determined and used in the management of the farm [8]. Environmental factors affecting variability in daily milk yield are widely documented in dairy cattle [9-11]. The 305 days milk yield of Holstein cows was 5905 kg in Tunisia [12], 5353 kg in Morocco [13], and between 4597 and 6464 kg in Turkey [14-16]. Environmental factors such as year of calving, season of calving and age at calving affect productivity [17]. Many researchers [6, 14] reported that the effect of calving season on 305 days milk yield was as significant and indicated that milk yield was higher in autumn and winter. Unlike, Bilgiç and Aliç [16] and Pelister et al. [18] reported that effect of calving season on 305 days milk yield was non-significant. Although, effect of lactation number on 305-days milk yield was reported [14, 15, 19] as significant. Opposing, Koçak et al. [6] and Bilgiç and Aliç [16] reported a non-significant effect of lactation number on 305 days milk yield. Effects of calving age on 305 days milk yield have been reported as significant [18, 20]. The lactation duration of Holstein cows was between 284.7 and 333.9 days in previous studies [14, 16, 21, 22]. The effect of calving season on lactation duration was reported as non-significant [6, 16, 18]. The effect of lactation number on lactation duration was reported as non-significant [14, 16, 19]. However, the effect of calving age on lactation duration was reported as significant [15, 18]. Zambrano et al. [23] reported that the effect of calving year on lactation duration was significant. The dry period of Holstein cows was between 73.34 and 82.1 days [14, 15, 22]. Koçak et al. [6] and Pelister et al. [18] reported a non-significant effect of calving season on dry period. However, Erdem et al. [14] stated a significant effect of age at calving on lactation duration. Similarly, the effect of lactation number on dry period was reported as non-significant [14, 15]. On the other hand, the effect of calving age on lactation duration was stated as significant [19]. Effects of age at calving on dry period have been reported as significant [18]. Many researchers [14, 15, 23] were found that effect of calving year on all milk yield traits (305 days milk yield, lactation duration, dry period) was significant. Inci et al. [15] reported that effects of calving year on 305 days milk yield and lactation duration were significant but non-significant on dry period. The present research work was designed to investigate non-genetics factors affecting milk yield, lactation length and dry period of Holstein cows raised under Tunisian conditions.

## 2. Materials and methods

The data used in the present study were taken from the Tunisian Livestock and Pasture Office (OEP). 260,241 test-day records of 5649 Holstein cows from Tunisian milk control

data were analysed. Data from lactations records of cows having their first calving between 1996 and 2003 were used. To evaluate the significant effects of calving year, season of calving, lactation number, and age at calving on different milk yield traits, eight groups for calving year were formed, between 1996 and 2003, three age groups of calving age (1) 2–4, (2) 5–6, and (3) 7 or older, four calving seasons were established; winter (December, January and February), spring (March, April and May), summer (June, July, August), and autumn (September, October and November) and five groups for parity. The 305 days milk yield was estimated from test milk yields collected once a month during all lactation periods [8, 24]. Lactations with less than five tests were not used in calculation. Milk yields were standardized to 305 days by using adjustment factors estimated by Çilek [25]. Environmental factors, which influenced lactation milk yield, lactation duration and dry period were investigated. Simple means and standard errors for the traits studied were estimated using SAS 9.13. The General Linear Model (GLM) was utilized for variance analyses of milk yield traits. Duncan's multiple range test (DMRT) was used for multiple comparisons of each trait.

The statistical model was as follows:

$$Y_{ijkl} = \mu + L_i + S_j + CY_k + AC_l + e_{ijklm}$$

Where,

$Y_{ijkl}$  = Observed trait at lactation number  $i$ , calving season  $j$ , calving year  $k$  and group of age  $l$

$\mu$  = Population mean for each trait,

$L_i$  = Effects of lactation number ( $i = 1, 2, 3, 4, 5$ ),

$S_j$  = Seasonal effects ( $j =$  spring, summer, autumn and winter),

$CY_k$  = Effect of calving year ( $k =$  years between 1996 and 2003),

$AC_l$  = Effect of group of age at calving ( $l = 1, 2$  and  $3$ ),

$e_{ijklm}$  = Random sampling error

### 3. Results

The lactation performance of dairy cattle is usually measured by determining the total milk yield per lactation or per year, average daily milk yield, lactation length, lactation persistency, and milk composition. The least squares means along with their standard errors for different traits analysed are presented in table 1. The overall average 305-days milk yield was  $5807.83 \pm 78.27$  kg, ranging between 2271 and 7013. Average lactation length and dry period were estimated to be as  $309.60 \pm 7.01$  and  $97.17 \pm 3.28$  days, respectively when minimums were forced to be at least 127 and 11 days and when 356 and 213 days as maximums values. Average age at first calving was  $1092.3 \pm 196.8$  days (range, 646–1588 days). The effect of year and season of calving and parity was significant ( $P < 0.01$ ) on milk traits. Effects of all factors (calving year, calving age, parity and calving season) on 305-days milk yield were significant ( $P < 0.001$ ).

Trait	Records	Means	SD	Minima	Maxima
305-days Milk yield (kg)	2147	5807.83	78.27	2271.53	7013.80
Lactation length (days)	1789	309.60	7.01	127.56	356.53
Dry period (days)	1789	97.17	3.28	11	213.71

**Table 1.** Records, means, standard deviations, minima and maxima of variation for milk yield, lactation length and dry period of Holstein cows

### 3.1. Sources of variation

The major sources of variation in milk production, lactation length and dry period are genotype, environment and the interaction between the two. The influence of environmental factors on dairy production has been well documented.

#### 3.1.1. Effect of calving year

Year of calving significantly influenced MY ( $P < 0.001$ ). The variation in milk yield from one year to other (Table 2) could be attributed to changes in herd size, age of the animals and good management practices introduced from year to another. The lowest 305 days milk yield ( $4879 \pm 117.89$ ) was seen in 1998 years, the highest milk yield ( $6251 \pm 185.72$  kg) was seen in 2003. Furthermore, the effects of calving year on lactation duration were statistically significant ( $P < 0.05$ ). Effects calving year on dry period were statistically significant ( $P < 0.001$ ). The dry period was lowest ( $96.57 \pm 5.57$  days) in 2002 and highest ( $113.29 \pm 3.78$  days) in 1996. Year wise means indicated that there was an increasing trend in lactation length from 1996 to 2003.

Calving year	305 days milk yield (kg)		Lactation length (day)		Dry period (day)	
	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE
1996	1147	5223 $\pm$ 118.74b	1147	269.9 $\pm$ 7.56a	1086	113.29 $\pm$ 3.78a
1997	1158	5396 $\pm$ 127.43a	1158	273.3 $\pm$ 9.78a	978	110.37 $\pm$ 4.56a
1998	2151	4879 $\pm$ 117.89c	2151	277.6 $\pm$ 7.67ab	1113	107.43 $\pm$ 4.28ab
1999	3146	5352 $\pm$ 123.91ab	3146	281.5 $\pm$ 7.23b	1275	103.73 $\pm$ 5.36bc
2000	4183	5609 $\pm$ 127.83ad	4183	288.3 $\pm$ 6.47b	1256	101.85 $\pm$ 5.78c
2001	4728	6051 $\pm$ 156.25d	4728	292.6 $\pm$ 8.46bc	1317	99.47 $\pm$ 6.32dc
2002	4689	6125 $\pm$ 158.36d	4689	312.7 $\pm$ 5.13c	1328	96.57 $\pm$ 5.57d
2003	3879	6251 $\pm$ 185.72d	3879	322.78 $\pm$ 6.44c	1278	98.23 $\pm$ 4.86d

a-d: differences between groups with same letter in the same column are non-significant, differences with different letter are significant ( $p < 0.05$ ).

**Table 2.** Least square means of milk yield traits by calving year

#### 3.1.2. Effect of calving season

The least squares analysis revealed that 305 days milk yield was significantly ( $P < 0.001$ ) affected by season of calving (table 3). The present results suggested that milk yield was sensitive to seasonal variation. The effect of calving season on milk yield was significant

and milk yield was high ( $5827 \pm 69.23$ ) in cows calving in winter. However, the effect of calving season on lactation duration was significant ( $P < 0.001$ ), but non-significant ( $P > 0.05$ ) on dry period. Then, season of calving affected both the lactation length and milk yield. As shown in table 3, least squares mean was higher for autumn calvers ( $307.6 \pm 4.57$  days) as compared to spring calvers ( $296.7 \pm 3.99$  days). Although, summer and winter calvers have similar lactation length ( $301.4 \pm 4.12$  and  $303.7 \pm 4.28$  days) but winter calvers have the highest milk yield ( $5827 \pm 63.17$  kg). Milk yield on the other hand had the opposite trend. Summer calvers produced 614 kg less milk ( $5213$  vs.  $5827$  kg) as compared to winter calvers.

Calving season	305- days milk yield (kg)		Lactation length (day)		Dry period (day)	
	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE
Spring	1235	5608 $\pm$ 62.36ab	1235	296.7 $\pm$ 3.99b	978	104.29 $\pm$ 2.78a
Summer	1117	5213 $\pm$ 73.17b	1117	301.4 $\pm$ 4.12ab	1109	98.23 $\pm$ 3.12a
Autumn	1227	5713 $\pm$ 69.23ab	1227	307.6 $\pm$ 4.57a	1089	96.48 $\pm$ 3.28a
Winter	1347	5827 $\pm$ 63.17a	1347	303.7 $\pm$ 4.28ab	1117	87.56 $\pm$ 2.97a

a, b, c: differences between groups with same letter in the same column are non-significant, differences with different letter are significant ( $p < 0.05$ ).

**Table 3.** Least square means of milk yield traits by calving season

### 3.1.3. Effect of age at calving

Total 305 days milk yields were lowest in 2-4 years of age at 5312 kg and highest in 7 years of age at 5611 kg. However, the effects of age at calving on lactation length were non-significant ( $P > 0.05$ ). Dry period increased with increase of age at calving. The lowest dry period was found in 2-4 years old age at 83.37 days and highest in 7 years old age at 99.71 days. The dry period was above the ideal value in all years. In order to make animals more profitable, it is essential they were made pregnant as soon as possible during the service period in order to shorten the dry period (Table 4).

Age at calving	305 days milk yield (kg)		Lactation length (day)		Dry period (day)	
	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE
1	1256	5312.54 $\pm$ 78.95a	1256	307.56 $\pm$ 4.78a	1127	83.37 $\pm$ 3.27a
2	1147	5517.23 $\pm$ 72.36ab	1147	303.47 $\pm$ 5.12a	1217	87.12 $\pm$ 3.54ab
3	1378	5611.17 $\pm$ 79.27b	1378	299.78 $\pm$ 4.56a	1236	99.71 $\pm$ 3.31b

a, b: differences between groups with same letter in the same column are non-significant, differences with different letter are significant ( $p < 0.05$ ).

**Table 4.** Least square means of milk yield traits by group of calving age

### 3.1.4. Effect of parity

The effects of parity on lactation duration were statistically significant ( $P < 0.001$ ). Lactation duration was shortest in lactation 5 at  $297.8 \pm 3.04$  days and longest in lactation 4 at  $317.5 \pm 4.17$  days. Lactation duration decreased with increase of lactation number. Effects of parity on

dry period were statistically significant ( $P < 0.001$ ). Dry period was longest in 5th lactation at  $113.28 \pm 3.25$  days and shortest in 3rd lactation at  $87.23 \pm 2.17$  days (Table 5).

Lactation number	305 days milk yield (kg)		Lactation length (day)		Dry period (day)	
	n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE
1	1457	5412.27 $\pm$ 52.41a	1457	311.7 $\pm$ 3.12a	1123	93.14 $\pm$ 2.34a
2	1353	5721.35 $\pm$ 54.74b	1353	307.1 $\pm$ 2.78ab	1099	89.27 $\pm$ 2.41b
3	1246	5614.23 $\pm$ 47.13bc	1246	303.3 $\pm$ 3.27c	987	87.23 $\pm$ 2.17b
4	1127	5417.58 $\pm$ 46.51a	1127	317.5 $\pm$ 4.17b	956	95.16 $\pm$ 3.04a
5	978	5123.47 $\pm$ 48.45c	978	297.8 $\pm$ 3.04ac	979	113.28 $\pm$ 3.25c

a, b, c: differences between groups with same letter in the same column are non-significant, differences with different letter are significant ( $p < 0.05$ ).

**Table 5.** Least square means of milk yield traits by parity

#### 4. Discussion

In this study, means of 305 days milk yield was  $5807.83 \pm 78.27$  kg. The findings of present study were in accordance with those of Ajili et al. [12], Boujenane [13], Erdem et al. [14], Inci et al. [15], Bilgiç & Aliç [16], Duru & Tuncel [21] and Kaya et al. [22]. As reported previously [7, 20, 26], the effect of calving season on milk yield was significant and milk yield was the highest in cows calving in winter. Similar finding have been reported by Javed et al. [27] and Tekerli et al. [28] in Holstein Friesian cows. Thorpe et al. [29] showed the effects of season of calving on production performance of dairy cattle in Kenya. Cows calving in winter have high milk yields, due probably to good feeding levels in the first 3 or 4 months of lactation. On the other hand, cows calving in summer have low milk yields due to their being subject to high environmental temperatures in the first 3 or 4 months of lactation. On the contrary many workers [30 - 32] observed that the season of calving had a non-significant effect on lactation milk yield in Holstein Friesian cows.

Analysis of variance revealed that 305-days milk yield is significantly ( $P < 0.05$ ) affected by age at calving. The result is closely in accordance with the results of Kaya et al. [22] and Catillo et al. [33]. The lowest milk yield was obtained from cows calving at 2 years of age and the highest from those calving at 7 years of age. The negative effect of early calving on milk yield could have been due to different factors, such as higher body weight gain before puberty. Milk yield decreased after 7 years of age. As reported in the literature [7, 20], this confirms that milk yield increases with age up to maturity and decreases thereafter.

305-days milk yield differed significantly ( $P < 0.05$ ) with calving year (Table 2). The lowest milk yield was obtained in 1998. After 1998, milk yield increased up to 2003. The reasons for this increase could be the use of bulls with high genetic capacity, selection for milk yield and culling in the herd and especially improvement in management and feeding conditions. The variation in milk yield observed in different years reflected the level of

management as well as environmental effects. Between 1996 and 2001, short lactation duration and consequently low milk yield may result from the deficiency of attention and feeding conditions. The significant effect of year of calving productive performance of dairy cows could be attributed to the changes in feeding and managerial systems and environmental conditions which occurred from year to another as well as to differences between years in the quantity and quality of forage available. 305-days milk yield differed significantly ( $P < 0.05$ ) with lactation order (Table 5). The 305-days milk yield in second lactation was significantly higher than in first lactation. This result is consistent with Munim et al. [34] who found significant ( $P < 0.05$ ) effect of parity on milk yield. Nevertheless, the result differed from that of Habib et al. [35] who found non-significant ( $P > 0.05$ ) effect of lactation number on milk yield. The significant effect of parity on productive performance may be due to the changes in managerial systems and environmental conditions among parties. The average lactation length calculated in this study was 309.6 days. This was very close to the ideal value (305 days). This length of lactation was longer than results reported by Sattar et al. [36] and Alim [37] who reported a lactation length of  $293 \pm 3$  and  $291.86 \pm 6.55$  days in Friesian cows in Libya and Pakistan, respectively. The lactation duration of Holstein cows was between 284.7 and 333.9 days in previous studies [14-16, 18, 21, 22]. The shorter lactation duration is 127.56 days it may be related to incomplete lactations when data were collected. Lactation duration decreased with increase of lactation number. Short lactation duration in the oldest cows (5th lactation number) may be related to incomplete lactations because of culling.

The average dry period was  $97.7 \pm 2.25$  days. Dry period was higher than the ideal value (80 days) but shorter than funding of Sattar et al. [36] who reported a longer (224.99 days) dry period. However, the dry period increased with calving age, as a result of increase of milk yield level with age in the herd. It can be said that if milk yield increases with calving age, dry period would decrease. Effect of calving year on all milk yield traits was significant. Differences among years may be related to management. It can be said that differences of management among years was the most important factor affecting milk yield traits.

Dairy cows are usually dried-off for two months prior to the next calving. This rest period is necessary to maximize milk production in subsequent lactation. It was reported that the dry period is required for the renewal of the udder glandular tissue [38, 39]. Nevertheless, the optimal dry period was established as 60 days. A significant increase in milk yield of the dairy cows caused a new attention in creating the optimum dry period [40]. Two months were accepted as a sufficient dry period for high-productive cows [41]. A research done in Poland by Borkowska et al. [42] and Winnicki et al. [43] indicated that in practice the dry period is extended or excessively shortened, which leads to a reduction in milk production as compared to the recommended optimum. Milk yield is usually reduced when the dry period is less than 40-60 days (25-40% less milk). Dry period longer than 60 days in length does not result in a significant increase in milk production. Long dry

periods decrease the average annual production of the cow by extending the calving interval beyond the normal 13-14 month interval and causing a decrease in the lifetime production of the dairy cow.

## 5. Conclusion

In this study, there was increase of milk yield level according to previous research [16]. This may result from improvement in breeding, feeding and management conditions (selection for milk yield and culling in the herd etc.). Although, lactation duration was found almost at ideal value, dry period was estimated as higher than the ideal value. In order to make animals more profitable, it is essential to make them be pregnant as soon as possible during the service period in order to shorten the dry period. It can be concluded that Holstein cattle is raised successfully for milk yield under Tunisian environmental conditions. It is concluded that milk yield and lactation length are affected by year and season of calving. Adjusted milk yield (adjusted for lactation length) and lactation length are affected by year into season of calving interaction but actual milk yield is not affected by year by season of calving interaction. Age within parity, also, affected lactation length and milk yield. Negative phenotypic trend in milk yield is alarming and needs further investigations.

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## Acknowledgement

The authors thank Professors Olfa Frouja Sayadi and Zeineb Dkhili for the language revision and for prompting this research. The authors also thank OEP for making the data available.

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# Heat Stress Management for Milk Production in Arid Zones

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Leonel Avendaño-Reyes

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/51299>

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## 1. Introduction

Arid and semi-arid zones account for one third of the earth's surface land area and cover most parts of the developing nations in the world including Latin America, Africa, and parts of India and South East Asia. Nevertheless, these ecosystems are not exclusive of developing countries because they also exist in developed nations such as United States of America, Spain, Australia, and Israel, among others. Arid zones are characterized by excessive heat loads and an insufficient and erratic pattern of precipitation. Also, actual evapotranspiration equals rainfall and recharge of groundwater is relatively infrequent. In general, summers are very hot and intense, but winters have commonly cold weather. In many countries with arid zones, high environmental temperatures during summer seasons may last up to 6 months, with average temperatures over 30°C. This is important because approximately one third of the cattle population in the world is located in arid zones, and according to IPCC predictions, the global average surface temperature may increase between 1.8 and 4°C by year 2100 (IPCC, 2007). The negative effects of global warming will be manifest in animal agriculture of both developed and developing countries, but the pressure will be greater on developing countries because of their deficiency of resources, their lack of veterinary and extension services, and their limitations on research technology development (FAO, 2007).

One of the chief problems facing dairy producers located in arid regions is thermal stress. In temperatures above 28°C, even without humid conditions, lactating cows show evidence of hyperthermia and emerges a condition called heat stress, so that the events feed intake, milk yield, milk fat and protein production, as well as fertility rate are reduced. Meanwhile, body temperature and respiration rate show a significant increased (West, 2003).

There are a number of options to assist in minimizing the negative effects of heat stress on dairy cows. Some of these options are feeding management, housing and facilities

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adjustments, and selection of tolerant breeds. Rations should be adjusted to increase energy and protein intake while maintaining rumen and cow health. The purpose is to increase the quantity of grain fed and decreased the quantity of forage in the ration. Also, environmental modifications that help to alleviate heat stress problems are structure orientation, structure ventilation, use of shades, and use of cooling systems in different sections of the dairy farm. Evaporative cooling means a combination of wetting and forced ventilation drying the cow's coat to maximize the cooling effect. Milking parlors with adequate holding pens can employ the use of subsequent sprinkling and forced air in the pens. In dairies with adequate drainage and housing, evaporative cooling can be provided above the feed bunks in addition to or instead of in the holding pen (Armstrong, 1994).

Therefore to reduce heat stress on dairy cattle it is required a multi-disciplinary approach and should include nutrition, environmental modifications, and management practices. This chapter will focus on these strategies with special reference to arid zones.

## **2. Global warming and dairy farming**

Emissions to the atmosphere of the gasses carbon dioxide, methane and nitrous oxide are believed to be a major cause of global warming. These gasses are able to absorb and emit infrared radiation, so they restrict the rate of thermal energy flowing out of the earth, causing the greenhouse effect. In addition, most of the observed increase in globally averaged temperatures since the last 200 years is very likely due to the increase in anthropogenic greenhouse gas concentrations. The animal agriculture sector is responsible for almost 40% of annual methane emissions that are consequence of enteric fermentation in ruminants and from farm animal manure (Koneswaran & Nierenberg, 2008). So is common to hear that livestock are important contributors to climatic change.

A resultant rise in the earth's temperature may boost the occurrence and concentration of severe climate events, as well as to intensify desertification of arid and semi-desert regions which results in warmer and more intense summers. The frequency and severity of extreme climatic events such as drought, flooding, and long heat waves would have substantial impacts on crop and livestock productivity, and therefore in food production and security. As a result of global warming, the prospective for food production from livestock is expected to decline because of high mortality, less productivity and more competition for animal resources (IFAD, 2010).

Dairy cattle are specially affected by climate change because most of the high production breeds were originated in cold regions. For instance, the breed Holstein was developed in Europe, in a cold region what is now The Netherlands, and then introduced to many ecological zones of the world such as tropical and desert regions. Because of that this breed is well adapted to cold environments, thus harsh ambient conditions like hot temperatures or elevated relative humidity make this breed difficult to reproduce and produce under these circumstances. Furthermore, Holstein breed is recognized as the world's highest milk

production cattle nowadays. So many approaches have been made to adapt this breed to adverse conditions like those prevalent in arid and semi-arid zones (Place & Mitloehner, 2009).

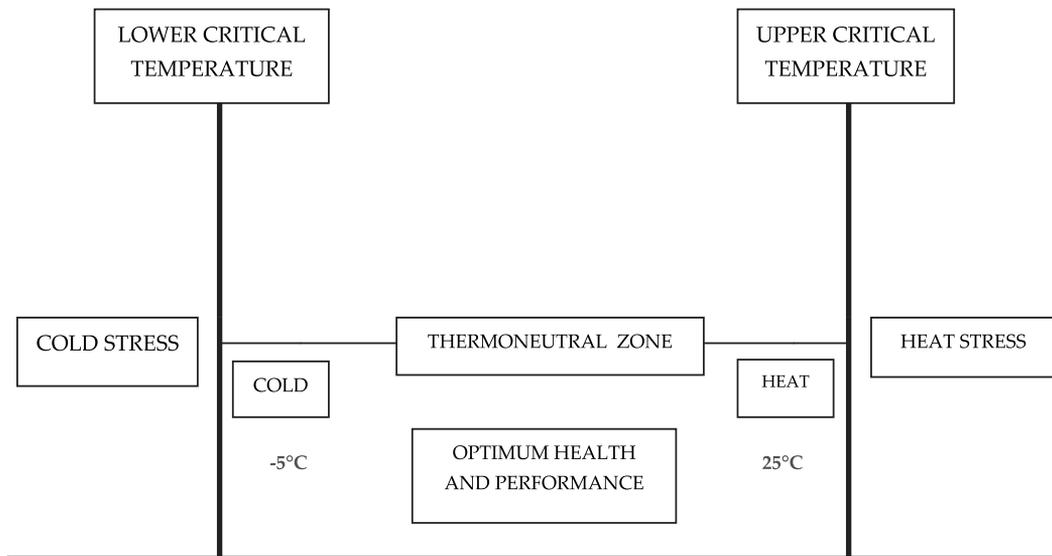
Climate change is projected to increase the number of days each year when dairy cows experience heat stress. Kadzere et al. (2002) defined heat stress in dairy cows as all temperature-related forces that encourage changes or adjustments which may occur from the cellular to the total animal level to help the cows stay away from physiological disorders and then to better adapt to an adverse thermal environment. Heat stress in dairy cows results in greater nutritional requirements, lower fertility, reduced milk production and milk quality, and increased frequency of health-related issues such as mastitis. Using simulation models in a dairy basin located in Australia, researchers estimated that by 2025, production of greenhouse gas emissions will increase 25% heat stress days, which could account for a decline of 35 to 210 kg of milk per cow per year. Projecting this scenario by year 2050, there will be a 60% increase in heat stress days, which may result in a decline from 85 to 420 kg of milk per cow per year (Crimp et al., 2010).

Few years ago, St. Pierre et al. (2003) conducted an extensive study on the economic losses from heat stress to USA livestock revealing convinced evidence to all dairy farmers of the importance of providing heat abatement practices for their cattle. For dairy cows, the equations to estimate the cost of heat stress on productivity included dry matter intake, milk production, change in days open, change in monthly reproductive cull rate, and change in monthly death loss. Equations were also developed for replacement heifers that considered dry matter intake loss, weight gain loss, and change in monthly death rate due to heat stress. It was estimated that hot weather costs dairy farmers \$ 900/million per year considering milk production and fertility. This economic loss was higher in dairy cattle compared to any other livestock specie in that country. The general conclusion was that for dairy cows some type of heat abatement is always economically justified across all states, and the optimum environmental strategy is the use of spray and fans. However, in regions where heat stress is more intensive, the use of high-pressure evaporative cooling chambers could be economically necessary.

### **3. Physiological changes in dairy cows attributable to heat stress**

Dairy cows are homoeothermic animals, so they exhibit optimum performance in their neutral environment which is known as thermoneutral zone (TNZ). For lactating dairy cows from European breeds, this TNZ ranges between -5 and 25°C, and are called lower critical temperature (LCT) and upper critical temperature (UCT). Within this temperature range, dairy cows require no additional energy above maintenance to cool or heat their body. LCT is the environmental temperature at which an animal needs to increase metabolic heat production to maintain body temperature. UCT is the environmental temperature at which the animal increases heat production as a consequence of a rise in

body temperature resulting for inadequate evaporative heat loss (Fuquay, 1981; Johnson, 1987). Figure 1 shows LCT and UCT for dairy cattle. Thermoneutral zone depends on the age, breed, feed intake, diet composition, previous state of temperature acclimatization, production, and housing and stall conditions, tissue (fat, skin) insulation and external (coat) insulation, and the behavior of the animal. As ambient temperature increases, the cow's body temperature will also increase. The physiological mechanisms for regulating body temperature are under the control of a region of the brain called the hypothalamus, which acts like a thermostat. There are two main mechanisms used by dairy cows to increase the amount of heat loss from the skin when heat stress is increasing internal heat production. The first is dilatation of the blood vessels in the dermis so that blood flows close to the skin surface and heat loss to the environment comes about. The second is by sweat production from the sweat glands (Willmer et al., 2004). The evaporation of sweat on the skin surface produces a cooling effect. However, dairy cows sweats at only 10 percent of the human rate, so that they are more susceptible to heat stress and need mechanical ways to reduce heat.



**Figure 1.** Critical temperatures and thermo-neutral zone in dairy cattle.

The physiological mechanisms for dealing with heat stress include sweating, more rapid respiratory rate, greater vasodilatation with increased blood flow to the skin surface, decreased dry matter (DM) and nutrient intake, reduced rate of metabolism, an altered water metabolism, and alterations of levels of numerous hormones. Maintenance of a high milk production during elevated ambient temperatures is determined primarily by the balance between metabolic heat production and heat loss. Metabolic heat production is relative to the amount of milk production plus the heat produced for maintenance. High producing cows exhibit more signs of heat stress than low producing cows because higher

producing cows generate more heat as they eat more feed for higher milk yield (West, 2003).

The best recognized effect of heat stress is an adaptive depression of metabolic rate associated with reduced appetite (Silanikove, 2000). Reduced DM consumption, and consequently heat generated during ruminal fermentation and body metabolism, assists in maintaining heat balance. Furthermore, an elevated environmental temperature reduces gut motility, rumination, ruminal contractions and thereby depresses appetite by having a direct negative effect on the hypothalamus (Chaiyabutr et al., 2008; Kadzere et al., 2002).

As ambient temperatures get higher, the respiratory rate rises with panting growing to open mouth breathing. As a result cow enters in respiratory alkalosis resulting from a rapid drop of carbon dioxide. The cow counterbalances this situation by increasing urinary output of bicarbonate, and rumen buffering is affected by a reduction in salivary bicarbonate reservoir. The risk is that lameness, with individual ulcers and white line disease may emerge in a few weeks to a few months after the heat stress takes place (Wheelock et al., 2010).

The best approach to conclude that cows are being affected by heat stress is to measure the rectal temperature. Normal body temperature of the cow is about 38.5°C, and a cow that has a rectal temperature of 39°C or higher during the afternoon, and it is not sick, is possible to be heat stressed. Determining rectal temperature on group of cows in the afternoon can be a quick way to get a precise judgment of the degree of heat stress and the efficiency of any cooling system integrated into cow housing (West, 2003; Willmer et al., 2004).

Joint genetic selection for heat tolerance and milk production can be a possible way to reduce heat stress. Also, identification of genetic traits which enhance heat tolerance without affecting milk yield in dairy cattle breeds. Some of these traits would be coat color, hair length and genes controlling heat shock resistance in cells (Hansen and Aréchiga, 1999).

#### 4. The temperature-humidity index (THI)

Usually, a reasonable assessment of cow's heat stress is the Temperature-Humidity Index, which combines ambient temperature and relative humidity to express an indicator of the degree of heat stress. This index was developed by environmental physiologists and it is shown in Table 1. It represents a general classification of different combinations of ambient temperature and relative humidity and is, at present, the most used stress index for use in animal production. There are different formulas to estimate the THI, being one of them as follows (Hahn, 1999):

$$\text{THI} = (0.8 \times T_{\text{db}}) + \left[ \left( \text{RH}/100 \right) \times (T_{\text{db}} - 14.4) \right] + 46.4,$$

Where  $T_{\text{db}}$  is the dry bulb temperature in degrees Celsius, and RH the relative humidity

Temperature, °C	Relative Humidity, %																					
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
23.3																72	72	73	73	74	74	
23.9															72	72	73	73	74	74	75	75
24.4															72	72	73	73	74	74	75	76
25.0															72	72	73	73	74	74	75	76
25.6															72	73	73	74	74	75	75	76
26.1															72	73	73	74	74	75	76	76
26.7															72	72	73	73	74	74	75	76
27.2															72	72	73	73	74	74	75	76
27.8															72	73	73	74	74	75	76	76
28.3															72	73	73	74	74	75	76	77
28.9															72	73	73	74	74	75	76	77
29.4															72	72	73	73	74	74	75	76
30.0															72	73	73	74	74	75	76	77
30.6															72	73	73	74	74	75	76	77
31.1															72	73	73	74	74	75	76	77
31.7															72	73	73	74	74	75	76	77
32.2															72	73	73	74	74	75	76	77
32.8															72	73	73	74	74	75	76	77
33.3															72	73	73	74	74	75	76	77
33.9															72	73	73	74	74	75	76	77
34.4															72	73	73	74	74	75	76	77
35.0															72	73	73	74	74	75	76	77
35.6															72	73	73	74	74	75	76	77
36.1															72	73	73	74	74	75	76	77
36.7															72	73	73	74	74	75	76	77
37.2															72	73	73	74	74	75	76	77
37.3															72	73	73	74	74	75	76	77
38.3															72	73	73	74	74	75	76	77
38.9															72	73	73	74	74	75	76	77
39.6															72	73	73	74	74	75	76	77
40.0															72	73	73	74	74	75	76	77
40.6															72	73	73	74	74	75	76	77
41.1															72	73	73	74	74	75	76	77
41.7															72	73	73	74	74	75	76	77
42.2															72	73	73	74	74	75	76	77
42.3															72	73	73	74	74	75	76	77
43.3															72	73	73	74	74	75	76	77
43.9															72	73	73	74	74	75	76	77
44.4															72	73	73	74	74	75	76	77
45.0															72	73	73	74	74	75	76	77
45.4															72	73	73	74	74	75	76	77
46.1															72	73	73	74	74	75	76	77
46.7															72	73	73	74	74	75	76	77
47.2															72	73	73	74	74	75	76	77
47.3															72	73	73	74	74	75	76	77
48.3															72	73	73	74	74	75	76	77
48.9															72	73	73	74	74	75	76	77
49.4															72	73	73	74	74	75	76	77
50.0															72	73	73	74	74	75	76	77

**Table 1.** Temperature-Humidity Index combining ambient temperature (°C) and relative humidity to determine the degree of heat stress (Adapted from Armstrong, 1994).

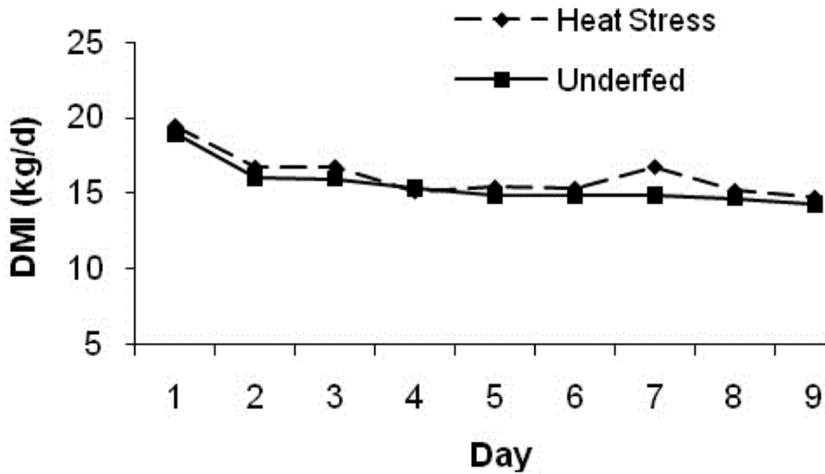
Below 72 units, which can be reached with 25 °C of ambient temperature and values below 50% of relative humidity, lactating dairy cows express their optimum productivity performance, so there are no evident signs of heat stress. Slight heat stress can be reached between 72 and 79 units of THI; dairy cows are likely to begin experiencing heat stress; cows start looking for shade to cover them from solar radiation, respiration rate increase but there is a minimum effect on milk yield. This heat stress level can be reached with combinations 25°C of ambient temperature and relative humidity values above 50%; or with 30°C and more than 30% of relative humidity. Moderate heat stress occurs from 80 to 89 units of THI, and cows show and increased in respiration and salivation rate. Reduction in feed intake is evident as well as an increase in water consumption. Body temperature increases and milk production and reproduction parameters are seriously affected. This level of heat stress can be reached with combinations of 35°C and 40% of ambient temperature and relative humidity respectively, or with 40°C of ambient temperature and 35% of relative humidity. The next level of heat stress ranges from 90 to 98 units of THI and is considered severe. Dairy cows feel very uncomfortable because of a dramatic increase in body temperature and respiration rate. Panting and drooling are common events under this level of heat stress and some cows even hang out her tongue. There are significant losses in milk yield and cows rarely become pregnant. When THI is above 98 units, heat stress is extreme and some dairy cows may die during this conditions, which are characterized by combinations of ambient temperature and relative humidity of 40°C and 60% or 49°C and 35% of relative humidity. These levels of heat stress are very excessive but not uncommon in arid zones during heat waves in summer months (Avendaño, 1998; Bohmanova et al., 2007).

## **5. Effects of heat stress on production and reproduction of dairy cattle**

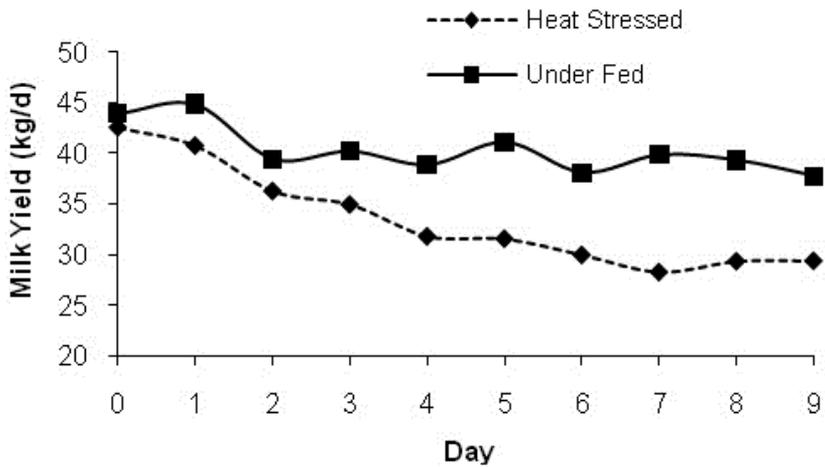
### **5.1. Effects on production**

Dairy cows automatically will reduce their feed intake during period of heat stress, and this reduction could increase as weather becomes hotter. Typically, early and high producing cows are more directly and severely affected than late or low producing cows. The reduction in nutrient intake has been identified as a major cause of decline milk synthesis because has been associated to a negative energy balance state, regardless of the stage of lactation but under heat stress conditions (Wheelock et al., 2010). Nevertheless, in order to know the exact contribution of reduction feed intake to the overall reduced milk yield during heat stress, Rhoads et al. (2009) used a group of thermo-neutral pair-fed dairy cows to eliminate the confounding effects of nutrient intake. The cows were in mid-lactation and were either subjected to a THI of 80 units for 16 h/d (cyclically heat-stressed) during 9 d or kept under a THI of 64 units during 24 h/d (constant thermoneutral conditions). Both groups of cows were pair-fed to maintain similar nutrient intake. Heat-stressed cows showed a rapid reduction of 5 kg/d of DMI, reaching the nadir in DMI by day 4, and keeping constant afterward (Figure 1). Milk production was reduced in 14 kg/d and production steadily declining in the first 7 d and then reaching a plateau (Figure 2). In summary, these results indicate that the reduction in dry matter intake can only account for about 40 to 50% of the

reduction in milk yield when cows are under heat stress conditions and that the remaining 50 to 60% could be explained by other changes induced by heat stress.



**Figure 2.** Effects of heat stress and pair-feeding thermoneutral lactating Holstein cows on dry matter intake (Adapted from Rhoads et al., 2007).



**Figure 3.** Effects of heat stress and pair-feeding thermoneutral conditions on milk yield in lactating Holstein cows (Adapted from Rhoads et al., 2007).

The mammary gland requires glucose to synthesize milk lactose, which is considered the primary osmoregulator and thus determinant of milk yield. However, in an attempt to generate less metabolic heat, the body (primarily skeletal muscle) appears to use glucose at an increased rate. As a result, the mammary gland may not receive adequate amounts of glucose; thus mammary lactose production and subsequent milk yield are reduced. This may be the primary mechanism which accounts for the additional reductions in milk yield that cannot be explained by decreased feed intake (Wheelock et al., 2010).

Acute stress in response to dehydration resulted in more intense inhibition of lactose and fluid secretion than of fat and protein secretion, which is reflected in increased fat and protein concentrations in milk, though these increases did not compensate for the overall reduction in their yields (Kadzere, 2002; Wheelock et al., 2010).

Silanikove (2000) states that heat stress stimulates a short-term rapid regulatory response, since in lactating cows under commercial conditions, the effects of heat stress that may be experienced under exposure to high ambient temperatures during the day appears to be alleviated when temperatures drop at night, and that lack of a cool night-time ambient temperature intensifies the reduction in milk yield.

## 5.2. Effects on reproduction

The detrimental effects of heat stress on reproduction processes of Holstein cattle have been well documented and include in cows: a) reduction in the intensity and duration of estrus, b) reduction in the pulse and amplitude of luteinizing hormone, c) reduced estradiol secretions, d) delayed ovulation, e) low progesterone concentrations, f) reduced quality of oocytes, g) decreased blood flow to the uterus, h) increased uterine temperature, i) higher follicular persistency, j) changes in endometrial prostaglandin secretions, k) increased embryonic mortality, and l) reduced fertility rates (Jordan, 2003). In bulls we have: a) hyperthermia of the scrotum, b) deterioration of semen quality as evidenced by reduced semen motility, semen concentration, percentage of motile sperm, and percentage of intact acrosome; as well as increased of abnormal sperm, c) decreased testosterone levels, and d) reduced spermatogenesis (Hansen & Arechiga, 1999; Wolfenson, 2009). So it is evident that the negative effects of heat stress on reproduction efficiency is the result of direct impact on reproduction functions and embryonic development, as well as indirect influences mediated by changes in energy balance. The negative energy balance is caused by a reduction in dry matter intake, and if this physiological status is prolonged may reduce plasma concentrations of insulin, IGF-1, and glucose, which finally can lead to retarded follicle development, poor estrus expression, and low quality of oocytes (Jordan, 2003). The effect of using cooling systems during summer on milk production performance and reproductive efficiency differs considerably, because of summer cooling is capable of substantially improves summer milk yield, while summer fertility is only slightly enhanced. Flamenbaum & Ezra (2003) conducted several trials during 4 consecutive summers in dairy herds located in an arid region of the Middle East to compare productive and reproductive traits during summer and winter. They found that milk production during summer months was almost similar (difference of 2 - 4%) to that during winter season, which means that cooling systems are capable of minimize the drop in milk production attributable to heat stress. In contrast, conception rates were only somewhat improved during summer, so that reproduction efficiency was still low in summer compared to the observed during winter. These results suggest that additional hormonal treatments are required during summer to further improved summer fertility. In addition, other studies in arid and semi-arid conditions have shown that fertility of Holstein dairy cows drop from 40 to 20% during summer months (Wolfenson, 2009).

Embryo transfer has been mentioned as a possible solution for improving summer fertility because it has shown a considerably progress in pregnancy rates during the summer months. This is because embryo transfer can escape the period in which the embryo is more susceptible to heat stress, considered before day 7 after AI (Jordan, 2003). However, embryo transfer is not a commonly adopted technique, consequently there is the necessity to improve events such as *in vitro* embryo production techniques, embryo freezing, timed embryo transfer, and decreasing the cost of commercially available embryos before this technique becomes a viable solution. In addition, altering biochemical properties of the embryo, or even its genetic modification before the embryo transfer, could be a possible way to improve thermo-tolerance and enhance summer fertility.

## **6. Nutritional and feeding strategies to minimize effects of heat stress in dairy cattle**

Dairy cows reduce their feed intake during heat stress, so more nutrients need to be consumed into a smaller volume of feed. Maintaining adequate nutrient intake becomes vital to avoid a reduction in milk production. Some alternatives to increase dietary nutrient density include feeding high quality forage, feeding more grain and use of supplemental fats. Reducing the forage to concentrate ratio may result in more digestible rations that may be consumed in greater amounts. However, feeding more concentrate would have problems of acidosis and cows stop feeding. Sodium bicarbonate may help buffer the rumen to adapt to a higher levels of concentrate. Other feed additives that have been successful in heat stress conditions to stabilize rumen health from dietary modifications are yeast, which improves fiber digestion, and fungal cultures and niacin, which improves energy utilization. As a practical recommendation, do not use together these additives (Escobosa et al., 1984; Zimelman et al., 2010).

Dairy cows experiencing heat stress often shows a negative N balance because of reduced feed intake. Increasing the level of crude protein may increase energy requirements and excess of dietary protein is converted to urea and excreted, causing problems of environmental pollution. It is suggested that during heat stress the level of crude protein in the diet should not exceed 18%, while the level of rumen-degradable protein should not exceed 61% of crude protein or 100 g/N/d (Huber et al., 1994).

The feed manager can provide shades in the feed bunk for added comfort to the cows while they are eating and feeding. It is also recommended to add a water sprinkler system and fans that are directed towards the cows to further reduce the heat felt in the place. Do not spray water on the feed as dry matter intake is important. As much as possible, keep the udders of the cows dry to reduce the possibility of having mastitis. Also make sure that the floor is still good enough for the cows to walk on and prevent injuries due to slipping.

Other strategies that dairy managers consider when feeding lactating dairy cows during heat stress periods are feeding frequency, time of feeding, and adequate feed bunk space. Feeding frequency consists of increasing an extra feeding or two during the day, obviously

these extra feedings should be provided during the cooler times of the day. This strategy promotes a reduction of flies around the feed, therefore decreasing the insect population in the dairy barn. Also, increasing the amount of feed during these cooler periods of the day (early morning or late evening) is another alternative to avoid the reduction in feed intake during summer. Providing between 60 to 70 percent of the ration from 20:00 to 08:00 h has proven to have a positive impact on milk yield during periods of hot weather. The objective to provide enough space in the feed bunk is that all dairy cows can eat together without crowding (Hahn, 1999; West, 2003).

The increase in respiration rate and perspiration can cause an excessive loss of water, therefore reducing mineral levels in the cow. The recommendation is to increase K content from 1.3 to 1.5% of the total dietary dry matter, Na to 0.3% and Mg to 0.5%. Feed complete mineral mixes with higher K and Na levels only to the milking cows. If fed to dry cows, these mineral mixes may cause increased udder edema.

Water is really a priority when the temperature rises. We can say that management and the feeding of the cows are also part of the process in reducing heat stress in dairy cows. Some responses of the cows, though, can help reduce heat production in them, like selective consumption of feeds and cooling strategies. The dairy cows can only do so much and the dairymen are the one controlling their environment.

Before performing any critical modification to the diet during heat stress periods, be sure to ask for advice from a feed consultant. This is because we have to remember that dairy cows are under severe heat stress and any drastic change could be detrimental. Maintaining cows comfortable is the key to hold them eating which is critical in keeping them productive.

## **7. Environmental modifications to minimize effects of stress in dairy cattle**

Use of environmental modifications such as shade and cooling systems is critical in arid and semi-arid zones affected by heat stress in order to maintain milk production, milk component levels, reproductive performance, and animal welfare.

The most basic attempt to reduce heat load from direct solar radiation in cattle is the use of shades. They can be from natural or artificial materials and are considered practical and economical ways for reducing heat stress. Trees are considered the most effective shade since they protect from the sun and capture radiation through the evaporation of humidity in the leaves (Avendaño, 1995). The wood or leaves of palms are materials also used for shade although corrugated steel sheets are the most widely used material because they last longer and of low maintenance costs (Armstrong, 1994).

Buffington et al. (1983) pointed out that painting of white color the upper part of the shade unit and installing a 2.5 cm thick of isolating material may considerable reduce solar radiation. For arid and semi-arid zones, areas of 3.5 to 4.5 m<sup>2</sup> per lactating cow are recommendable. A more reduced area could provoke lesions in the udder due to competition of cows for shade

space, while an area greater than 4.5 m<sup>2</sup>/cow have little or no benefit (Armstrong, 1994; Berman, 2006). Height of shades in the corral must be from 3.6 to 4.2 m in order to guarantee reduction in solar radiation. However, the shade structure should be high enough from the ground to allow circulation and tractor access for corral cleaning.

Orientation of shades is also important to minimize heat load during summer months. North-South orientation will expose the surface to the sun under the shade during the morning and the afternoon, helping to maintain it dry, but under extremely hot conditions and low rainfall (10 to 12 cm), the East-West orientation could be preferable, although it requires greater labor for maintaining the surface under the shade in dry conditions. In any case, shades must be placed in the center of the corral and should avoid accumulation of humid material under the structure. If concrete floors are used, shade orientation is indistinct (Avendaño, 1995). Figure 4 shows an example of a shade for dairy cattle made from artificial material.



**Figure 4.** A group of cows seeking for shade during hot weather. Shade structure is from artificial materials.

Cooling systems alleviate heat load from dairy cows by using the principle of evaporation, combining water misting and forced ventilation through use of spray and fans, and are frequently placed inside free-stall barns or under shades in open space corrals (Berman, 2006). Even though responses have varied, cooling techniques have consistently improved feed intake and milk production in areas with high environmental temperatures (Armstrong, 1994; Ryan et al., 1992).

In general, cooling systems based on spray and fans consist of conventional fans of variable diameter (60 to 90 cm) suspended from the ceiling of the shade. There are lines with water sprinklers in front of them, that creates a kind of breeze with small water droplets which completely moisture the cow surface and skin and support loss of heat by evaporation. These cooling systems can operate at different time intervals which vary according climatic conditions. One disadvantage is the accumulation of moisture under the cooling area because cows spend hours under this area, so urine and feces build up very easily. More labor is required to maintain clean resting areas where the cooling is installed (Avendaño, 1995). Figure 5 shows a cooling system based on spray and fans located in a free stall barn to improve comfort of cows during feeding.



**Figure 5.** A cooling system based on spray and fans placed over the head-locks, next to the feedbunk.

Traditionally, dry cows are provided little or no protection against heat stress because they are not producing milk and it is erroneously assumed they are less prone to heat stress. However, dry cows are experiencing many physiological changes (milk-producing tissue formation, colostrum secretion, accumulation of antibodies, and final fetal growth) that may increase their susceptibility to hot weather and have a critical impact on postpartum cow health, milk yield

and reproduction (Avendaño-Reyes et al., 2010a). Avendaño-Reyes et al. (2006) allocated a group of dry cows in a pen with a cooling system based on spray and fans and compared them to a group with just shade in the pen. Cooled dry cows showed better physiological status than control dry cows. Fat content and fat-corrected milk production at eight week postpartum was significantly higher in cooled cows, as well as conception rate; however, calf birth weight and milk yield showed a trend to be higher in the cooled group. In addition, culling rate was higher in the control group and there was a benefit for using the cooling system during 60 d prepartum in Holstein cows. The physiological rationale for improved postpartum productivity in response to prepartum cooling is not entirely clear, but heat stress was found to negatively influence secretory function of the udder by decreasing mammary blood flow, thereby reducing the efficiency of energy utilization for milk fat precursor synthesis (Kadzere et al., 2002).

Using a cooling system based on spray and fans and installed in the holding pen, previous to the milking parlor, in an arid zone with extreme hot temperatures, Avendaño-Reyes et al. (2010b) provided 1, 2 or 3 h of cooling to a mid-lactating Holstein cows bringing the cows to that site. They found that even though cows under the cooling management system with the highest time of cooling per day showed better milk yield (+2 kg/d of milk), their physiological status did not correspond to a those non-heat stressed lactating cows. So they conclude that is necessary to increase the time of cooling to effectively reduce heat load during severe summer heat conditions. Figure 6 illustrates an installation of a cooling system on the roof of the parlor holding pen, and Figure 7 exemplifies a cooling system based on evaporative environmental chambers.



**Figure 6.** A cooling system based on spray and fans installed in the roof of the holding pen, prior to the milking parlor.



**Figure 7.** A cooling system based on evaporative chambers. Note at the curtains in one side of the corral to avoid water misting goes out the shade.

With milk production increases between 5 and 10% the benefits for investment in cooling equipment (spray and fans) is from 2 to 3 years. As production increases approach to 20%, the profit on cooling investment could be one year or even less. Management strategies that reduce the impact heat stress has on milk peak production can produce large economic returns to cows that are in their second or higher lactation. The benefit of reducing heat stress in first lactation cows is considerably less because of their inherent lower productivity (Avendaño, 1995; Berman, 2006).

In Table 2 are presented several results of milk production from the use of three environmental modifications against heat stress in different regions of the world. It can be noted that the milk production from the use of spray and fans has a obvious advantage over the use of just shades in the corrals. However, there is no clear evidence that cooling chambers has an advantage over the use of spray and fans, recalling that the cooling chambers are characterized by a considerable investment cost.

In general, Livestock Environmental Management is an emerging area in Animal Science that is getting more acceptance due to the Climatic Change. This new area is an attempt to avoid adverse environmental impacts on animal production systems and is also an effort to minimize the need for expensive environmental protection measures for domestic animals.

Place	Shade	Spray and fans	Evaporative cooling chambers	THI	Cooling time	Reference
Missouri	23.3 <sup>a</sup>	25.3 <sup>b</sup>	--	76	24	Igono et al., 1987
Israel	37.2 <sup>a</sup>	40.7 <sup>b</sup>	--	80	9	Wolfenson et al., 1988
Saudi Arabia	--	26.8 <sup>a</sup>	27.7 <sup>b</sup>	88	12	Ryan et al., 1992
Mexicali	27.0 <sup>a</sup>	31.0 <sup>b</sup>	--	89	8	Correa et al., 2002
Arizona	31.0 <sup>a</sup>	39.1 <sup>b</sup>	37.9 <sup>b</sup>	85	11	Correa et al., 2004
Mexicali	19.1 <sup>a</sup>	21.1 <sup>b</sup>	--	88	4	Avendaño-Reyes et al., 2010b
Arizona	--	38.3 <sup>a</sup>	42.2 <sup>b</sup>	76	12	Burgos et al., 2008

<sup>ab</sup> Milk yield means with different superscript differ ( $P < 0.05$ )

**Table 2.** Milk production of Holstein cows cooled with different environmental modifications in several studies in arid and semi-arid zones of the world.

## 8. Conclusions

It is clear that farm animals are not the cause of climate change. If livestock is managed and their feed production properly, cattle actually can help us take carbon out of the air and store it in the soil. Environmental issues will become more important and we need to make sure people understand that farm animals are part of the solution and not part of the problem. Tell people how cows are helping our farming systems to be more environmentally friendly and more sustainable.

The productive and reproductive efficiency of dairy cattle is notably reduced under conditions of high temperature. The temperature-humidity index is an indicator of the degree of heat stress on the animal. Heat stress is a heavy load for the cow's zootechnical performance and health status that costs the dairy industry millions of dollars every year. Implementing adapted herd management strategies as early as possible before the problems are visible at production level is the key. These management strategies include primarily diet manipulation and environmental modifications. Summer environmental adjustments in arid zones mean to provide shades and cooling systems to efficiently reduce the negative effects of heat stress. Those environmental strategies have demonstrated to increase production performance during heat stress periods. However, an economical analysis helps to determine the best cooling system for a specific production system in arid ecosystems.

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# The Course of Machine Milking in Small Ruminants

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50802>

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## 1. Introduction

### 1.1. Machine milking of small ruminants: A review

During the last 20 years the reported worldwide goat populations have increased by 52% (56% in the developing countries and 17% in the developed countries), while the sheep populations worldwide decreased by 3% (6% in the developed countries, while increasing by 14% in the developing countries). Dairy goat and sheep farming are important economically in many developed countries, especially in the Mediterranean area, such as France, Italy, Spain and Greece (Haenlein, 2001). Many of the benefits of dairy sheep and goat farms are also recognized in the developing countries, that provide a significant proportion (from 2 to 7%) of sheep and goat milk of the total world milk production. Milk production by small ruminants accounts for about 3.5 percent of world milk production; however, this percentage is higher in the developing countries (7.5%) than in the developed countries (1.5%) (Boyazoglu & Morand-Fehr, 2001). Milk of small ruminants is a valuable raw material for the production of yoghurt, milk powder and UHT milk, cheese brine and ripening hard cheeses (Haenlein, 2001; Olechnowicz & Jaśkowski, 2004; Olechnowicz & Jaśkowski, 2005). The quality of these products is assessed in relation to their hygienic, sanitary, dietary and nutritional value, as well as flavor (Boyazoglu & Morand-Fehr, 2001). Deterioration of sensory attributes and shortening of the shelf life of finished products is associated with an excessive number of somatic cells in bulk tank milk, i.e.; bulk tank somatic cell count – BTSCC (Boyazoglu & Morand-Fehr, 2001; Haenlein, 2001; Gonzalo et al., 2005; Zweifel et al., 2005). BTSCC is the main indicator of udder health in small ruminants (Fernández et al., 1999; Muehlherr et al., 2003; Diaz et al., 2004). Factors that influence log BTSCC include herd and breed, lactation stage, the course of the drying-off period of animals and milking system (Zeng & Escobar, 1996; Diaz et al., 2004; Zweifel et al., 2005). In machine milking log BTSCC is also influenced by factors such as vacuum level, pulsation

frequency and pulsator ratio in milking installations (Billon et al., 1999; Fernández et al., 1999; Sinapis et al., 2000; Peris et al., 2003a, 2003b). In sheep and goats daily milk yield and composition (fat, protein and lactose percentage contents) as well as log BTSCC are also affected by the frequency of milking (Knight & Gosling, 1995; Synapis et al., 2000; Negrão et al., 2001; Salama et al., 2003; Capote et al., 2008) and the use or omission of machine stripping (Knight & Gosling, 1999; Molina et al., 1999; McKusik et al., 2000, 2003). In the course of machine milking in small ruminants it is also important to monitor the health status of mammary glands by diagnostic tests and dipping of teats after milking has been completed (Bergonier & Berhelot, 2003; Bergonier et al., 2003). Improvement of hygienic conditions during milking is important in assessing the microbiological quality of milk. Zweifel et al. (2005) reported that in Switzerland the standard plate counts (SPC) for bulk tank milk from small ruminants was median SPC of 4.71 log cfu/ml (mean SPC 6.86 log cfu/ml). For goat's milk median SPC was 4.68 log cfu/ml (mean SPC was 6.92 log cfu/ml), whereas for ewe's milk median SPC was 4.79 log cfu/ml (mean SPC 6.05 log cfu/ml). The highest median SPC (5.24 log cfu/ml) was found in June. Microbiological quality of milk from small ruminants was significantly influenced by the month of sample collection, the number of milkings from which milk was contained in the bulk tank, the technique of milking, and flock size. *Enterobacteriaceae* were detected in 212 (61.6%) goat's milk and 45 (71.4%) ewe's milk samples, whereas *Staphylococcus aureus* were detected in 109 (31.7%) samples of goat's milk and 21 (33.3%) samples of ewe's milk (Muehlherr et al., 2003). At present breeders pay increasing attention to the level of milk yield and the clinical status of the udder in small ruminants. The values of genetic and phenotypic correlation coefficients between somatic cell counts and morphological traits of the udder suggest that some of the latter, such as e.g. the depth, shape and attachment of the udder, and the location and the size of teats, need to be taken into consideration in the evaluation of suitability of small ruminants for machine milking (Fernández et al., 1999; Peris et al., 1999; Dzidic et al., 2004; Legarra & Ugarte, 2005; Marie-Etancelin et al., 2005). In relation to the influence of many factors on bulk tank somatic cell counts (BTSCC) it seems justified to show the course and consequences of machine milking in small ruminants on milk quality and health of the teat end.

## 1.2. Physiological aspects of machine milking in small ruminants

Milk in the udder of cows is usually stored in the alveolar compartment (around 80%) and only up to 20% is stored in the cistern. In contrast, in small ruminants the cisternal fraction amounts to more than 50% (Bruckmaier et al., 1994; Bruckmaier & Blum, 1998). In dairy ewes and goats after a normal 12-hour milking interval the cisternal milk represents between 50 and 80% (Marnet & McKusik, 2001). These larger cisterns play an important role in milk collection and storage and have a significant influence on milk ejection during milking. The cisternal milk fraction is available for machine milking or to suckling before the occurrence of milk ejection. In animals, in which the mammary glands do not have the capacity to store milk (e.g. rodents) the milk let-down reflex is necessary to maintain the secretory function, while in ruminant animals exclusion parts of the innervation of

glandular sinuses have no effect on the end of lactation (Marnet et al., 1998). It follows that the release of oxytocin is not crucial for the maintenance of milk production in small ruminants (Bruckmaier et al., 1997; Marnet et al., 1998, Marnet & McKusik, 2001). As a result of stimulation of the teats and mammary glands either by the sucking young or machine milking followed by the transmission of nerve impulses to the anterior pituitary, which secretes oxytocin, it is transported to the mammary gland and its myoepithelial cells, which surround the alveoli and small intralabular ductules. Contraction causes a flattening of the alveolar lumen and results in the transfer of milk through the ductules to the cistern and teat for milk removal (Lollivier et al., 2002). In contrast to cows, the time of oxytocin release during milking in goats does not influence significantly the model curve of milk flow and an increasing pressure of milk in the cisterns probably induces milk ejection (Bruckmaier et al., 1994; Marnet et al., 1998). On this basis one can identify goats with high milk production and high rate of milk flow (Marnet et al., 1998). The release of oxytocin is significantly diversified in goats and the curve of milk flow during milking often has two or three peaks (Bruckmaier et al., 1994; Marnet et al., 1998). In White German Goats higher rates of milk flow (ml/min) were reported in the group of goats without stimulation of teats when compared with goats, who were subjected to teat stimulation and goats stimulated by 2 kids (Mueller & Kaufmann, 2005). In ewes there is latency in milk removal when applied to the teat milking cups, but a lack of stimulation before milking, similarly as in goats, has no effect on the model curve of milk flow during milking (Bruckmaier et al., 1994; Marnet et al., 1998). The release of oxytocin in ewes, however, is necessary for the removal of milk during a short time of milking, although blood oxytocin concentration is not related to milk yield. However, determination of blood oxytocin concentrations in ewes may be useful in determining the efficiency of milking machine providing optimal stimulation of mammary glands. On the other hand, oxytocin stimulates milk flow from the lumen of alveoli to the cistern between milkings (Marnet et al., 1998). Moreover, 92% Lacaune ewes demonstrate significant increases in plasma oxytocin concentrations in response to machine milking; however, large variations are noted in oxytocin concentration between ewes, ranging from 10 to more than 150 pg/ml (Marnet et al., 1998). In ewes of this breed oxytocin concentration significantly increased within 0.5 minutes after the start of stimulation or milking, while in East Friesian ewes low levels of oxytocin or a lack of its release were reported. Stimulation of the teats before milking resulted in the release of oxytocin within 1-2 minutes after the start of milking, indicating a delayed response while waiting for the onset of milk flow (Bruckmaier et al., 1997). Anatomically mammary glands in ewes and in goats differ slightly; however, in ewes the outlet duct of the teat is not always located on the edge of the mammillary part of the cistern, from which proportionally to the amount of milked milk the stripping fraction is obtained (Labussi re, 1988; Bruckmaier et al., 1997). This anatomical peculiarity is the cause of obtaining a large quantity of milk from machine stripping. On the other hand, the apparent latency in the release of oxytocin in ewes results in a situation when the curves have two milk peaks, with the second peak referring to the alveolar fraction of milk, which is not always milked due to the short time of milking (Bruckmaier &

Blum, 1998). In small ruminants milk is readily transferred from the alveoli to the cistern during the period between milkings. This phenomenon of milk transfer during the periods between milkings is not fully understood (Marnet & McKusik, 2001). There are several theories on this subject. It seems that the variations in the microanatomy of the mammary gland may affect milk transfer. On the other hand, it is possible that a spontaneous contraction of the smooth muscle causes the removal of milk out of the alveoli and small ducts to the cistern. Oxytocin was also found to have a considerable effect as a promoter of milk transfer from the alveolar lumen to the cisternal cavity between milkings (Marnet et al., 1998). Ewes with a considerable cistern volume well tolerated milking once a day, but were not adapted to the increased frequency of milkings (Labussière, 1988). More recent data indicate that an accumulation of proteins synthesized in the gland (FIL – feedback inhibitor of lactation) reduces the synthesis of milk in the alveoli. The inhibition of milk ejection may occur as a result of emotional stress. In most species (except for cattle)  $\beta$ -endorphin plays an important role. The milk ejection rate is regulated by the adrenergic system and peripheral inhibition of milk ejection may be caused by the adrenergic receptor stimulation or an oxytocin receptor blockade (Wellnitz & Bruckmaier, 2001). An increased frequency of milkings in ewes positively affected the secretion of milk (Labussière, 1988; Knight & Gosling, 1995; Negrão et al., 2001). Multiparous Lacaune ewes breed between 60. and 65. days of lactation were milked at different frequencies during the day (1x, 2x, 3x, 4x, 5x, and 7x). To determine the concentration of oxytocin during milking, blood samples were taken 30 s before milking and 30, 60 and 120 s after the start of milking. Milking cups were applied to the teats at time 0 (with no teat washing or the massage) and were removed from the teats after 90 s. Basic levels of oxytocin (30 seconds before milking) were low at all milking frequencies, while a significant increase in oxytocin levels was noted in all groups of ewes after the start of milking. Single milking in ewes induced higher levels of oxytocin in comparison to all the frequencies of milking (Negrão et al., 2001). A hypothesis was proposed on the hindering of milk flow through the blocking of oxytocin release from the pituitary gland, as well as a local blocking of its influence in mammary glands. Blood concentration of noradrenaline in blood in East Friesian ewes at above 300pg/ml, and above 700 pg/ml in Lacaune ewes stops the release of oxytocin. In small ruminants the management system can have a significant impact on milk ejection. Dairy ewes and goats are frequently suckled by the young for a period of 30 to 60 days, and after that period they are only machine-milked. In the period of adaptation to the milking machine a significant decrease (about 30%) is observed in total milk production (Labussière, 1988). The decrease in milk production during this period is affected by three important physiological factors. An important factor in the transitional period is connected with a less frequent udder evacuation from many sucklings per day, compared with the udder being emptied only twice per day at milking. Another factor influencing the lower milk production during the transitional period is the less effective stimulation of the udder by the machine milking compared to the presence and suckling stimulus by the young. This is also linked with a long time of the release of oxytocin during suckling compared to milking, i.e. 5 vs. 2

minutes, respectively (Marnet & McKusik, 2001). The third factor is the milking environment-induced stress, when ewes and goats are milked in the milking parlor as compared to suckling in the barn (Marnet & Negrão, 2000). In addition to these factors an important role is also played by management factors, such as a mixed management system, which allows for lambs to suckle their mothers for 8 to 12 hours per day, then they are separated from their mothers at night, and the ewes are machine-milked in the morning (McKusik et al., 2000). The release of oxytocin in ewes milked in the mixed management system does not differ from the baseline levels, indicating a total inhibition of the milk ejection reflex (Marnet & Negrão, 2000). If ewes were milked in the presence of their lambs, the release of oxytocin would be normal; however, if lambs are not present during the milking, oxytocin release is again inhibited. This inhibition of oxytocin release only slightly influences the recuperation of the cisternal milk fraction, while the alveolar fraction is retained in the udder (McKusik et al., 2000). The mother-young bond plays an important role in the modulation of maternal endocrinology also in goats (Hernández et al., 2002). In addition, a prolonged period of maternal nursing may have positive effects on the welfare of the mothers and their young (Hernández et al., 2007). Dairy ewes produce 25% of total milk production in lactation during their first month of lactation. Taking into account the fact that in ewes milk yield is increased up to approximately 24 days after parturition, when ewes are not machine milked, the mixed management system seems to be beneficial both for the growth of the lamb and also due to the commercial nature of milk production (McKusik, 2000). This inhibition in the release of oxytocin and the related decrease in milk production persist until several days following weaning. If the adopted management system used in a suckling period of more than 30 days, then a higher proportion of ewes does not show normal milk ejection following weaning. During the first few weeks of adaptation to the machine milking a gradually increasing secretion of oxytocin and better release synchronization of the oxytocinergic system are observed (Marnet & McKusik, 2001).

### 1.3. Technique and course of machine milking

Ewes are most often milked at the high pulsation frequency (from 120 to 180 cycles/minute), a low level of negative pressure (from 32 to 40 kPa) and a 50% pulsator rate. In the least years a tendency was observed to reduce the weight of the milking cluster as well as to lower the level of negative pressure in milking installations (to 34 – 36 kPa) and to apply a 50% pulsator rate (Billon et al., 1999). High frequency pulses are used to ensure an optimal milk let-down reflex and accurate milking of ewes (Marnet et al., 1998; Marnet, 2002). In recent years a trend towards a further reduction in milking vacuum (to 34 - 36 kPa) has been observed due to the decreasing mass of milking clusters (Billon et al., 1999). In a study on Manchega ewes creasing the pulse frequency from 120 to 180 cycles per minute at 36 kPa vacuum and a pulsator rate of 50% did not have a detrimental impact on the health status of the udder and teat end condition (Peris et al., 2003b). Optimal conditions of machine milking for goats include pulsation frequency of 70 – 90 cycles/minute, at the negative pressure in milking installation of 36 – 44 kPa and a 65% pulsator rate (Sinapis et al., 2000).

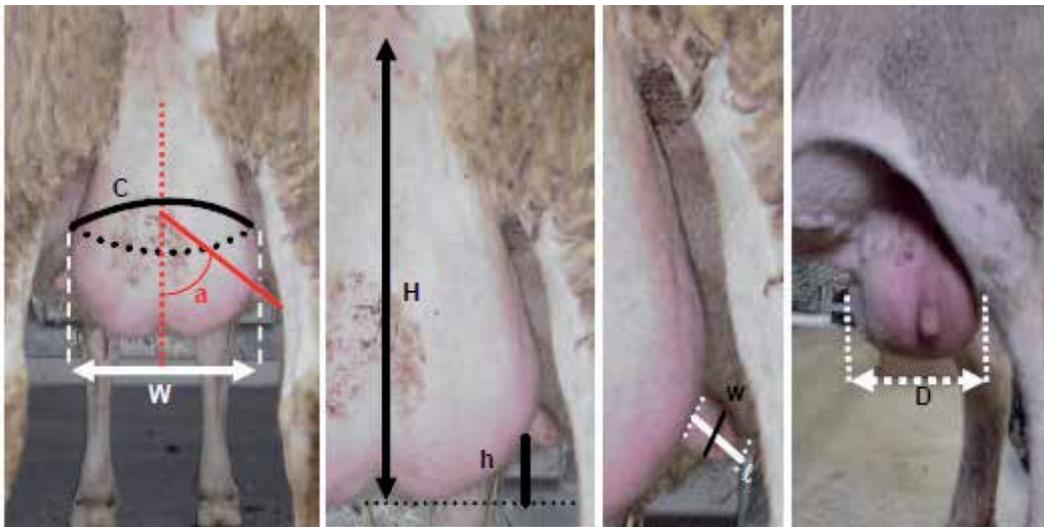
In the Alpine and Saanen breeds high frequency pulses (90 and 120 cycles/minute with a high pulsator rate of 60%) reduced the milking time (higher average milk flow rate), while low frequency pulses (60 cycles/minute) and pulsator rate of 50% lengthened the milking time and decreased the average milk flow rate (Billon et al., 2005). The milking installation in the milking parlor mounted at more than 1.26 m from the level of the position is the high-level system (HL), and below 1.25 m – midlevel (ML), while a milk pipeline placed below the position of the milked sheep is regarded as the low level milking system (LL). ML and LL milking systems in Manchega ewes do not have a significant influence on milk production, the volume of individual milk fractions, the frequency of falling teatcups, milking time, somatic cell counts or milk composition (Diaz et al., 2004). A stable vacuum in the milk system milk and a pipeline diameter of 76 cm positively influenced the milking routine, that was not interrupted by linear slips (Caria et al., 2008). In Assaf, Awassi, Churra and Castelana ewes, being hand- and machine-milked, log BTSCC was higher in hand-milked (6.07) than in machine-milked animals (5.94). In ewes milked in the ML and LL systems log BTSCC was 5.88 and 5.94, respectively, and that was less than in the bucket system (6.04). High frequency pulses (180 cycles/minute) and low levels of vacuum are considered equally important as the optimum conditions of ewe milking determining udder health (Gonzalo et al., 2005). In dairy goats of the Alpine and Nubian breeds the milking system (machine, bucket and hand milking) did not significantly influence log SSC, which was 5.94; 5.97 and 6.01, respectively (Zeng & Escobar, 1996). In that study milk of hand-milked goats was more infected (standard plate count, log SPC  $3.62 \text{ cfu/ml}^{-1}$ ) than that of machine-milked and bucket-milked goats, for which log SPC was  $2.97 \text{ cfu/ml}^{-1}$  and  $2.44 \text{ cfu/ml}^{-1}$ , respectively. The SPC results in bulk-tank milk of small ruminants are significantly influenced by the month of sample collection, the number of milkings contained in the bulk-tank, milking technique and flock size. The introduction of the milking machine in place of hand milking in dairy ewes will improve the work conditions of breeders, because the milking procedure accounts for 40-50% of all the work on the farm. The mechanization of milking not only improves the working conditions of farmers, but also improve the hygienic quality of milk (Sinapis, 2007). The main indicators of milking performance of sheep are the proportions of various milk fractions, i.e. milked, stripping and residual milk fractions. These values depend on the breed, routine milking and technical characteristics of the milking equipment, and are as follows: milk from the milking of 60 to 75, and stripping of milk from 10 to 20 and residual milk from 10 to 15% (Labussi re, 1988; Fern andez et al., 1999; Molina et al., 1999). In a study by McKusik et al. (2000) the waiting time to the onset of milk flow in crossbred East Friesian ewes in mid-lactation (90 days) was determined to be 13.1 s, while the peak milk flow rate (ml/minute) was found after 34.3 s milking. According to those authors the time of milk flow is 105.9 s (0.92 l), time of machine stripping is 26 s (0.22 l) and the total milking time (without overmilking time) is 132 s (1.14 l), respectively. In a study on Saanen goats by Bruckmaier et al. (1994) the waiting time to the onset of milk flow after attaching the clusters on teats was 12.09 s, and was characterized by low variability ( $\text{sd}=0.45 \text{ s}$ ) at individual milkings. The average milk flow rate is significantly correlated with milk yield and the stage of lactation (Mottram et al., 1994). In Alpine goats the waiting time for milk flow was 12.8 s, and time milk flow was 236.3 s. Small ruminants

are generally milked in groups, thence homogeneity in milkability of the whole flock is also essential (Casu et al., 2008). The main objective of machine milking in small ruminants is to obtain large quantities of valuable milk in a short time with few manual interventions (McKusik et al., 2003). Manual or machine stripping is not widely used in Lacaune ewes in France, while it is practiced in most dairy ewes in the United States and Canada (McKusik et al., 2003). The amount of milk obtained from stripping, i.e. the massage of the udder with the clusters still attached, depends on the breed of sheep, formation of the udder (morphology traits), lactation rank, and vacuum level, and accounts for 10 to 30% of the average amount of milk yield (Labussière, 1988). Stripping milk yield depends also on blood oxytocin concentration during milking (Bruckmaier et al., 1997). The factor limiting the use of one or two milkings a day in dairy ewes should be the capacity of the cisternal storage (Negrão et al., 2001). It seems that the release of oxytocin is not a limiting factor in milk production when high milking frequencies are used. Increasing the frequency of milkings from 5 to 7 a day gave modest gains in milk production, hence probably the limiting factor was the milk synthesis rate. The results of a study by Negrão et al. (2001) indicate that a better interaction between continuous milk synthesis and alveolar milk ejection was found at 3 milkings daily. Given the profitability of milk production from dairy ewes and goats the results suggest that an increase of milking frequency can improve the income from milk production in many breeds. In New Zealand in view of the considerable amount of work connected with double milking the afternoon milking and machine stripping were abandoned in ewes of such breeds as Poll Dorset, Romney, Coopworth and Perendale (Knight & Gosling, 1995). In East Friesian dairy ewes the omission of machine stripping during mid- and late lactation reduced milk yield by approx. 14%; however, it did not affect milk composition, lactation length or SCC (McKusik et al., 2003). Such an action improves parlor throughput by eliminating machine stripping and significantly decreases the incidence of overmilking. Through a simplified procedure and milking routine additional ewes may be added to the flock, which can compensate for potential losses in milk production. In crossbred East Friesian ewes the frequency of milkings was associated with the system of rearing lambs, and the periods between milkings  $\leq 16$  hours during the mid- and late lactation did not affect significantly the yield and composition of milk (McKusik et al., 2000). In Murciano-Granadina goats, regardless of differing periods between milkings (8, 16 and 24 hours), milk production in the alveolar fraction increased after milking time from 8 to 16 hours, but a longer interval did not result in any increase in milk production. The glandular area index for follicular sinuses (measured by ultrasound) increased with the length of the periods between milkings (from 57 : 43 to 75 : 25), but the difference was significant at an 8-hour interval between milkings (Salama et al., 2004). A single daily milking in goats of this breed moderately reduced milk yield, whereas it did not affect milk composition and udder health. Due to the higher losses in milk production, a single milking is not recommended for goats in early lactation and for dairy goats at less than four parities (Salama et al., 2003). Increasing milking frequency from once to twice a day in Tinerfeña dairy goats admittedly statistically significantly affected machine stripping milk (MSM) and residual milk (RM) and fat content; however, it did not improve milkability and did not affect milk yield (Capote et al., 2008). Machine milkability of dairy ewes tends to deteriorate

with the lactation stage and parity. Milk emission traits show a high individual variability, suggesting that they are genetically determined and can be improved through selection (Casu et al., 2008).

#### 1.4. Udder morphology and machine milking ability

Healthy and well-shaped udders of small ruminants, suitable for machine milking, should have the following characteristics: a great volume, a globose shape and clearly defined teats, soft and elastic tissues, with palpable cisterns inside, moderate height, not surpassing the hock, a marked intermammary ligament, and teats of medium size, implanted near the vertical position (Labussière, 1988). Figure 1 illustrates mammary traits, in the rear and lateral view (Rovai et al., 2004).



**Figure 1.** Mammary traits, rear and lateral view. **C:** udder circumference, **a:** teat angle, **W:** udder width, **H:** udder depth, **h:** cistern height, **l** and **w:** length and width of the teat and **D:** udder depth

Lactation stage significantly influences the dimensions of the udder in small ruminants (Fernández et al., 1995). On the other hand, the impact of breed on the length of the udder and the distance between the teats was not significant; similarly, there was little effect of lactation on the teat angle and length of the udder. Breed and parity of animals are significantly associated with the length of teats, and with the width and height of cisterns (Labussière, 1988). Positive and significant correlations between morphology traits of the udder are included in the evaluation of the udder and selection of animals (Fernández et al., 1995). Valuation of the udders is performed as part of testing in ewes of the following breeds: Churra, Manchega, Latxa and Lacaune (Marie-Etancelin et al., 2005). Cisternal size and udder morphology traits are correlated with milk secretion rate and milk emission kinetics during machine milking in dairy ewes (Labussière, 1988; Marnet & McKusik, 2001; Ayadi et al., 2011). As it was stated by Ayadi et al. (2011) in Tunisia Sicilo-Sarde ewes are adapted to machine

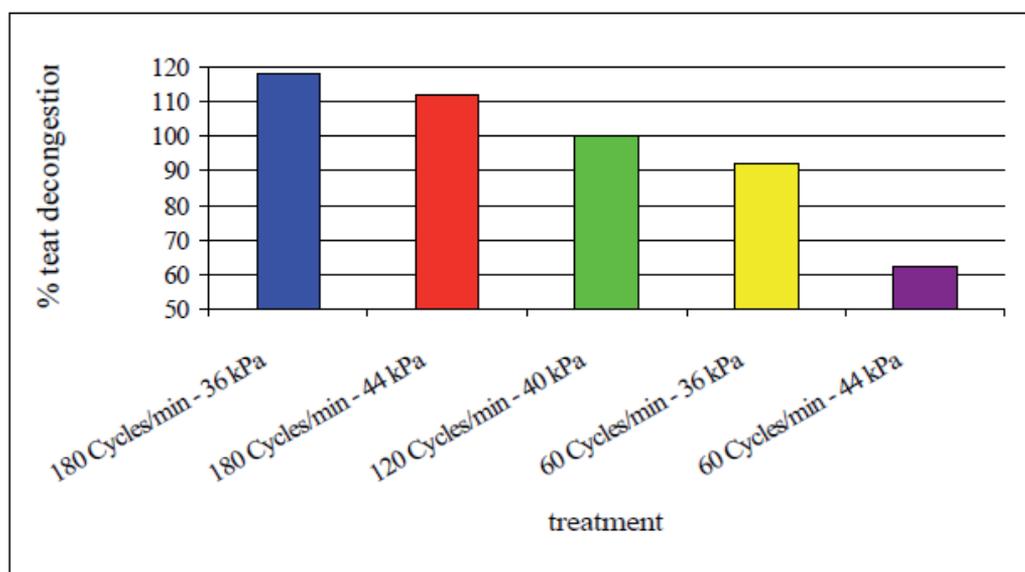
milking in terms of the morphological traits of the udder, because of their medium-sized cisterns and teats. According to those authors udder morphology traits showed positive correlations with milk yield. Another study also showed a moderate association between the udder measurements and milk production in Frizarta dairy sheep (Kominakis et al., 2009). In ewes of that breed the most important predictors for milk yield include udder circumference, udder width, udder height and teat length. Associations of morphological traits of the udder with quantitative and qualitative milk production and milking ability are of particular importance to machine milking of ewes. Similar conclusions are presented in a study by Iñiguez et al. (2009) for two Awassi sheep genotypes and their crosses. Genetic correlation coefficients for Latxa ewes between traits of the udder in the first and following lactations, ranging from 0.85 to 0.95, indicate that they are almost identical. Genetic correlations between milk yield and the depth of the udder, suspension of the udder and the location and size of the teat amounted to 0.43, 0.10, - 0.25 and - 0.10, respectively (Diaz et al., 2004). Genetic correlations between somatic cell counts in milk in the evaluation scoring (somatic cell score - SCS) and the depth of the udder, suspension of the udder, and the location and size of the teat were 0.10, - 0.27, - 0.01 and 0.29, respectively. In another study it was found that linear udder traits present a favorable genetic correlation with SCS (Casu et al., 2010). A well-formed udder in sheep is less prone to subclinical inflammation. Ewes with large and vertically located teats, as well as those with a greater amount of milk obtained from machine stripping (portion machine stripping - PMS) had a higher log SCC. In PMS a significant effect was found for teat size ( $r_p = 0.177$ ) and suspension of the udder ( $r_p = - 0.205$ ), and form of the udder ( $r_p = - 0.141$ ) (Legarra & Ugarte, 2005). Few significant relationships between suspension of the udder and milk production suggest that in recent years there has been a deterioration in some of the characteristics, especially the depth of the udder and suspension of the udder (Legarra & Ugarte, 2005; Marie-Etancelin et al., 2005). It is possible, however, to combine important morphological traits of the udder with its health status in a selection index (Marie-Etancelin et al., 2005). More recent data show relationships between udder morphology traits and udder health (Casu et al., 2010). According to those authors ewes with deep and pendulous udders and with high implanted teats are more prone to udder inflammation or *mastitis*. Bearing in mind the hygienic status of the flock, these traits of the udder should be an indication for culling of these sheep from the flock. Dzidic et al. (2004) in their study stated that the capacity of the udder in Istrian ewes significantly affects the volume of milk production, milking time, average and peak milk flow rates, whereas the teat angle has a negative effect on all traits characterizing the course of machine milking in ewes). As it was reported by Peris et al. (2003a, 2003b), Murciano-Granadina goats rearing twins had a larger capacity of the udder (2.86 l) and a shorter teat-floor distance (23.57 cm) than in the case of nanny goats rearing single kids, where it was 1.60 l and 26.39 cm, respectively. According to the above mentioned authors, the capacity of the udder is positively correlated with body weight ( $r_p = 0.80$ ) and milk production ( $r_p = 0.69$ ). Those authors also found phenotypic positive correlations between teat length and milk flow rate ( $r_p = 0.55$ ) and between residual milk yield and teat length in early and mid-lactation ( $r_p = 0.47$ ), and teat diameter ( $r_p = 0.58$ ). Murciano-

Granadina goats are adapted to the milking machine and do not require increased selection criteria. Selection of Lacaune ewes should improve milking traits, and consequently increase milk production, while the latency time is shortened and the phase of high flow is extended, thereby improving milking ability (Marie-Etancelin et al., 2006). The key adaptations of sheep to the milking machine include the relatively high occurrence of bimodal and plateau milk flow curves, which are very important in the assessment of milkability, because it is assumed that they ensure milk ejection during milking (Mačuhova et al., 2008). Such observations were conducted on Tsigai sheep, improved Valachian sheep and their crosses with the Lacaune breed. One can assume that in around 69% milkings the sheep released oxytocin during machine milking. The assessment of milkability in these breeds of sheep delivers complete and precise information on milk production related to milk flow kinetics throughout lactation in the East European breeds (Tančin et al., 2011). High production of milk and similar average and peak flow rates were characteristic of Istrian crossbred ewes with an advantageous udder shape, which shows good adaptation to the milking machine (Dzidzic et al., 2004). In that study the udder volume of those sheep positively influenced all milking characteristics however, the teat angle had a negative effect on milking time and milk yield, but not milk flow rate. The milk flow and udder morphology traits mainly influenced milking efficiency and milk yield, while Istrian dairy sheep should be included in breeding programs for further improvement of their milk yield. Ewes with a more horizontal teat position and larger teats had higher SCC. These ewes are more prone to develop subclinical *mastitis* (Margetin et al., 2005). Ewes producing more milk within 30 and 60 s (with a quicker milk ejection) had lower log SCC. Conversely, ewes with a higher machine stripping rate had higher log SCC. The level of milk production in high-yielding dairy sheep depends mainly on the capacity of their udder cisterns and greater storage of milk between milkings (Rovai et al., 2008). In that study ewes of two breeds, Manchega and Lacaune, produced approximately the same amount of alveolar milk; however, Lacaune ewes had greater volumes and larger areas of the cisterns, and greater milk yield when compared with Manchega ewes. According to those authors, their results reduce the importance of the alveolar and indicated a greater role of the udder cisterns. For a thorough assessment of udder compartments in high-yielding ewes, such as the Lacaune sheep, in order to prevent spontaneous milk ejection when entering the milking parlor an oxytocin receptor blocking agent should be used. The occurring genetic antagonisms between udder conformation and milk production in primiparous Lacaune ewes suggests that it should be taken into account in the current selection the udder-type traits, the more that there were positive associations between udder traits and SCC in milk (Marie-Etancelin et al., 2005). Results indicated a possibility to combine four traits for conformation and health in a global udder selection index.

### **1.5. Adverse consequences of machine milking**

Irregular and cyclical fluctuations in the vacuum level in the milking cluster result in a situation when the drain stream of milk hits back with some force at the end of the teat, or even milk is pumped back to the teat canal, transferring pathogens. Mainly during machine stripping the air getting into the milking cluster results in a stroke back of milk, which also

occurs when the milking clusters are removed from the udder without the vacuum being shut down. In France, over 50% of farmers practiced sporadically machine stripping, and 65% did not turn off the vacuum before removing the clusters from the udder (Bergonier & Berthelot, 2003). The use of machine stripping in small ruminants causes a significant increase of overmilking time, which occurs in 33% and 92% ewes, at two and single milkers, respectively (McKusik et al., 2003). Extended milking time results in a daily interaction of overmilking on the teat tissue and the simultaneous entry of milk into the teat canal, transferring bacteria (Molina et al., 1999; McKusik et al., 2003). Overmilking causes congestion, swelling and dysfunction of the sphincter muscle of teats, and damages the epithelium and the mouth of the teat canal (Bergonier & Berthelot, 2003; Bergonier et al., 2003). This results in a limited capacity to produce keratin by the epithelium of the teat canal and an incomplete closure of the teat canal (Bergonier et al., 2003). In the California Mastitis Test increasing the overmilking time in two groups of machine-milked sheep at different pulse frequencies and different levels of vacuum (180 cycles/minute; 34kPa, and 120 cycles/minute; 40 kPa) did not influence the health status of the udder. A two-fold higher SCC, however, was found in the milk of ewes milked at a lower frequency of pulsation and a higher level of vacuum,  $1598.8 \times 10^3$  cells/ml and  $770.4 \times 10^3$  cells/ml, respectively. At a high frequency of pulsation (180 cycles/minute) and different levels of vacuum (36kPa and 42 kPa) the time of overmilking extended from 1.5 to 2 minutes in the half of the udder throughout 35 days did not influence the frequency of new infections. The percentage of new infections in halves of the udder with overmilking at vacuum levels of 36 kPa and 42 kPa was 10% and 13%, respectively, while in halves without overmilking it was 7% and 11%, respectively (Peris et al., 2003a; 2003b). A high frequency of pulsation (180 cycles/minute) and a low level of vacuum (36 kPa) significantly decreased irritation of the teat end during milking (Fig. 2).



**Figure 2.** A relationship between teat end condition and pulsation rate and working vacuum level (Marnet, 2002).

Many observations conducted in France on different breeds of sheep showed that pulsation rate should not be less than 150 cycles per minute, while 180 cycles/min should be the pulsation rate applied for high-yielding ewes, such as the Lacaune breed. To improve the quality of machine milking in ewes of the Greek Boutsiko breed, the use of vacuum level of 38 kPa is better, wherein machine stripped milk % is low and irritation of teats is reduced, without affecting the health status of the udder (Sinapis et al., 2006). The results indicate that the low vacuum level of 16.5 kPa is required for the opening of the teat sphincter in ewes of this breed (Sinapis et al., 2007). However, low vacuum significantly modifies the kinetics of milk ejection. The vacuum level of 28 kPa causes an extended latency time for the first milk emission, reduces the average milk flow rate and peak flow rate, and prolongs by about 17% the milking time for milking of a single ewe (Caria et al., 2008). In small ruminants SCC is a good indicator of subclinical inflammation of the udder. Healthy mammary glands in ewes and goats in regular studies during lactation should produce less  $500 \times 10^3$  cells/ml of milk (Bergonier & Berhelot, 2003). For Murciano-Granadina goats at such a physiological threshold only 62.3% of milk samples could be correctly classified. At the level of the flock (SCC above 1 million/ml) BTSCC was a good indicator detecting the degree of mammary gland infections ( $r^2 = 845$ ). During the drying-off period in ewes and goats an antibiotic therapy effectively reduces the proportion of infected mammary glands (Bergonier & Berhelot, 2003). Regular maintenance and inspection carried out every year in terms of the technical parameters of milking machines significantly contribute to the health of the udder and hygienic quality of milk; however, in a survey of milking machine maintenance in different countries Billon et al. reported that only 40-60% of milking machine installations are controlled every year (Billon et al., 2005). The etiological factor of infection in mammary glands in small ruminants is connected with staphylococci (Ameh & Tari, 2000; Bergonier et al., 2003). *Staphylococcus aureus* is the most common organism isolated from milk in cases of clinical mastitis, while in sub-clinical mastitis it is coagulase-negative staphylococci (CNS). According to Bergonier & Berhelot (2003), the proportion of clinical mastitis typically does not exceed 5% and it is associated with the start of the milking machine, whereas the percentage of subclinical inflammation ranges from 10% to 50%. The causes of damage to the end of the teat in small ruminants during lactation (apart from the overmilking time) include machine stripping and the period of suckling when lambs or kids try to suckle other females (Bergonier & Berhelot, 2003). Halves of udders with damaged teats are infected more frequently when compared with the halves with healthy teats (Ameh & Tari, 2000; Bergonier & Berhelot, 2003). In goats teat size and their distance from the floor do not influence the percentage of subclinical inflammation in the mammary glands (Ameh & Tari, 2000). Omission of machine stripping in the middle and late lactation decreases milk production by 14%; however, it eliminates the incidence of overmilking time. The elimination of machine stripping does not affect milk composition, length of lactation, or SCC in milk, while it increases the number of milked sheep from 15 to 28/h, at one or two milkers, respectively (McKusik et al., 2003). In Poll Dorset ewes omitted afternoon machine milking and machine stripping in the 8-week period of milking (Knight & Gosling, 1995). The consequence was a decrease of 19.4% (7.9 l/ewe) in milk production in that period. For Murciano-Granadina goats the reduction in the frequency of milkings from 2x to 1x daily

had a negative quantitative and qualitative effect on the milk production and health status of mammary glands (Mottram et al., 1994).

## **2. Machine milking of small ruminants: based on the results of a study by Olechnowicz (2006)**

### **2.1. Introduction**

The rationale of this study is to determine the relationship between the organization and course of milking sheep and goats on the Zlotniki farm belonging to the University of Life Sciences in Poznan and qualitative and quantitative levels of milk production. In this farm one milker operates six milking clusters, which can have a significant impact on the course of milking, mainly through prolonged overmilking time. Such an organization of milking may result in an increased SCC in milk, infection of mammary glands, and teat-end damage in small ruminants. The results obtained can be used in practice, the recommendation for the milking milker to shorten or to eliminate overmilking time. On the other hand, the results will be used when selecting individuals to operate milking machines. The aim of the study was to evaluate milking of small ruminants and its effect on some characteristics of milk (SCC and composition). Additional objectives were to determine the influence of some factors on the course of milking, assess the degree of teat end damage and contamination of milk, evaluate dimensions of the udder and their relationship with the course of milking. Correlation coefficients between the phenotypic traits of the udder and milking characteristics were also calculated. Such ratios were also calculated between parameters of machine milking course and morphological traits of the udder and selected milk characteristics.

### **2.2. Material and methods**

The study was conducted at the Zlotniki farm (Poznan University of Life Sciences, Poland). On the farm machine milking is performed on dairy sheep of line 05 with the shares of East-Friesian genes in their genotype amounting to 50 – 75%, and 76 – 90% (13/16 East-Friesian sheep and 3/16 Polish Merino), a prolific ewe sheep 09 (25% of the Finnish sheep, 31% East-Friesian and 44% Polish Merino), white-headed and black-headed sheep, and meat and dairy goats of the White Improved breed. In the years 2000 - 2002 a total of 755 sheep and 207 goats were analyzed. Ewes were milked for 16 weeks in two calendar seasons: spring - summer (March to June) and summer - autumn (July to October). Milking was performed on ewes with clinically healthy udders, after two months of lamb nursing. Milking performance of ewes was tested at monthly intervals and milk production was described from both morning and evening milkings (ml); additionally, milk samples were collected before the morning milking for laboratory testing. Nanny goats with clinically healthy udders were selected for milking after 70 days of rearing kids. Goats were milked from May till November. At monthly intervals each year six milking tests were carried out, with measurements of both morning and evening

milking production (ml). Milk samples were collected for laboratory analyses before the morning milking. The technical condition of the milking machine was tested annually in accordance with the Polish Standards. The measurements of pulsation frequency and pulsator coefficients were taken with the use of a Milko Test 2000 electronic pulsograph (Bilgery). Ewes and goats were milked in 14 and 11 stands, respectively, of a milking parlor (Westfallen). The ewes were milked at milking vacuum of 41 kPa, the pulsation rate of individual clusters ranged from 121.7 to 126.7 pulses/min and pulsation ratio was  $50 \pm 5\%$ . Milking of goats was conducted at a vacuum level of 41 kPa, pulsation rate of 69.9 to 76.7 pulses/min and pulsation ratio of  $60 \pm 5\%$ . During three or four sheep milking performance tests, as well as two and five goat milking performance tests, milk samples of approximately 15 ml each were collected for bacteriological tests under sterile conditions. Directly after the samples had been collected they were cooled down to the temperature of  $4^{\circ}$ , and next, after freezing ( $- 20^{\circ}\text{C}$ ), they were delivered to the microbiological laboratory of the State Veterinary Institute in Pulawy (Branch in Bydgoszcz). Microbiological determination was carried out according to the laboratory diagnostics of *mastitis*. At each milk test day before the morning milking, after fore-stripping, washing and drying of the teats, approximately 50 ml of milk (preserved with a CC preparation) were collected from ewes and nanny goats from their udder halves in order to determine the percentage contents of fat, total protein and lactose, as well as SCC. Analyses of milk samples were carried out at the Laboratory of Milk Evaluation in Krotoszyn. The basic milk composition was determined with the use of a MilkoScan apparatus, while SCC was analyzed with Fossomatic appliances. All parameters were measured to monitor the course of milking in seconds. Measurements of activities related to milking were taken every month for 3 days (milk test day  $\pm 1$  day) during the morning milking. Measurements of the duration of individual milking tasks (the time of milk flow from udder halves, the time of overmilking of udder halves, the time of machine stripping, and the total milking time) were recorded using electronic timers for the same three-person team. One person supervised milking time simultaneously in two sheep/goats. Udder zoometric measurements were carried out on sheep and goats on milk test days accurate to 0.1 cm applying both zoometric callipers and a measurement tape. The following parameters were measured in ewes and nanny goats: length, width, depth and circumference of udder, teats dimensions and teat length. Additionally, in goats the distance of teats from the floor was measured and teats were evaluated in terms of their morphology, distinguishing the following types: cylindrical, funnel-shaped, pear-shaped and bottle-shaped. Directly after milking the clinical condition of the teat ends was examined, adopting the following criteria: teats without injuries – canal of teat impalpable, flabby and thin, teat end injuries of the first group – livedo or white ring in the region of escape of teat canal; the teat canal lightly pachynsic and perceptible, teat end injuries of the second group – escape of teat canal enclosed with grommet pachyepidermi, hypertrophy of the epidermis and the circular muscular layer; the canal of teat more perceptible. Winter feeding of ewes was based on haylage, maize silage, mangolds, meadow hay, and all-mash, while the basis for summer feeding of sheep was green fodder from lucerne and cereal grain. The estimated nutritive value of the ration for milked ewes

was 11 MJ energy and 320 g crude protein in 2.3 kg DM. During lactation goats were fed green lucerne forage (7-8 kg), concentrate (0.8 kg), meadow hay (0.3 – 0.5 kg) and fodder straw (0.4 kg). The study used mathematical models, applying the statistical package by SAS, Version 8 (2000).

## 2.3. Results

### 2.3.1. *Parameters of the course of milking*

The results of measurements of the course of machine milking in ewes and goats are shown in Table 1. In the groups of ewes with a greater number of somatic cells (over 250 000 in 1 ml), shorter times of milk flow from both halves of the udder were reported, overmilking time was the longest in the groups of sheep with injuries to the end of the teat. In these ewes longer milking times were also observed. In the course of machine milking in ewes observed high variability of the studied traits, which indicates the necessity of improving the ewes (by selection) towards better milkiness.

The time of milk flow and time of overmilking depend on a calendar year, and indicate a variety of external environmental conditions in particular years. The level of nutrition of ewes, as well as the quality of feed were also likely to vary in subsequent years. Ewes' milking season (March-June and July-October) had a significant effect on the time of milk flow from both halves of the udder, and the probable cause was the quality of feed used in the nutrition of ewes in both milking seasons. The month of lactation had a significant effect on all parameters of the course of machine milking. At the start of milking longer times of milk flow from udder halves, and longer times of overmilking from both halves were observed, which resulted in a simultaneous extension of stripping and milking times. It should be noted that the time of stripping was prolonged with the number of lambs reared, and was longer in the ewes with the number of somatic cells of less than 250 000 in 1 ml. This table contains also the values of these parameters in the groups of goats evaluated for the degree of teat end injuries, the degree of milk contamination and the type of teats. The descriptive statistics of the parameters characterizing the average milking in goats show large standard deviations, indicating a lack of improvement towards milking performance. On the other hand, the long and variable time of overmilking for the halves of the udder depends on the organization of machine milking (one milker operates 5 clusters), and the fact that nanny goats occupy different positions during subsequent milkings. Variability in assigning milking stations to the animals and the sequence of goat milking results in an increase or decrease in overmilking time. The time of milk flow, stripping time and milking time were significantly different in both years of the study (2001 and 2002). External environmental conditions affect the course of milking in goats, mainly through the variable forage base resulting from weather conditions. In the successive months of lactation there were no statistically significant differences in milk flow time, overmilking time, stripping time, and milking time. Nursing a greater number of kids by nanny goats had a considerable effect on the prolonged milk flow time and extended the milking time.

Milking parameters in ewes (s)								
Somatic cell count in 1ml	Number of udder halves	Milk flow from udder half		Overmilking of udder half		Stripping of udder	Milking	
		left	right	left	right			
≤ 250 000	474	56.7±23.2	55.6±23.7	147.4±110.2	145.9±111.5	33.7±18.9	235.1±116.2	
> 250 000	281	53.9±26.8	51.7±28.6	131.1±111.0	133.2±110.8	30.0±16.1	215.3±117.8	
Total	755	55.0±24.7	54.2±26.1	141.4±110.6	141.2±111.1	32.3±18.1	228.0±118.1	
Case-sensitive degree of teats end injuries								
≤ 250 000	168	49.9±21.5	47.3±21.9	204.6±149.1	196.3±113.0	37.2±22.8	228.0±132.6	
> 250 000	81	37.1±16.2	36.3±15.7	211.7±114.6	212.3±106.9	33.6±30.2	283.6±120.9	
Total	249	45.7±20.2	43.7±20.4	206.9±136.5	201.5±110.2	36.0±25.7	286.6±126.8	
Case-sensitive degree of milk infection								
≤ 250 000	120	59.2±32.8	52.9±38.6	143.3±118.2	145.3±122.9	32.5±18.5	236.4±182.3	
> 250 000	60	46.1±26.7	49.2±34.9	151.4±97.4	148.4±93.6	27.0±12.7	224.9±100.4	
Total	180	54.8±31.8	51.6±30.2	146.0±110.9	146.3±113.8	30.7±17.0	231.8±118.3	
Milking parameters in goats (s)								
	207	129.2±46.7	116.3±48.2	95.9±98.7	107.9±101.4	33.3±24.6	258.5±99.3	
	414	122.7±47.1		101.9±97.3		33.3±23.7	258.5±99.3	
	Case-sensitive degree of teat end injuries							
	92	106.8±49.7	96.0±46.3	100.3±87.2	109.3±93.0	26.7±20.2	234.3±106.0	
	184	101.4±47.3		104.8±88.3		26.7±19.9	243.3±103.3	
	Case-sensitive degree of teat end injuries							
	67	126.3±53.4	107.3±45.8	102.2±113.8	121.2±117.3	32.4±23.4	261.0±122.3	
	134	115.9±50.3		110.9±114.1		32.4±22.5	261.0±119.9	
	Case-sensitive types of teats							
	184	101.4±47.3		104.8±88.3		26.7±19.9	243.3±103.3	

**Table 1.** Descriptive statistics of ewes' and goats milking course parameters (arithmetic averages in seconds ± standard deviation)

The results of the effect of selected factors, including injuries to the teat end of milked ewes and goats are given in Table 2. The degree of injury to the end of the teats in ewes in the group with the number of somatic cells below 250 000 in 1 ml had no effect on the course of milking; however, longer times can be seen trailing the times of milk flow and overmilking of teat end injuries. In the second group of ewes (SCC above 250 000/ml) a significant difference was found in the time of overmilking in right halves between the ewes with teat injuries from the first and second groups, amounting to 172.5 and 359.0 s, respectively. The long time of overmilking was associated with a significantly longer time of milking in ewes. In all the groups of sheep longer times of milk flow were recorded in halves with teat end injuries (especially with injuries of the second group) when compared with halves of the udder, which teats sustained no injuries. At the same time shorter times of machine stripping were found for halves with teat end injuries. The longer times of milk flow from halves with teat injuries may indicate disturbances in the milk flow, which may be due to

internal damage of the teat canal. The long times of overmilking are caused by injuries of teat ends.

Factor	Milking parameters in ewes (s)							
	Milk flow from udder half		Overmilking of udder half		Stripping of udder halves		Milking of udder halves	
	left	right	left	right	left	right	left	right
Somatic cell count in milk below 250 000 in 1 ml (n = 336)								
Season of milking	**	**	ns	ns	ns	ns	ns	ns
Month of lactation	**	**	ns	ns	ns	ns	*	*
Number of reared lambs	ns	ns	ns	ns	ns	ns	ns	ns
Breed of ewes	ns	**	ns	ns	*	**	**	**
Teats end injuries of:								
- the first group	52.5	55.2	212.7	189.7	37.1	36.0	300.6	290.2
- the second group	59.3	49.9	264.3	212.1	24.1	36.2	258.1	299.1
- teats without injuries	47.5	43.6	192.7	196.4	39.0	37.8	287.0	285.3
Somatic cell count in milk above 250 000 in 1 ml (n = 162)								
Season of milking	**	**	ns	ns	ns	ns	ns	ns
Month of lactation	*	ns	*	*	**	**	*	*
Number of reared lambs	ns	ns	ns	ns	ns	ns	ns	ns
Breed of ewes	ns	ns	ns	ns	ns	ns	ns	ns
Teats end injuries of:								
- the first group	51.1	40.1	211.3	172.5 <sup>A</sup>	36.8	26.7	298.4	239.6 <sup>A</sup>
- the second group	59.1	44.7	263.8	359.0 <sup>A</sup>	23.1	9.3	260.7	413.0 <sup>A</sup>
- teats without injuries	46.2	35.2	194.1	213.5	38.7	36.1	287.1	286.4
Milking parameters in goats (s) n = 92								
Teat end injuries of:	ns	ns	ns	ns	ns	ns	ns	ns
The first group	105.8	92.0	103.0	116.3	28.2	30.6	235.7	240.6
The second group	90.5	89.0	193.5	195.3	37.0	37.0	321.0	321.0
Teats without injuries	108.1	99.3	95.2	100.6	25.5	23.4	230.2	226.0
Month of lactation	*	**	*	*	ns	ns	**	**

**Table 2.** The effect of teat end injuries on the course of milking in ewes' and goats. Means in columns designated with identical capital letters differ significantly at  $P \leq 0.01$ ,  $*P \geq 0.05$ , ns – non-significant difference

Despite the small number of udder halves with teat end injuries of the second group ( $n = 2$ ), the overmilking time for those halves was doubled when compared with halves of the udder and teats without injuries. No statistically significant impact of injuries to the teat end on the course of machine milking could be explained by large standard deviations and the small number of half exchange with teat end damage.

The degree of microbial contamination of milk in the first and second groups did not significantly influence the course of machine milking in ewes (Table 3). The degree of microbial contamination of milk by major and minor pathogens did not affect significantly the parameters of machine milking in ewes. However, longer times of milk flow from infected halves of the udder were reported when compared with the times of milk flow from healthy halves of the udder. The reason for the lack of significant differences in milking of ewes from these groups might have been connected with the small number of sheep, whose milk was contaminated by the major pathogens.

Milk contamination with microorganisms:	Number of udder halves		Milking parameters in ewes (s)							
			Milk flow from udder half		Overmilking of udder half		Stripping of udder halves		Milking of udder halves	
	left	right	left	right	left	right	left	right	left	right
Somatic cell count below 250 000 in 1 ml (n = 240)										
- major pathogens <sup>1</sup>	6	2	78.7	89.0	140.3	160.0	25.7	13.5	244.7	262.5
- minor pathogens <sup>2</sup>	37	43	66.6	57.4	139.4	134.3	34.9	34.0	241.9	237.6
- milk samples without bacterial growth	77	75	54.1	49.7	145.4	151.7	31.9	31.6	231.4	233.5
Somatic cell count above 250 000 in 1 ml (n = 120)										
- major pathogens <sup>1</sup>	1	2	11.0	57.0	164.0	165.0	22.0	22.5	197.0	244.5
- minor pathogens <sup>2</sup>	30	27	52.7	43.4	148.0	151.8	29.1	29.1	227.4	224.1
- milk samples without bacterial growth	29	31	40.5	53.7	154.5	144.4	25.4	25.4	223.2	224.3
Total (n = 360)										
- major pathogens <sup>1</sup>	7	4	69.0	73.0	143.7	162.5	25.1	18.0	237.9	253.5
- minor pathogens <sup>2</sup>	67	70	60.4	52.0	143.2	141.1	31.2	32.1	235.4	232.4
- milk samples without bacterial growth	106	106	50.3	50.9	147.9	149.5	30.7	29.8	229.2	230.8
Milking parameters in goats (s) n = 67										
- major pathogens <sup>1</sup>	9	5	95.6	120.4	104.1	133.4	28.0	38.4	228.3	292.2
- minor pathogens <sup>2</sup>	12	12	129.7	126.3	80.2	131.4	42.8	49.2	252.7	307.0
-milk samples without bacterial growth	46	50	113.4	101.5	107.6	117.5	30.6	27.8	269.5	246.8

**Table 3.** The effect of degree of contamination in milk from udder halves on the course of milking in ewes. <sup>1</sup>Staphylococcus aureus and Escherichia coli, <sup>2</sup>Coagulase negative staphylococcus, Micrococcus, Corynebacterium and Bacillus.

Similarly, there was no significant effect of the contamination of milk on the course of milking in goats. Milk flow time and stripping time were found to be about 15 seconds longer in case of the halves infected by minor pathogens when compared to the healthy halves of the udder, but these differences are not statistically significant, probably due to the high variability of parameters that characterize milking.

### 2.3.2. Milk composition, somatic cell count and milk yield

The results concerning the effect of overmilking time on the number of somatic cells in milk, milk composition and the level of small ruminants milkiness are shown in Table 4. Overmilking time for both halves of the udder of ewes did not have a significant effect on somatic cell counts. In early lactation of ewes (four to six weeks) longer overmilking times were associated on the one hand with longer stripping times, while on the other hand, with the organization adopted for the farm milking machine (one milker handled six milking clusters). With such an organization of machine milking the ewes, in which overmilking lasted longer, were characterized by both higher milk production and a lower percentage of fat and protein in milk. Ewes with somatic cell counts  $\leq 250\ 000$  w 1 ml a trend towards a higher lactose content and a higher milk production was observed. Evident differences in milk composition (with an increase in overmilking time) are visible in the group of ewes with lower somatic cell counts in milk (below 250 000 ml/ml). In the group of ewes with high somatic cell counts (above 250 000/ml), and including the two above mentioned groups of sheep such differences were not reported. Overmilking time of halves of the goats udder had no effect on daily milk yield, composition and SCC. Goats with a higher daily milk production were characterized by a longer milk flow and longer stripping time, as well as lower somatic cell counts in milk. In these goats, however, overmilking time was slightly longer.

In the milk of goats from udders halves with short milk flow times (up to 1.5 minutes) somatic cell count was twice as high (1 153 000/ml) when compared with milk of goats with a longer milk flow times (91-180 and 181 - 317s, 615 000 and 572 000 cells/ml, respectively). Shorter milk flow times were significantly associated with a lower daily milk production, a greater percentage of fat and protein in milk, and lower lactose content ( $P \leq 0.01$ ). Stripping time for halves of the udders was associated with daily milk production. The results indicate the need for improvement in terms of raising goats, obtaining large quantities of milk from milking, and a lower machine stripping yield. The selection should be aimed at increasing the capacity of the cisterns.

Ewes					
Trait	Overmilking time (s)				
	0 - 60	61 - 120	121 - 180	181 - 240	> 240
Somatic cell count below 250 000 in 1 ml (n = 988)					
Number of udder halves	300	176	167	132	213
Somatic cell count (log SCC)	4.70	4.72	4.77	4.68	4.68
Fat content (%)	5.17	5.59 <sup>AB</sup>	5.11	4.70 <sup>B</sup>	4.72 <sup>A</sup>
Protein content (%)	6.13 <sup>AB</sup>	6.26 <sup>CD</sup>	6.01 <sup>EF</sup>	5.68 <sup>BCE</sup>	5.66 <sup>ADF</sup>

Lactose content (%)	5.11 <sup>A</sup>	5.02 <sup>CE</sup>	5.07 <sup>BD</sup>	5.24 <sup>ABC</sup>	5.21 <sup>DE</sup>
Milk production (ml)	983.7 <sup>AB</sup>	963.8 <sup>DE</sup>	1 059.6 <sup>C</sup>	1 105.3 <sup>BE</sup>	1 218.7 <sup>ACD</sup>
Somatic cell count above in 1 ml (n = 522)					
Number of udder halves	155	121	109	56	81
Somatic cell count (log SCC)	6.10	6.06	6.13	6.15	6.14
Fat content (%)	6.12	6.20	6.35	6.43 <sup>A</sup>	5.49 <sup>A</sup>
Protein content (%)	6.55 <sup>A</sup>	6.48 <sup>B</sup>	6.43 <sup>B</sup>	6.30	6.0 <sup>ABC</sup>
Lactose content (%)	4.54	4.45	4.41	4.57	4.65
Milk production (ml)	799,0 <sup>A</sup>	754.9 <sup>B</sup>	767.2 <sup>C</sup>	804.5 <sup>D</sup>	1 042.0 <sup>ABCD</sup>
Total (n = 1510)					
Number of udder halves	455	297	276	188	294
Somatic cell count (log SCC)	5.18	5.26	5.31	5.11	5.08
Fat content (%)	5.52	5.84	5.60	5.22	4.93
Protein content (%)	6.28	6.35	6.18	5.86	5.75
Lactose content (%)	4.92	4.79	4.82	5.04	5.06
Milk production (ml)	920.8	878.7	944.1	1 015.7	1 170.0
Goats					
Number of udder halves	183	95	61	34	41
Somatic cell count (log SCC)	5.94	5.85	5.81	5.83	5.80
Fat content (%)	3.01	3.16	2.84 <sup>A</sup>	3.19 <sup>A</sup>	3.09
Protein content (%)	3.00	3.13 <sup>A</sup>	2.88 <sup>A</sup>	2.99	2.99
Lactose content (%)	4.21	4.31	4.23	4.20	4.28
Milk production (ml)	2 850.8	2 988.4	2 928.7	3 135.3	3 104.1

**Table 4.** The effect of overmilking time on milk somatic cell count and composition, and small ruminants milkiness level. Means in rows designated with the same capital letters differ significantly at  $P < 0.01$ .

### 2.3.3. Teat end injuries and milk infection

Damage to teats of ewes during machine milking can affect the degree of milk contamination. The use of machine stripping is associated with a significant increase of overmilking time. A long overmilking time with an inadequate vacuum level and pulsation ratio may influence teat ends causing injuries. Damaged teats contribute to the penetration of the mammary gland by microorganisms, that cause inflammation of the mammary glands, and milk microbial contamination. Table 5 shows the results of the effect of selected factors, depending on teat end injuries, on milk somatic cell count, composition and production.

In the group of ewes, which were characterized by a smaller number of somatic cell count ( $SCC \leq 250\ 000/\text{ml}$ ) teat end injuries did not differentiate significantly the number of somatic cells, milk composition or production levels. A greater percentage of fat and protein content in milk was observed in the milk of both halves of the udder with healthy teats. The halves of the udder from the first group of teat injuries ( $SCC > 250\ 000/\text{ml}$ ) yielded a higher percentage of milk fat, probably resulting from lower milk production. A comprehensive comparison of the results showed no effects of teat end injuries on milk somatic cell count,

Factor	Number of udder halves		SCC from udder half (log SCC)		Percentage content in mil from udder half						Milk production and degree of teat injuries (ml)	
	left	right	left	right	fat		protein		lactose		left	right
					left	right	left	right	left	right		
Somatic cell count below 25 000 in 1 ml												
Teat end injuries of:	168	168	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
The first group	45	44	4.64	5.30	4.61	4.83	5.88	5.73	5.05	4.95	1 078.9	1 126.1
The second group	15	18	4.78	5.12	4.90	4.81	5.62	5.56	5.07	4.99	965.0	1 016.7
Teats without injuries	108	106	4.76	4.96	5.25	5.64	5.87	6.00	4.98	4.84	1 068.7	1 043.4
Season of milking	168	168	ns	ns	**	**	**	*	**	**	ns	ns
Month of lactation	168	168	ns	ns	**	**	**	**	**	**	**	**
Number of reared lambs	168	168	ns	ns	*	*	*	**	ns	*	*	*
Breed of ewes	168	168	ns	ns	ns	ns	ns	ns	ns	ns	**	**
Somatic cell count above 250 000 in 1 ml												
Teat end injuries of:	81	81	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
The first group	17	13	5.98	5.94	8.18 <sup>A</sup>	7.51	6.53	6.09	3.94	4.24	570.06	600.0
The second group	4	3	5.96	5.46	4.95 <sup>A</sup>	6.19	5.85	5.99	4.29	4.77	512.5	500.0
Teats without injuries	60	65	6.00	5.58	6.16	6.71	6.46	6.40	4.51	4.23	803.3	779.2
Season of milking	81	81	ns	ns	**	**	**	**	*	ns	*	*
Month of lactation	81	81	ns	ns	**	**	**	**	**	**	**	**
Number of reared lambs	81	81	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Breed of ewes	81	81	ns	ns	*	*	*	ns	ns	ns	**	**

**Table 5.** The effect of selected factors in terms of teat end injuries on milk somatic cell count and composition, and ewes' milkiness level. Means in columns designated with the same capital letters differ significantly at  $P \leq 0.01$ . \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , ns – non-significant difference.

composition and the level of milk production; however, a marked reduction in milk yield was observed in ewes with teat end injuries in the group with higher somatic cell counts ( $SCC > 250\ 000/ml$ ). The result is a greater concentration of fat and less protein and lactose in milk. The results of the impact of teat end injuries on somatic cell count, composition and production of goats milk are given in Table 6.

Injuries to the teat end do not differentiate the log SCC, the composition and level of milk production in goats. At the same time it needs to be stressed that significant differences were found in the percentage of milk fat, at higher fat content in the right halves of the udder, while in the left it was lower ( $P < 0.01$ ). The volume of daily milk production in goats with damaged and healthy teats was similar. Month of lactation had an effect on contents of fat and protein in milk, as well as milk production; however, no such effect was observed on somatic cell count and lactose content in milk. Table 7 presents the results concerning the effect of the degree of contamination of ewe and goats milk on somatic cell count, composition and milk production.

Factor	Number of udder halves		Somatic cell count in milk from udder half (log SCC)		Percentage content in milk from udder half						Milk production (ml)		
					fat		protein		lactose				
					left	right	left	right	left	right			
	left	right	left	right	left	right	left	right	left	right	left	right	
Teat end injuries of:													
The first group	92	92	ns	ns	**	**	ns	ns	ns	ns	ns	ns	ns
The second group	35	39	5.84	6.06	3.40 <sup>A</sup>	3.55 <sup>A</sup>	3.22	3.32	4.38	4.05	2 794.3	2 835.9	
Teats without injuries	2	2	5.77	6.08	4.09	4.14	3.47	3.53	4.39	4.19	3 250.0	3 250.0	
Month of lactation	55	51	6.05	6.14	3.02 <sup>A</sup>	3.98 <sup>A</sup>	2.87	2.99	3.95	3.77	2 607.3	2 560.8	
Month of lactation	92	92	ns	ns	**	**	**	**	ns	ns	**	**	

**Table 6.** The effect of teat end injuries on somatic cell count in milk, basic milk composition and goats' milkiness levels.

Means in columns designated with the same capital letters differ significantly at  $P \leq 0.01$ , ns – non-significant difference.

Microbial infections of mammary glands cause great economic losses in dairy sheep farms due to reduced milk production and adverse changes in its composition. The incidence of clinical inflammatory conditions generally does not exceed 5%, and most micro-organisms isolated from milk are *Staphylococcus aureus* bacteria. The etiological agents in subclinical inflammation of mammary glands in most cases are *Coagulase negative staphylococci*, and especially *Staphylococcus epidermidis*. The above-mentioned results do not indicate an association between milk contamination and milk composition and productivity levels, probably due to the low number of milk samples with increased contents of major pathogens. In ewes which are characterized by a smaller number of somatic cells (< 250 000/ml) infection caused by minor pathogens were associated with a smaller increase in somatic cell counts, whereas in ewes with greater numbers of somatic cells in milk (> 250 000/ml) this increase was larger. In the milk of ewes with infected mammary glands the concentration of fat and lactose was lower, at a higher concentration of protein when compared with milk from healthy mammary glands. These ewes produced also less milk.

No significant effect of microbial milk contamination by major and minor pathogens was found on the number of somatic cells (log SCC), milk composition and daily milk production in dairy goats. Somatic cell count in milk from both halves of the udder contaminated by major pathogens was greater (log 5.93, i.e. 858 000 cells/ml and log 6.21 i.e. 1 628 000 cells/ml) than that from both halves of the udder contaminated by minor pathogens (log 5.74 i.e. 548 000 cells/ml and log 5.72 i.e. 522 000 cells/ml). In milk from healthy halves of the udder somatic cell count was similar to that in milk from udder halves infected by minor pathogens (log 5.75 i.e. 561 000 cells/ml and log 5.85 i.e. 705 000 cells/ml). Less fat, protein and lactose was found in milk from infected halves of the udder when compared with the percentage of these components in milk from healthy halves of the udder, but these differences were not statistically significant. The daily milk yield from infected and healthy halves of the udder was similar in goats.

Ewes												
Factor	Number of udder halves		SCC from udder half (log SCC)		Percentage content in milk from udder half						Milk production and degree of udder half infection (ml)	
					fat		protein		lactose			
	left	right	left	right	left	right	left	right	left	right	left	right
Somatic cell count in milk below 25 000 in 1 ml (n = 240)												
Major pathogens <sup>1</sup>	6	2	4.52	5.64	4.90	4.62	6.08	6.02	5.42 <sup>A</sup>	5.01	1 004,2	835.4
Minor pathogens <sup>2</sup>	37	44	4.69	5.15	5.57	6.03	6.38	6.36	5.11	4.98	855.1	967.3
Without bacteria	77	74	4.68	4.85	6.12	6.26	6.22	6.22	4.95 <sup>A</sup>	4.90	968.5	943.1
Somatic cell count in milk above 250 000 in 1 ml (n = 120)												
Major pathogens <sup>1</sup>	1	2	5.94	6.02	7.19	5.18	7.42	7.03	4.17	4.94	450.0	500.0
Minor pathogens <sup>2</sup>	30	27	6.20	5.91	6.64	7.43	6.63	6.66	4.35	4.26	768.3	686.1
Without bacteria	29	31	6.03	5.70	7.65	7.41	6.98	6.86	3.95	4.44	762.1	841.1
Goats												
Major pathogens <sup>1</sup>	9	5	5.93	6.21	2.67	2.96	2.76	3.10	3.84	4.39	2 677.8	2 820.0
Minor pathogens <sup>2</sup>	12	12	5.74	5.72	2.93	3.14	2.94	2.93	4.36	4.23	3 233.3	3 116.7
Without bacteria	46	50	5.75	5.85	3.04	2.92	3.02	3.05	4.52	4.15	2 810.9	2 814.0
Calendar year	67	67	ns	**	**	*	ns	ns	*	**	*	*
Month of lactation	67	67	*	ns	**	**	**	**	ns	ns	*	*

**Table 7.** The effect of the degree of milk contamination on milk somatic cell count and composition, and the level of milk production. Means in columns designated with identical capital letters differ significantly at  $P \leq 0.01$ . <sup>1</sup>*Staphylococcus aureus* and *Escherichia coli*, <sup>2</sup>*Coagulase negative staphylococcus*, *Micrococcus*, *Corynebacterium* and *Bacillus*. \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , ns – non-significant difference.

#### 2.3.4. Phenotypic correlations

Table 8 presents the correlation coefficients between the phenotypic characteristics of udders and the parameters of machine milking in ewes and in goats.

Ewes with greater udder dimensions were characterized by a longer time of milk flow. Similarly, a longer time of milk flow was recorded in ewes with a greater distance between the teats. Correlation coefficients between the overmilking time for both halves of the udder and the udder dimensions were small, but significant. Ewes with a greater depth and circumference of udders were characterized by a longer stripping time. Milking time was significantly correlated with traits of the udder and distance of the teats. Teat length was not related with the course of machine milking.

Ewes							
Parameter (s)	Udder dimensions (cm)				Teats dimensions (cm)		
	Length	Width	Depth	Circumference	Distance	Teat length	
						left	right
Milk flow from the left udder half	0.356**	0.115**	0.121**	0.415**	0.177**	0.019	-
Milk flow from the right udder half	0.331**	0.171**	0.179**	0.414**	0.194**	-	0.027
Overmilking of the left udder half	0.114**	0.093**	0.165**	0.118**	0.167**	- 0.007	-
Overmilking of the right udder half	0.094**	0.090**	0.164**	0.108**	0.157**	-	- 0.021
Stripping of udder	0.046	0.118**	0.250**	0.146**	0.194**	0.053	0.070*
Milking	0.158**	0.119**	0.224**	0.188*	0.188*	0.002	- 0.014
Goats							
Milk flow from the left udder half	0.107	0.297**	0.405**	0.433**	0.460**	0.072	0.076
Milk flow from the right udder half	0.116*	0.287**	0.444**	0.390**	0.370**	0.105	0.231**
Overmilking of the left udder half	0.076	- 0.004	0.022	0.115*	0.117*	- 0.095	- 0.043
Overmilking of the right udder half	0.076	0.007	0.020	0.146**	0.172**	- 0.098	- 0.104
Stripping of the udder	0.169**	0.014	0.146**	0.259**	0.157**	- 0.097	- 0.142
Milking	0.181**	0.131*	0.259**	0.362**	0.363**	- 0.073	- 0.045

**Table 8.** Coefficients of phenotypic correlations between udder traits and parameters of the course of milking in ewes (n = 747) and in goats (n = 621), \*P<0.05, \*\*P<0.01.

There were significant and positive correlation coefficients between milk flow times from both halves of the goats udders and the width, depth and circumference of udders ( $r_p$  from 0.29 to 0.44). Statistically significant correlation coefficients were also calculated between the milk flow time and distance between teats ( $r_p = 0.46$  and  $r_p = 0.37$ ). Goat teat length was not related to the milk flow time. Longer milk flow times were recorded for udder halves with a shorter distance from the teat end to the floor. Overmilking time was slightly dependent on the circumference of the udder and distance between teats. Stripping time of the udder was positively and significantly correlated with the length, depth and circumference of the udder. Milking time was significantly dependent on all udder dimensions, with the exception of teat length. The calculated correlation coefficients indicate a slight relationship to the udder dimensions on overmilking time. The probable cause for the long overmilking time was the use of stripping of the udder, often in excess of 30 s, and milking organization adopted on the farm (one milker operating six clusters). Table 9 shows correlation coefficients between udder dimensions and ewes' milkiness parameters.

The number of somatic cells in case of both halves of the udder (log SCC) was negatively correlated with udder dimensions and the distance between the teats, but the calculated values of correlation coefficients were small. Similarly, negative and significant correlation coefficients were calculated between the percentage of fat and protein in the milk and all the dimensions of the udder. Ewes with a large circumference of the udder have less fat and protein in milk, and this trait was also associated with a higher milk production and lower concentrations of these components in milk. Lower contents of these components in milk were also found in ewes with longer teats. The lactose content in milk from both halves of the udder was positively correlated with all dimensions of the udder. The calculated values of correlation coefficients between the dimensions of the udder, teat length and the amount of milk produced were positive and statistically significant. Table 10 shows the correlation

coefficients between phenotypic traits of the udder and the traits of goats' milkiness. Phenotypic correlation coefficients between all the dimensions of the udder and the number of somatic cells in milk from both halves of the udder were negative and statistically significant. A similar interdependence was found between the distance between the teats and log SCC for both halves of the udder. The greater distance from the teats end from the floor was associated with higher somatic cell counts in milk. The dimensions of the udder and the distance and the length of the teats were negatively and significantly correlated with percentage contents of fat and protein in milk ( $P \leq 0.05$ ).

Parameter (s)	Udder dimensions (cm)				Teats dimensions (cm)		
	Length	Width	Depth	Circumference	Distance	Teat length	
						left	right
Somatic cells in the left udder half (log SCC)	-0.091**	-0.081*	-0.051	-0.146**	-0.076*	0.007	-0.082*
Somatic cells in the right udder half (log SCC)	-0.126**	-0.118**	-0.092**	-0.164**	-0.163**	-	-
Fat content in milk from the left udder half (%)	-0.441**	-0.207**	-0.148**	-0.544**	-0.341**	-0.208**	-0.123**
Fat content in milk from the right udder half (%)	-0.279**	-0.180**	-0.127**	-0.510**	-0.323**	-	-
Protein content in milk from the left udder half (%)	-0.403**	-0.191**	-0.160**	-0.474**	-0.372**	-0.102**	0.010
Protein content in milk from the right udder half (%)	-0.340**	-0.155**	-0.112**	-0.458**	-0.347**	-	-
Lactose content in milk from the left udder half (%)	0.326**	0.249**	0.166**	0.490**	0.342**	0.172**	0.095**
Lactose content in milk from the right udder half (%)	0.324**	0.274**	0.138**	0.459**	0.349**	-	0.105**
Ewes' milkiness (ml)	0.323**	0.372**	0.408**	0.509**	0.413**	0.132**	

**Table 9.** Coefficients of phenotypic correlations between udder traits and ewes' ( $n = 747$ ) and milkiness parameters, \* $P \leq 0.05$ , \*\* $P \leq 0.01$ .

Parameter	Udder dimensions (cm)				Teat dimensions (cm)				
	length	width	depth	circumference	distance	Teat length		Distance of teats from floor	
						left	right	left	right
Log SCC, left udder	-0.376**	-0.248**	-0.233**	-0.601**	-0.446**	0.007	0.036	0.245**	0.136*
Log SCC, right udder	-0.282**	-0.168**	-0.297**	-0.524**	-0.380**	0.010	-0.0046	0.176**	0.201*
Fat, left udder, %	-0.214**	-0.107**	-0.288**	-0.414**	-0.358**	-0.180**	-0.152**	0.005	0.075
Fat, right udder, %	-0.342**	-0.016	-0.418**	-0.417**	-0.385**	-0.217**	-0.151**	-0.007	0.093
Protein, left udder, %	-0.469**	-0.074	-0.484**	-0.472**	-0.411**	-0.192**	-0.082	0.098	0.196**
Protein, right udder, %	-0.382**	-0.104	-0.448**	-0.423**	-0.382**	-0.208**	-0.152**	0.049	0.221**
Lactose, left udder, %	0.083	0.213**	0.024	0.326**	0.353**	-0.240**	-0.256**	-0.037	0.153*
Lactose, right udder, %	0.086	0.196**	0.143**	0.313**	0.238**	-0.071	-0.071	0.152**	0.074
Goats' milkiness, ml	0.439**	0.383**	0.516**	0.787**	0.586**	0.079	-0.007	-0.186**	-0.207**

**Table 10.** Coefficients of phenotypic correlation between udder traits and goats' milkiness parameters ( $n = 621$ ). \*\* $P \leq 0.01$ , \* $P \leq 0.05$ .

The width and circumference of the udder and the distance between teats were significantly positively correlated with the percentage of lactose in milk ( $P \leq 0.05$ ). Teat length was not related to the content of lactose in milk. All dimensions of the goats' udder, mainly circumference, were positively correlated with daily milk production. The greater distance from the teat end to the floor was associated with lower milk production.

## 2.4. Conclusion

A long overmilking time of udder halves in ewes is closely connected with teat end injuries. The injuries bring about a certain reduction of ewe milk production and an increased fat content, while protein and lactose contents are lower. In order to reduce the overmilking time or to eliminate it from the milking process the author suggests employing two milkers in the milking process. Injuries of goat teats do not influence milk yield, SCC or milk composition. The degree of milk infection in small ruminants does not influence the levels of production either in qualitative or quantitative terms. In future studies on the course of machine milking in small ruminants and its implications for quantitative and qualitative levels of milk production it is proposed to investigate the milking schedule and increase the overmilking time at different stages of lactation.

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# Water Salinity Under Heat Stress in Grazing Conditions

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/51249>

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## 1. Introduction

Water is essential for animal survival under any environmental condition. Furthermore, it is becoming a limiting factor at a global level. Cattle water needs can be satisfied in three ways: 1. Metabolic water, from tissue and organic substrates oxidation; 2. Feed water; and 3. Drinking water.

Under any circumstances, drinking water is the most important source, mainly during summer months. When animals are in a hot environment, any factor limiting access to good quality water will directly affect milk production, which will dramatically fall, especially in high producing cows. Water restricted animals show higher body temperature, increasing heat stress, and immune system alterations. Besides, water restriction affects feed intake, since water and dry matter intakes are strongly related. This is true even under grazing conditions, regardless of the high water contents many fresh pastures have. Also, under hot conditions, ingestion of high volumes of water contributes to improve animal comfort, since reticulo-rumen temperature decreases.

It should be pointed out that water quality alone is not enough to avoid the effects of heat stress on lactating milking cows during hot weather. Other nutritional, as well as environmental strategies can be implemented to improve grazing dairy cattle performance and mitigate heat stress, and will also be discussed.

## 2. Dairy water intake and environment

No doubt water is the most essential element for the survival of animals. Water requirements for livestock can be met in three ways:

1. Metabolic water, derived from the oxidation of organic substrates and tissue
2. Water contained in food

### 3. Drinking water

In any event the latter route is the most important in the quantitative sense and in summer is by far the largest source. During this season of the year, any factor that limits access to water directly affect the production of milk, which will fall sharply, mainly in high-producing cows. Cows with water restrictions manifest higher body temperature, with a degree of heat stress higher than normal. Furthermore, water restriction causes a greater reduction in the consumption and ingestion of water and dry matter intake are closely related (National Research Council (NRC), 2001). Also, under intense heat, ingestion of large volumes of water affects comfort by reducing the temperature of the rumen reticulum.

Dairy cows normally drink large amounts of water, but with intense heat they could take more than 120 L/day. In a landmark study conducted in climatic chambers, it was recorded water consumption of lactating cows increasing by 29% when the temperature rose from 18 to 30°C. Concomitantly, fecal water loss decreased 33%, but losses via urine, skin and respiratory tract increased by 15, 59 and 50% respectively.

Regarding minerals, heat-stressed cows increase their need for Na<sup>+</sup> and K<sup>+</sup>, due to the electrolyte imbalance generated at the cellular level. The higher needs of Na<sup>+</sup> are attributed to increased secretion of urine that reduces the plasma concentration of aldosterone. Instead, the increased demands for K<sup>+</sup> are attributable to an increased removal of this element with sweat.

In lactating cows fed a diet based on corn silage, hay and concentrates, typical of many production models, it was found that the main factors that determined water intake were: dry matter consumed; the level of milk production, temperature and Na<sup>+</sup> intake. The following equation (NRC, 2001) shows these relationships:

$$WI = 16 + [(1.58 \pm 0.271) * (DMI)] + [(0.9 \pm 0.157) * (MP)] + [(0.05 \pm 0.023 * (Na^+))] + [(1.20 \pm 0.106) * (T_{md})],$$

where

WI = Water intake (kg/day)

DMI = Dry matter intake (kg/day)

MP = Milk production (kg/day)

Na<sup>+</sup> = sodium (g/day)

T<sub>md</sub> = daily minimum temperature (°C)

### 3. Dairy water quality and milk production

The quality of drinking water is often one of the causes limiting its intake. Water quality is measured in chemical, bacteriological and physical terms, through laboratory tests. To avoid significant production losses each of these aspects must be carefully and regularly evaluated.

Regarding chemical composition, the concentration of total dissolved solids (TDS) and the prevalent salts represent the quality factors that can seriously limit milk production in many

regions. There is controversy regarding the maximum levels of salts that affect the performance of dairy cows. Water with TDS > 7000 mg/L would not be suitable for high producing cows (>35 L/day), but would have little effect on low-producing animals (<25 L/day) (Bahman et al. 1993; NRC, 2001). Experiments conducted in Israel (Solomon et al., 1995) showed that water with TDS above 4000 mg/l produced negative effects on cows producing an average 35 l/day, when temperature was above 30°C.

All sulfate salts ( $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ), when exceeding 1500 mg/L, can decrease productivity because of their laxative effect, the most potent being sodium sulfate (Socha et al., 2003). However, livestock drinking water high in sulfates (1000 to 2500 mg/L) initially suffer diarrhea, but then a process of habituation begins. Moreover, ingestion of "light" water, i.e. very low in TDS, is also considered detrimental to productivity, especially when levels of sodium chloride are very low.

The temperature of drinking water could be another factor limiting intake. For example, in an experiment conducted in Texas (Wilks et al., 1990) it was observed that cows drinking water cooled to 10°C presented lower respiration rate (70 VS. 81 rpm), lower rectal temperature in the afternoon (39.8 vs. 40.2°C) and higher milk production (26.0 vs. 24.7 L/cow/day), as compared to animals drinking water at 27°C.

#### 4. Water quality under Argentine grazing conditions

A recent study (Pérez Carrera et al., 2005) performed in the milking area of Cordoba (Argentina), showed that 37% of the samples from groundwater were non adequate for dairy cattle as assessed in terms of TDS. A similar situation was found in large areas of the Central Santa Fe milking region (Revelli et al., 2002). In the latter, 53% of the samples taken from dairy operations were considered unsuitable for lactating dairy cows and, therefore, were not recommended for animal intake. Both Cordoba and Santa Fe are within the most important milking region in Argentina. However, the information available in Argentina regarding lactating cows (Taverna et al. 2001; Valtorta et al., 2008) indicates that under grazing conditions, water with 7000-10000 mg/L of TDS, with 20-30% of sulfate, had little effect on productivity, for cows producing below 30 L/d.

Particularly, the trial by Valtorta et al. (2008) was performed at the Dairy Unit at Rafaela Experimental Station (INTA), Santa Fe, Argentina (31°11'S) from January 6<sup>th</sup> until April 2<sup>nd</sup>, 2005. Eighteen multiparous lactating Holstein cows, 9 ruminally cannulated, average days in milk 136.1±14.6 days, were randomly assigned to three treatments, consisting of water containing different levels of TDS (mg/L): Treatment 1=1,000; Treatment 2=5,000 and Treatment 3=10,000. Cows were balanced for milk production during the week previous to the beginning of the trial (31.9±4.1 L/cow/day), body weight (BW, 521±61 kg/cow) and body condition score (BCS, 2.3±0.24). Animals were arranged in a randomized complete block design with three 28-day experimental periods, which consisted of 3 weeks for water adaptation and one week for measurements.

Animals were milked twice a day, at 04:00 h and 16:00 h. From the pm to the am milking all cows were on an alfalfa pasture, in a daily strip grazing system. All experimental groups

grazed within the same paddock and were separated by electric fences in a sub-paddock, where cows had access to their respective treatment water *ad lib*. Since the trial was performed during summer, when radiation and temperatures are high, each group was sent to a pen where the treatment water *ad lib* and shade were available, from 9:00 until the pm milking. There, the animals also received alfalfa hay and cottonseed wholes with lint. A mixed concentrate was offered in the milking parlor, during both milkings.

In order to formulate the water for the different treatments, the normal available water (2880 mg/L TDS) was treated with a reverse osmosis equipment (OSMOTIKA® Model OI-7.0-F; Entre Ríos, Argentina). The water for TDS 1,000 was prepared by mixing completely desalinated water with normal water, to obtain 1,000 mg/L TDS. On the other hand, treatments 5,000 and 10,000 mg/L TDS were obtained by adding and mixing controlled amounts of salts to the equipment refusal water (3.51 mg/L TDS). Drinking waters were formulated to have not less than 100, 850 and 2000 mg  $\text{SO}_4^{2-}$ /L for treatments 1,000; 5,000 and 10,000 mg/L TDS, respectively. Samples were taken every week in order to analyze TDS and concentrations of sulfate, bicarbonate, chloride, sodium, calcium and magnesium ions.

Individual water intake was recorded during two non-consecutive days by pairing cows in sub-groups, both on paddock and in the shaded pen. The volumes of water offered to and refused by every pair of cows were estimated from the height the water reached in each drinker, together with the drinker dimensions. The difference between both estimates (offered and refused) represented the total drunk water. Daily water group consumption was also recorded by measuring the volumes offered and refused, as described above.

Individual pasture dry matter intake (DMI) was estimated during two non-consecutive days on 40 m<sup>2</sup> paddocks (9 in total), where pairs of cows were located. Within each paddock, 5 samples of 0.10 m<sup>2</sup> of pre- and post-grazing pasture mass were taken, as described in Gallardo et al. (2005). The DMI of concentrate, hay and cottonseed were assessed every day, as the difference between the amounts offered and refused.

Water samples were taken from the drinkers, in 1,000-mL sterilized plastic bottles. Total soluble salts were determined by means of a Water Quality Checker U-10 Horiba (Kyoto, Japan), and  $\text{SO}_4^{2-}$ ,  $\text{CO}_3^{2-}$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  by Colorimetric and Volumetric methods (Merck, Darmstadt, Germany).

Representative pre-grazing pasture samples were taken by “plucking” for chemical analyses, following a protocol similar to that described by Roche et al. (2005). Pasture, hay, cotton seed and concentrate samples were analyzed for DM, CP, ash, and fat (AOAC, 1990), NDF, ADF, and lignin (Van Soest et al., 1991). Energy concentration ( $\text{NE}_L$ /kg DM) of the diet was estimated according to NRC (2001).

At the beginning of the study, and on day 28 of each experimental period, BW was measured and body condition was scored by three experienced independent observers using the five-point BCS scale (1 = thin, 5 = fat; Edmonson, 1989).

Milk production was recorded daily during the measurement periods by Waikato® milk meters (New Zealand). Milk samples were collected from 10 milkings (sequence am – pm) during the 7-day sample collection period and analyzed for fat, total protein, lactose, and milk urea nitrogen (MUN) with an infrared spectrophotometer (Foss 605B Milk-Scan; Foss Electric, Hillerød, Denmark).

For two consecutive days, 50-ml liquid samples were obtained from the rumen via a tube introduced in the ventral sac, at 08:00 h (immediately before feeding; time 0) and at times 3, 6, 12, 18 and 24. On those samples, pH was measured with a glass electrode and ammonia was analyzed by a colorimetric technique.

Sub-samples were utilized for VFA analyses. The sub-samples were filtered through two layers of gauze, acidified with m-phosphoric acid (24%) in 3 N H<sub>2</sub>SO<sub>4</sub> and kept at -20°C till analysis. Volatile fatty acids were determined with a Shimadzu gas chromatograph GC-14B (Shimadzu Corporation, Kyoto, Japan) using a 2 m glass column packed with 10% polyethylene glycol and 3% H<sub>3</sub>PO<sub>4</sub> in chromosorb AW, and fitted with a flame ionization detector (Erwin et al., 1961). The working temperatures were 155°C, 185°C and 190°C for the column, injector and detector, respectively. A Shimadzu CR6A integrator was used for peak quantification and identification. The internal standard was 2-methyl valeric acid. For enumeration of protozoa, sub-samples from times 0, 3 and 6 samples were utilized. Equal parts of rumen fluid and a saline-formalin solution (20% formalin in 0.85% NaCl solution) were mixed and stored. Prior to counting, a 2 mL aliquot of the fixed rumen sample was stained for at least 4h with 2 mL of methyl green-formalin solution (Ogimoto and Imai, 1981). Protozoa quantification and generic composition were determined using a 1 mL counting chamber (Hausser Scientific Partnership, cat. No. 3800), following the procedures described by Dehority (1993).

At time 0, samples of rumen contents were collected for bacterial enumeration. Rumen solids and liquid (100 g + 100 mL) were homogenized under a CO<sub>2</sub> atmosphere and filtered through two layers of gauze. Samples were diluted in decimal series (10<sup>-1</sup> to 10<sup>-10</sup>). For total bacterial concentration, 10<sup>-6</sup>, 10<sup>-7</sup> and 10<sup>-8</sup> dilutions were inoculated into 10 mL of RGCSA medium according to the procedure described by Grubb and Dehority (1976), which follows the roll tube procedure of Hungate (1966). Inoculated roll tubes were incubated for 5 d at 39°C and counted under a dissecting microscope. Cellulolytic and amylolytic bacterial concentrations were estimated with a most probable number (MPN) procedure, using a basal medium with either cellulose (filter paper) or starch as the only added carbohydrate source (Bryant et al., 1958; Bryant and Robinson, 1961). All tubes were incubated at 39 °C. Amylolytic bacteria were measured after 7 days, using Lugol's iodine reaction to determine starch digestion (Persia et al., 2002). After 15 d incubation, cellulolytic bacterial concentrations were determined by observing the disappearance of filter paper.

Air temperature and relative humidity data were obtained from a meteorological station located about 500 m from the experimental dairy farm. Average daily temperature humidity index (THI) was calculated after Armstrong (1994).

Data were analyzed using in cross-over randomized complete block design.

Table 1 presents the composition of the diet offered during the trial to animals in all treatments. It represents a typical grazing system diet, except for the addition of cottonseed wholes. The latter were included because of their high fat contents and, therefore, their beneficial effect for summer diets (Grummer, 1992).

<b>Ingredient (% on a DM basis)</b>	
Alfalfa pasture	57.7
Alfalfa hay	4.7
Cottonseed wholes with lint	7.4
Concentrate mixture <sup>(1)</sup>	30.2
<b>Composition</b>	
Dry matter (%)	31.0±2.75
Crude protein (%)	16.2±1.65
Neutral detergent fiber (%)	39.3±6.5
Acid detergent fiber (%)	21.0±4.1
Non-fibrous carbohydrates <sup>(2)</sup> (%)	34.7±6.15
Ether Extract (%)	4.7±0.7
NEL <sup>(3)</sup> (Mcal/kg DM)	1.56±0.17

<sup>(1)</sup> Ingredients: 87.3% corn grain; 9.5% corn germ; 3.2% mineral and vitamins premix: Calcium carbonate: 31.5%; Magnesium oxide: 18.5%; Di-calcium phosphate: 38.4%; Salt: 11.6% Vitamins-micro-minerals = Vit. A: 4620 UI/kg; Vit. D3: 920 UI/kg; Vit. E: 12 UI/kg; Cu: 4.5 mg/kg; Zn: 31 mg/kg; Fe: 33 mg/kg; I: 0.6 mg/kg; Se: 0.12 mg/kg; Co: 0.375 mg/kg

<sup>(2)</sup> NFC = 100 - (ash + CP + NDF + Fat)

<sup>(3)</sup> Net energy estimated according to NRC (2001)

**Table 1.** Composition of the diet offered during the trial, for treatments containing different amounts of total dissolved salts: 1,000; 5,000 and 10,000 mg/L in the drinking water.

More than 50 % of the diet was fresh grazed alfalfa, which usually has high levels of highly degradable protein and low fiber. Chemical composition of the water utilized during the trial is shown in Table 2.

Component (mg/L)	T R E A T M E N T					
	1,000		5,000		10,000	
	Mean	SD	Mean	SD	Mean	SD
Total solids	1100	84	5280	390	9220	545
SO <sub>4</sub> <sup>2-</sup>	125	18	883	196	2088	253
CO <sub>3</sub> <sup>2-</sup>	19	31	57	86	125	40
Na <sup>+</sup>	335	40	1628	186	2767	316
Cl <sup>-</sup>	115	18	1425	124	2775	361
Ca <sup>2+</sup>	9	09	64	6	85	9
Mg <sup>2+</sup>	9	3	103	7	211	13

**Table 2.** Chemical composition of the water utilized during the trial, for treatments containing different amounts of total dissolved salts: 1,000; 5,000 and 10,000 mg/L in the drinking water.

Sulfates represented about 11% TDS in treatment 1,000; 17% in treatment 5,000 and 23% in 10,000. In treatment 1,000, Na<sup>+</sup> and Cl<sup>-</sup> together represented about 40% TDS, while they were 60% TDS in treatments 5,000 and 10,000.

Table 3 presents pasture, concentrate and total DM intake for each treatment. No significant differences were observed in response to level of salinity. However, pasture dry matter consumption was significantly lower during the third experimental period, regardless of the water salinity level. During periods 1 and 2, DM intake averaged  $10.6 \pm 1.85$  kg/cow/day, while in period 3 it was  $8.8 \pm 0.6$  kg/cow/day.

Item	Treatment		
	1,000	5,000	10,000
Pasture (alfalfa based)	$10.4 \pm 1.0$	$9.8 \pm 2.7$	$9.7 \pm 1.7$
Concentrate <sup>(1)</sup>	7.63	7.63	7.63
Total	$18.03 \pm 1.0$	$17.43 \pm 2.7$	$17.33 \pm 1.7$

<sup>(1)</sup> Concentrate composition: 71.5 % concentrate mix; 17.5 % cottonseed wholes with lint; 11 % alfalfa hay

**Table 3.** Pasture, concentrate and total dry matter intake (kg /cow/day; mean  $\pm$  SD), for treatments containing different amounts of total dissolved salts: 1,000; 5,000 and 10,000 mg/L in the drinking water.

Water intake data per treatment and period are presented in Table 4. It ranged between 97.5 and 202, 2 L/cow/day, with animals in treatment 10,000 showing the highest levels.

The water produced for each treatment presented the expected characteristics, as assessed in terms of TDS and SO<sub>4</sub><sup>2-</sup> concentrations. According to the guidelines for TDS (NRC, 2001), treatment 1,000 represents a safe water for animal drinking. On the other hand, water containing 5,000 mg/L TDS should be avoided for pregnant or lactating animals, if maximum performance is the target, while water containing over 7000 mg/L TDS should never be offered to dairy animals, since they could present health problems or a poor production.

Pasture intake was lowest in the third period. This response could have been affected by the lower quality of the pasture offered in this period. Protein and NDF were 17.1 and 51.1%, as compared to 21.8 and 49.5% and 19.5 and 49.8% for periods 1 and 2, respectively. Also, during that period rainfall was much higher than during the previous ones (317.6 mm vs. 177.6 and 39.7 mm for periods 1 and 2, respectively). This environmental situation could have affected paddock conditions, so as to render grazing more difficult for the cows.

Surprisingly, animals in treatment 10,000 drunk more water than the others in all three periods. These results disagree with other reports where it was found that water intake for cows drinking desalinated water was higher, as compared to animals receiving salty water, defined as water presenting >1,000 mg/L TDS (Solomon et al., 1995). However, in that report TDS and ion composition differed from the treatments in the present work.

In Argentina, Revelli et al. (2005), found similar levels of water intake for animals drinking water with 1,000 and 10,000 mg/L TDS. However, their data were not obtained during the

summer season. Warm environmental temperature (e.g., heat stress) is an important factor when evaluating water nutrition. Water intake increases as environmental temperature goes up (NRC, 2001; Holter & Urban 1992).

Week	Treatment		
	1,000	5,000	10,000
1: Jan 27 <sup>th</sup> - Feb 2 <sup>nd</sup>	97.5 ± 23.4 <sup>a</sup>	123.2 ± 12.6 <sup>b</sup>	169.6 ± 18.3 <sup>c</sup>
2: Feb 24 <sup>th</sup> - Mar 2 <sup>nd</sup>	110.9 ± 32.1 <sup>a</sup>	127.1 ± 9.5 <sup>a</sup>	193.9 ± 22.93 <sup>b</sup>
3: Mar 25 <sup>th</sup> -Mar 31 <sup>st</sup>	108.4 ± 41.0 <sup>a</sup>	114.9 ± 8.0 <sup>a</sup>	202.2 ± 28.2 <sup>b</sup>

Within row different superscripts represent statistical significance ( $P < 0.05$ )

**Table 4.** Water intake during the three measurement weeks (L/cow/day; mean ± SD), for treatments containing different amounts of total dissolved solids: 1,000; 5,000 and 10,000 mg/L in the drinking water.

The meteorological data recorded during the 1-week measuring periods are shown in Table 5. Average temperatures corresponding to complete 28-days experimental periods were 26.1 ± 3.7, 24.3 ± 2.6 and 23.2 ± 3.6 °C, for periods 1 to 3. The respective rainfall values were 177.6; 39.7 and 317.6 mm .

Week	Average temperature (°C)			Average THI
	Mean	Max	Min	
1: Jan 27 <sup>th</sup> - Feb 2 <sup>nd</sup>	22.5 ± 5.9	31.3 ± 7.2	13.7 ± 4.6	70.9 ± 6.3
2: Feb 24 <sup>th</sup> - Mar 2 <sup>nd</sup>	24.1 ± 3.2	29.3 ± 3.9	17.0 ± 3.5	72.9 ± 5.8
3: Mar 25 <sup>th</sup> -Mar 31 <sup>st</sup>	22.1 ± 2.6	28.0 ± 3.8	17.2 ± 1.8	70.4 ± 4.1

**Table 5.** Temperature and temperature humidity index (THI) during the three measuring weeks, for treatments containing different amounts of total dissolved solids: 1,000; 5,000 and 10,000 mg/L in the drinking water.

Cows producing 20 L milk/day would intake about 90 L water/day at 16°C and about 105 L water/day at 26°C (Beede,1992). In the present study, the results for cows in treatment 1,000 fell within this range. Regarding treatments 5,000 and 10,000, it can be pointed out that diets high in salt, sodium or protein appear to stimulate water intake (Holter & Urban, 1992). Furthermore, sodium intake alone was found to increase water intake by 0.05 kg/day per gram of sodium intake (Murphy et al, 1983). The authors derived a prediction equation for water intake, where minimum temperature and sodium intake were among the predicting variables. On the basis of that equation, estimated overall average water consumption in the present trial resulted 91, 115 and 185 kg/cow/day, for treatments 1,000; 5,000 and 10,000, respectively. These values compare quite well with the actual overall averages: 106, 122 and 189 L/cow/day, for the respective treatments.

Table 6 presents milk production and composition and BCS change. No treatment effects were observed in any parameter.

Grazing diets generally tend to be unbalanced, because cows present a selective habit. Concentrate and cottonseed wholes were included to solve this problem, and to obtain a

better balanced ration, as shown by the levels of milk yield. Milk yield and composition were not affected by treatment. Solomon et al. (1995) reported higher yields and milkfat percentages for cows receiving desalinated water, as compared to the levels obtained by animals drinking natural salty water. Those results disagree with the present report, where no treatment effects were detected on milk production and composition. However, that trial was performed in a desert climate on non-grazing cows and average milk production was higher than the levels obtained in the present study.

Item	Treatment			SEM	Effects	
	1,000	5,000	10,000		Treat	Period
Milk yield (kg/cow/day)	24.23	24.81	24.55	1.79	0.6304	<0.0001
Milk fat (%)	3.27	3.23	3.36	0.21	0.1939	0.0628
Protein (%)	3.40	3.34	3.36	0.17	0.6450	0.0004
Lactose (%)	4.92	4.90	4.91	0.13	0.9835	0.0662
MUN (mg %)	7.54	7.48	7.01	2.35	0.7641	<0.0001
BCS, change <sup>(1)</sup>	-0.11	0.05	-0.06	0.09	NS	NS

<sup>(1)</sup> Final BCS – Initial BCS

**Table 6.** Milk yield and composition and body condition score change for treatments containing different amounts of total dissolved salts: 1,000; 5,000 and 10,000 mg/L in the drinking water.

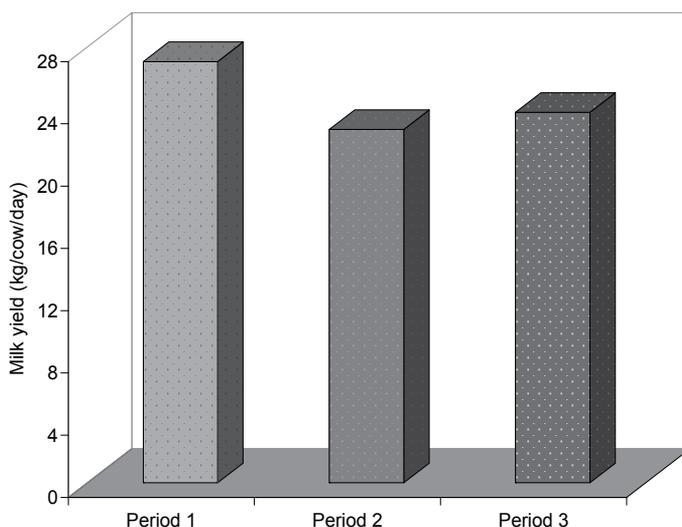
Under non-grazing conditions, Sanchez et al. (1994) found that milk production was reduced during the summer months in response to increasing intakes of chloride and sulfate. They also found that feeding high amounts of sodium does not reduce milk production or lactation performance.

Milk production was affected by period, the highest yield being recorded in period 1 (Figure 1). Different variables could have determined the period effects on milk production. First, total consumption was lower during period 3, as compared to the other periods. On the other hand, there is a natural trend to decrease in yield as lactation progresses. In any event, the levels obtained are quite good if considering the grazing based production system and the season. Also, the conversion efficiency was high: approximately 750 g DM/kg milk, with no BCS lost (Table 3).

Milkfat and protein presented low concentrations in all treatments. Similar results were obtained by Revelli et al. (2002, 2005). In treatments 1,000 and 5,000 fat and protein values were reversed. This response could indicate low effective fiber content in the ingested forage, possibly affected by pasture intake behavior, since grazing animals select leaves and tender stems.

Rumen bacteria and protozoa (Table 7), as well as pH, ammonia and VFA (Table 8), were not affected by treatment.

Rumen parameters and microbiology were not affected by water salinity. Those results show the incredible rumen buffer capacity, probably because of the effects of fresh alfalfa pasture, an important protein source, in the diet. The buffering system in the rumen includes not only the saliva, but also the feed (Van Soest, 1994). In the present trial, average



**Figure 1.** Milk yield for the three experimental periods in a trial with treatments containing different amounts of total dissolved salts (TDS): 1,000; 5,000 and 10,000 mg/L in the drinking water. Periods lasted 28 days each, and the different treatment waters were formulated to have not less than 100, 850 and 2000 mg  $\text{SO}_4^{2-}/\text{L}$  for treatments 1,000; 5,000 and 10,000 mg/L TDS, respectively. All animals were subjected to all treatments, since data were obtained and analyzed in a cross-over design.

Item	Treatment			Effects	
	1,000	5,000	10,000	T	P
Amylolytic bacteria ( $\times 10^9$ )	3.4	3.4	3.6	0.89	0.98
Cellulolytic bacteria ( $\times 10^6$ )	20.5	31.9	14.5	0.55	0.81
Protozoa ( $\times 10^3/\text{ml}$ )	9.3	13.8	12.9	0.46	0.25

**Table 7.** Ruminal amylolytic and cellulolytic bacteria and protozoa at sampling time 0 for treatments containing different amounts of total dissolved salts: 1,000; 5,000 and 10,000 mg/L in the drinking water.

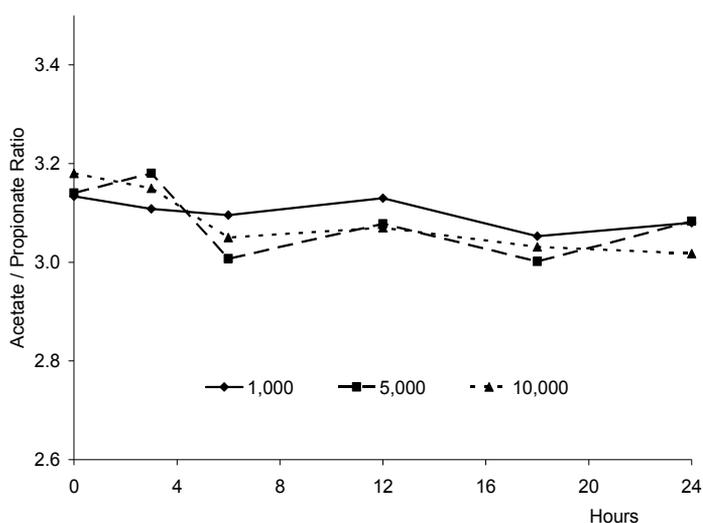
Measurement	Treatment			Contrast				
	1,000	5,000	10,000	Per	Col	Treat	Hour	TxH
VFA, $\mu\text{mol}/\text{mL}$ :								
Acetate (A)	76.51	74.03	75.29	0.42	0.46	0.71	<0.0001	0.90
Propionate (P)	24.7	24.4	23.3	0.16	0.17	0.66	<0.0001	0.98
Isobutyrate	1.61	1.74	1.45	0.14	0.92	0.32	0.0025	0.30
Butyrate	11.55	11.26	11.17	0.34	0.63	0.89	0.0002	0.94
Isovalerate	1.72	1.60	1.41	0.31	0.69	0.18	<0.0001	0.94
Valerate	1.21	1.20	1.07	0.10	0.76	0.45	0.0004	0.94
Total	117.5	114.6	113.9	0.27	0.35	0.79	<0.0001	0.95
pH	6.37	6.37	6.36	0.30	0.71	0.41	<0.0001	0.98
Ammonia, mg/dL	7.65	8.07	8.41	0.39	0.68	0.49	<0.0001	0.94

**Table 8.** Ruminal volatile fatty acids, pH and ammonia concentration for treatments containing different amounts of total dissolved salts: 1,000; 5,000 and 10,000 mg/L in the drinking water.

pH was quite constant and also relatively low, near 6. However, the values recorded for rumen ammonia (Table 8) agree with MUN (Table 6), and both indicate no excess in degradable protein in the diet.

There are very few reports on the effects of water salinity on rumen parameters. Potter et al. (1971) found no effects on VFA concentration when offering chaffed rations to sheep receiving either fresh water or a 1.3% sodium chloride solution. However, sheep are known to tolerate high amounts of salt in their drinking water (Peirce, 1957).

Figure 2 shows the temporal patterns of the Acetate/Propionate ratio, for all treatments. The values varied around 3 at every measuring time. Treatment 1,000 tended to be less variable.



**Figure 2.** Acetate/Propionate Ratio in the rumen of cows in treatments containing different amounts of total dissolved salts: 1,000; 5,000 and 10,000 mg/L in the drinking water. All animals were subjected to all treatments, since data were obtained and analyzed in a cross-over design.

The lack of effect of drinking water salinity on milk production and composition and on rumen parameters is striking, especially if considering that treatment 10,000 had a TDS quite above the levels considered to be limiting for lactating dairy cows. These results indicate that the single consideration of TDS would be not enough to characterize drinking water quality. More studies should be performed in commercial farms in order to assess the impact of natural salty water on lactating dairy cow performance.

## 5. Modifications of the environment under grazing conditions. Animal response

### 5.1. Shades

During summer, the operations should consider the strategic enclosure in a shaded pen between milkings (Valtorta et al., 1996), so as to reduce the heat load and reduce the walking

distances. In addition, the adequacy of milking schedules within this scheme would take advantage of both peaks as grazing pasture at night (Davison et al., 1996).

In a study performed in the central dairy area of Argentina (Valtorta et al., 1996) four groups of cows were compared. Two of them were locked between 09:00 and 16:00 in a pen adjacent to the parlor, which possessed an artificial shade structure and water *ad libitum*. The other two had no access to shade. Within each treatment, with and without shade, one of the groups received supplementation with concentrate, 3.5 kg / v cow/ d of corn grain. The strategic provision of shade improved the comfort of the grazing animals. The increase in rectal temperature between morning and afternoon had an average of 0.28 ° C for animals with access to shade and 1.1 ° C for those exposed to the sun. As for breathing rate, the differences were 10.5 and 23.4 rpm, respectively.

The strategic provision of shade had a similar impact to the energy supplementation, and the combination of both practices significantly increased milk production. The concentrate also produced an increase in the concentration of milk protein (Table 9).

Shade	Concentrate	MP, l/c/d	F, %	P, %
NO	NO	15.3	3.55	2.81
NO	YES	16.8	3.69	2.96
YES	NO	16.9	3.49	2.77
YES	YES	19.2	3.61	2.,85

**Table 9.** Milk production (MP) and milk fat (F) and protein (P) in milk of multiparous cows in late lactation, managed with and without access to shade (strategic shading from 09:00 to 16:00), and with or without concentrate in their ration (3.5 kg conc/c/d)

In this study, the grazing patterns adapted to confinement. Grazing time recovered during the peaks, especially during the early hours of the day.

The average maximum temperature was 29 ° C and relative humidity 72%. The activity was concentrated in two well-marked periods: from dawn, at 05:00, and 09:00 and between 16:00 and 22:00. Enclosure time was offset by increased activity in those periods. Evening grazing, of somehow greater relative importance, ended after sunset, indicating some degree of nocturnal activity.

## 5.2. Animal cooling

With respect to the direct cooling of the animal, using a system as described, in Argentina the effectiveness of pre-milking refrigeration has been evaluated (Valtorta & Gallardo, 2004). Cows were cooled for 20 min prior to both milkings through a combination of sprinkling and continuous ventilation. Sprinklers produced large droplets that penetrated the coat, their water consumption being 30 l/h. The cooling system improved cow comfort, measured in terms of the significant decrease in rectal temperature and respiratory rate.

Cooled cows produced more milk with higher fat content and yield and protein (Table 10).

Production	NR	R	Difference, %
Milk, kg/c/d	22.14	23.18	4.69
Fat, %	3.44	3.75	9.01
Fat, kg/d	0.755	0.870	15.23
Protein, %	3.22	3.35	4.03
Protein, kg/d	0.713	0.784	9.96

**Table 10.** Productivity of cows with (R) or without (NR) a 20 min refrigeration in the holding pen before milkings

In Israel cows are cooled using a similar system, on the basis of increasing evaporation from the body surface and the respiratory tract. In that case, they use the combination of large drops that penetrate the animal coat, produced by sprinkler consuming 300 to 500 l / h and forced ventilation, both in the holding pen and in the resting area. The cooling is done in cycles in which combine spraying (30 sec) followed by ventilation (4.5 min), in cycles of 30-45 min. This system is used in Israel at 2-3 hours intervals, 6-10 times per day. High producing cows are maintained in situation of normal body temperature for most of the day. Also, significantly increases in milk production and reproductive efficiency are obtained (Flamenbaum, 2010, 2008; Flamenbaum & Ezra, 2007, 2003).

According to Flamenbaum (2008) in Israel it has being shown that this intensive cooling system, applied in transition cows, can reduce the loss that causes the hot season in the level of milk production and pregnancy rate.

During summer, the combination of a proper cold treatment with an adequate body condition at calving and a good feeding management to early lactation have the potential to enable production and fertility levels almost similar to those obtained in winter. In high production herds productive summer performance is 96 to 100% of that obtained in winter, while, if not intensive cooling is applied, this ratio varies between 86 and 88% (Flamenbaum, 2008).

The implementation of these management strategies in most dairy farms in Israel have had the potential to level up the supply of milk to the market throughout the year. These measures help to increase the efficiency of milk production, giving the Israeli dairy industry a greater degree of competitiveness against the threat of importing milk powder in the summer. In connection with the modification of environmental factors, they have tried to determine if intensive cooling can prevent productive and reproductive losses in high-producing cows (Flamenbaum & Galon, 2010). The results are presented in Table 11.

The results show that intensive cooling during summer reduced the decrease in conception rate by about 50%, even in extremely high production cows. Over the years the Israeli extensionists found the need to develop tools to monitor the effectiveness of cooling systems.

Also, if during late gestation, or dry period, the environment is manipulated, so as to ease the stress of summer, cows can increase the later milk production. In a study by Amaral et al. (2009), dry advanced pregnant cows that underwent a refrigeration system increased the subsequent production, as compared to untreated animals. In this study, cows were

subjected to daily refrigeration for a period of 46 days pre-calving. After calving all cows were managed together in a barn equipped with sprinklers and fans. With this management cow milk production was significantly higher during the first 30 weeks of lactation.

Cooling intensity	Production level			
	High		Low	
	I	M	I	M
<b>Winter, corrected milk (kg/day)</b>	41-43	39-40	35-38	33-36
<b>Summer production, as related to winter</b>	.96-1.00	.86-.88	.97-1.03	.84-.90
<b>Average corrected milk (kg/day)</b>				
Winter	42.0	39.1	37.1	35.3
Spring	42.3	39.2	39.1	36.2
Summer	42.0	35.7	38.0	32.0
Fall	42.1	36.9	38.1	34.1
<b>Conception rate (%)</b>				
Winter	39	39	40	39
Summer	19	12	25	3

**Table 11.** Milk production and conception rate of low and high production cows with intensive (I) or moderate (M) cooling in Israel

Although the physiological mechanisms involved in such responses are not fully understood (Avenida-Reyes et al., 2010), various hormonal actions may be implicated.

In Argentina, these management systems may have special connotations, given the trend towards intensification in the dairies.

### 5.3. Combination of feeding and environmental management

Since both nutritional and environmental factors affect the performance of dairy cows in the central basin of Argentina, a trial was designed to evaluate the combined effects of diet and pre-milking cooling with sprinklers and fans (Gallardo et al., 2005). Responses of rectal temperature, respiratory rate, and milk production and composition were evaluated. Cows were assigned to four treatments, consisting of the combination of two diets: control (CD) and balanced (BD) with two levels of cooling before milkings: Sprinklers and fans (SF) or nothing (NSF).

In order to obtain different Forage: concentrate (F:C) ratios (about 80:20 in CD and 70:30 in BD) grazing in the DB group was restricted. The CD was prepared according to common practices in the area, while the DB was calculated to obtain better protein, energy and lipids balance. Based on the quality of its components, the energy density of diets was 1.48 Mcal of NEL / kg DM and 1.60 Mcal ENL / kg DM for CD and BD, as calculated according to NRC (2001).

In addition, SF animals received a combination of spray and ventilation for 20 min before the morning milking and 30 min before the afternoon milking in the holding pen.

Rectal temperatures (RT) and respiratory rate (RR) were recorded before and after the afternoon milking. As a result of cooling, both RT and RR were lower after milking in the SF groups, compared to non-refrigerated or NSF. The production and milk protein concentration were higher for the BD. The authors speculated that this increase in production could be due to the higher density of the diet, which would provide enough energy to increase production under conditions of heat stress. Similar results were observed by Drackley et al. (2003) when offering diets with 1.60 Mcal NE<sub>L</sub>/kg DM to cows in mid lactation. The controls received a diet with 1.52 Mcal NE<sub>L</sub>/kg DM during the summer.

No effects on the variation of body condition were detected, which would indicate that the factors acted in a way that energy was derived more efficiently to produce milk.

The diet did not affect urea-N in milk. However, this parameter was affected by cooling. Probably the cooling produced a decrease in the demand of energy to remove extra body heat, leaving more energy available for milk production. Also, the balance of the diet by manipulating the ratio F: C could have given greater availability of energy for microbial protein synthesis that may result in increased milk protein. It is possible that there was an increased use of ammonia in the rumen, also considering the increased consumption of protein in the BD. On the other hand, there might have been less use of amino acids as a source of energy in the refrigerated treatments.

These results show that under grazing conditions, the effects on production and milk composition are enhanced when diets are specially formulated for warm periods. All this environmental managements, together with the provision of large amounts of water, help improve the efficiency of water use in dairy cattle during hot periods.

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# Milk Removal: The Key to Maintaining Milk Production and a Tool to Enhance Efficiency

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Emma Wall and Thomas McFadden

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50773>

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## 1. Introduction

Manipulation of the lactation curve by increasing peak milk production and (or) lactation persistency has consistently been a focus of both researchers and dairy producers. One of the key findings that has enabled manipulation of lactation performance is that the milk yield of dairy cows is responsive to demands of offspring or milk removal; hence milk production can be increased by frequent milking or suckling. Early studies illustrated the galactopoietic effect of frequent milking during the entire lactation, with three times daily milking increasing milk yield by up to 20% relative to twice daily milking. Research using nurse cows revealed a long-term increase in milk production when cows and heifers were allowed to suckle a calf during the first 8 to 10 wk of lactation (Everitt & Phillips, 1971; Edmunds, 1977; Fulkerson, 1981). The results of these experiments laid the groundwork for current research, which has identified a time during early lactation wherein the mammary gland of dairy cows is especially receptive to the stimulus of frequent milking. More recently, it has been established that frequent milk removal (three or more times daily) for a short duration within the first three weeks of lactation can increase milk production through the remainder of lactation (Hale et al., 2003; Dahl et al., 2004b; Wall & McFadden, 2007b). Since the establishment of the galactopoietic effect on milk production, several experiments have been conducted to identify the factors that regulate the milk yield response. These reports have documented consistent responses to increased milking frequency; however, questions remain about the mechanisms involved in regulation of milk production efficiency.

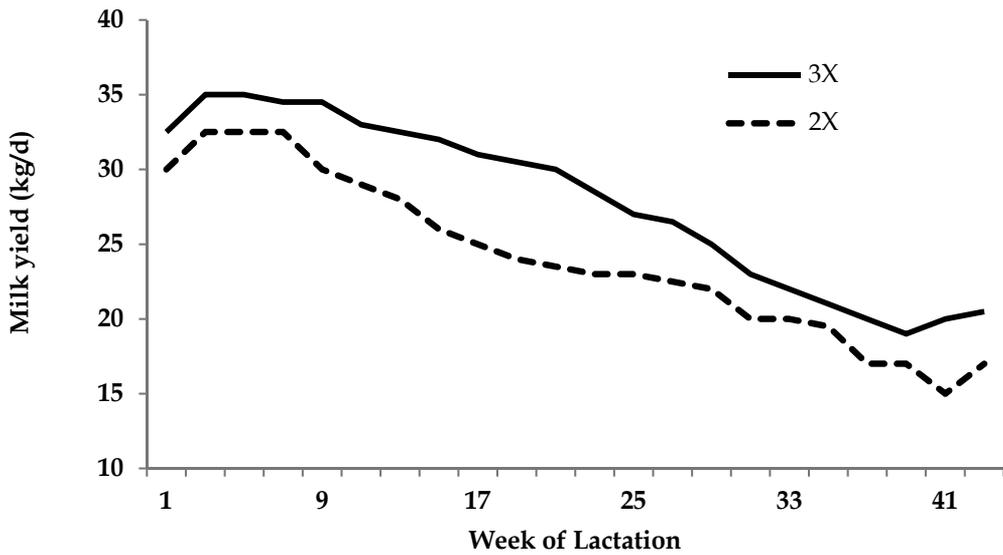
### 1.1. Frequent milking or suckling increases milk production

As previously indicated, frequent milking of dairy cows has emerged as an effective management tool for dairy farmers to increase milk production efficiency. Although it is a

relatively novel management practice, the original interest and research in this area dates back to the late 1800's (Hills, 1890; 1898). Despite considerable variation in the magnitude of the milk yield response, it was recognized long ago that thrice-daily milking (3X) increased milk production relative to twice-daily milking (2X), and that frequent milking could be a profitable management tool if costs associated with the extra milkings are outweighed by the value of additional milk obtained (Riford, 1922; Dahlberg, 1924). Cows milked 3X generally produced about 20% more milk than those milked 2X, and milk production could be increased another 7% by milking four times daily (4X) instead of 3X (Woodward, 1931). Modern-day adjustment factors used to compare milk production of cows milked 2X to those milked 3X range from 12-14%, depending on the parity of the cow (VanRaden et al., 1999).

Much of the work on frequent milking for the entire lactation was conducted during the 1980s and 1990s, when there was great interest in switching milking regimes from 2X to 3X in order to increase milk production efficiency (Table 1). A typical response to 3X milking is illustrated in Figure 1, which shows the lactation curves of cows milked 2X or 3X for the entire lactation (re-drawn from Amos et al., 1985). Three-times daily milking increased milk production both at peak and through the entire lactation. Persistency of the lactation curve was also slightly increased, but this effect disappeared after approximately 180 DIM. In summaries of DHIA records, the increase in milk production with 3X was 13, 12, and 16% compared to 2X (Allen et al., 1986; Gisi et al., 1986; Smith et al., 2002). These reports were obtained from mostly Holstein herds, or combined Holstein and Jersey herds. Culotta and Schmidt (1988) suggested that smaller dairy breeds do not respond as well to frequent milking as larger breeds. Consistent with that hypothesis were observations of Campos et al. (1994), that relative to 2X, 3X increased milk production by 17.3 and 6.3% in Holsteins and Jerseys, respectively. In contrast, Copeland (1934) observed an impressive 21 and 19% increase in milk and fat production, respectively, from Jerseys milked 3X compared to those milked 2X. In addition, they reported a correlation (+.64) between the amount of milk cows produced prior to frequent milking and the magnitude of the response to frequent milking (Copeland, 1934). This led to speculation that higher producing cows better responded to increased milking frequency than did lower producing cows. The existence of such a relationship, however, has not been established. To the contrary, Erdman and Varner (1995) and Stockdale (2006) reviewed the literature on frequent milking, and reported no correlation between previous milk production and the response to changes in milking frequency. Instead, those researchers concluded that there was an incremental milk yield response. Relative to 2X, this fixed milk yield response was -6.2, +3.5 and +4.9 kg/d for once daily milking (1X), 3X, or 4X, respectively (Erdman & Varner, 1995). In agreement with this, Peel et al. (1979) estimated that the post-suckling increase in milk production of cows suckled for as little as one week during early lactation was 4.3 kg/d. They went so far as to provide the readers with the following equation to allow prediction of the milk yield response to various suckling regimes:

$$\text{Increase in milk yield (\%)} = 4.3 \pm 0.8 \text{ (mean } \pm \text{S.E.)} \times \text{number of weeks suckled.}$$



**Figure 1.** The effect of frequent milking on milk production of dairy cows. Lactation curves of multiparous cows milked twice (2X) or thrice (3X) daily for the entire lactation (re-drawn from Amos et al., 1985).

Reference	Parity	Breed	Duration of FM	Change in milk yield <sup>b</sup> (2X vs. 3X <sup>c</sup> )	Change in milk yield (2X vs. 4X)
Riford, 1922	≥ 1	Holstein; Guernsey	Unknown	+ 4.6 kg/d	
Woodward, 1931	≥ 1	Holstein	Full lactation	+ 20%	
Copeland, 1934	≥ 1	Jersey	Through late lactation	+ 21%	
Rao and Ludri, 1984	≥ 2	Brown Swiss x Sahiwal	50 to 130 DIM <sup>d</sup>	+ 1.34 kg/d	+ 1.73 kg/d
DePeters et al., 1985	≥ 2	Holstein	Full lactation	+ 17%	
	1	Holstein	Full lactation	+ 6% (NS)	
Amos et al., 1985	≥ 2	Holstein	Full lactation	+ 18.5%	
	1	Holstein	Full lactation	+ 25.2%	
Allen et al., 1986	≥ 2	Holstein	Full lactation	+ 13.4%	
	1	Holstein	Full lactation	+ 19.4%	
Gisi et al., 1986	≥ 2	Holstein	Full lactation	+ 12%	
	1	Holstein	Full lactation	+ 14%	
Barnes et al., 1990	1	Holstein	Full lactation	+ 14%	
Campos et al., 1994	1	Holstein	Full lactation	+ 17.3%	

Reference	Parity	Breed	Duration of FM	Change in milk yield <sup>b</sup> (2X vs. 3X <sup>c</sup> )	Change in milk yield (2X vs. 4X)
	1	Jersey	Full lactation	+ 6.3%	
Klei et al., 1997	≥ 1	Holstein	Full lactation	+ 10.4%	
Smith et al., 2002	≥ 1	Holstein	Full lactation	+ 16%	

<sup>a</sup>Reports on frequent milking or suckling during early lactation or on half-udders are represented in tables 3 and 5.

<sup>b</sup>Numbers in columns represent the increase in milk yield observed with increased milking frequency.

<sup>c</sup>2X = twice daily milking; 3X = thrice daily milking; 4X = four times daily milking.

<sup>d</sup>DIM = days in milk

**Table 1.** Summary of selected literature reports on the effects of frequent milking (FM) on milk yield of dairy cows<sup>a</sup>.

Importantly, the increase in milk yield in response to frequent milking or suckling is not specific to dairy cows; milk production was increased with frequent milking or suckling of other dairy ruminants, including goats (Wilde et al., 1986), sheep (Geenty & Davison, 1982; Negrao et al., 2001; Nudda et al., 2002), and buffalo (Dash et al., 1976), as well as various cross breeds (Little et al., 1991; Krohn, 2001; Sidibe-Anago et al., 2008; Alvarez-Rodriguez et al., 2010).

## 1.2. Effects of frequent milking or suckling on milk composition and cow health

Reported effects of frequent milking on milk composition, somatic cell count (SCC) and herd health have been inconsistent. Many researchers have observed no effect of frequent milking on milk composition (Poole, 1982; Rao & Ludri, 1984; Amos et al., 1985; DePeters et al., 1985; Gisi et al., 1986), whereas some have observed a decrease in fat percentage (Allen et al., 1986; Smith et al., 2002). Due to the increase in milk production in response to frequent milking, however, there is often an increase in the total yield of fat and protein (Klei et al., 1997; Dahl et al., 2004b). With respect to SCC, some reports have indicated an association between frequent milking and decreased SCC, and these authors concluded that frequent milking may improve mammary health (Poole, 1982; Armstrong et al., 1985; Smith et al., 2002; Dahl et al., 2004b). Others have reported no effect of frequent milking on SCC (Waterman et al., 1983; DePeters et al., 1985; Gisi et al., 1986; Bar-Peled et al., 1995; Klei et al., 1997; Hale et al., 2003; Patton et al., 2006; Wall & McFadden, 2007a; Shields et al., 2011; Wright et al., 2011). The inconsistencies in the above reports may be the result of variations in timing and methods of sampling across experiments. Suckling of cows during early lactation has consistently been associated with a decrease in SCC and a decrease in the incidence of clinical mastitis, in some cases by up to 50% or more (Walsh, 1974; Edmunds, 1977; Little et al., 1991; Krohn, 2001). In fact, Walsh (1974) suggested that the increase in milk production elicited by suckling was probably due to the additional stimulus of the gland as well as the markedly improved mammary health of suckled animals.

Discrepancies also exist in the reported effects of frequent milking on reproductive performance and herd health. Some researchers have observed decreased reproductive

performance in 3X cows compared to 2X cows (Ludwin, 1942; Armstrong et al., 1985; DePeters et al., 1985; Smith et al., 2002), whereas others have observed no effect (Poole, 1982; Amos et al., 1985; Gisi et al., 1986) or an improvement (Allen et al., 1986) in reproductive performance with 3X. Armstrong et al. (1985) suggested that any negative effects of frequent milking on herd health or reproductive performance may be associated with poor herd management. Well-controlled field studies using standardized sampling procedures will be necessary to verify the impact of milking frequency on milk composition, SCC and herd health, as well as identifying the interaction between frequent milking and herd management on these factors.

Similarly, suckling of a calf during early lactation of the cow is associated with increased weight loss and an increase in the days to first estrus (Margerison et al., 2002). In most cases, however, the delay in resumption of estrous cyclicity is offset by an increase in conception rate (Little et al., 1991; Krohn, 2001). Consequently, the effects of suckling on reproductive performance appear to be negligible, or in some cases positive (Table 2). In addition, Perez-Hernandez et al. (2002) reported that exposure of cows to a teaser bull decreased the effects of suckling on days to first estrus. Therefore, in agreement with the suggestion by Armstrong et al. (1985), simple changes in management - when feasible - can be used to overcome any potential negative impact of frequent milking or suckling regime on reproductive performance. Table 2 summarizes the findings of Little et al. (1991), who looked at lactation and reproductive performance of cows allowed to suckle calves for the first 90 days of lactation.

	<b>Suckled &amp; machine milked</b>	<b>Machine milked</b>
Total milk production (kg)	1,894.10	1,644.60
Lactation length (d)	341	305
Dry matter feed intake (kg/d)	13.25	13.14
Days to estrus	101	41
Services per conception	1.3	1.9

**Table 2.** Productivity characteristics of crossbred cows subjected to suckling compared to machine-milked herdmates. Cows were allowed to suckle their calves for the first 90d of lactation and were also machine milked twice daily for the entire lactation (Little et al., 1991).

In addition to the increase in milk yield, suckling during early lactation has also been associated with an increase in lactation length (Everitt & Phillips, 1971; Little et al., 1991; Krohn, 2001). This is particularly relevant since some producers are interested in incorporating an extended lactation management scheme into their herd. Extended lactation offers the benefit of decreased health risk to the cow because there is less exposure to risk of metabolic disease, which highest during the transition period. This, in turn, decreases costs to the producer associated with treating sick cows and veterinary expenses. The use of frequent milking or suckling offers a tool for increasing lactation persistency during

extended lactations. Indeed, Sorensen et al. (2008) reported that the use of increased milking frequency makes an extended lactation cycle economically viable for the producer, in addition to an observed improvement in the health of the cow.

### 1.3. Effects of suckling on calf health and performance

There are health benefits of keeping the calf with the cow and allowing the calf to suckle. Edmunds (1977) suggested that suckling calves generally perform better than those raised on milk replacer. Little et al. (1991) reported an increase in growth rate from birth to weaning of 0.2 kg/d for calves that were allowed to suckle vs. those that were raised on milk replacer. Similar observations were made by Alvarez-Rodríguez et al. (2010). In some cases, however, there is wide variation in the daily weight gain of suckling calves, especially if there are multiple calves on a single cow. Therefore, depending on the suckling scheme used, calves allowed to suckle don't always have higher growth rates than calves raised on milk replacer (Krohn, 2001). In a review of the literature, Edmunds (1977) reported that suckling calves had superior daily weight gain, a decrease in scours, and minimal incidence of other diseases compared to bucket-reared herdmates. The author suggested that this was a result of decreased stress associated with separation from the cow, since suckling calves are allowed almost constant contact with their mothers.

An interesting study conducted by Bar-Peled et al. (1997) reported on the performance of first lactation heifers that were allowed to suckle as calves, and compared them to heifers that were reared on milk replacer. Their findings are summarized in table 3, and have major implications regarding the effects of suckling on performance as adults; suckling clearly improved production efficiency in several areas.

	Suckled	Milk replacer
Body weight at conception (kg)	358.6	327.2
Average daily gain (kg)	0.82	0.68
Age at conception (d)	394	426
Conception rate (%)	83.4	74.2
Calving age (d)	669	700
Milk production (kg/300d)	9624	9171

**Table 3.** Productivity characteristics of heifers that were allowed to suckle as calves compared to herdmates raised on milk replacer. Heifers were allowed to suckle as calves for the first six weeks of life. Herdmates were raised on milk replacer (Bar-Peled et al., 1997).

### 1.4. The effect of parity on the response to frequent milking

It has been reported that the milk production response to frequent milking was more pronounced in animals with smaller udder capacity, such as heifers (Woodward, 1931; Copeland, 1934; Lush & Shrode, 1953). Copeland (1934) speculated that this may be a

function of increased udder pressure associated with less udder capacity. In agreement with this, Allen et al. (1986) observed that relative to 2X, 3X increased milk production by 13.4 and 19.4% in cows and heifers, respectively. In the previous year, however, another group reported that mature cows respond better to frequent milking than primiparous cows (DePeters et al., 1985). Of the studies that measured the milk yield response in heifers separately, most reported that heifers responded to frequent milking similarly to or better than multiparous cows. Therefore, no relationship has been established between parity and the magnitude of the milk yield response to frequent milking. To the contrary, we observed that that relative to multiparous cows, heifers respond similarly to increased milking frequency during early lactation (Wright et al., 2011). Similar observations have been made on the response of primi- vs. multi-parous cows to suckling during early lactation (Everitt & Phillips, 1971).

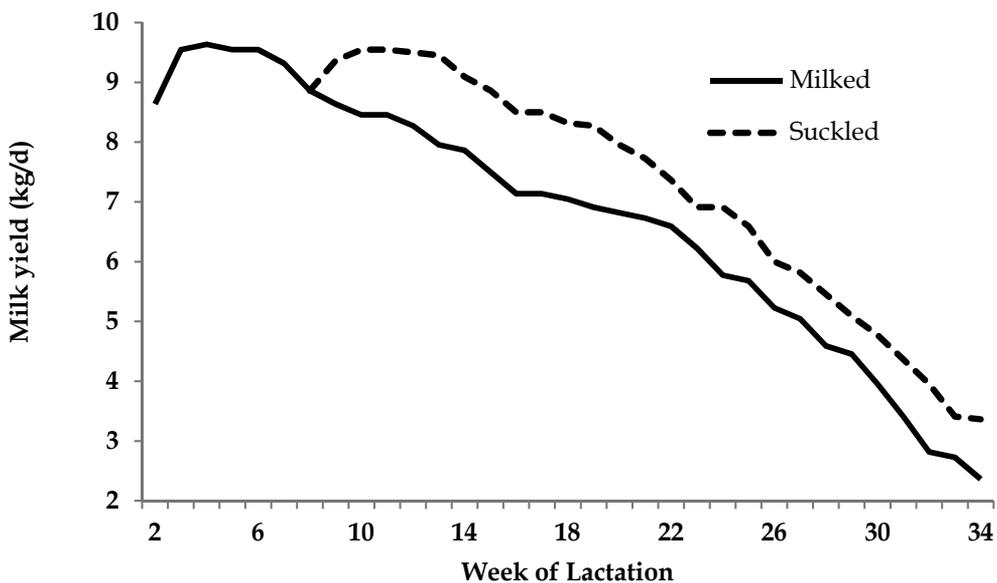
### **1.5. Economic impact of frequent milking or suckling**

Several research groups have characterized the economic impact of frequent milking. Factors contributing to the profitability of frequent milking were labor, herd size, herd health, management, feed costs, and milk price (Armstrong et al., 1985; Culotta & Schmidt, 1988; Maltz et al., 2003). Rao and Ludri (1984) reported that 3X increased net income by 21% relative to 2X. More recently, we estimated a net increase of approximately \$93/cow/yr when cows were milked 4X for the first 3 wk of lactation and milked 2X thereafter (Table 4; Wall & McFadden, 2007b). With respect to suckling, McKusick et al. (2001) estimated an increase in net income of \$25 per ewe/suckling lamb pair in a mixed rearing system (in which ewes are suckled *and* machine milked until lambs are weaned) relative to ewes solely machine milked and lambs raised on milk replacer. The increase in profitability of suckling management systems comes from eliminating the expenses associated with purchase of milk replacer, and also the increase in milk production of the dams after the suckling period. This, combined with the observation of Bar-Peled et al. (1997) that suckling calves perform better as adults, indicates multiple areas of economic gain with a suckling regime. Therefore, when there are no negative effects on animal health or reproductive performance, frequent milking or suckling has the potential to be a very profitable management tool.

## **2. Frequent milking or suckling during early lactation: a window of opportunity**

An exciting development for both dairy producers and dairy scientists was the finding that the timing of implementation can influence the milk yield response to frequent milking. During middle and late lactation, frequent milking increased milk production; however cessation of frequent milking resulted in an immediate decrease in milk yield to pre-treatment levels (Elliott, 1961; Morag, 1973a, b; Svennersten et al., 1990). During early lactation, however, frequent milking for a short duration can stimulate milk production and the effect persists through the remainder of lactation, even after less frequent milking is resumed. This effect was originally observed in experiments designed to determine the

milk yield loss associated with the use of nurse cows. Using identical twin cows, Everitt and Phillips (1971) discovered that suckling by calves in addition to machine milking during the first 8 to 10 weeks of lactation was associated with increased milk production after weaning and throughout the remainder of lactation in both primi- and multiparous cows (Figure 2). Shortly after this report, similar observations were made in both cows (Edmunds, 1977; Moss & O'Grady, 1978; Thomas et al., 1978; Fulkerson, 1981) and heifers (Fulkerson et al., 1978; Peel et al., 1979). Pearson et al. (1979) assigned cows to 3X for the first 143 d of lactation, followed by 2X thereafter. Although they did not report the full lactation curves, they measured milk yield for the entire lactation and reported that relative to 2X, cows that were milked 3X for the first 143 d of lactation produced more milk through 280 DIM (Pearson et al., 1979). Subsequently, it has been observed in numerous experiments that frequent milking during early lactation was associated with both acute and persistent increases in milk production (Table 5). These findings presented an opportunity for dairy producers; that an initial investment in labor could increase milk production efficiency for the remainder of lactation. Poole (1982) speculated that the practice might not be adopted, however, because producers would be discouraged by the partial decrease in milk production upon cessation of frequent milking, despite the significant carry-over effect.



**Figure 2.** The effect of suckling during early lactation on milk production of dairy cows. *Ad libitum* suckling for the first 8 weeks of lactation increases milk production through late lactation (re-drawn from Everitt and Phillips, 1971). Cows were either milked 2X during the entire lactation (solid line), or were suckled by calves until 8 weeks of lactation, followed by 2X milking thereafter (dashed line).

Milking routine <sup>b</sup>	Feed cost during FM <sup>c</sup>	Feed cost after FM <sup>d</sup>	Labor <sup>e</sup>	Miscellaneous cost <sup>f</sup>	Extra milk income/cow/yr <sup>g</sup>	Total net income/cow/yr	100-cow herd <sup>h</sup>
4X 1 to 21	\$19.28	\$98.00	\$84.00	\$0.50	\$294.75	\$92.94	\$9,293.60
4X 1 to 14	\$12.86	\$100.75	\$56.00	\$0.34	\$210.29	\$40.33	\$4,032.85
4X 7 to 21	\$12.86	\$98.00	\$56.00	\$0.34	\$239.33	\$72.12	\$7,212.35

<sup>a</sup>Cows were assigned to unilateral frequent milking (twice daily milking of the left side, four times (4X) daily milking of the right side) for days 1 to 21, 1 to 14, or 7 to 21 of lactation (n = 10 cows per treatment). The differential milk yield response was adjusted to a whole-udder basis, and additional milk yield per cow was estimated (Wall & McFadden, 2007b).

<sup>b</sup>Four times daily milking for days 1 to 21, 1 to 14 or 7 to 21 of lactation, followed by twice daily milking for the remainder of lactation.

<sup>c</sup>Additional feed to support increased milk production during frequent milking (FM), estimated at \$0.92/cow/d.

<sup>d</sup>Additional feed to support increased milk production after frequent milking, estimated at \$0.39/cow/d.

<sup>e</sup>Additional labor associated with extra milkings and animal handling during 4X milking, approximately \$4/d

<sup>f</sup>Cost associated with extra milkings, including inflation replacement, teat dip, and towels; approximately \$0.025/d

<sup>g</sup>Extra milk income is based on \$12/cwt.

<sup>h</sup>Total net annual income for a 100-cow operation.

**Table 4.** Potential economic return of milking four-times daily during early lactation.<sup>a</sup>

Reference	Treatment duration <sup>d</sup>	Parity	Change in milk yield (kg/d; 2X vs. 3X)	Change in milk yield (kg/d; 2X vs. 4X)	Change in milk yield (kg/d; 3X vs. 6X)
Pearson et al., 1979	1 to 150	≥ 2	+ 2.2		
Poole, 1982	1 to 140	≥ 2	+ 4.4 (acute)		
			+ 1.84 (pers.)		
		1	+ 2.17 (acute)		
			+ .65 (pers.)		
Bar-Peled et al., 1995	1 to 42	≥ 2			+ 7.3 (acute)
					+ 5.1 (pers.)
Sanders, 2000	1 to 42	≥ 2			+ 6.0 (acute)
					+ 3.7 (pers.)
		1			+ 1.7 (acute)
Hale et al., 2003	1 to 21	≥ 2		+ 8.6 (acute)	
				+ 2.6 (pers.)	
Dahl et al., 2004b	1 to 21	≥ 2			+ 14 (acute)
					+ 3.7 (full lactation)

<sup>a</sup>Reports on frequent milking during the entire lactation or on half-udders are represented in Tables 1 and 6.

<sup>b</sup>Numbers in columns represent the increase in milk yield observed with increased milking frequency

<sup>c</sup>2X = twice daily milking; 3X = thrice daily milking; 4X = four times daily milking; 6X = six times daily milking.

<sup>d</sup>Numbers in columns represent days in milk.

<sup>e</sup>Pers. = the persistent increase in milk production after cessation of frequent milking.

<sup>f</sup>Acute = the increase in milk production during frequent milking.

**Table 5.** Summary of select literature reports on the effects of frequent milking during early lactation on milk yield of dairy cows<sup>a</sup>

In an attempt to minimize additional costs associated with frequent milking, and to investigate the response of dairy cows to frequent milking or suckling during a short interval of time in early lactation, Bar-Peled et al. (1995) compared 3X to 6X or 3X+suckling for the first 6 wk of lactation, followed by 3X of all cows. Relative to cows milked 3X during the entire lactation, 6X and 3X + suckling acutely increased milk production by 7.3 and 14.7 kg/d, respectively (Bar-Peled et al., 1995). Cessation of frequent milking or suckling was associated with a partial decline in milk production; however, a carry-over effect was observed in 6X cows (+5.1 kg/d relative to 3X; Bar-Peled et al., 1995). In a similar experiment, Sanders et al. (2000) observed an acute increase of 6 kg/d and a carry over response of 3.7 kg/d in 6X cows relative to 3X cows. In heifers, the acute response to 6X was lower in magnitude (+1.7 kg/d), and no carry over effect was observed (Sanders et al., 2000).

A summary of literature reports on the effect of suckling during early lactation on milk production is presented in Table 6. The results of subsequent experiments have further narrowed down the ‘window’ during early lactation wherein frequent milking can increase milk production for the remainder of lactation. Hale et al. (2003) assigned cows to 2X or to 4X for the first 3 wk of lactation, followed by 2X. Four times daily milking was associated with an acute increase of 8.8 kg/d and a carry over effect of 2.6 kg/d for the remainder of lactation. A treatment interval of 1 to 21 DIM was also used in a field study by Dahl et al. (2004b), who observed similar effects of frequent milking during early lactation. In contrast, VanBaale et al. (2005) assigned cows to 3X or 6X for the first 7, 14, or 21 d of lactation and reported that 6X did not increase milk production relative to 3X. Their observations were inconsistent with previous reports, and the authors speculated that facility logistics may have influenced their results because 6X cows were housed farther away from the milking parlor and spent a considerably longer time away from their pen than 3X cows (VanBaale et al., 2005). With the exception of one negative report (VanBaale et al., 2005), and one abstract (Fernandez et al., 2004), it is generally accepted that frequent milking increases milk yield, and that frequent milking or suckling during early lactation can increase milk production for the remainder of lactation (see Tables 5 and 6). The mechanistic basis for the milk yield response to frequent milking, however, is poorly understood. Even less understood are the mechanisms involved in the persistent effect on milk yield of frequent milking during early lactation.

Reference	Treatment Duration (DIM)	Parity	2X vs. 1X + suckle	2X vs. 2X + suckle	3X vs. 3X + suckle
Everitt and Phillips, 1971	1 to 70	2+		(+) 1.2 kg/d (pers. <sup>a</sup> )	
		1		(+) .87 kg/d (pers.)	
Walsh, 1974	1 to 100	2+		(+) 11.3% (acute <sup>b</sup> )	
				(+) 7.7% (pers.)	
Moss and O’Grady, 1978	1 to 56	2+		(+) 3.3 kg/d	
				(no pers.)	
Thomas et al., 1978	1 to 56	2+		(+) 1.68 kg/d (acute)	

Reference	Treatment Duration (DIM)	Parity	2X vs. 1X + suckle	2X vs. 2X + suckle	3X vs. 3X + suckle
				(+) 1.77 kg/d (pers.)	
Fulkerson et al., 1978	1 to 56	1	(+) 16% (full lactation)		
Pearson et al., 1979	1 to 150	2+			
Peel et al., 1979	1 to 28	1	(+) 13% (pers.)		
Fulkerson et al., 1981	1 to 7	2+	(+) 9% (pers.)		
Teeluck et al., 1981	1 to 90	2+	(+) 2.2 kg/d	(+) 3.33 kg/d	
Poole, 1982	1 to 140	2+			
Little et al., 1991	1 to 94	5	(+) 15% (full lactation)		
Bar-Peled et al., 1995	1 to 42	2+			+ 14.7 kg/d (acute)

<sup>a</sup>Pers. = the persistent increase in milk production after cessation of suckling, if reported

<sup>b</sup>Acute = the increase in milk production during suckling

**Table 6.** Summary of select literature reports on the effects of suckling during early lactation on milk yield of dairy cows

### 3. Local regulation of the milk yield response to frequent milking

Shortly after the report in the 1930s that frequent milking increased milk production, several studies utilized unilateral frequent milking (UFM) to investigate the effect. Half-udder designs are extremely powerful because they eliminate variation between animals due to environment, nutrition, and genetics. In addition, both udder halves are theoretically exposed to the same systemic factors, hence responses to frequent milking are strictly at the level of the mammary gland. A summary of the milk yield response to frequent milking in selected half-udder experiments is presented in Table 7. The early reports provided strong evidence for local regulation of milk production, and increases in milk yield from 8.4 to 32% in the frequently-milked udder half were observed (Ludwick et al., 1941; Cash & Yapp, 1950; Agarwala & Sundaresan, 1955; Claesson et al., 1959). Morag (1973b) reported that the increase in milk production in response to UFM occurs within 24 h, and the magnitude of the response was independent of previous milk production. In addition, heifers respond to UFM; Hillerton et al. (1990) milked udder halves 2X or 4X for 4 wk during mid-lactation. In both cows and heifers, milk production of 4X udder halves increased by 10.4% relative to 2X udder halves (Hillerton et al., 1990).

Reference	Stage of Lactation	Duration of UFM	Parity	Change in milk yield (%; 2X vs. 3X)	Change in milk yield (2X vs. 4X)
Cash & Yapp, 1950	-	Full lactation	≥ 2	+ 32	
Agarwala & Sundaresan, 1955	Early	25 d intervals	≥ 2	+ 8.4	
Hillerton et al., 1990	Mid-late	28 d	≥ 1		(+) 10.4%
Knight et al., 1992	Mid	14 d	1		(+) 14%

Reference	Stage of Lactation	Duration of UFM	Parity	Change in milk yield (%; 2X vs. 3X)	Change in milk yield (2X vs. 4X)
Knight, 1992	Early	42 d	≥ 1	+ 10.4	
Norgaard et al., 2005	Mid	7 d	≥ 1		(+) 18%
Wall & McFadden, 2007a	1 DIM <sup>d</sup>	21 d	≥ 2		(+) 3.5 kg/d (acute <sup>e</sup> )
					(+) 1.8 kg/d (pers. <sup>f</sup> )
Wall & McFadden, 2007b	1 DIM	14 d	≥ 2		(+) 3.7 kg/d (acute)
					(+) 1.2 kg/d (pers.)
	7 DIM	14 d	≥ 2		(+) 2.9 kg/d (acute)
					(+) 1.5 kg/d (pers.)
Wright et al., 2011	1 DIM	21 d	1		(+) 2.8 kg/d (acute <sup>e</sup> )
					(+) 0.9 kg/d (pers.)
Shields et al., 2011	1 DIM	21 d	≥ 2		(+) 3.4 kg/d

<sup>a</sup>Reports that did not use half-udders are represented in Tables 1 and 5.

<sup>b</sup>Numbers in columns represent the increase in milk yield observed with increased milking frequency.

<sup>c</sup>2X = twice daily milking; 3X = thrice daily milking; 4X = four times daily milking.

<sup>d</sup>DIM = days in milk.

<sup>e</sup>Acute = the increase in milk production during frequent milking.

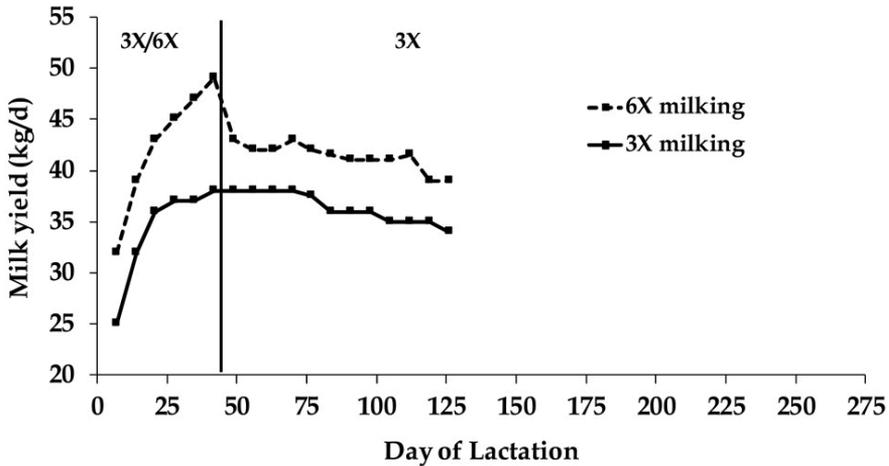
<sup>f</sup>Pers. = the persistent increase in milk production after frequent milking, if reported.

**Table 7.** Summary of select literature reports on the effects of unilateral frequent milking (UFM) on milk yield<sup>a</sup>

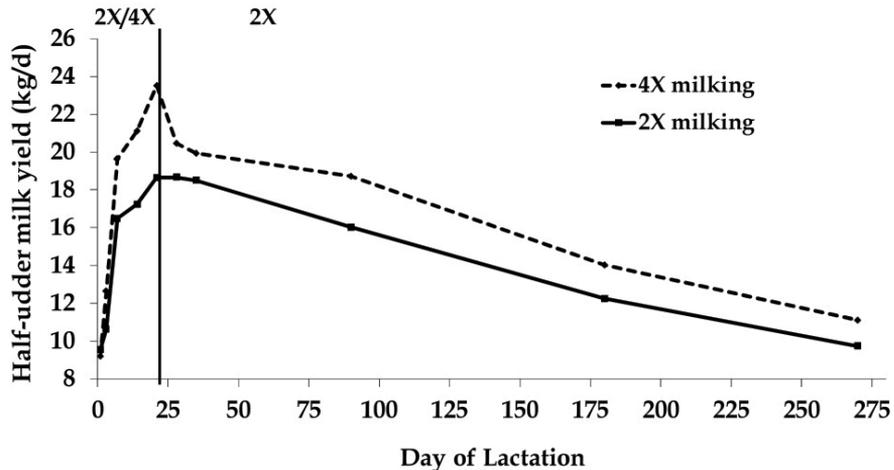
As mentioned previously, an emerging theme in these experiments has been that the effects of frequent milking during early lactation on milk production persist even after a lower milking frequency is resumed (Bar-Peled et al., 1995; Hale et al., 2003; Dahl et al., 2004b). Although this persistent milk yield response has been consistently observed (Table 5), it was unknown whether the response was regulated by hormones, by local factors within the mammary gland, or by the combination of the two. To investigate this question, we used a half-udder model and assigned cows to UFM (4X of the right udder half, 2X of the left udder half) for d 1 to 21 of lactation, followed by 2X for the remainder of lactation (Wall & McFadden, 2007a). When the half-udder milk yields were adjusted to the equivalent of a whole udder basis, the acute and long-term milk yield responses to frequent milking that we observed were consistent with those reported by Hale et al. (2003). Therefore, our results indicated that both the acute and persistent effects of frequent milking during early lactation are regulated by local factors within the mammary gland. This is illustrated in Figure 3A and B. Figure 3A (re-drawn from Bar-Peled et al., 1995) shows the milk yield response of multiparous cows to 6X for the first 6 wk of lactation, followed by 3X. We observed a similar effect using a half-udder experiment (Figure 3B), and the milk yield response lasted through 270 DIM. This finding presents some intriguing questions and research opportunities. First, what are the local factors that regulate milk production capacity of the mammary gland? Once the factor(s) have been identified and pathways understood, how can we refine our approach to maximize milk production efficiency of dairy cows? Now that it is established that the factors are indeed local, the problem has become relatively simplified. Extremely

powerful, within cow experiments that are less sensitive to the influence of environment, genetics and nutrition can now be designed to ask such mechanistic questions.

A



B



**Figure 3.** A. Six-times daily milking for days 1 to 42 of lactation increases milk production through late lactation (re-drawn from Bar-Peled et al., 1995). B. Unilateral four-times daily milking for days 1 to 21 of lactation increases milk production for the remainder of lactation (Wall and McFadden, 2007).

On the road to refinement, one theme that has transpired is the existence of a 'window' of time wherein the mammary gland is especially responsive to frequent milking. The duration of this window has been shortened from the first 10 wk of lactation (Moss & O'Grady, 1978; Thomas et al., 1978) to the first 6 wk of lactation (Bar-Peled et al., 1995; Sanders et al., 2000), and shortened further still to the first 3 wk of lactation (Hale et al., 2003; Dahl et al., 2004b;

Wall & McFadden, 2007a). It was unknown whether a shorter duration or altered timing of frequent milking during early lactation would still elicit a persistent effect on milk production; however, since any costs associated with extra labor are increased only during frequent milking, it was of great interest to shorten the duration of frequent milking if a persistent increase in milk yield could still be observed. To answer this question, we assigned cows to UFM (4X of the right udder half, 2X of the left udder half) for d 1 to 14 or d 7 to 21 of lactation (Wall & McFadden, 2007b). We observed an acute milk yield response in both treatments; and a significant carry-over effect in the d 7 to 21 group. There was a numerical carry-over for the d 1 to 14 group; however it was not significant. Our results indicate that within the first 21 DIM, an interval of frequent milking as short as 2 wk can elicit a persistent increase in milk production. As mentioned previously, similar observations have been made on the response of cows and heifers to suckling during early lactation (Fulkerson et al., 1978; Peel et al., 1979). Further narrowing of this “window” within the first 21 of lactation, as well as characterization of the cellular response could provide insight into the mechanisms underlying the receptiveness of the mammary gland to stimulus during this time.

#### **4. Endocrine response to frequent milking or suckling**

It has long been thought that the hormones released at milking may be involved in regulating the galactopoietic effects of frequent milking on milk production. Indeed, multiple hormones are released during milking including glucocorticoids, oxytocin, and prolactin (Tucker et al., 1975; Carruthers & Hafs, 1980; Akers & Lefcourt, 1982). Oxytocin is responsible for milk ejection. Cows suckling calves are thought to have more efficient milk ejection due to increased secretion of oxytocin elicited by the presence of the calf. In fact, on dairies using cross breeds and cows not bred for high milk production, the calf is often used as a facilitator of milk letdown during milkings (Little et al., 1991; Krohn, 2001; Bruckmaier & Wellnitz, 2008). In addition, treatment with exogenous oxytocin was associated with increased milk production of both dairy cows (Nostrand et al., 1991; Ballou et al., 1993; Lollivier & Marnet, 2005) and sheep (Zamiri et al., 2001). Therefore, it is possible that oxytocin is involved in regulating the increase in milk production elicited by frequent milking or suckling, perhaps by allowing for more complete milk removal and a decrease in negative feedback on the gland.

Along with enhanced milk production, Bar-Peled et al. (1995) observed increased concentrations of growth hormone, insulin-like growth factor-1, oxytocin and prolactin in circulation of cows that were frequently milked or suckled. In addition, the magnitude of milking-induced PRL release declines concomitantly with the decrease in milk production as lactation progresses (Koprowski & Tucker, 1973). Consequently, PRL has been hypothesized as a candidate regulator of the effects of frequent milking on milk production (Dahl et al., 2004a). In an attempt to determine whether milking-induced PRL release indeed mediates the effects of frequent milking on milk production, we assigned cows to 2X, 4X, or 2X + twice daily injections of PRL (Crawford et al., 2004; Wall et al., 2006). Four times daily milking or PRL injections increased milk production relative to 2X (Crawford et al., 2004);

however our results indicated that PRL injection or frequent milking exerted distinct effects on mammary cell growth and gene expression, thus probably increased milk production via separate mechanisms (Wall et al., 2006). The response to unilateral frequent milking during early lactation supports this concept; frequent milking may stimulate milk production via local factors, whereas PRL injections may increase milk yield through a more systemic pathway.

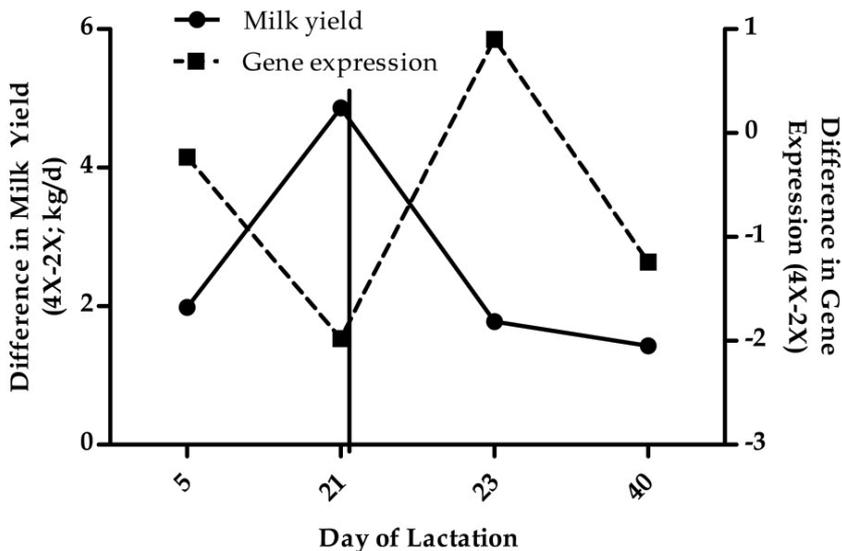
## 5. Cellular response to frequent milking

Several authors have speculated that frequent milking increases milk yield via an increase in mammary cell number and (or) activity (Bar-Peled et al., 1995; Stelwagen & Knight, 1997; Sanders et al., 2000; Hale et al., 2003), both of which are critical to improved lactation performance (Capuco et al., 2003). Hillerton et al. (1990) observed an increase in activity of mammary enzymes, protein and lactose synthesis (in heifers only), DNA synthesis, and alveolar area in response to increased milking frequency, and concluded that cellular differentiation and proliferation were optimized with frequent milking. Hale et al. (2003) reported an increase in mammary cell proliferation at 7 DIM in cows that were milked 4X for the first 3 wk of lactation compared to cows milked 2X; however, differences in proliferation were only observed in one of the two frequently milked cow groups. In contrast to those experiments, Norgaard et al. (2005) reported that despite an increase in milk yield (+18%), there was no effect of frequent milking on cell death, proliferation, or enzyme activities in the mammary gland. In agreement with that report, we have observed across multiple experiments that relative to 2X, 4X did not affect mammary cell proliferation or apoptosis (Wall et al., 2006; Wall & McFadden, 2010; Wall et al., 2011b), indicating that changes in milking frequency influence milk yield through an alternative mechanism.

Using a unilateral frequent milking model and a functional genomics approach, we determined that the increase in milk yield associated with frequent milking is regulated by changes in gene expression elicited by removal of milk from the gland (Wall et al., 2011a). We then used a sequential biopsy approach and obtained mammary tissue at various times during and after exposure to unilateral frequent milking and determined that the temporal expression of 64 genes was co-regulated by unilateral frequent milking (Wall et al., 2011b). Importantly, the pattern of differential expression of the 64 genes was negatively correlated with differential milk yield (Figure 4); therefore, we hypothesize that we have identified a pathway for the autocrine regulation of milk production. Furthermore, this transcriptional signature appears to be malleable and adaptable to the needs of the offspring (mimicked by changes in milking frequency), since expression of some of the genes was still different between udder halves nearly three weeks after cessation of treatment (Figure 4). Future experiments will clarify the role of these genes in the mammary gland and their involvement in the autocrine regulation of milk production.

What is unique to early lactation, when the stimulus of frequent milking for a short duration can elicit a persistent increase in milk production? This question remains

unanswered, but work by Stelwagen and Knight (1997) has provided some clues. Using a half-udder model, they compared 1X to 2X of cows in early or late lactation and reported a more dramatic increase in milk secretion efficiency in response to 2X during early lactation compared to late lactation (Stelwagen & Knight, 1997). In agreement, Walsh (1974) observed different effects of suckling during early vs. late lactation on mammary health. During early lactation, suckling of a calf was associated with a 27% decrease in clinical mastitis, whereas suckling during late lactation had no effect (Walsh, 1974). Taken together, the observations of Walsh (1974) and Stelwagen and Knight (1997) indicate that there are distinct differences in the cell population during early vs. late lactation. It is possible that during early lactation, there are more secretory cells present in the mammary gland, and these cells may have more potential to respond to stimulus than cells present in late lactation. Frequent milking may prevent otherwise unused cells from undergoing apoptosis, or may provide the stimulus to push the cells to reach higher levels of differentiation and secretory capacity. These scenarios could result in an increase in the number of cells in the gland throughout lactation, an increase in the activity of cells throughout the lactation, or both. Shorten et al. (2002) proposed a hypothetical model by which frequent milking for the entire lactation increases the number of active alveoli by reducing the rates of quiescence and senescence in the mammary gland. If such an event occurs with frequent milking during early lactation, this could permanently increase the number of actively secreting alveoli and enhance milk production potential for the remainder of lactation. Many of the biopsy studies that have been previously conducted could have captured changes in mammary cell activity, but would not have captured changes in total cell number or in rates of quiescence and senescence within the gland.



**Figure 4.** Unilateral four-times daily milking for days 1 to 21 of lactation is associated with coordinated changes in mammary expression of 64 genes, and this is negatively correlated with differential milk yield. Solid vertical line represents cessation of unilateral frequent milking.

## 6. Conclusions

Research in the area of frequent milking of dairy cows has established a robust milk yield response to increased milking frequency or suckling, and has identified a window of time during early lactation wherein the mammary gland is especially responsive to the stimulus of frequent milk removal. In addition, there is now evidence that this response is regulated within the mammary gland. Consequently, the concept of ‘use it or lose it’ is becoming more clearly established, that is, the stimulus of frequent milking or suckling during early lactation permanently increases the milk production capacity of the mammary gland. Exciting research opportunities now present themselves, and ongoing experiments seek to identify the local factor(s) that are involved in the regulation of milk production efficiency of dairy cows. The opportunity now exists for dairy scientists to identify the mechanisms involved in local regulation of milk production potential, and for dairy producers to further refine milking management practices to maximize milk production efficiency of their operations.

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# **Regulation of Mammary Development as It Relates to Changes in Milk Production Efficiency**

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Emma Wall and Thomas McFadden

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50777>

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## **1. Introduction**

### **1.1. Mammary development and function**

The development and function of the mammary gland occurs through a cyclical process that changes during the physiological states of pregnancy, lactation, and involution. During pregnancy, maternal hormones in the circulation are responsible for stimulation of mammary gland development and this ensures a sufficient number of mammary cells to produce milk during lactation. Immediately prior to parturition, lactogenic hormones stimulate differentiation of mammary cells, and they adopt a secretory phenotype. In addition to the influence of hormones, the mammary gland itself is thought to be involved in the regulation of mammary cell proliferation, differentiation, and response to hormones. Ultimately, milk yield is dependent on the number and metabolic activity of secretory cells; however, both of these factors are tightly regulated by the endocrine system and physiological state, and also by local factors including the uptake of nutrients by the mammary gland, the connective tissue surrounding the epithelium, and the frequency and degree of milk removal. The following sections will briefly discuss each of the above areas of regulation during lactogenesis and lactation, with emphasis on ruminant species.

### **1.2. The ultimate determinants of milk production potential: mammary cell number and activity**

Milk production potential is a function of the number of mammary epithelial cells in the gland, as well as the secretory activity of those cells (Akers, 2002; Capuco et al., 2003; Boutinaud et al., 2004). Therefore, improved lactation performance can be achieved under conditions that enhance mammary cell proliferation (or decrease apoptosis), biochemical and structural differentiation of mammary epithelium, and synthesis and secretion of milk

components. Moreover, any factors involved in the regulation of these processes can directly impact mammary function and milk yield (Akers, 2002).

The majority (~80%) of mammary cells are formed during pregnancy and prior to lactation; however, cell proliferation during established lactation has been observed in both rodents (Tucker, 1969) and ruminants (Knight & Peaker, 1984; Capuco & Akers, 1990). Because the DNA content per mammary cell nucleus remains relatively constant during pregnancy and lactation, total DNA is considered an accurate indicator of mammary cell number (Tucker, 1987). Mammary cell secretory activity can be estimated by quantification of mammary RNA, and the ratio of RNA to DNA (Paape & Tucker, 1969). Measurements of both DNA and RNA content have provided insight into the relationship between mammary cell number, secretory activity, and milk yield.

It is well established that total mammary cell numbers and milk yield are positively correlated in both ruminants (Linzell, 1966; Keys et al., 1989) and rodents (Tucker, 1969; Nagai & Sarkar, 1978). The secretory activity of these cells is also an important factor involved in determining milk production potential. During lactogenesis, the mammary epithelium becomes highly differentiated. This period is associated with an overall increase in the size and metabolic activity of each cell, closure of tight junctions between cells, an increase in mitochondrial size, and development of the endoplasmic reticulum (Nickerson & Akers, 1984). During established lactation, any new cells that are formed are thought to become differentiated almost immediately (Tucker, 1969). The increase in milk yield during early lactation is associated with an increase in mammary DNA, followed by an increase in mammary cell secretory activity (Knight & Peaker, 1984). In addition, enhanced milk production potential is associated with increases in both mammary epithelial cell number and secretory activity (Keys et al., 1989). In rodents, successful rearing of pups and high rates of litter weight gain are both associated with an increase in mammary DNA, RNA, and ratio of RNA to DNA (Hackett & Tucker, 1969; Paape & Tucker, 1969). Consistent with the observed effects of mammary cell number and secretory activity on milk yield, the declining phase of lactation has been associated with losses in both mammary cell number and metabolic activity (Tucker, 1969; Knight & Peaker, 1984).

Taken together, these observations illustrate the importance of mammary cell number and secretory activity in determining milk yield. Therefore, to improve lactational performance of dairy cows, it is critical to understand the factors involved in the regulation of mammary development and differentiation. Indeed, novel management strategies based on discoveries in mammary gland biology have proven highly successful for use in improving milk production efficiency (Dunlap et al., 2000; Dahl et al., 2004). Some of these techniques will be discussed in more detail later in this chapter.

## **2. Hormonal regulation of mammary function**

One of the major roles of the endocrine system is to coordinate mammary function with the reproductive state of the animal. This physiological synchronization is a very complex process that involves the action and interaction of multiple hormones, as well as the

interplay between hormones in the circulation and local regulation of the mammary response to these hormones. Although much of this chapter will be focused on local regulation of mammary function, it is important to appreciate the role of hormones in regulating mammary function and milk yield. During lactation, several key hormones are involved in the regulation of mammary cell number, secretory activity, and consequent milk production potential.

## **2.1. Hormones involved in lactogenesis and lactation**

### *2.1.1. Prolactin*

As the name indicates, prolactin (**PRL**) is known as the hormone of lactation. Accordingly, it has received much attention from lactation biologists studying the hormonal regulation of mammary function. In ruminants, PRL and glucocorticoids provide the primary stimulus for lactogenesis (Akers, 1985). A role for PRL in the onset of lactation was indicated by a peak in concentrations of the hormone in circulation immediately prior to parturition (Ingalls et al., 1973). Akers et al. (1981a; 1981b) used a dopamine agonist to inhibit periparturient PRL secretion in dairy cows, and this resulted in failure of the mammary epithelium to reach complete structural differentiation. The inhibition of cellular differentiation was accompanied with a 35% reduction in mammary RNA content, a decrease in rates of lactose and fatty acid biosynthesis, and a 40% reduction in milk yield (Akers et al., 1981a). In addition, cytological analysis revealed that inhibition of PRL secretion resulted in a decrease in the size of the metabolic machinery of the cell, including the rough endoplasmic reticulum and the Golgi Apparatus (Akers et al., 1981b). These effects were reversed by treatment with exogenous PRL; therefore, periparturient PRL secretion is essential for complete biochemical and structural differentiation of the mammary gland.

During established lactation, PRL is released during milking or suckling, indicating a role for the hormone in the maintenance of milk production (Koprowski & Tucker, 1973b; Akers, 1985). Indeed, PRL has been shown to maintain both the structural integrity and the functional activity of the mammary epithelium during lactation in rodents (Tucker, 1969; Flint & Gardner, 1994). In addition, PRL maintains and enhances lactation performance in rabbits (Cowie, 1969). Reports on the effect of PRL on milk yield in dairy cows, however, have been inconsistent (Plaut et al., 1987; Wall et al., 2006; Lacasse et al., 2008; Titus et al., 2008). It is generally accepted that PRL is not involved in galactopoiesis (the maintenance of milk production) in ruminant species (Tucker, 2000; Akers, 2006).

As mentioned previously, PRL and glucocorticoids are the major mediators of lactogenesis in many species. In addition to a well-established role in the structural differentiation of the mammary gland, PRL initiates lactogenesis by stimulating the mammary expression and secretion of milk proteins. Using explant culture, Guyette et al. (1979) reported that PRL and glucocorticoids stimulated the expression of casein mRNA within 1 hr of treatment. Similar observations were subsequently made for  $\alpha$ -lactalbumin gene expression (Goodman et al., 1983). Subsequent research has confirmed that indeed, PRL and glucocorticoids elicit an

increase in mRNA and protein expression, as well as a decrease in the degradation of milk protein gene transcripts (Vonderhaar, 1987; Rosen et al., 1999). The ability of PRL and glucocorticoids to regulate casein gene expression is due to the presence of response elements in the promoter region of the casein gene (Rosen et al., 1986).

The action of PRL in the mammary gland is mediated through its receptor, which activates the Janus kinase/signal transducers and activators of transcription (**JAK/STAT**) pathway (Hennighausen et al., 1997a). Stimulation of casein and  $\alpha$ -lactalbumin gene expression by PRL is mediated mainly by STAT5a, which is essential for both mammary gland development and lactation (Hennighausen et al., 1997b; Hynes et al., 1997; Horseman, 1999). Expression of the PRL receptor is also critical for normal mammary development and differentiation. In rodents, the number of PRL receptors in the mammary gland is positively correlated with milk yield and litter weight gain (Sakai et al., 1985). Additional evidence supporting a direct role for PRL receptor in the mammary gland was reviewed by Ormandy et al. (2003). The results of knockout experiments have revealed that there is a minimum requirement for PRL receptor expression in the mammary epithelium of mice, and this is critical for normal mammary development, lactogenesis, and lactation.

### 2.1.2. *Glucocorticoids*

Cortisol is the main glucocorticoid in cattle, and, as mentioned previously, its major function is to enhance the action of PRL in stimulating differentiation of the epithelium and milk protein gene expression in the mammary gland during lactogenesis (Akers, 2002). In addition, glucocorticoids are involved in the regulation of tight junction closure (Stelwagen et al., 1998) and uptake of glucose by the mammary gland (Paterson & Linzell, 1974) during lactogenesis. In pregnant dairy cows, administration of exogenous glucocorticoids resulted in parturition and subsequent induction of lactation (Tucker & Meites, 1965).

During established lactation, glucocorticoids are released during milking or suckling in both rodents and ruminants (Koprowski & Tucker, 1973a; Ota et al., 1974). Interestingly, however, treatment with exogenous glucocorticoids is galactopoietic in rodents (Thatcher & Tucker, 1970) but not dairy cattle (Braun et al., 1970). It is thought that the galactopoietic effect of glucocorticoids in rodents is mediated via an increase in mammary cell secretory activity (Akers, 2002).

The action of glucocorticoids is mediated by its receptor, which is located in the cytosol of the mammary epithelial cell (Gorewit & Tucker, 1976). Upon binding to its receptor, the complex is translocated to the nucleus of the mammary epithelial cell, where it stimulates milk protein gene expression (Tucker, 1985; Li & Rosen, 1994). In addition, the glucocorticoid receptor has been observed to interact with PRL-activated STAT5 molecules to enhance the action of PRL in inducing  $\beta$ -casein gene expression (Wyszomierski et al., 1999). Surprisingly, however, local expression of glucocorticoid receptor was not critical for normal function during lactogenesis and lactation of mice (Kingsley-Kallesen et al., 2002). Therefore, although glucocorticoids enhance the action of PRL during lactogenesis, their direct action on the mammary gland is not essential for normal lactation in rodents. It is

unknown whether expression of glucocorticoid receptor is required for normal mammary function during lactation of ruminants.

### 2.1.3. *Growth hormone*

Growth hormone (**GH**) is widely known for its galactopoietic effect in lactating dairy cattle. The first evidence of this was reported by Asimov and Krouze (1937), who observed that injections of dairy cows with pituitary extracts was associated with increased milk production. Although these findings represented an opportunity for increasing milk production efficiency of dairy cows, it was not practical to harvest and purify pituitary GH for commercial use until the 1980's, when the discovery of recombinant DNA technology made it possible to synthesize large quantities of GH. The recombinantly-derived bovine GH (**rbGH**) was subsequently used extensively for research, and was eventually approved for commercial use on dairy operations (Bauman, 1999). A galactopoietic effect of GH in rodents has not been observed (Tucker, 1985; Hadsell et al., 2007); however, Allan et al. (2002) suggested that GH is involved in maintaining mammary cell number during lactation of mice.

In ruminants, the action of GH on the mammary gland is thought to be mediated mainly by the insulin-like growth factor (**IGF**) signaling axis (Eherton, 2004). Treatment with exogenous GH increases the concentrations of IGF-1 in the circulation (Purup et al., 1993), which acts directly on the mammary gland (Shamay et al., 1988; Baumrucker & Stemberger, 1989). In addition to systemic IGF, locally produced IGF, as well as mammary expression of IGF receptor may influence mammary function and the response of the mammary gland to GH (Plath-Gabler et al., 2001; Akers, 2002). Indeed, the effect of GH on the mammary gland of ruminants varied with physiological state. During early lactation, treatment with exogenous GH had no effect on mammary cell proliferation in goats (Sejrsen et al., 1999). When administered during mid-lactation, however, GH was associated with an increase in mammary cell proliferation in cows (Capuco et al., 2001) and an increase in total volume of secretory tissue in goats (Knight et al., 1990). Because local production of IGF, as well as expression of IGF receptors are also physiologically regulated (Sinowatz et al., 2000; Plath-Gabler et al., 2001), this may explain the differences in the response to GH across physiological states.

Although the action of GH is mediated mainly through the IGF axis, there is evidence that GH may act independently of IGF-I to stimulate milk production (Hadsell et al., 2008). In addition, expression of GH receptor has been detected in mammary tissue (Knabel et al., 1998; Sinowatz et al., 2000; Plath-Gabler et al., 2001). The GH receptor belongs to a superfamily of transmembrane receptors, of which PRL receptor is a member (Postel-Vinay & Kelly, 1996). Therefore, the signaling pathway of GH is very similar to that of PRL: binding of GH to its receptor leads to activation of the JAK/STAT signaling pathway, which stimulates changes in gene expression in target tissues (Postel-Vinay & Kelly, 1996). Unlike PRL receptor, however, expression of GH receptor in mammary epithelium is not required for normal mammary development and function in rodents (Kelly et al., 2002). Instead,

expression of GH receptor in the mammary stroma is critical for normal mammary development, supporting the concept that the action of GH on the mammary epithelium is indirect and may be mediated by locally-produced IGF from the stroma (Kelly et al., 2002).

## **2.2. Other hormones**

### *2.2.1. Leptin*

Leptin is a hormone produced mainly by adipose tissue and is involved in appetite regulation. Although it is primarily associated with appetite regulation, leptin and its receptors are expressed in the mammary gland so it is thought to act locally to influence mammary development (Laud et al., 1999; Chilliard et al., 2001). Indeed, treatment of human mammary epithelial cells with leptin elicited a marked increase in cell proliferation (Hu et al., 2002). In contrast, treatment of bovine (Silva et al., 2002) or mouse (Baratta et al., 2003) mammary epithelial cells with leptin was associated with a decrease in proliferation. In fact, it is thought that leptin mediates the negative effects of a high-energy diet on mammary development of dairy heifers (Silva et al., 2002). In addition to the involvement of leptin in mammary development, it has also been proposed to work synergistically with prolactin to regulate mammary function and inflammation (Motta et al., 2004). More recently, it has been reported that leptin specifically induces expression of its long form receptor in goat mammary gland, and influences mammary development and function through several distinct JAK pathways (Li et al., 2010). Therefore, although the action of leptin in the mammary gland is not fully understood, it clearly plays a role in development and function and may directly influence changes in lactation efficiency by acting locally within the gland.

### *2.2.2. Melatonin*

Melatonin is secreted by the pineal gland during exposure to dark and is involved in the circadian rhythm of many biological functions. For over 30 years, melatonin has had an implicated role in mammary development due to its association with the incidence of breast cancer (Cohen et al., 1978). Indeed, a direct negative relationship between melatonin treatment or presence of the pineal gland and the development of mammary cancer was reported long ago (Tamarkin et al., 1981), and it has subsequently been well documented that melatonin inhibits mammary cancer (For reviews see Cos & Sanchez-Barcelo, 2000; Sanchez-Barcelo et al., 2003; Sahar & Sassone-Corsi, 2007; Pandi-Perumal et al., 2008). Because melatonin is secreted during the dark, and has a negative effect on breast cancer risk, the incidence of breast cancer is increased in night-shift workers and people with sleep disturbances (Stevens, 2006; Blask, 2009), and decreased in the blind (Feychting et al., 1998). It is thought that melatonin exerts its effects on breast cancer possibly by modulating estrogen receptor binding activity (Danforth et al., 1983; Cos et al., 2006; Hill et al., 2009).

Melatonin has also been observed to act directly on the mammary gland to inhibit growth in both rodents (Sanchez-Barcelo et al., 1990) and ruminants (Sanchez-Barcelo et al., 1991; Asher et al., 1994). As will be discussed in a later section of this chapter, exposure of

lactating dairy cows to long day photoperiod (16h light; 8h dark) increases milk production, and exposure of late-pregnant cows to short day photoperiod (8h light; 16h dark) increases milk production in the subsequent lactation (Dahl et al., 2000; Dahl & Petitclerc, 2003). It was initially thought that this effect was mediated by melatonin. Because feeding melatonin did not mimic the effect, however (Petitclerc et al., 1998), alternative mechanisms have been proposed (Dahl, 2008). Nevertheless, melatonin plays a clear role in mammary development and function, and it may work together with other hormones, such as prolactin, to mediate the effects of varying daylength on milk production efficiency.

### 2.2.3. *Oxytocin*

Oxytocin is a peptide hormone that is secreted as part of the neuroendocrine response to milking or suckling (Goodman & Grosvenor, 1983). Once secreted into the bloodstream, oxytocin acts on the mammary gland to elicit the ejection of milk from the alveolar tissue so that it can be removed by the offspring or by the milking machine. Although it is mainly associated with milk ejection, treatment with exogenous oxytocin was associated with increased milk production of both dairy cows (Nostrand et al., 1991; Ballou et al., 1993; Lollivier & Marnet, 2005) and sheep (Zamiri et al., 2001). During milk stasis in lactating mice, treatment with exogenous oxytocin delays the onset of apoptosis and subsequent involution of the mammary gland (Akers, 1985). The action of oxytocin is mediated by its receptor, which is located on the membrane of myoepithelial cells in the mammary gland (Soloff, 1982; Reversi et al., 2005).

Local regulation of the response of the mammary gland to oxytocin has been observed. In lactating rats, milk stasis was associated with a decrease in the response of the mammary gland to exogenous oxytocin (Kuhn et al., 1973). Similarly in dairy cattle, Linnerud et al. (1966) observed that treatment with exogenous oxytocin did not increase milk yield in the absence of milk removal. In addition to the effects of mammary fill with milk, locally produced hormones are thought to influence the effects of oxytocin on the mammary gland of ruminants (Peaker et al., 1995).

### 2.2.4. *Ovarian hormones*

Estrogen and progesterone are both secreted by the ovary, as well as the placenta of pregnant animals, and these hormones are mainly involved in the growth and development of the mammary gland during puberty and pregnancy (Erb, 1977; Schams et al., 1984; Tucker, 1985). Both hormones, however, have additional roles during lactogenesis and lactation. Prior to parturition, estrogen is one of the first hormones to increase in circulation, indicating a role for estrogen in lactogenesis (Akers, 2002). Indeed, administration of exogenous estrogen has been used to induce lactation in both pregnant and non-pregnant dairy cattle (Meites, 1961; Smith & Schanbacher, 1973; Howe et al., 1975; Collier et al., 1977). Estrogen also stimulates the anterior pituitary gland to secrete PRL, and it increases the expression of PRL receptors in the mammary epithelium (Tucker, 2000). During established lactation, estrogen decreases milk yield by interfering with milk ejection (Bruce & Ramirez,

1970), and by inducing mammary involution (Athie et al., 1996; Bachman, 2002). Similar to GH, the action of estrogen on the mammary epithelium is thought to be mediated locally by the mammary stroma and by local production of growth factors (Imagawa et al., 2002; Cunha et al., 2004; Parmar & Cunha, 2004).

Prior to parturition in dairy cattle, progesterone inhibits the synthesis of  $\alpha$ -lactalbumin, casein, and lactose and consequently inhibits the onset of lactogenesis (Goodman et al., 1983; Wilde et al., 1984; Akers, 1985; Tucker, 2000). Once lactation has been established, however, progesterone has no effect on mammary function or milk production, probably because expression of progesterone receptor in lactating mammary gland is very low (Tucker, 2000).

#### 2.2.5. *Relaxin*

Relaxin is a protein hormone that is involved in relaxing the pelvic ligaments around the time of parturition of several species (Sherwood et al., 1993). Although not classically considered to be involved in mammary development, research has shown that it is critical for normal mammary development in rodents (Bani et al., 1986), ruminants (Cowie et al., 1965), and pigs (Hurley et al., 1991; Bagnell et al., 1993). Relaxin is also thought to be involved in the inhibition of lactation prior to parturition (Harness & Anderson, 1975). Wahab and Anderson (1989) suggested that relaxin works synergistically with estrogen and progesterone to stimulate mammary growth in pregnant rats, and similar observations have been made in pigs (Winn et al., 1994). In mice, however, relaxin appears to work independently of sex hormones to stimulate nipple development (Kuenzi et al., 1995). Of particular relevance to milk production efficiency, the stimulus of suckling by piglets appears to overcome any effects of relaxin deficiency on lactation performance of lactating pigs (Zaleski et al., 1996). In mice, however, deletion of the relaxin gene resulted in death of pups due to insufficient nipple development and the inability of the pups to suckle (Zhao et al., 1999). Therefore, although relaxin clearly plays a role in mammary development and function, it is still unclear what role, if any, it plays during lactation. In addition, there are clear differences in the role of relaxin across species.

#### 2.2.6. *Thyroid hormone*

Thyroid hormones have no clearly established role in mammary development (Tucker, 2000), but they are galactopoietic in dairy cows. In addition, they may enhance the effect of other lactogenic and galactopoietic hormones such as PRL and GH (Capuco et al., 1989; Akers, 2002). Leech (1950) investigated the effects of exogenous thyroxine on milk yield of dairy cows, and reported that the hormone was galactopoietic in a dose-dependent fashion. The author speculated that thyroxine functions to increase mammary cell secretory activity; however, the treatment only transiently increased milk yield and upon cessation of treatment, milk yield decreased below pre-treatment levels (Leech, 1950). Consequently, although the milk yield response was investigated further (Stanley & Morita, 1967; Schmidt et al., 1971), treatment with exogenous thyroid hormone to increase milk production of dairy cows was never adopted by the dairy industry.

Hormone	Role in Mammary Gland During Lactation
Prolactin	Lactogenesis; cellular differentiation; galactopoiesis (rodents)
Glucocorticoids	Lactogenesis; cellular differentiation; galactopoiesis (rodents)
Growth Hormone	Mammary development; galactopoiesis (ruminants)
Leptin	Mammary development and function
Melatonin	Inhibition of mammary development
Oxytocin	Milk ejection; cellular differentiation; galactopoiesis
Estrogen	Lactogenesis; involution
Progesterone	None
Relaxin	Mammary development; suppression of lactation
Thyroid Hormone	Galactopoiesis (ruminants)

**Table 1.** The role of various hormones on mammary function during lactation

Clearly, the endocrine system plays an important role in the regulation of mammary function and milk yield across many species. In addition, there is substantial evidence for local regulation of the response of the mammary gland to the endocrine system. This local regulation includes changes in the expression of specific hormone receptors in the gland, as well as the local production of growth factors that mediate or enhance hormonal effects on mammary function. Moreover, there are regulatory mechanisms in the mammary gland that are thought to act separately from, and may sometimes interact with, the effects of the endocrine system.

### 3. Local regulation of mammary function

In addition to the influence of hormones, mammary function is also under the regulation of local factors. It is essential that sufficient nutrients are taken up by the mammary gland to support the synthesis of milk components. Although the mammary epithelium is the site of milk synthesis, it is highly responsive to and largely dependent on the dynamics of the surrounding connective tissue and extracellular matrix. Finally, removal of milk from the gland is involved in the regulation of mammary cell number, secretory activity, and milk yield.

#### 3.1. Mammary blood flow

An extensive vascular system provides the mammary gland with the nutrients required for milk synthesis. Uptake of nutrients, and subsequent synthesis of milk components, is dependent on the rate of blood flow through the capillaries surrounding each alveolus, and also the exchange of nutrients across the capillary wall (Prosser et al., 1996). During pregnancy, the number of blood vessels in the mammary gland gradually increases as the gland prepares for copious milk production (Yasugi et al., 1989; Matsumoto et al., 1992; Djonov et al., 2001). During established lactation, the vasculature is maintained and then slowly regresses with advancing lactation and involution. Consequently, changes in milk yield are usually associated with changes in blood flow to the mammary gland (Prosser et al., 1996).

Local regulation of mammary blood flow in rodents was originally observed by Silver (1956). He observed that within 100 h of sealing selected teats and subsequent engorgement with milk, mammary involution had taken place, and capillaries were empty and collapsed. This occurred even when contralateral glands were suckled, indicating that mammary blood flow is indeed under the control of local factors and not hormones (Silver, 1956). When pups were allowed to resume suckling of the previously sealed teats, the capillary bed was promptly re-filled with blood and mammary function was restored (Silver, 1956). In agreement with those observations, Mao and Caruolo (1973) reported that mammary blood flow was inversely related to the amount of milk accumulated in the gland, and that decreased milk secretion during milk stasis may be mediated by a decrease in availability of nutrients to the mammary gland. Similarly, during extended milk stasis in lactating goats, blood flow to the mammary gland decreased linearly over 36 h (Stelwagen et al., 1994). Stelwagen et al. (1994) suggested that during milk stasis, the decline in mammary blood flow may be the result of negative feedback from the gland due to a reduction in demand for milk precursors. Farr et al. (2000) reported that extended milk stasis in lactating goats resulted in a 50 to 75% decrease in mammary blood flow and capillary permeability, as well as a marked regression of the vasculature, in agreement with previous observations in mice (Silver, 1956). The results of this research support the concept that during milk stasis, blood flow to and metabolic capacity of the mammary gland is impaired (Farr et al., 2000).

In contrast to the negative effect of milk stasis on mammary blood flow, a positive relationship has been observed between mammary blood flow and frequent milk removal. During hourly milking of lactating goats, blood flow to the mammary gland increased (Farr et al., 2000). In addition, milk yield of lactating goats increased within 2 h of an experimental increase in mammary blood flow via vasodilatation (Prosser et al., 1990). After the treatments stopped, however, milk yield decreased to pre-treatment levels. Despite these observations, frequent milk removal does not always stimulate an increase in mammary blood flow (Maltz et al., 1984), and an increase in mammary blood flow does not always elicit an increase in milk yield (Prosser et al., 1994; Lacasse & Prosser, 2003). Therefore, although mammary blood flow and milk yield are closely associated, they are not always causally linked. This indicates that although mammary blood flow sometimes influences milk yield, other limiting factors are involved.

### **3.2. Extracellular matrix**

As discussed above, the development and differentiation of the mammary gland requires stimulation by hormones. However, an important local mediator of cellular function is the environment surrounding the epithelium. This surrounding environment contains the extracellular matrix (**ECM**), which acts directly on the mammary epithelium to regulate cell differentiation, growth, gene expression, and response to hormones (Wilde et al., 1984; Lee et al., 1985; Streuli et al., 1991).

Suard et al. (1983) reported that the nature of substratum used in culture had a marked effect on both proliferation and differentiation of primary epithelial cells harvested from rabbit

mammary glands. Whereas cells cultured on a floating collagen gel were able to synthesize and secrete caseins in response to PRL, cells embedded in collagen were observed to secrete caseins in response to PRL and also to proliferate (Suard et al., 1983). In contrast, cells cultured on an attached collagen gel were able to proliferate only and did not synthesize or secrete caseins in response to PRL. Moreover, the cells cultured on the attached collagen gel did not express casein mRNA. The authors concluded that cell surface conditions, as well as cell-ECM interactions regulate cellular proliferation and differentiation (Suard et al., 1983). Similar conclusions had been previously made based on work with primary mouse mammary cells (Shannon & Pitelka, 1981). They speculated that the function of a mammary cell is directly linked to its shape, which is dictated by the nature and flexibility of the substratum. Subsequent work using mouse mammary cells has confirmed that the nature and physical state of ECM regulates cell shape, as well as the mRNA expression, synthesis, degradation, and secretion of caseins (Lee et al., 1984; Lee et al., 1985; Bissell & Hall, 1987; Schmidhauser et al., 1990; Streuli & Bissell, 1990). Mouse mammary cells cultured on floating collagen gels expressed up to ten-fold more casein mRNA than those cultured on plastic. In addition, caseins that were synthesized by cells cultured on plastic were degraded intracellularly, whereas those synthesized by cells on floating gels were secreted into the culture media (Lee et al., 1985). This research also provided evidence that milk proteins are differentially regulated, because synthesis and secretion of some non-milk proteins was not affected by the culture substratum (Lee et al., 1984). In no culture conditions has there been any significant expression of  $\alpha$ -lactalbumin (and, consequently, there is no synthesis of lactose), indicating that expression of this protein is regulated by an alternative mechanism within the mammary gland (Lee et al., 1984; Wilde et al., 1984; Bissell & Hall, 1987). More recently, it has been observed that in addition to the presence of ECM, adhesion of the mammary epithelium to ECM is critical for cellular differentiation (Zoubiane et al., 2004). Based on their observations, these authors suggested a role for the integrins in coordinating the cytoskeleton and regulating the induction of cellular differentiation by PRL. To regulate cellular proliferation and mammary growth, ECM influences the cellular response to steroid hormones (Wilde et al., 1984), and also appears to interact with local growth factor signaling pathways (Berry et al., 2003). In addition, remodeling of ECM and mammary involution is induced by decreased milking frequency and milk stasis in ruminants (Weng et al., 2008).

The local environment and ECM surrounding the mammary epithelium play a critical role in regulating cellular development and function. As discussed above, the action of ECM on the epithelium influences the mammary response to hormones, and this interaction has a marked effect on the number and secretory activity of mammary cells in the gland. This, in turn, determines the milk production potential of the animal. A deeper understanding of stromal-cell interactions and how they influence and limit milk production may provide the means to promote a desirable local environment to improve milk production efficiency.

### **3.3. Milk removal**

Across many species, regular removal of milk from the mammary gland during established lactation is critical to maintaining mammary cell number, activity, and consequent milk

production. The local mechanisms regulating the mammary response to milk removal are poorly understood, although several have been proposed. In addition to the effect of milk removal on mammary blood flow and uptake of nutrients for milk synthesis, the mammary response to milk removal may involve changes in the extracellular matrix, negative feedback by factors present in the milk or milk fat, and changes in intramammary pressure.

### *3.3.1. The effect of milk removal on mammary cell number and activity*

Local regulation of mammary cell number and secretory activity was originally observed in experiments using teat ligation in lactating rats. In those experiments, selected teats were ligated and pups were allowed to continue suckling intact glands. Tucker and Reece (1963b) observed that coincident with 24h of milk stasis, the ratio of RNA to DNA in ligated glands decreased by 31%, indicating a decrease in mammary cell activity. The authors suggested that during milk stasis, intact (suckled) glands were able to take up more nutrients and hormones from the circulation than the sealed glands, and that this may explain the observed increase in mammary cell secretory activity (Tucker, 1966; Tucker et al., 1967). In contrast to the effect of milk stasis on mammary cell activity, increased suckling frequency was linearly associated with an increase in mammary cell number, activity, and litter weight gain (Tucker, 1966; Tucker et al., 1967; Tucker & Thatcher, 1968). The increase in mammary cell secretory activity was observed within 24 h of increased suckling intensity, indicating rapid local regulation in response to increased demand of the offspring (Tucker, 1966). Similar experiments have revealed local regulation of lactogenesis and cellular differentiation in ruminants (Akers et al., 1977; Guy et al., 1994), as well as mammary involution and cellular apoptosis (Goodman & Schanbacher, 1991; Quarrie et al., 1994). Taken together, these observations support the concept that mammary cell proliferation and differentiation can be regulated locally by factors within the mammary gland. Moreover, milk removal from the gland can elicit a stimulatory effect on these processes. The mechanisms underlying this response, however, are unclear.

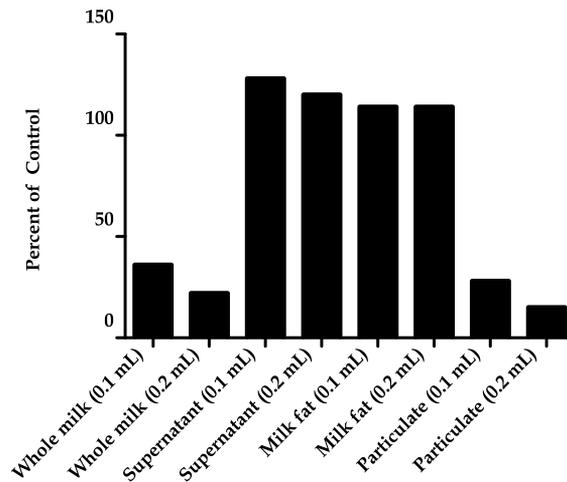
### *3.3.2. Local regulation of mammary function by factors in milk*

It has been hypothesized that a chemical in milk negatively regulates milk secretion in the absence of milk removal (Linzell & Peaker, 1971). Subsequently, a small glycoprotein in milk was reported to reversibly inhibit casein and lactose synthesis in a dose-dependent manner (Wilde et al., 1987). This glycoprotein has been named feedback inhibitor of lactation (FIL). It is both synthesized and secreted by mammary epithelial cells, and is located in the whey fraction of milk. Therefore, it is thought that FIL may be involved in autocrine regulation of milk secretion and the adjustment of milk production to meet (but not exceed) the nutritional demands of the offspring (Peaker & Wilde, 1987). Similar observations have been made in lactating women (Daly et al., 1993; Daly & Hartmann, 1995; Wilde et al., 1995). The mechanisms underlying this regulation are unclear; however, it has been suggested that FIL inhibits milk production by interfering with the casein secretory pathway (Rennison et al., 1993; Burgoyne & Wilde, 1994). In addition, Peaker and Wilde (1987) proposed that the mammary gland responds to removal of FIL in a sequential manner consisting of an

immediate response that increases milk secretion within hours of milk removal; an acute response that increases mammary cell differentiation after several days of frequent milk removal; and finally a long-term response that increases mammary cell proliferation after several weeks or months of frequent milk removal. Unfortunately, no experiments have been conducted to determine the mechanism by which FIL inhibits milk secretion. To the contrary, research on this protein has not been pursued since the 1990's; therefore, the identity of this protein and its role in the mammary gland have yet to be confirmed.

### 3.3.3. Negative feedback on milk fat synthesis

Before the reports on FIL, it was observed that the synthesis of fatty acids by the mammary gland was regulated by a factor within the milk fat itself (Levy, 1963, 1964). This research, however, received much less attention than the FIL literature. Levy (1964) observed an accumulation of fat within 12 h of weaning and a consequent diminution of fatty acid synthesis in the mammary gland of lactating rats. By 24 h, fatty acid synthesis was inhibited by 90%, and lactose was reabsorbed into the bloodstream. The synthesis of fatty acids was restored, however, when pups were returned to the mother to suckle (Levy, 1964). Teat-ligation experiments showed that the regulation occurred at the level of the individual mammary gland, since intact (suckled) glands continued to synthesize milk and milk fat (Levy, 1964). In an attempt to identify the factors involved in the inhibition of fatty acid synthesis, Levy (1964) used tissue explants from rat mammary gland and observed that whole milk markedly inhibited the synthesis of fatty acids in a dose-dependent response. Subsequent analysis revealed that the inhibitory activity was acting on acetyl CoA carboxylase, and was not associated with milk fat itself but was located in the particulate fraction of milk (Figure 1; Levy, 1964). Levy (1964) speculated that the inhibitor was bound to microsomes in the milk.



**Figure 1.** Inhibition of fatty acid synthesis by milk fractions. Mammary glands were removed from lactating rats, incubated with various milk fractions, and assayed for incorporation of  $^{14}\text{CO}_2$  as described by Levy (1964).

More recently, inhibition of mammary lipogenesis by medium chain fatty acids has been observed (Agius & Williamson, 1980; Heesom et al., 1992). Heesom et al. (1992) suggested that FIL may regulate lactose and casein synthesis, whereas fat synthesis may be regulated by a negative feedback mechanism involving medium chain fatty acids. To test this hypothesis, Peaker and Taylor (1994) investigated the effect of milk fat on litter weight gain in mice. Infusion of whole milk (which contains milk fat globules) into the mammary glands of lactating mice inhibited litter growth, whereas skim milk (which contains FIL) or fractions of milk fat globules alone had no effect. The authors concluded that there is no negative feedback mechanism located in the milk fat (1994). This conclusion, however, seemed particularly dismissive, as their results did not prompt them to question a role for FIL, which had no apparent effect on litter weight gain in this experiment. Perhaps coincidentally, that report was one of the last published primary research articles investigating a role for FIL in the mammary gland.

Certainly, there is substantial evidence for the existence of at least two types of chemical negative feedback mechanisms involved in the regulation of milk synthesis and secretion. Moreover, it is probable that there are other feedback mechanisms that have yet to be discovered. These factors may act on distinct components of milk, or there may be some redundancy in their activity. It makes biological sense that a costly metabolic process such as lactation would be tightly regulated by a variety of local mechanisms to prevent overproduction in the absence of milk removal.

#### 3.3.4. *Intramammary pressure*

Because accumulation of milk elicits an increase in pressure within the mammary gland, it is not surprising that intramammary pressure has been investigated as a potential regulator of mammary blood flow and milk secretion. Infusion of air or milk into the mammary glands of goats was associated with an increase in intramammary pressure and a linear decrease in mammary blood flow (Pearl et al., 1973). The infusion of only one udder half revealed that this response is regulated locally within the gland, as adjacent glands were unaffected (Pearl et al., 1973). Peaker (1980) reported that loss of mammary cell secretory activity during milk stasis of lactating goats was caused by an increase in intramammary pressure, and not to a decrease in mammary blood flow. An increase in intramammary pressure, however, did not always result in a decrease in milk production (Henderson & Peaker, 1984). Therefore, the relationship between intramammary pressure, mammary blood flow, and milk removal remains unclear. It is possible that intramammary pressure may indeed be a local mediator of mammary function, but its role may change with physiological state, metabolic status, and stage of lactation.

Interestingly, fur seals do not undergo inhibition of milk secretion or mammary involution during prolonged absence of milk removal (reviewed by Sharp et al., 2006). During lactation, these animals go through cycles of suckling their young on land, and foraging for food for up to 30 d at a time. During foraging, milk secretion continues and mammary function is maintained so that the seals can suckle their young when they return to shore. It

has been suggested that fur seals have adapted to override the influence of local negative feedback mechanisms to accommodate their foraging cycles and continue to rear their offspring successfully (Sharp et al., 2006). Moreover, this adaptation is thought to be regulated at the transcriptional level (Sharp et al., 2008). This is an exciting and active area of study. Once the mechanisms of local regulation and negative feedback are understood, and the genes involved are identified, there may be an opportunity to identify limits on milk secretion and improve milk production efficiency of dairy animals.

#### **4. Lactation persistency**

The performance of lactating animals is assessed by examination of the lactation curve, which depicts milk production over time during a complete lactation. A typical lactation curve consists of 3 phases: a phase of increasing milk yield during early lactation, followed by a phase of peak milk production, and finally a phase of gradually decreasing milk yield which occurs post-peak and continues throughout the remainder of lactation (Wood, 1967). One of the key aspects of the lactation curve, and a general indicator of lactation performance, is lactation persistency. Lactation persistency is defined as the degree to which peak milk yield is maintained throughout lactation. As expected, animals with persistent lactations are highly desirable, as they have the ability to attain exceptional milk production efficiency.

The shape of the lactation curve and lactation persistency is influenced by many factors, including mammary cell number and secretory activity, hormones, and nutritional status (McFadden, 1997; Sorensen & Knight, 2002; Capuco et al., 2003; Hadsell et al., 2007). As mentioned previously, milk yield is ultimately a function of the number of secretory cells in the mammary gland, and the metabolic activity of these cells. In lactating goats, the increase in milk yield during early lactation was associated with an increase in mammary cell number, followed later by an increase in mammary cell activity (Knight & Peaker, 1984). In dairy cattle, however, the increase in milk yield during early lactation appears to be a result of increased secretory activity of mammary cells, and not an increase in cell number (Capuco et al., 2001). In both species, the decrease in milk production during the declining phase of lactation was associated with a decrease in mammary cell number only (Knight & Peaker, 1984; Capuco et al., 2001). If the lactating animal was pregnant, however, a decrease in secretory cell activity was also observed (Knight & Peaker, 1984). Similar results have been observed in rodents, such that mammary cell number and activity decline during advancing lactation despite continued milk removal (Tucker & Reece, 1963a; Thatcher & Tucker, 1968; Hadsell et al., 2007). Therefore, several researchers have suggested that lactation is a transient process, and that the declining phase of lactation is a programmed response (McFadden, 1997; Capuco et al., 2003; Hadsell et al., 2007).

##### **4.1. Hormonal regulation of lactation persistency**

The role of hormones in regulating persistency of lactation is not thoroughly understood, but some hormones are clearly involved. Whereas concentrations of PRL, glucocorticoids,

and GH are high during early lactation and decrease with advancing lactation, concentrations of oxytocin are low during early lactation and increase with advancing lactation (Koprowski & Tucker, 1973a, b; Tucker, 1985). In addition, glucocorticoids, oxytocin, and prolactin are released during milking (Tucker et al., 1975; Carruthers & Hafs, 1980; Akers & Lefcourt, 1982). Treatment with exogenous PRL increased milk yield in rabbits (Cowie, 1969), but reported effects of PRL on milk yield of dairy cattle have been inconsistent (Plaut et al., 1987; Wall et al., 2006; Lacasse et al., 2008; Titus et al., 2008). Oxytocin was galactopoeitic in cattle (Nostrand et al., 1991; Ballou et al., 1993; Lollivier & Marnet, 2005) and sheep (Zamiri et al., 2001), but had no effect on milk yield of rodents (Thatcher & Tucker, 1970). As mentioned previously, GH is galactopoeitic in ruminants (Knight, 1992; Etherton & Bauman, 1998; Baldi, 1999). Estrogen also plays a role in lactation persistency: during established lactation in pregnant dairy cows, increasing estrogen concentrations are associated with the onset of declining lactation and mammary involution (Akers, 2002; Bachman, 2002; Capuco et al., 2003).

#### **4.2. Effect of parity on lactation persistency**

The lactation curve of primiparous dairy cows is more persistent than that of multiparous cows; however the mechanisms involved are unknown (McFadden, 1997). Miller et al. (2006) compared mammary cell dynamics, milk yield and milk composition of primi- vs. multiparous cows and reported that the extent of mammary cell differentiation was lower in primiparous than multiparous cows. Mammary cell renewal, however, was greater in primiparous cows (Miller et al., 2006). Because loss of mammary cells is associated with the declining phase of lactation, the authors concluded that primiparous cows are more persistent than multiparous cows because they maintain the population of secretory cells (Miller et al., 2006). Concentrations of IGF-1 in circulation were approximately 20% higher in primiparous cows, and this may have elicited a mitogenic effect on the mammary gland. Interestingly, the percentage of lactose in milk was constant during the lactation of primiparous cows, whereas it decreased during lactation of multiparous cows (Miller et al., 2006). A similar relationship between maintained lactose concentration in milk and lactation persistency was reported by Sorensen et al. (2008). The synthesis of lactose by the mammary gland is a marker for cellular differentiation during lactogenesis (Akers, 2002). Taken together, these observations support the concept that to increase lactation persistency, the population of functionally active secretory cells must be maintained. Clarification of the mechanisms involved in maintenance of secretory cell number and metabolic activity could lead to improved lactation persistency in multiparous animals and consequent enhancement of lactation efficiency.

#### **4.3. The effect of pregnancy on lactation persistency**

Dairy cows are typically pregnant for most of their lactation. This is a standard management practice to optimize generation of replacement animals, and also to ensure that the cow will continue to lactate. Unfortunately, however, lactation persistency is decreased by concurrent

pregnancy (McFadden, 1997; Sorensen et al., 2008). After about the 5<sup>th</sup> month of pregnancy, concentrations of estrogen in the circulation increase, and this is associated with a decline in milk yield (Bachman, 2002), as well as reductions in both mammary cell number and secretory activity (Capuco et al., 2003). Similar observations have been made in rodents, that pregnancy decreases mammary function and milk yield (Paape & Tucker, 1969). Therefore, despite continued milk removal, mammary involution does occur as the gland prepares for the next lactation.

#### **4.4. Manipulation of lactation persistency**

Management interventions such as long-day photoperiod, supplementation with rbGH, and increased milking frequency have been used to change the shape of the lactation curve and increase milk production efficiency (Bauman, 1999; Dunlap et al., 2000; Stelwagen, 2001). It has been suggested that these management practices stimulate an incremental increase in milk yield (Erdman & Varner, 1995; Stockdale, 2006). Whether these interventions actually increase lactation persistency, however, is questionable (McFadden, 1997).

##### *4.4.1. Manipulation of photoperiod*

Seasonal effects on mammary development and function have been extensively studied, and it is well established that manipulation of day length influences mammary development and milk production in dairy cattle (reviewed in Dahl et al., 2000). Exposure of dairy cows to long day photoperiod (16 h light: 8 h dark) during established lactation was associated with increased milk production (Peters et al., 1981; Evans & Hacker, 1989; Miller et al., 1999). In contrast, exposure to short day photoperiod (8h light: 16h dark) during the last 2 mo of pregnancy was associated with an increase in milk yield in the subsequent lactation (Miller et al., 2000). Because serum PRL concentrations change with photoperiod, researchers have focused on PRL signaling as a potential mediator of the milk yield response (Auchtung et al., 2005; Wall et al., 2005; Dahl, 2008). The results of this research have indicated that although PRL signaling may be involved in the effects of photoperiod on non-lactating cows, the IGF-1 axis may be more important during established lactation. In addition to changes in the concentration of hormones in the circulation, manipulation of photoperiod is also associated with changes in mammary gene expression (Auchtung et al., 2005; Wall et al., 2005; Dahl, 2008). This indicates that local regulation of mammary function is involved in the response of the mammary gland to changes in photoperiod.

During established lactation, manipulation of photoperiod appears to influence lactation persistency (Peters et al., 1981; Evans & Hacker, 1989; Miller et al., 1999), whereas manipulation of photoperiod during the non-lactating period had no effect on subsequent lactation persistency (Miller et al., 2000). Despite the uncertainty with respect to the underlying mechanisms, and whether there is truly an effect on lactation persistency, manipulation of photoperiod has emerged as an effective management strategy to improve milk production efficiency of dairy cows (Dahl & Petitclerc, 2003).

#### 4.4.2. *rbGH*

As mentioned previously, GH is galactopoietic in dairy cows (Bauman et al., 1985). Since it became commercially available for use on dairy operations, the effects of rbGH on lactation performance and animal health have been extensively studied (Crooker & Otterby, 1991; Bauman, 1999; Dohoo et al., 2003). Treatment of dairy cattle with rbGH is associated with a decrease in lipogenesis and an increase in gluconeogenesis by the liver, which increases the availability of fatty acids and glucose to the mammary gland for the synthesis of milk fat and lactose (Akers, 2002). This shift in metabolism and nutrient utilization is thought to be mediated by interactions between GH, insulin, and the IGF axis (Molento et al., 2002). In addition to altered nutrient metabolism to support increased milk production, treatment with rbGH is associated with an increase in blood flow to the mammary gland (Breier et al., 1991; Prosser et al., 1996). The mechanisms underlying the milk yield response, however, remain unclear. Capuco et al. (2001; 2003) suggested that bGH increased lactation performance by increasing the population of mammary epithelial cells, possibly via the IGF signaling axis, and similar observations have been made in rodents (Allan et al., 2002). Because the milk yield response to bGH is acute and disappears upon cessation of treatment, however, it seems more likely that the mechanism works to enhance the milk synthetic activity, rather than the number, of secretory cells (Akers, 2002; Yang et al., 2005).

<b>Intervention</b>	<b>Timing of implementation</b>
Manipulation of photoperiod	Lactation; dry period
Increased milking frequency	Early lactation; full lactation
Suckling (with or without machine milking)	Early lactation; full lactation
rBST	Lactation
Genetic selection	Selection of cows for breeding; purchase of semen

**Table 2.** Various management interventions that can increase milk production efficiency.

#### 4.4.3. *Frequent milking*

Another management strategy that increases milk production is frequent removal of milk from the mammary gland by either suckling or increased milking frequency. Frequent milking (3 or more times daily) has been adopted on many dairy farms and has proven to be a highly successful approach to increase milk production efficiency. Reports on the effects of frequent milking on lactation persistency, however, are inconsistent. Whereas several researchers have observed an increase in persistency in response to frequent milking (Pearson et al., 1979; Poole, 1982; Amos et al., 1985; Hillerton et al., 1990; Sorensen & Knight, 2002), others have reported no effect (Allen et al., 1986; Gisi et al., 1986). The discrepancy in results may be due to differences in the definition or measure of persistency, the nature and duration of frequent milking treatment, or to the physiological state of the animals (pregnancy status, stage of lactation). Like photoperiod treatment and rbGH, the mechanisms underlying the milk yield response to increased milking frequency are unknown. It has been suggested, however, that use of the three interventions combined will elicit additive effects on milk production (Dunlap et al., 2000). This indicates that distinct mechanisms may be involved in each of the responses.

## 5. Conclusions

Milk production potential is dependent on the number of secretory cells in the mammary gland, as well as the metabolic activity of those cells. Both of these factors are greatly influenced by the endocrine system, by local regulatory mechanisms within the mammary gland, and by the interaction between endocrine and local regulation. Moreover, interventions that perturb the endocrine system or the local mammary environment can result in changes in mammary cell number, secretory activity, and consequent milk production potential. The mechanisms underlying the response of the mammary gland to those interventions are unknown. Research focused on determining the mechanisms involved will improve the knowledge of mammary gland biology and regulation of mammary function, and could lead to novel management strategies to further optimize milk production efficiency. Our companion chapter provides an extensive review of the literature on frequent milking or suckling as they influence milk production and mammary function in dairy animals.

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# **Dairy Cattle Welfare Status Measured by Animal-Linked Parameters Under Tunisian Rearing Conditions**

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/28287>

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## **1. Introduction**

To enhance food production and maintain the competitiveness of Tunisian animal agriculture in the global economy, it is imperative that the agricultural industry has access to cutting edge scientific information on animal welfare. The issue of animal welfare has received significant attention from major grocery and food service companies in the world. Animal welfare is also an important issue for consumer confidence in animal production. There are many definitions of the animal welfare. The welfare of an animal has been defined by Fraser and Broom (1990) as its state at it seeks to cope with its environment. Welfare principally concerns both the physical and psychological wellbeing of an animal, which is largely determined by the standard of stockman ship, the system of husbandry and the suitability of the animal f or the environment (FAWC, 2009). Nowadays, the evolution of the worldwide agriculture has come to raise new aspects, and animal welfare is one of them. Public concern about farm animal welfare has steadily grown during recent years. In this context, welfare assessment has many roles such as identifying current welfare problems, checking farm assurance, indicating risk factors leading to a welfare problem, testing the efficacy of interventions, researching tools for evaluating and comparing production systems, environments, management systems, animal genotype etc. (Whay, 2007). Hristov et al. (2008) reported that there is major public demand for improvements in animal welfare, housing conditions and health aspects. The assessment of welfare at farm level can be used as an advisory tool by farmers, as source of information for legislation and as a component of quality assurance schemes for consumers (Napolitano et al., 2005; Webster, 2005; Vučinić, 2006). Welfare is multidimensional and it cannot be measured directly, rather it is inferred

from external parameters. Therefore, different methods of on-farm monitoring of animal welfare have been developed (Johnsen et al., 2001). Animal welfare (AW) can vary substantially between similar production systems indicating the major influence of management and it needs to be assessed through indirect indicators (Rousing, 2003; Sørensen et al., 2003). In fact, productivity can be used as an indirect measure of animal welfare (Waiblinger et al., 2002; Breuer et al., 2003). In high-performing dairy herds, cattle that have a positive relationship with their handlers tend to move more quickly into the milking parlor, have smaller flight zones, and are less nervous and more settled (Breuer et al., 2000; Hemsworth et al., 2000; Waiblinger et al., 2002). Adopting this approach to animal care and management can result in greater ease and efficiency of management as well as reduced production losses and, in some cases, increased productivity. A decrease in productivity, such as a drop in milk yield, can indicate a welfare problem. Likewise, decreases in reproductive rates or increases in mortality or morbidity should be clear signs that the welfare of livestock is declining. Illness and injury can indicate poor welfare. Other symptoms of problems are changes in behavior; animals that are lethargic, unwilling to move, or that have become aggressive are unlikely to be doing well (Pawelek & Cronney, 2003). The physiological and behavioral responses of dairy cattle to stress can reduce their productivity, their health and their welfare. Dairy cattle that have been selected for high milk production seem particularly susceptible to stress and are at more risk of behavioral, physiological and immune problems and so require higher levels of care and management (Oltenucu & Algers, 2005). Therefore, the main aim of this research was to characterize animal welfare issues under Tunisian conditions by measuring welfare of Holstein population cows through some animal-related measures and testing reactions of cows towards humans on the hypothesis that these reactions reflect validly the human–animal relationship on these farms.

## **2. Material and methods**

The animal-based parameters include observations of physical conditions, animal behaviour observations and examination of the farm's recording. Each selected parameter was included on either the animal observation or record data collection forms. Different approaches for assessing animal welfare at farm level have been developed often with quite different purposes (Johnsen et al. 2001). The scientific assessment of the well-being of an animal involves finding indicators of three broad criteria: 1) a high level of biological functioning; 2) freedom from suffering in the sense of prolonged fear, pain, and other negative experiences; and 3) positive experiences such as comfort and contentment.

### **2.1. Farms and animals**

Information was collected during farm visits to 35 dairy farms located in four Tunisian provinces (Nabeul, Sousse, Monastir and Mahdia). Farms were selected from a sample of 50 cattle farms that responded to a questionnaire. Selection criteria for farm visits were a

minimum herd size of 10 Holstein cows, and participation in milk recording. The herd size of the farms ranged from 10 to 50 lactating animals. The sample was then taken randomly from the farms that fulfilled these criteria. The study was carried out in the Tunisian Sahel. Thirty five farms with horned dairy cows in loose housing were selected for the investigation. There were three types of loose housing: cubicle housing (16), straw bedding pen (15) and straw flow pen (4). On all farms, the rearing method was similar (artificial insemination, calves being separated from the mother at the age of 1 to 7 days and fed by man). Thus all cows were artificially reared and suckled by man, giving all cows a certain degree of habituation to and contact with farmers. A total of 350 Tunisian Holstein cows (46%) heifers (H) and (54%) cows (C) were included in the study.

## **2.2. Assessment of animal welfare indicators**

Welfare assessment systems, for use in dairy farms may differ according to both the definition of animal welfare, and the purpose of the welfare assessment. Thus choice of welfare indicators and methods of measurement reflects the basic considerations of how animal welfare is understood. If the farmer wants to improve animal welfare he needs a method to assess animal welfare at herd level. A relevant welfare assessment system should describe the welfare of the animals in the herd, and allow the farmer to assess the development over time and to respond appropriately. Many indicators may possibly be relevant for inclusion in an operational welfare assessment system. So far, assessments of animal welfare relied mainly on resource-based parameters, i.e. measures taken regarding the environment in which the animals are kept, while animal-based measures aim to directly measure the actual welfare status of the animal and thus include indirectly the effect of resource and management factors as well, because of their effect on the animal. Performance and behavior measurements and behavior tests were performed to show whether the animals were adapting to the production system or whether the animals showed any signs of strain. Animal behavior of 10 cows randomly assigned was recorded through one visit in each farm.

### *2.2.1. Milk yield*

A key issue is the extent to which genetic selection for increased production affected the ability of the animals to adapt to the environment in which they find themselves. Reviewing the negative side-effects of selection for high production, Rauw et al. (1998) concluded that “when a population is genetically driven towards high production, fewer resources will be left to respond adequately to other demands like coping with stressors”. The key problem as noted by Rauw (2008) is that high productivity in farm animals could mean that there are insufficient resources for adequate coping and hence poor welfare whenever resources are limiting. Data on milk traits (production, fat and protein) of seven consecutive years (2002-2008) were obtained from the official recordings of the farm. Cows which have more than 10 records during complete 305-days lactation were considered. Milking was carried out twice daily.

### 2.2.2. *Fertility*

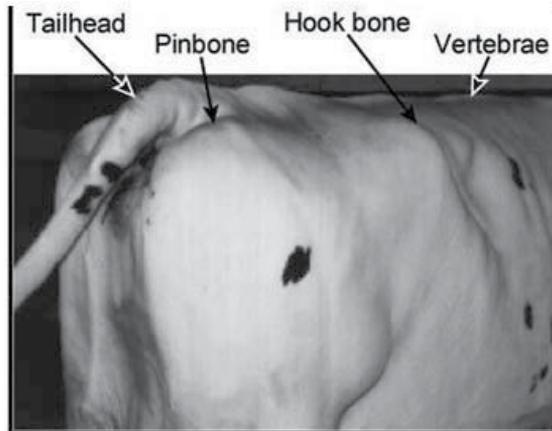
There are strong motives for including reproduction in selective programs, both economical and welfare related (Berglund, 2008). Female fertility cannot be easily defined as a single trait as it comprises different aspects. Some of these aspects are related to the prompt resumption of cyclicity and the showing of recognizable oestrous behavior, while others are related to the ability of the cow to become (and remain) pregnant with a limited number of inseminations (Groen et al., 1997). In addition, cows should have good calving ability and give birth to viable calves (Berglund, 2008). Calving to first service interval (CFSI), calving interval (CI), calving to conception interval (CCI), and number of services per conception (NSC) were extracted from the records of individual cows in each farm. Farmers were also surveyed about aspects of their management system relating to age at first calving of heifers and their management of reproductive health and fertility.

### 2.2.3. *Mastitis and Somatic Cell Counts (SCC)*

Data for individual cows were extracted from the farm records and edited to include records from the first three lactations. Data contained multiple somatic cell count (SCC) measurements made during the lactation months for each cow and the number of cases of clinical mastitis. SCC was log-transformed. The lactation number, milk yield, stage of lactation and season of calving are all factors known to affect somatic cell count (Dürr et al. 2008), so they were all included in the analysis. The age of the cow at which it enters each lactation is also known to affect SCC. The total number of cases of mastitis and the number of cows which were treated twice or more were calculated. As many cows received repeated treatments for mastitis, it was necessary to use a criterion to define what a new case was, and what a repeated treatment was. Any treatment started on a new quarter was considered a new case. Any re-treatment of a single quarter within a period of 8 days was considered a repeated case, and greater than 8 days was considered a new case. The number of cases was converted to cases/cow-year for analysis.

### 2.2.4. *Body condition scoring*

Every dairy producer has cattle that are too fat or too thin for their stage of lactation. The scoring method involves a manual assessment of the thickness of fat cover and prominence of bone at the tail head and loin area. Methods for assessing energy reserves, the role of assigning BCS in dairy management, and the impact of varying BCS on animal productivity, health, and reproduction are explored from a whole-system viewpoint. Most body condition scoring (BCS) systems in dairy cattle use the 5-point scoring system with quarter point increments. The scale used to measure BCS differs between countries, but low values always reflect emaciation and high values equate to obesity. Visual body condition scores were recorded for all milking cows on the farms. A body condition score is assigned by visual observation of the cow's rump area—primarily the region delimited by the hip bones, the pinbones and the tailhead as shown in figure 1.



**Figure 1.** Identification of some body parts used to assign body condition scores (Ferguson et al., 1994).

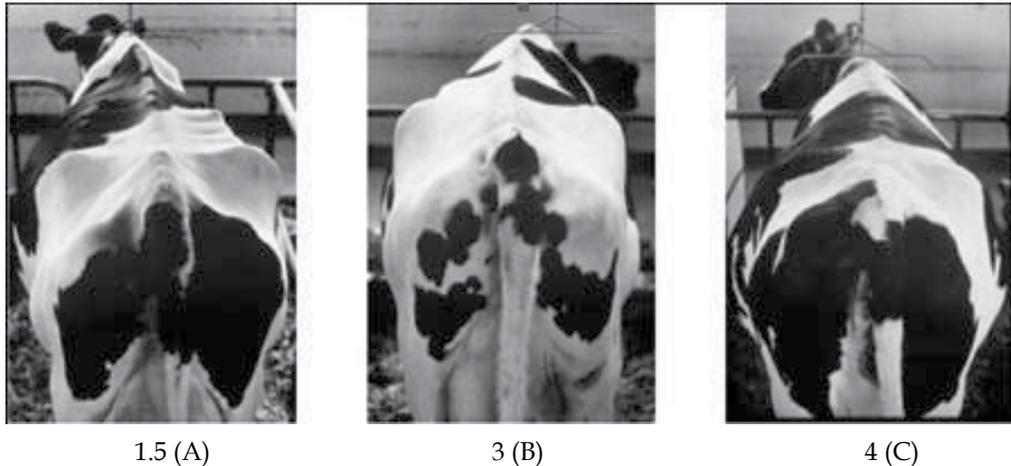
Cows are usually ranked on a scale from 1 to 5. Extremely thin cows are assigned a score of 1 and extremely fat cows, a score of 5 (Fig. 2).

Body Condition Score	Vertebrae at the middle of the back	Rear view (cross-section) of the hook bones	Side view of the line between the hook and pinbones	Cavity between tailhead and pinbone	
				Rear view	Angled view
1 Severe underconditioning					
2 Frame obvious					
3 Frame and covering well balanced					
4 Frame not as visible as covering					
5 Severe overconditioning					

**Figure 2.** Dairy Cattle Body Condition Scoring Chart (Edmonson et al. 1989)

A body condition score of 1.5 one or two months after calving is not desirable because it indicates severe lack of adequate nutrition (negative energy balance, Fig. 3a). A body condition score of about 3.0 (Fig. 3b) should be typical of a cow recovering body reserves in

mid-lactation (Sprecher et al. 1997). In late lactation and during the dry period, a body condition score of 3.5 may be the most desirable. This body condition score gives the cow sufficient body reserves to minimize the risk of complications at calving while maximizing milk production in early lactation. As milk production declines in late lactation, cows gain body weight efficiently. Overfeeding concentrate is common management mistake. Cows fed too much concentrate in the later part of lactation tend to become obese (Fig. 3c). These cows are likely to have difficult calving and to develop other disorders (fat cow syndrome).



**Figure 3.** Examples of cows with body condition scores of 1.5 (A), 3 (B) and 4.5 (C) (Sprecher et al. 1997)

### 2.2.5. Avoidance distance test

The human–animal relationship is an important issue when assessing animal welfare on farms. In many farm animal species, the relationship to humans affects their welfare considerably. A feasible, reliable methodology for assessing responses of cows to humans would be helpful for large scale surveys on this topic. Measuring avoidance distance to assess animals' relationship to humans was shown to be a feasible and stable measure in dairy cow herds (Waiblinger et al. (2003). The measure of avoidance distance was inspired from the method of Waiblinger et al. (2003) and it consists of estimating this distance at the feeding rack (ADF) and inside the stall (ADS). The test person approaches slowly to the animal and the distance was calculated at the moment of withdrawal of the animal or at the moment of touching.

### 2.2.6. Lameness scoring

Dairy lameness is a very visible well-being issue as well as a production and economic issue. A locomotion score is a qualitative index of a cow's ability to walk normally. Locomotion scoring is a relatively quick and simple qualitative assessment of the ability of cows to walk normally. Visual locomotion scoring of cows is normally used in lameness research as a method to identify lameness. Visually scored on a scale of 1 to 5 (Table 1), where a score of 1

reflects a cow that walks normally and a score of 5 reflects a cow that is three-legged lame, a locomotion score is made in a few seconds per cow. Generally locomotion scores of 2 and 3 are considered to represent subclinically lame cows whereas locomotion scores of 4 and 5 represent those cows that are clinically lame. A locomotion score higher than 1 is not an indication of why the cow's gait is affected, merely the degree of lameness that she is showing (Sprecher et al. 1997).

Score	Clinical description	Description
1	Normal	Stands and walks normally with a level back. Makes long confident strides.
2	Mildly Lamé	Stands with flat back, but arches when walks. Gait is slightly abnormal.
3	Moderately Lamé	Stands and walks with an arched back and short strides with one or more legs. Slight sinking of dew-claws in limb opposite to the affected limb may be evident.
4	Lamé	Arched back standing and walking. Favoring one or more limbs but can still bear some weight on them. Sinking of the dew-claws is evident in the limb opposite to the affected limb.
5	Severely Lamé	Pronounced arching of back. Reluctant to move, with almost complete weight transfer off the affected limb.

**Table 1.** Description of the scale used for scoring lameness (Sprecher et al. 1997)

### 2.3. Statistical analysis

The data obtained was statistically analyzed using the SAS statistical package, version 9.1 for Windows (SAS Institute Inc, 2006). Spearman correlation was used to determine relationships between variables. Differences in mean values and proportions were respectively examined with t-test and Fisher's exact test, and Kruskal-Wallis test was used for pair-wise comparisons. Analysis of variance (ANOVA) using the General Linear Models procedure with t-test (least-significant-difference, LSD) was used for comparison of avoidance distances. Differences of  $P < 0.05$  were considered statistically significant. Non parametric tests (Spearman rank correlation, and Kruskal-Wallis test) were used due to the non-normality of the data and the small sample size of the farms ( $n = 35$ ). By using the Kruskal-Wallis test and Mann-Whitney U-tests, it was investigated if farms differed significantly in distribution of age (based on the average herd age). Furthermore, the Kruskal-Wallis test was used to evaluate if farms differed significantly with respect to avoidance distances. For lameness score, a screening process was used whereby each explanatory variable was tested in a univariate analysis. For SCC, hock damage and some aspects of behavior, a LMM (Linear Mixed Models) were used (data had normal distributions, or could be transformed to give a normal distribution).

### 3. Results

Many welfare problems are the result of animals not being fully adapted to the production system. The consequences of poor welfare include those of disease, injury, starvation, beneficial stimulation, social interaction, housing conditions, deliberate ill treatment, human handling, transport, laboratory procedures, various mutilations, veterinary treatment or genetic change by conventional breeding or by genetic engineering (Broom, 1996). The rapidly changing conditions prevent animals to adjust and cope with the changes (Halverson, 2001). The overview should give the farmer a clear picture of the actual welfare status of the farm. This is a prerequisite when determining the priority of animal welfare considerations in a whole farm framework.

#### 3.1. Milk production

The increase in production has been accompanied by declining ability to reproduce, increasing incidence of health problems, and declining longevity in modern dairy cows. Genetic selection for increased milk yield increasingly is viewed as increasing profit at the expense of reducing animal welfare. The average 305-d lactation milk yield was 5953 kg (with 3.46 and 3.16% content of milk fat and protein, respectively). On average, milk yield at the peak was about 25 kg, and there were a few cows with production exceeding 35 kg. We noted that average milk production varies with herd size. Indeed, according to this study, larger herds showed serious losses in production as herd size increased. In opposition, smaller herds were less affected ( $P < 0.001$ ) as herd size varied. On the other hand the lower value of fat composition indicated a poor health and therefore a poor welfare. Multivariate analyses with the GLM procedure revealed herd size as significant influence on milk production (coefficient of determination  $r^2 = 0.504$ ) as shown on table 2.

#### 3.2. Somatic cell count

Somatic cell counts (SCC) have long been used as a way of measuring milk quality. And high SCC levels in the milk cause deterioration of the milk quality. The average somatic cell counts amounted to  $427.3 \pm 90.12 \times 1000$  cells/ml. Smaller farms had a lower somatic cell count. SCC increased with lactation number ( $P < 0.001$ ) and varied with stage of lactation in a quadratic manner ( $P < 0.001$ ). SCC was highest in the autumn period ( $P < 0.001$ ) and it was associated with cow milk yield ( $P < 0.001$ ). The size of the groups that the animals were housed in also affected SCC, with larger group sizes having the lowest cell counts ( $F = 3.20$ ,  $P < 0.05$ ). However, the season of calving was not significant ( $P = 0.09$ ). Today, mastitis is considered to be a multifactorial disease, closely related to the production system and environment that the cows are kept in. Mastitis risk factors or disease determinants can be classified into three groups: pathogen, host and environmental determinants.

#### 3.3. Fertility

Reproductive performance in dairy cows remains one of the most intriguing issues in cattle production, not in the least because of the complex interactions between different systems

resulting in certain fertility and of the continuous challenges to improve (herd) fertility results. There has been a gradual decline in dairy cow fertility. Fertility traits were  $444 \pm 101.5$ ,  $154 \pm 78.4$ ,  $82 \pm 56.8$  days and  $2.1 \pm 1$ , respectively for CI, calving to conception interval (CCI), calving to first service interval (CFSI), and NSC. Cows were on average  $6.0 \pm 1.0$  years old. (Table 2). This decline of fertility can be considered an indication of the health costs of the milk production of today's dairy cows.

	All farms	1-10	11-20	>20
Cows (n)	35	16	12	7
MY (Kg)	5953	5678a	6054b	6247b
SCC (1000 cells/ml)	427.3	447a	387b	378b
CI (days)	444	478a	437b	435b
CFSI (days)	82	87a	78b	73b
CCI (days)	154	159a	147b	145b
NSC	2.1	2.3a	1.8ab	1.6b
Age (years)	6	6.3a	6ab	5.8b
Culling rates (%)	23.5	23.8ab	21.8a	27.01b

Different letters indicate significant differences within that part of the column ( $P < 0.05$ )

MY= Milk yield; SCC= Somatic cell count; CI= Calving interval; CFSI= Calving to First Service Interval; CCI=Calving to Conception Interval; NSC= Number of Services per Conception.

**Table 2.** Animal-related parameters and selected key features (possible influences) of investigated farms

### 3.4. Body Condition (BC) scoring

Condition scoring is a technique for assessing the condition of livestock at regular intervals. The purpose of condition scoring is to achieve a balance between economic feeding, good production and good welfare. The body condition score (BCS) of a dairy cow is an assessment of the proportion of body fat that it possesses, and it is recognized by animal scientists and producers as being an important factor in dairy cattle management. Body condition score (BC) ranged from 1.25 to 4 (lactating cattle). The majority of cows were BC score 2.5 (50% cows). The majority of dry cows were BC score 2.75 (65% cows), ranging from BC score 1.5 to 4. We considered a BC score of 2 or less to be classified as 'thin'. The mean number of lactating cows in this category on all farms was  $18.9 \pm 1.9\%$ , however, this ranged from 1% to 57% of the herd. Body condition affects productivity, reproduction, health and longevity of dairy cows.

### 3.5. Avoidance distance

The variation in the response of animals to the avoidance distance test is shown in table 4. Individual avoidance distances ranged from 0 to 1.5m, and the percentage of animals that could be touched on a farm ranged from 41 to 97%. Farms differed significantly with respect to individual avoidance distances ( $P < 0.001$ ) with a minimum farm median of 0.05 m and a maximum farm media of 0.15 m. There was a small but significant correlation between the avoidance distances of individual animals and age ( $r = -0.14$ ,  $P = 0.015$ ). At farm level, none of the ADF farm measures was significantly related with mean age of cow ( $P > 0.05$ ). (Table 3)

	Mean	Median	S.D.	Min-Max	25%- 75%	n
<b>Individual level</b>						
ADF(m)	0.13	0.07	0.141	0- 1.5	0.05 – 0.2	-
<b>Farm level</b>						
ADF mean (m)	0.13	0.14	0.034	0.08 – 0.18	0.11 – 0.16	10
ADF median(m)	0.08	0.07	0.035	0.05 – 0.15	0.05 – 0.10	10
ADF % touch	61.45	50.75	10.72	39.8 – 70.9	42.5 – 62.5	10
ADF% > 0.2 m	17.4	18.7	8.9	1.8 – 29.2	9.1 – 25.1	10

S.D. standard deviation, 25% and 75% percentile.

**Table 3.** Descriptive statistics of the different measures calculated for the avoidance distance at the feeding place test (ADF)

Analysis of variance showed a significant difference ( $P<0.05$ ) between cows and heifers regarding avoidance distance. We conclude that cows have an ADF of  $0.33 \pm 0.17$  m which is considered short compared to those of heifers ( $0.56 \pm 0.37$  m), but no significant difference ( $P=0.11$ ). Regarding ADS, indeed they have similar behavior in the stall. The proportion of animals with ADF 0 were 22 % and 31 % in heifers and cows, respectively and those of animals that tolerated to be touched for 3 seconds and more ( $ADF0 \geq 3s$ ) were 24 % and 50 % in heifers and cows, respectively. (Table 4)

	Avoidance distance (m)		
	Low	Medium	High
ADF-H	$0.63 \pm 0.07$	$0.45 \pm 0.15$	$0.47 \pm 0.08$
ADF- C	$0.28 \pm 0.01$	$0.35 \pm 0.06$	$0.26 \pm 0.14$
ADS-H	$1.05 \pm 0.10$	$1.09 \pm 0.44$	$1.01 \pm 0.9$
ADS- C	$0.89 \pm 0.16$	$0.74 \pm 0.17$	$0.88 \pm 0.12$

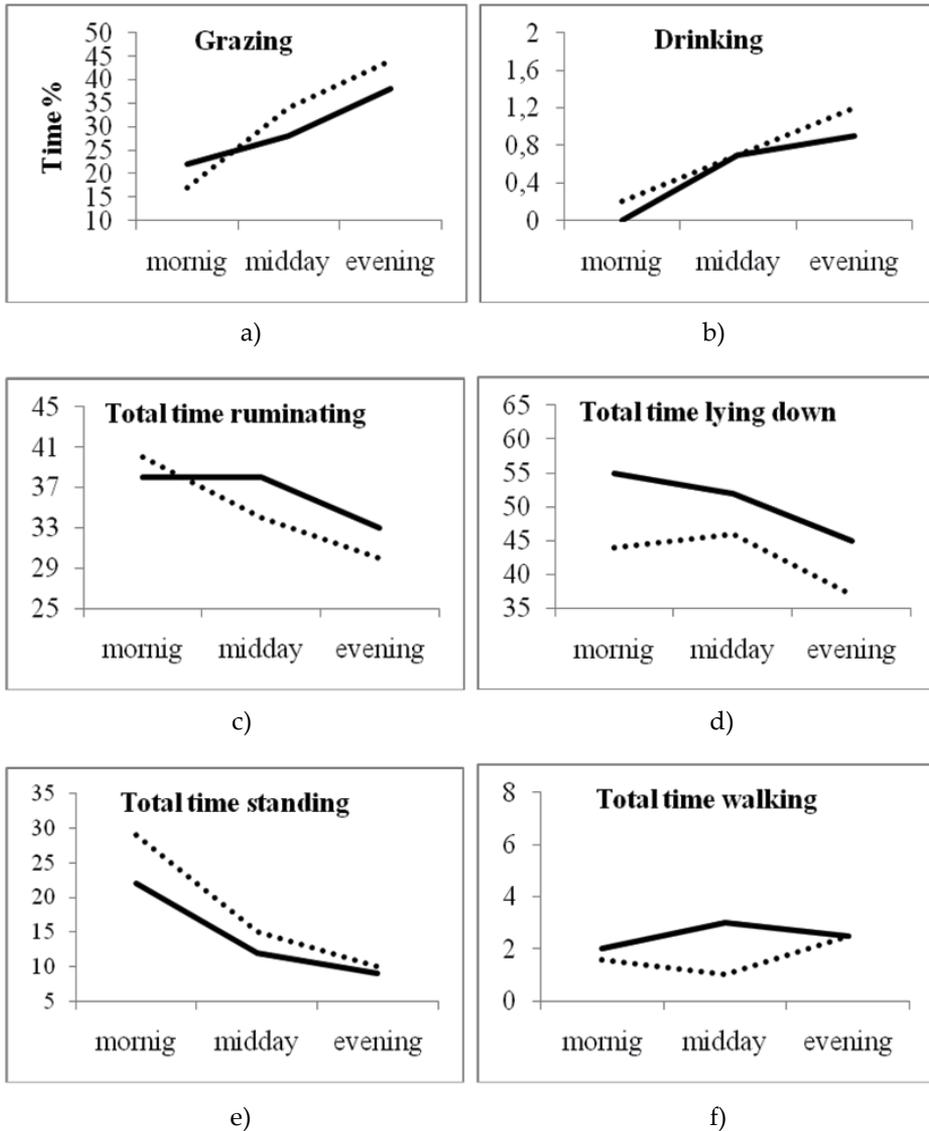
No differences were found with Proc GLM (t-test + LSD) ( $P<0.05$ ); H heifers and C cows.

**Table 4.** Avoidance distance dairy heifers (H) or cows (C) when tested in the feeding rack (ADF) or inside the stable (ADS) (means  $\pm$  SEM).

### 3.6. Lameness

Disease can be regarded as an important welfare indicator, because it is in many cases associated with negative experiences such as pain, discomfort or distress. One indicator in a welfare assessment, at farm level, may be the prevalence and intensity of certain health problems in the herd. Lameness in dairy cattle is an important welfare issue. It certainly stands out as a consequential and complex welfare problem in dairy cattle. Furthermore, the Farm Animal Welfare Council (1997) considers lameness among the best welfare indicators for dairy cattle. The complexity arises because lameness is an obvious sign of many clinical, environmental and management problems (Logue et al., 1998; Ward, 2001). Many factors influence hoof health including genetics, conformation, diet, contagious agents, and hygiene, housing system, animal behavior and management. Regarding lameness, it had a reduced proportion, only 19 cows of 350 (5.4%) showed moderate lameness. A strong increasing trend in the proportion of cows with painful lesions was detected. In both lame

and nonlame cows, the greatest proportion of time was spent grazing (~34%), followed by lying with or without ruminating (approximately 29 and 18%, respectively), with <10% time spent in each of the remaining behavioral states (Fig.4). Throughout, lame and nonlame cows spent similar proportions of time grazing, drinking, or ruminating, but lame cows spent less time elevated on their feet (includes standing with or without ruminating, drinking, grazing and walking) and lay down for longer (includes lying with or without ruminating). In both lame and nonlame cows, from early morning to midday to evening, the



**Figure 4.** Daily time budgets for lame or nonlame during the morning, midday, and evening, including proportion of scan samples (%) spent a) grazing, b) drinking, c) ruminating, d) lying down, e) standing, and f) walking.

proportion of time spent grazing or drinking increased, whereas time for totals of ruminating, lying, or standing decreased; walking was unaffected by period of day (Fig. 4).

Wet bedding reduced the time that cows spent lying by 5 hours per day and increased the time spent perching with just 2 feet in the stall. Reduced amounts of bedding and/or replacing the bedding less also often leads to cows standing for longer periods of time. Factors that increase the time cows spend standing also increase the stress on the hooves.

## **4. Discussion**

### **4.1. Milk yield**

The results of the current study showed that milk production varies due to improvement selection goal, feeding strategies, milking systems, health programs and breeding systems and management. Significant correlations have been found between human-animal interactions and milk yield in dairy cows, this agrees with results of some studies (Breuer et al., 2000; Hemsworth et al., 2000; Waiblinger et al., 2006). The main items that influence the comfort of a dairy cow include housing condition (Hristov et al., 2006), bedding, flooring, and ventilation (Hristov et al., 2007), nutrition, water quality, sanitation (DEFRA, 2003; Webster, 2005) and milking equipment. However, many welfare problems are the consequence of a non-adaptation of the animal to the production system. Comfort and cleanliness of animals is dependent not only on amount and type of bedding, but also in animal stocking density, type of shelter, temperature and humidity levels.

### **4.2. Mastitis**

The results of our study have shown that mastitis remains a great problem in Tunisian dairy farms as well as many other countries. Ferguson et al. (2007) reported the prevalence of mastitis in Sicily (35.4 %), Tenhagen et al. (2006) in Germany (26.4 %) and Pitkälä et al. (2004) in Finland (30.6 %). In this study we noticed associations between hygiene scores and udder health parameters and an interaction between stockperson and mammary gland health. Hence, mastitis, however it occurs, is a severe welfare problem. In a 1990 study of 370 cow herds and 45,133 cows, Oltenacu et al. (1990) found that trampled teats and udder injuries were the most serious risk factors for clinical mastitis in tied cows. Oltenacu & Ekesbo (1994), studying Swedish Friesian cows, found that high production predisposed cows for mastitis and that the risk of mastitis was greater for calving in July and August and increased with age at calving. Castillo-Juarez et al. (2000) and Kearney et al. (2004) showed that the magnitude of the antagonistic genetic correlations between milk yield and somatic cell score and between milk yield and conception rate were significantly higher in a poor environment relative to a good environment. The genetic antagonism between mastitis resistance and production traits has been well established. In their review, Mrode and Swanson (1996) reported a weighted-average genetic correlation between Somatic Cell Score (SCS) and milk yield in first lactation of 0.14. Pryce and Brotherstone (1999) and Rupp and Boichard (1999) reported similar results.

### 4.3. Fertility

The mean calving interval of Holstein cows has increased considerably. This prolongation is mainly caused by the lengthening of the calving to first insemination interval (Moreels, 2002). Realizing the complex nature of fertility, it is not surprising to find that ideal fertility criteria are extremely difficult to reach. As selection has led to higher milk production per cow, there have been steady increases in reproductive problems. This result was confirmed by Moberg (2000) and Kaltas & Chrousos (2007) who concluded that during stress, the reproductive axes may be inhibited at several levels. Royal et al. (2000) noted that the calving rate of the modern dairy cow is declining at approximately 1% per year and first service conception rates are below 40%. Washburn et al. (2000) noted a marked decline in reproductive performance in dairy herds over the past 25 to 30 years. They described a 1998 report on over 70 Kentucky dairy farms in which average days open had increased by 27 days between 1976 and 1996 and the number of services per pregnancy increased from 1.62 (with a 62% conception rate) to 2.91 (with a 34% conception rate).

### 4.4. Avoidance distance

Management practices associated with fear and pain are also viewed very negatively by animal scientists and veterinarians (Heleski et al., 2004, 2005). The analysis of variance showed a significant difference in avoidance distance between cows and heifers. This difference can be explained by a good habituation and adaptation of cows through farmer's attitudes during milking and feeding practices and the intensity of visits and treatment of the animal. These results are in agreement with those of Garcia (2009) and Waiblinger et al. (2003) who did not find consistent influence of age on avoidance distance, since there were herds with positive and negative Spearman correlation, yet most of them were very low and not significant. ADS correlated moderately with ADF (0.49,  $P < 0.05$ ), supporting the reliability of the two tests, although Windschnurer et al. (2008) found a stronger correlation (0.7-0.9) in a study on 16 commercial dairy farms. The greater distances in ADS test were expected, since ADS was tested immediately after ADF on the same animal. Waiblinger et al. (2003) found a strong relationship between animals' reactions to humans, particularly avoidance distance inside the stable, and the continuity, quality and quantity of daily contact and handling, and with the frequency of friendly interactions with the farmer (human-animal interactions). Other authors also revealed negative associations between avoidance distances and positive behavior of farmer in dairy farms (Hemsworth et al., 2000; Windschnurer et al., 2009). Accordingly, there are several evidences that positive interactions ease handling and milking (increase productivity) and can reduce mastitis by promoting adequate milk flow, which has, additionally to improved welfare, an economic impact (EFSA, 2009). Comparing the results of the present study with the ones from a protocol developed by Whay et al. (2003), where the shortest distance between observer and cow at moment of withdrawal, average flight distance categories A (best) to E (worst), were used to grade the welfare of 53 dairy farms in this case, mean avoidance distances (ADF and ADS) would be included in the A category (0.6 – 1.1 m). Even though, a margin of progression seems to exist, since some animals showed strong avoidance. Programs that aim

to improve stock people's attitude and behavior toward dairy cattle can reduce flight distance from humans and increase milk (protein and fat) yield (Hemsworth et al., 2002). Furthermore, the attitude of the stockperson towards interacting with farm animals is an important determinant of the stockperson's behavior and thus the animal's fear of humans (Hemsworth, 2004; Waiblinger et al., 2006). The results confirm our hypothesis, that the avoidance distance validly reflects the human–animal relationship. This is in line with earlier results, where avoidance distance was correlated with the behavior of the farmer (Waiblinger et al., 2002). In experimental studies, avoidance reactions of cattle were influenced by previous experience of positive or negative handling (Munksgaard et al., 2001; Hemsworth, 2004; Waiblinger et al., 2006). The average age of the cows did not confound the assessment of human–animal relationship on the farms in our study. Also within farms, there was no consistent influence of the age of the cows on avoidance distance.

#### **4.5. Body condition scoring**

Body condition is a subjective assessment of the amount of fat, or amount of stored energy, a cow carries. Body condition changes throughout the lactation cycle. Cows in early lactation are in negative energy balance and losing body condition (mobilizing body reserves). Our findings are in agreement with those of Studer (1998) who explained that high producing cows whose body condition score declines by 0.5 to 1.0 during lactation often experience anoestrus. However, a loss of condition score of about 1.0 during lactation was normal in the review presented by Broster & Broster (1998) and Popescu et al. (2009). An ideal body condition score is 3.0. Dechow et al. (2001) found that higher body condition scores were favorably related genetically to reproductive performance during lactation. While higher body scores during lactation were moderately negatively related to milk production, both genetically and phenotypically.

#### **4.6. Lameness**

Lameness is a crucial welfare issue in modern dairy production (Vermunt, 2007). It indicates a painful state and discomfort and is regarded as one of the most serious welfare problems in cattle. In our study, a significant percentage of dairy cattle (59) have severe lameness, this can be a sign of poor overall welfare standards within the herd. Hristov et al. (2008) noticed that lameness is indisputably the major welfare problem for the dairy cow. Our findings are in agreement with those of Webster (2005) who reported that half the cows go lame in any one year and 20% are lame at any one time. Lameness in any cow is usually a sign that they are in pain, ill-health and discomfort. It clearly affects cow welfare, as well as their performance and production (Bergsten, 2001; Ward, 2001; DEFRA, 2003; Hristov et al., 2008). Lameness in dairy cows impacts negatively on herd welfare and productivity. It is thought to be closely associated with avoidance of pain caused by limb lesions and, particularly in dairy cattle, by hoof lesions (Dyer et al 2007). It certainly stands out as a consequential and complex welfare problem in dairy cattle (Bergsten, 200; Rajkondawar et al., 2001; Ward, 2001). Leach et al. (2008) advise that a limited number of available cubicles are a high risk factor for lameness; in addition, deep bedding and soft lying surface play a key role

promoting comfort and reducing lameness. Comparing lameness prevalence in this study with the one from a protocol developed by Whay et al. (2003), where categories A (best) to E (worst) graded the welfare of 53 dairy farms, the E category (lameness prevalence of 30–50 %) would be the most adequate to classify the studied sample if only cows (59%, 95% CI = 42–75%) were considered, or D category (24–30%), if both cows and heifers were counted (27%, 95% C= 18–38%). Lameness prevalence was the major welfare problem identified within the studied parameters. Silva et al. (2008) have also pointed out hock lesions as a major welfare problem in a study of 50 Northwest Portuguese dairy farms. The current study demonstrated that lame cows spend less time elevated on their feet, due in part to spending less time standing and walking compared with non lame cows. This is in agreement with the results of Almeida et al. (2008) and Gonzales et al. (2008) who found that lameness significantly decreases feeding time. As shown in many other studies, the age of the cow and the time of year have a large effect on levels of lameness. Lameness prevalence was 12–87 % with the mean value of  $27 \pm 17$  %. Esslemont & Kossaibati (1996) reported 24 % lameness in a survey of 90 herds in 1992–1993, while in another survey (Kossaibati & Esslemont, 1999), performed on 50 farms during 1995–1996, lameness reached 38%. Herd lameness has been estimated at 22 % by recent studies in the UK (Whay, 2003) and Wisconsin, USA (Cook, 2003) and Clarkson et al. (1996). Our findings of lameness (23%) are in accordance with these authors. Herd lameness has been estimated at 22% by studies undertaken in the UK (Whay, 2002) and Wisconsin, USA (Cook, 2003). Whay et al. (2003) report that there has been little improvement in herd lameness levels over the last decade and the FAWC (1997) claim lameness is a greater problem now than it was 40 years ago.

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## Acknowledgement

The Authors are grateful to Pr. FEIDI Hakim for the grammar revision and to farmers for their help and their contribution in this research.

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# Alleviating Heat Stress Leads to Improved Cow Reproductive Performance

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Siriwat Suadsong

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50881>

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## 1. Introduction

Thailand, a country situated in the south eastern part of Asia, is situated in between 15° 00' North latitude and 100° 00' East longitude and located in a tropical area with high temperature and humidity. Crossbred Holstein dairy cattle are popular because, during times of high environmental thermal stress, their milk production and reproductive efficiency is not depressed as it is with purebred Holstein cattle. However, these crossbred cattle have been inseminated with purebred Holstein frozen semen to improve milk production. Although the genetic potential for milk yield has improved, the predominant dairy breed has now become Holstein and the impact of heat stress on production and reproduction has increased.

This chapter will be showed the finding of our studied that show the impact of heat stress on postpartum reproductive performance and milk production and evaluate the effects of utilizing an evaporative cooling system for improving cow comfort, energy balance, postpartum reproductive performance and milk production of early lactating dairy cows under hot and humid climatic conditions.

Climatic conditions in the tropics are such that the hot season is relatively long, there is intense radiant energy for an extended period of time, and there is generally high relative humidity. Thus heat stress is chronic in nature, there is often little relief from the heat during the night, and intense bursts of combined heat and humidity depress performance. Lactating dairy cows create a large quantity of metabolic heat and accumulate additional heat from radiant energy. Heat production and heat accumulation, coupled with a compromised cooling capability, because of environmental conditions, causes heat load in the cows to increase to a point when body temperature rises, feed intake declines and ultimately the cow's productivity falls.

During period of elevated temperature, animals show less physical activity and seek shelter to decrease radiant heat exposure. Elevated body temperatures will rapidly trigger adaptive mechanisms to restore body temperature to normal. These adaptations, including panting, sweating, reduced feed intake and lowered metabolism, may be necessary for survival, but they are not generally favorable to milk production [1]. Moreover, because of their relative size and their high metabolic rate, associated with milk production, dairy cows are particularly susceptible to the effects of heat stress.

Heat stress has a significant impact on dairy cattle in hot and humid climates. Environmental factors, which contribute to heat stress, include high ambient temperatures, radiant energy, and high humidity, all of which compromise the cow's ability to dissipate body heat. When the cow cannot dissipate sufficient heat to maintain thermal balance, body temperature rises and heat stress occurs. Ambient temperature is a major component of heat stress, however humidity must also be considered because evaporative heat loss is more effective when humidity is low. The temperature-humidity index (THI) combines these two factors into an indicator of cow comfort. Cows are beginning to be stressed when the THI exceeds 72 [2].

Dairy cows have several mechanisms to help dissipate body heat and maintain body temperature, such as; conduction, convection, radiation and evaporation. Conduction, convection, and radiation depend on a relatively large differential between the body and the environmental temperature, and evaporation works best at a low relative humidity. When the environmental temperature nears the cow's body temperature and is coupled with high relative humidity, all the cow's cooling mechanisms are impaired. As a result the cow's body temperature rises and the cow exhibits physiological responses to hot weather. Cows in hot climates generally produce additional heat, compared to those in cool climates, because of greater physical activity (such as panting) which is necessary to enhance cooling in hot conditions. In addition lactating dairy cows produce large amounts of heat from both ruminal fermentation and metabolic processes. As production increases, the total amount of heat produced increases. In order to maintain body temperature within the normal range, dairy cows must exchange this heat with the environment. The most noticeable response to heat stress is reduced feed intake, reduced milk yield, reduced activity, and increased respiration rate and water intake.

## **2. Effects of heat stress on production**

Temperature and humidity combine to decrease dry matter intake (DMI) in dairy cows as a physiological means of regulating internal body temperature. This is accomplished by decreasing rumen fermentation and the metabolic rate [3,4]. A reduction in DMI decreases the nutrients available for milk synthesis, milk production declines and many lactation parameters are affected [5-7]. High environmental temperatures also increase the respiration rate and the water intake, which consequently reduces DMI due to gut full [8]. Because of many dairy cows in hot weather are unable to consume enough feed to meet energy demands during early lactation, they typically mobilize body reserves to maintain their milk

production until the intake of feed can match or exceed nutritional requirements [9,10] thus, entering a state of negative energy balance (NEB).

In heat stressed dairy cows there is a reduction in DMI [11], which prolongs the period of negative energy balance. Negative energy balance leads to decreased plasma concentration of insulin, glucose and insulin-like growth factor-I (IGF-I), and increased plasma concentrations of growth hormone (GH) and non-esterified fatty acid (NEFA) [12,13]. All of these metabolic hormones can affect reproduction. Metabolic hormones acting on the hypothalamo-pituitary axis and the ovary probably mediate the inhibitory effects of negative energy balance on postpartum fertility.

### **3. Effects of heat stress on reproduction**

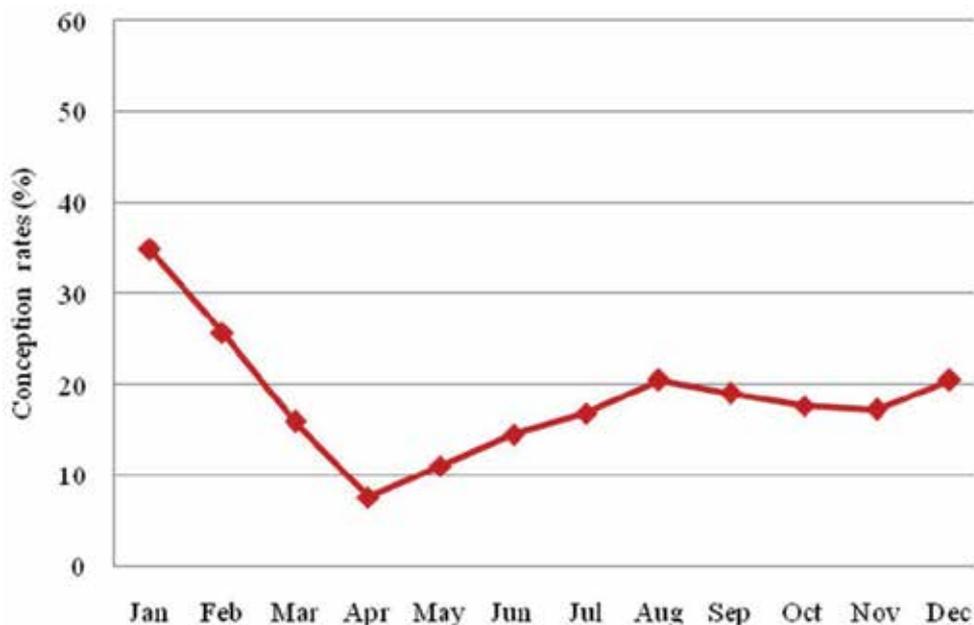
Heat stress affects reproductive performance both by direct action on reproduction and by indirect actions mediated through alterations in energy balance. There is an interaction between DMI, stage of lactation, milk production, energy balance and heat stress, that results in reduced luteinizing hormone (LH) secretion and a decreased diameter of the dominant follicle in the postpartum period [14], this results in reduced oestradiol secretion from the dominant follicle, leading to poor expression of oestrus. The postpartum anovulatory interval of dairy cow, is characterized by a variable period of negative energy balance that is reported to modulate the recrudescence of ovarian cyclicity [9,15,16]. In heat stressed cows, motor activity and other manifestations of oestrus are reduced [17] and the incidence of anoestrus and silent ovulation are increased [18].

There is a decrease in fertility in lactating dairy cows during summer in hot climate [19]. The magnitude of the depression depends on the geographical location and the milk yield [20-22]. In tropical climates, high ambient temperatures and humidity are important determinants of reproductive performance. Heat stress decreases the intensity and duration of oestrus, which in turn reduces both the number of inseminations and the pregnancy rates [23]. Heat stress alters the concentration of circulating hormones by increasing the circulating concentration of corticosteroids [24] and by reducing progesterone concentration [25]. The viability of pre-fixation embryos is reduced [26], and the uterine environment is altered by a decreased blood flow [27] and increased uterine temperature [28]. These changes are associated with increased early embryonic loss and a reduced proportion of successful inseminations. Cows exposed to heat stress have a high incidence of early embryonic mortality [26,29], and some of this effect is due to the direct effect of elevated temperature on the embryo [30].

Lactating dairy cows are susceptible to heat stress because of the elevated internal heat production which is associated with lactation. During periods of heat stress, milk production, feed intake, and physical activity are decreased [11]. At the same time, reproductive ability is compromised [31,32]. The exposure of lactating cows to heat stress has been shown to cause a decrease in follicular growth and to reduce serum estradiol [33], which also concluded that decreased follicular size or decreased dominant follicle function occurred in lactating cows that were exposed to heat stress [34,35]. Some of the reproductive losses, in heat stressed cattle, are associated with decreased expression of oestrus caused by anoestrus and silent ovulation

[36,37]. Heat stress delays follicle selection and lengthens the follicular wave having potentially adverse effects on oocytes quality [34] and follicular steroidogenesis [35].

Heat stress cause infertility and represented a major source of economic loss in dairy cows under tropical conditions. In recent study, the conception rate of dairy cows in Thailand decrease 20-30 % in hot season. The conception rate was lower in April and May when compared to other month, which lowest in summer and highest in winter (Figure 1). The effects of heat stress can be directly related to the increase in body temperature of dairy cow, which affects the reproductive function and embryonic development.



**Figure 1.** Conception rates of dairy cows in commercial farm located in the central part of Thailand.

The detrimental effects of heat stress on the reproductive performance of dairy cows have been well documented. These include a suppressed intensity of oestrus, a reduced preovulatory LH surge and decreased secretion of luteal progesterone [25], altered ovarian follicular development [33], decreased embryo development [38] and lower fertility [39]. In an attempt to minimize these effects, modifications to dairy cattle housing environments have been implemented to alleviate thermal stressors and improve cow comfort, through the use of shade, fans, sprinklers, and evaporative cooling [11,37,40]. These methods can enhance pregnancy rates significantly [24,41].

#### **4. Effect of environmental modification for dairy cows under tropical conditions**

Reducing heat stress in dairy cattle requires a multi-disciplinary approach. It involves breeding for improved heat tolerance, improved nutrition and improved reproductive

management for the animals, and improved the structural design and the environmental control of their housing.

Heat stress reduces milk production and reproductive efficiency. In an attempt to minimize these effects, modifications to dairy cattle housing environments have been implemented to alleviate thermal stressors and improve cow comfort [11,37,40,42]. The major objective of any cooling system is to keep the cow's body temperature as close to normal for as much of the day as possible. An acceptable range in rectal temperature is 38.5-39.3 °C. There are two general approaches to cooling dairy cows. One is to modify the environment to prevent heat stress or to utilize methods that increase heat dissipation from the skin surface of cattle. The easiest and most obvious way to help heat-stressed cows is to provide shade. Direct sunlight adds a tremendous heat load to the cow and can be blocked by shades, but shade alone is inadequate to reduce the effect of heat stress. A more economical method to reduce the effect of heat stress is by evaporative cooling. Evaporative cooling can be accomplished by two approaches; 1) direct evaporation from the skin surface of the cows (fan and sprinkler combinations) and 2) indirect evaporation which involve cooling the micro-environment of the cows, with cooling pads and fans, in an enclosed barn. When water evaporates it absorbs heat, thereby reducing the temperature and increasing heat dissipation from the skin of cattle. When water evaporates it also increases the relative humidity, due to the increased level of water vapor present. In hot and humid regions, evaporative cooling always requires the use of forced ventilation.

A number of studies have shown that housing systems in hot climates can be modified by the use of evaporative cooling to improve both milk production and reproductive efficiency of dairy cows [43-46]. There is a great potential to reduce temperature and THI. However, as relative humidity increases and or temperature decreases, the potential for evaporative cooling to modify the environment decreases. In hot and humid climates, high relative humidity reduces the potential of evaporative cooling. Therefore, there are questions regarding the effectiveness of evaporative systems in climates with high relative humidity. This chapter showed the impact of heat stress on postpartum cow performances and evaluated the effects of utilizing an evaporative cooling system for improved cow comfort and cow performance of early lactating dairy cows in a hot and humid climate.

## **5. Effect of evaporative cooling system on the environmental condition and physiological response of dairy cows**

The upper critical temperature for heat stress to begin was between 25 and 26°C [47]. Climatic conditions in the present study are such that the hot season is relatively long, and generally accompanied by high relative humidity. Thus heat stress is chronic in nature and there is little relief from the heat during the evening through to the morning, and also includes intense bursts of combined heat and humidity which further depresses performance.

Environmental modifications by evaporative cooling system equipped with tunnel ventilation in this study led to a decrease in the ambient temperature and an increase in the relative humidity, during the day. The air temperature in the tunnel barn was up to 6 °C

cooler ( $P<0.05$ ) during the daytime than that in the outside barn, while the relative humidity increased by up to 16%. As air was drawn through the wet cooling pads, water was evaporated into the air causing the temperature to be reduced while increasing the air moisture level. The amount of initial moisture in the outside air will directly impact on how much reduction in air temperature might be expected. Therefore, when the outside air is initially very high humid (relative humidity greater than 70%), the reduction in air temperature will be minimal (less than 2.8 to 5.6°C).

The average daily minimum and maximum temperatures were  $24.2\pm 0.1$  and  $28.4\pm 0.1$ °C, respectively in the evaporative, cooled tunnel, ventilated barn and  $24.7\pm 0.1$  and  $34.4\pm 0.1$ °C, respectively in the outside barn, indicating less variation and more consistency in this cooling system. This evaporative, cooled tunnel, ventilated barn reduced daily fluctuation in the ambient temperature, relative humidity and THI during hot and humid climatic conditions. Although, this system reduced ( $P<0.05$ ) afternoon barn temperature, but relative humidity increased ( $P<0.05$ ) when compared to the barn without a supplemental cooling system.

Although, high environmental relative humidity reduced the cooling capacity of the evaporative cooling, but on the days when animals were observed it reduced heat stress of the lactating dairy cows. This study shows that evaporative cooling with tunnel ventilation reduced the severity of afternoon heat stress in dairy cows. THI in the tunnel ventilated barn was decreased when compared to the outside barn. There was no thermoneutral zone ( $\text{THI}<72$ ) during the study but exposure to conditions of moderate heat stress ( $\text{THI}\geq 79$ ) that occurred outside was decreased by utilizing the evaporative cooling system. This difference in environmental conditions had a dramatic effect on the physiological response of the cows, as THI was highly correlated with both rectal temperature and respiration rate. The average rectal temperature and respiration rate in the cooled cows was lower ( $P<0.05$ ) than in the uncooled cows. Changing in cow body temperatures was most sensitive to same day climatic factors [48]. These results suggest that evaporative cooling and tunnel ventilation has the potential to decrease exposure to heat stress and alleviate the symptoms of heat stress.

The average daily THI in the morning and afternoon in the evaporative, cooled tunnel, ventilated barn were lower ( $P<0.05$ ) than that in the outside barn. However, the mean THI exceeded the critical point of 72 at daytime and nighttime, suggesting that the cows were exposed continuously to conditions conducive to mild heat stress for the cooled cows and moderate heat stress for the uncooled cows. In hot, arid conditions this system would work well and evaporative cooling has already been used very successfully to cool such dairy cows [46], but in high humidity locations its effectiveness would be limited by the evaporation potential. In this study the air temperature increases during the day and decreases in the evening until the next morning. As the air temperature rises during the day, the relative humidity will decrease. Accordingly during the hottest portion of the day, the outside relative humidity dropped to a level that allowed for maximum evaporation potential, making the system effective for reducing the severity of heat stress.

	Cooled (inside)	Uncooled (outside)	P-value
Number of animals (n)	17	17	
Environmental measurement :			
Daily minimum temperature (°C)	24.2±0.1	24.7±0.1	*
Daily maximum temperature (°C)	28.4±0.1	34.4±0.1	*
AM milking			
Daily mean temperature (°C)	25.3±0.1	25.6±0.1	*
Daily mean relative humidity (%)	88.5±0.3	91.6±0.2	*
Daily mean THI	76.0±0.2	76.7±0.2	*
Rectal temperature (°C)	38.4±0.0	39.2±0.1	*
Respiratory rate (breaths/min)	42.7±1.4	54.5±1.4	*
PM milking			
Daily mean temperature (°C)	26.5±0.1	31.4±0.1	*
Daily mean relative humidity (%)	86.5±0.3	74.6±0.8	*
Daily mean THI	78.0±0.2	83.1±0.2	*
Rectal temperature (°C)	38.7±0.0	39.7±0.1	*
Respiratory rate (breaths/min)	46.3±1.8	68.3±2.2	*

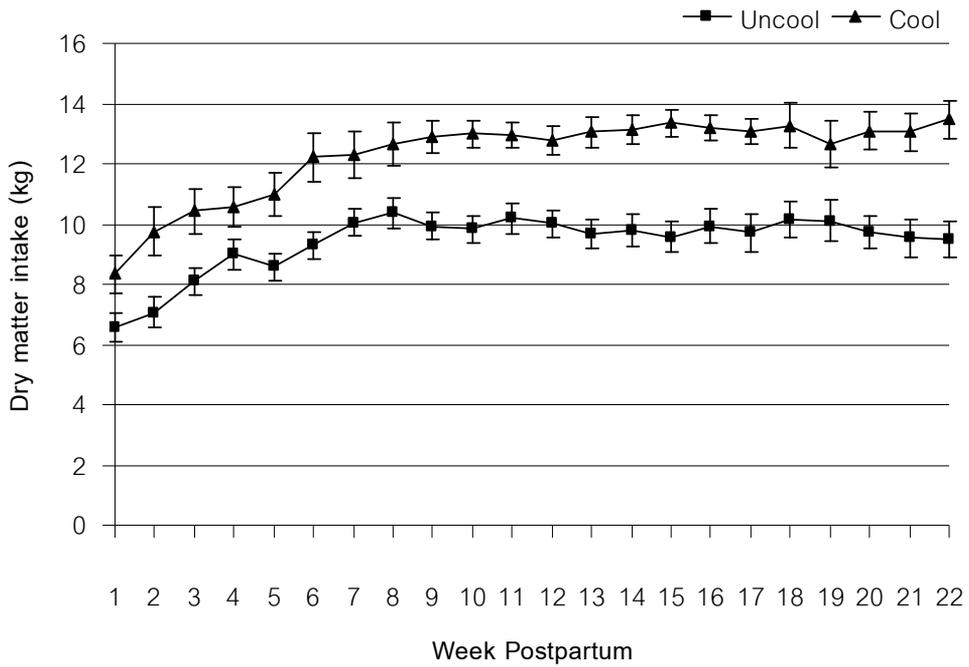
\* Means differ ( $P<0.05$ ) between groups

**Table 1.** The environmental measurement and physiological responses of dairy cows in the experimental housing. (Mean±S.E.M.)

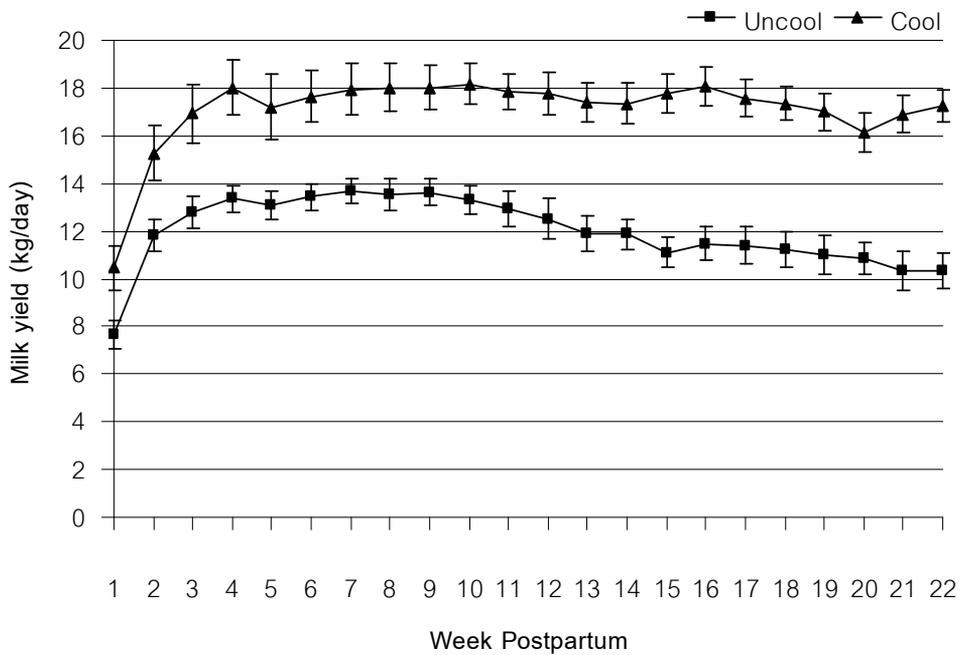
Air temperature had the greatest impact on physiological measurement, while radiation was second in importance, followed by vapor pressure and air movement [49]. Increasing the air temperature reduces the temperature differential between the cow's body temperature and the ambient temperature and decreases the transfer of heat to the environment. As ambient temperatures increase in the presence of low or high relative humidity, the cooling mechanisms employed by the cows shifted from a nonevaporative processes (convective, conductive, and radiation) to evaporative (sweating and panting) [48], this demonstrate that the percentage of cooling originating from the non evaporative processes declines as ambient temperature increases, while the evaporative process increase. As a result the uncooled cows had greater ( $P<0.05$ ) rectal temperatures and respiration rates than the cooled cows.

## 6. Effect of evaporative cooling system on dry matter intake and daily milk yield of dairy cows

Dry matter intake, expressed as kilograms per day (kg of DM/day) was greater ( $P<0.05$ ) in cooled ( $12.0 \pm 0.2$  kg/d) than uncooled cows ( $9.1 \pm 0.2$  kg/d). DMI increased ( $P<0.05$ ) from wk 1 to 22 of lactation in both groups of cows but treatment x week postpartum did not affect ( $P>0.05$ ) it (Figure 2) [50].



**Figure 2.** Weekly changes in average daily DMI for the cooled and uncooled cows during the first 22 week postpartum.



**Figure 3.** Weekly changes in average daily milk production for the cooled and uncooled cows during the first 22 week postpartum.

Daily milk yield was greater ( $P<0.001$ ) in cooled ( $16.9 \pm 0.3$  kg/d) than uncooled ( $12.6 \pm 0.2$  kg/d) cows. Daily milk yield increased ( $P<0.001$ ) from wk 1 to wk 22 of lactation in both groups of cows but treatment  $\times$  week postpartum did not affect ( $P>0.50$ ) daily milk yield (Figure 3). The 4% FCM also differ ( $P<0.001$ ) between cooled and uncooled cows. Cooled cows had more persistent milk production than uncooled cows. Milk composition did not differ ( $P>0.50$ ) between the groups of cows over the 12 week study (Table 2) [50].

	Cooled (inside)	Uncooled (outside)	P-value
Dry matter intake (kg/d)	$12.0 \pm 0.2$	$9.1 \pm 0.2$	0.001
Milk yield (kg/d)	$16.9 \pm 0.3$	$12.6 \pm 0.2$	0.001
Fat (%)	$3.3 \pm 0.6$	$3.4 \pm 0.6$	0.810
Protein (mg/ml)	$3.2 \pm 0.3$	$3.1 \pm 0.3$	0.650
Lactose (mg/ml)	$5.0 \pm 0.2$	$5.0 \pm 0.2$	0.571
Solid not fat (mg/ml)	$8.9 \pm 0.4$	$8.8 \pm 0.4$	0.450

**Table 2.** Dry matter intake, milk production and milk composition of cooled and uncooled cows during the 12 week study.

In this study, rectal temperature was positively correlated with air temperature and THI but negatively correlated with DMI. Dry matter intake was positively correlated with milk production. Milk yield and DMI exhibited a significant decline when maximum THI reached 77 [51]. There is a significant negative correlation between THI and DMI [52,53], and the effect of THI is probably mediated through the effects of increasing body temperature on cow performance.

Mean air temperature had the greatest influence on milk yield for Holstein cows under hot conditions [48]. The mean daily ambient temperature was highly correlated with the p.m. rectal temperature and milk yield was highly correlated with the cows p.m. rectal temperature. The elevated p.m. rectal temperatures were associated with concomitant reductions in DMI and milk yield [54]. Uncooled cows, in this study, had rectal temperatures exceeded  $39^{\circ}\text{C}$  at both the a.m. and the p.m. milking, when cow temperatures should have been near their lowest and highest points, respectively. Such consistently elevated rectal temperatures, result in a significant decline in DMI and milk yield when compare with the cooled cows which had a lower rectal temperature.

The negative effects of heat stress on milk production could be explained by decreased nutrient intake and decreased nutrient uptake by the portal drained viscera of the cow [55]. Blood flow which moves to peripheral tissues for cooling purposes may alter nutrient metabolism and contribute to lower milk yields during hot weather. Providing cows with supplemental shade and cooling to mitigate heat stress has been evaluated in economic terms using the increase in milk production data [2]. Adding sprinklers and fan cooling increased the feed intake (7.1 to 9.2%) and milk production (7.1 to 15.8%), and decreased rectal temperature ( $-0.4$  to  $-0.5^{\circ}\text{C}$ ) and respiration rate (17.6 to 40.6 %) [56].

In this study the cooling system improved cow comfort and milk production. The cooling system decreased ambient temperatures and THI. The cows with this cooling system had decreased rectal temperatures and respiration rates, and increased feed intake (30.9 %) and milk production (42.5%). In addition, an analysis of the economics in the this study showed that the rate of return on investment in cooling equipment and additional feed plus electric costs of this cooling system, showed it was profitable in hot and humid conditions.

## 7. Economic analysis of the utilizing evaporative cooling system

There are questions that arise regarding the cost effectiveness of an evaporative cooling system over a period of years. In this study, an economic analysis of the evaporative cooling system showed that cows in the tunnel ventilation barn produced 5.1 kg/cow/day more than the cows housed outside. The milk price was 11.50 baht/kg. Therefore, the use of tunnel ventilation cooling increased revenue by 58.65 baht/cow/day and increased feed costs by 22.17 baht/cow/day. Thus, income over feed cost was 36.48 baht/cow/day.

The cost of the fan and water pump operation was 6.88 baht/cow/day. The total equipment and supply cost to build the tunnel barn facility was 125,000 baht (for 18 cows), which, when depreciated over the expected life of the equipment (including maintenance costs of 25,000 baht over 5 years), was 4.11 baht/cow/day. Thus, the cows housed in the tunnel barn earned 25.49 baht/day or 3823.50 baht more than the cows housed outside over the 22-week study (Table 3).

Item	Difference	Income-Cost (Baht/cow/day)
Milk yield (kg/cow/day)	5.1	58.65
DMI (kg/cow/day)	2.9	-22.17
Electric cost (fan & water pump operation)		-6.88
Equipment and maintenance cost (5 years)		-4.11
Total		25.49

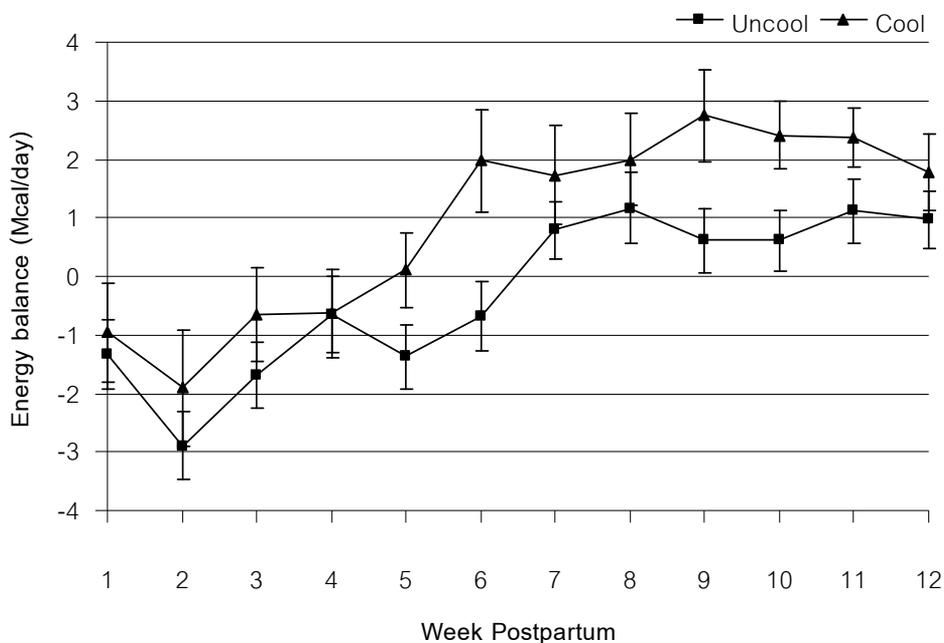
**Table 3.** The economic analysis of the evaporative cooling system over the first 22 week of lactation in dairy cows.

## 8. Effect of evaporative cooling system on energy balance and body weight of dairy cows

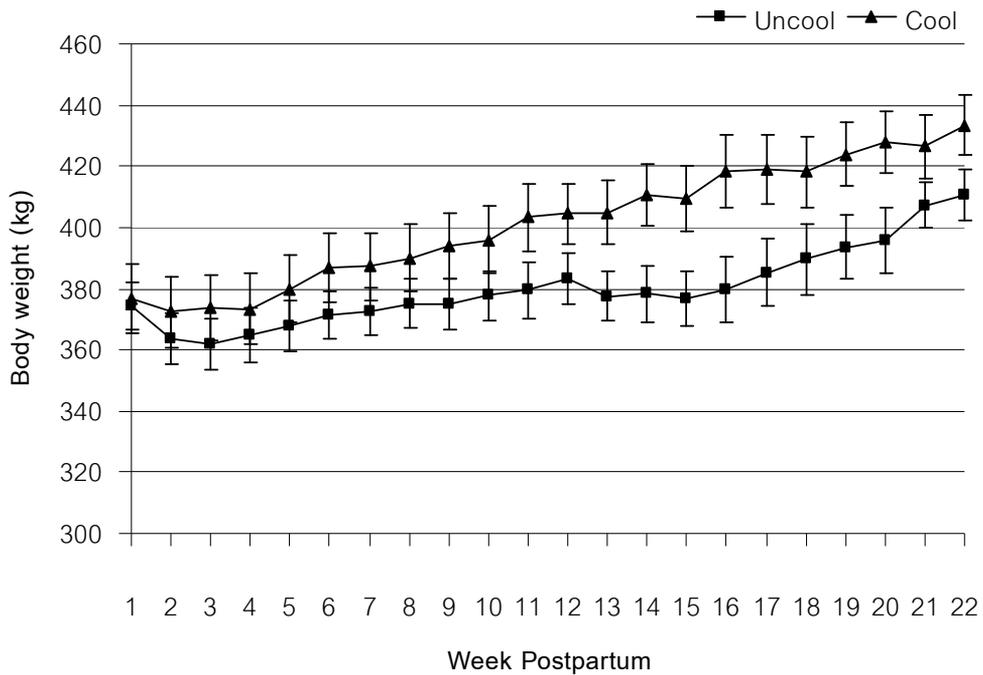
Responses of dairy cow to heat stress include panting and sweating. If these are not successful in alleviating the heat load, body temperature will rise. Increased body temperature will result in reduced feed intake, higher maintenance requirements (panting) and less efficient productive ability. The higher maintenance requirements dictates that cows need to increase feed intake to maintain milk production. However, this not possible as feed intake declines when ambient temperatures exceeded 26°C. For uncooled cows in this study, as a result of this increase in requirement and a decrease in intake, milk

production may decline as much as 25-30%, and typically they mobilize body reserves and lose body weight to maintain milk production until the intake of feed can match or exceed nutritional requirements [9,10], thus entering a state of negative energy balance.

The energy balance and body weight of dairy cows is presented in Figure 4 and Figure 5. During 12 wk of lactation, average body weight of postpartum cows was greater in cooled than uncooled cows. In both groups of cows, body weight decreased between wk 1 and 4, and increased between wk 5 and 22. Week postpartum ( $P<0.001$ ) and treatment ( $P<0.001$ ) affected EB but treatment  $\times$  week postpartum was not significant. Cows in both groups entered into NEB immediately after calving. Averaged EB was greater ( $P<0.001$ ) in cooled ( $0.916 \pm 0.194$  Mcal/day) than uncooled cows ( $-0.268 \pm 0.195$  Mcal/day). During the 12 week study, the week of EB nadir was at wk 2 in both groups and the degree of EB nadir did not differ significantly ( $P>0.50$ ) between the groups, although the average was lower in uncooled than cooled cows. After reaching the EB nadir, uncooled cows required more days to reach a positive energy balance than the cooled cows. The first week that EB was greater than zero was at wk 5 in cooled cows and at wk 7 in uncooled cows. Because of dry matter intake in uncooled cows was lower ( $P<0.05$ ) than in cooled cows. Resulting in uncooled cows having a prolonged period of negative energy balance and postpartum body weight in these cows were lesser ( $P<0.05$ ) than in cooled cows [50]. The negative energy balance is directly related to the postpartum interval to first ovulation, and follicle size was adversely affected by negative energy balance in early postpartum dairy cows [9]. The average EB during the first 4 week of lactation was negatively correlated to the postpartum interval to first ovulation [57].



**Figure 4.** Weekly changes in energy balance for the cooled and uncooled cows during the first 12 week postpartum.



**Figure 5.** Weekly changes in average body weight for the cooled and uncooled cows during the first 22 week postpartum.

## 9. Effect of evaporative cooling system on synchronization of follicular development and ovulation

The exposure of lactating cows to heat stress has been shown to cause a decrease in follicular growth and to reduce concentrations of serum estradiol [33]. In this study the cooled cows had a greater ( $P < 0.05$ ) average diameter of the largest ovulatory follicle than the uncooled cows. Heat stress inhibited follicular growth and dominance during the preovulatory period. Abnormal ovarian function in heat stressed cows was manifested as a decrease in the proestrus rise in estradiol, and the smaller size of the second wave dominant follicle [33]. Circulating estradiol concentration during the preovulatory period is necessary to produce an LH surge and ovulation. In addition, a reduced estradiol peak may also alter aspects of the LH surge that could account for some types of anovulation in lactating cows [58]. A reduction of the endogenous LH surge by heat stress was reported in heifers [59]. It has been suggested that these differences are related to preovulatory estradiol levels because the amplitude of tonic LH pulses and GnRH-induced preovulatory plasma LH surges are decreased in cows with low plasma concentrations of estradiol but not in cows with high plasma concentrations of estradiol [60]. Therefore, the synchronization rate in the uncooled cows tended to lower than in the cooled cows. In addition, the ovulation rate in response to a second injection GnRH of Ovsynch was reported to be between 87% [61] and 91% [62] in cycling cows. Therefore a second GnRH injection after the PGF<sub>2 $\alpha$</sub>  treatment might be used to improve the ovulation rate in dairy cows under heat stress.

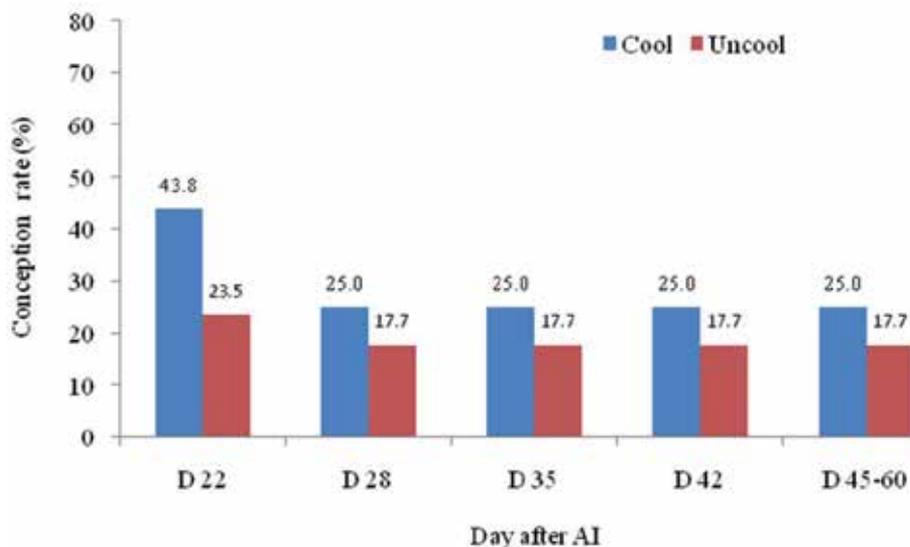
	Cooled	Uncooled	P-value
Number of animal (n)	17	17	
Synchronization rate (%)	82.4(14/17)	52.9(9/17)	0.08
Interval to new follicular wave emergence (day)	2.2±0.1	2.3±0.2	0.55
Size of the largest ovulatory follicle at PGF <sub>2α</sub> (mm)	11.5±0.6	10.2±0.5	0.12
Maximal size of the largest ovulatory follicle (mm)	14.6±0.5	14.2±0.4	0.57
Growth rate of the largest ovulatory follicle after PGF <sub>2α</sub> (mm/d)	0.9±0.1	1.1±0.1	0.17
Interval from PGF <sub>2α</sub> treatment to ovulation (h)	83.6±5.1	88.0±6.9	0.63

**Table 4.** The effect of evaporative cooling and tunnel ventilation system on follicular development, time of ovulation and the response rates of synchronized cows to GnRH and PGF<sub>2α</sub>. Results are expressed as mean±SEM.

## 10. Effect of evaporative cooling system on embryonic development and conception rate of dairy cows

Heat stress can compromise reproductive performance by decreasing the expression of oestrus behavior, altering follicular development, affecting oocyte competence, inhibiting embryonic development due to a reduced synchronization of ovulation response, lowering fertilization rates, and reducing embryo quality. In this study the modification of the barn environment and fixed TAI in lactating dairy cows in a hot and humid climate resulted in higher initial conception rates compared to cows housed in a barn without a supplemental cooling system (43.8 and 23.5%, respectively) (Figure 6). The results indicate that this method has the potential to attenuate some of the detrimental effects of heat stress on embryo survival during this period. Although, initial conception rates in cooled cows was higher than uncooled cows, it was still compromised by heat stress as the conception rate decreased further in both groups, indicating that embryo mortality may still have occurred after that. This was probably due to environmental heat stress [63], which causes maternal body temperature to rise leading to the impairment of embryo survival, or this cooling was not sufficiently cool to protect embryos from direct effect of high temperatures, or early embryos during this period might be sensitive to elevated temperature.

Climatic factors that may influence the degree of heat stress include: temperature, humidity, radiation and wind [32]. The upper critical temperature for heat stress begins between 25 and 26°C [47]. When environment temperatures exceed 30°C the day after insemination, pregnancy rates were adversely affected in lactating dairy cows than in heifers [64]. Maternal hyperthermia is detrimental to embryonic development and survival [65-67]. The oocyte and early cleavage stage embryo are the most sensitive to heat stress, while embryos that are 3 d or older are more tolerant [26,29]. Conception rates decline from 61 to 45% when rectal temperature 12 h, post breeding, increased by 1°C [68]. Furthermore, cattle with a rectal temperature of 40°C, as a result of exposure to 32.2 °C ambient temperatures for 72 h after inseminating, had conception rates of 0%, compared with a conception rate of 48% when rectal temperature was 38.5°C, for cows in an ambient temperature of 21.1 °C [68]. Given that exposure to conditions of high environmental temperature and humidity has been shown to elevate rectal and uterine temperatures [28].



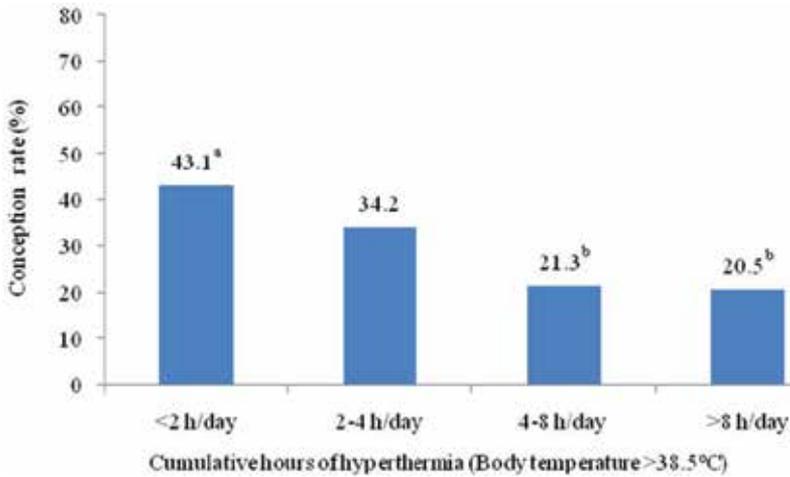
**Figure 6.** The conception rate after the synchronization of ovulation and fixed time AI of cooled and uncooled cows.

The modified environment had been used to reduce the effect of heat stress, however, this approach has not eliminated all problems. Timed AI might be particularly effective during heat stress periods because of the decreased incidence of missed oestrus, but heat stress has also a direct effect on the development of the embryo. Conception rates have ranged between 31 to 42 % in cows following the Ovsynch protocol [69-73]. However, conception rates in heat-stressed cows following the Ovsynch protocol were lower than in non stressed cows.

Timed AI programs based on follicular recruitment by synchronized ovulation have been developed [74]. The submission rates and pregnancy rates between d 27 and 30 were enhanced when a TAI program was used. However, the advantage was lost between d 40 and 50 due to increased embryonic mortality in the cows bred using TAI [75]. These results may indicate that cows were successfully induced to ovulate and subsequently conceive but had a reduced ability to maintain pregnancy. Previous studies also showed high (9.3 to 16.8 %) pregnancy losses between 28 and 56 d after AI [76,77].

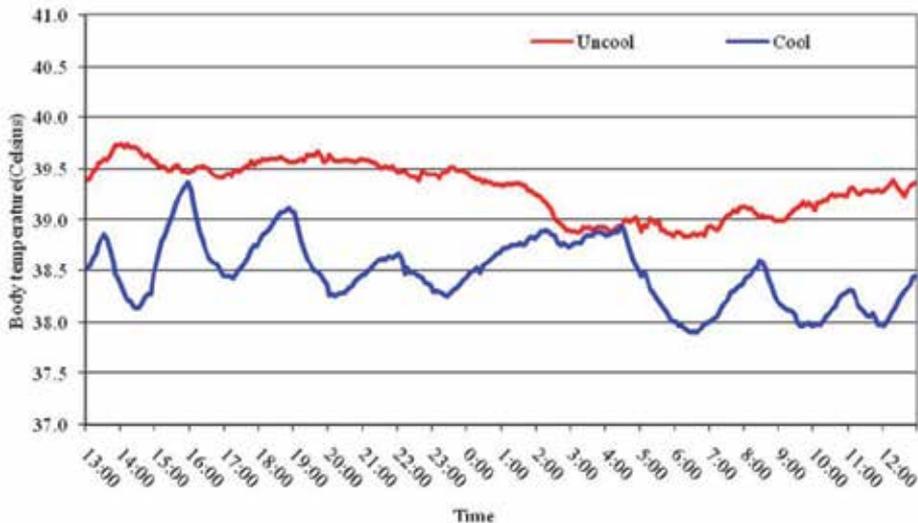
In recent study, reported that dairy cow had a cumulative hours of body temperature greater than 38.5°C more than 4-8 h per day, significantly ( $P < 0.05$ ) decline (Figure 7) in conception rate [78]. This study indicated that long period of hyperthermia (body temperature  $> 38.5^{\circ}\text{C}$ ) had an adverse effect on dairy cows and suggested that dairy cows in the tropical area need additional cooling system to completely eliminate heat stress result in close to normal fertility of dairy cows depend on the severity of the local environment conditions. In addition, *in vitro* heat stress during the critical stage of early embryo development significantly increases the incidence of early embryonic mortality [79]. These results indicate that increased maternal body temperatures adversely affect embryo quality and the conception rate. An increase in maternal body temperature may result in an increase

in the ambient temperature of oocytes, zygotes and embryos in the oviduct or the uterus. At temperature increase of  $0.5^{\circ}\text{C}$  above the basal body temperature has been associated with a decreased pregnancy rate [28]. These studies indicated that elevated environmental temperature lead to hyperthermia in lactating cows. Intensive cooling cows have the potential to eliminate the decline in conception rate of dairy cows under tropical conditions.



<sup>a, b</sup> Means with different superscripts differ ( $P<0.05$ )

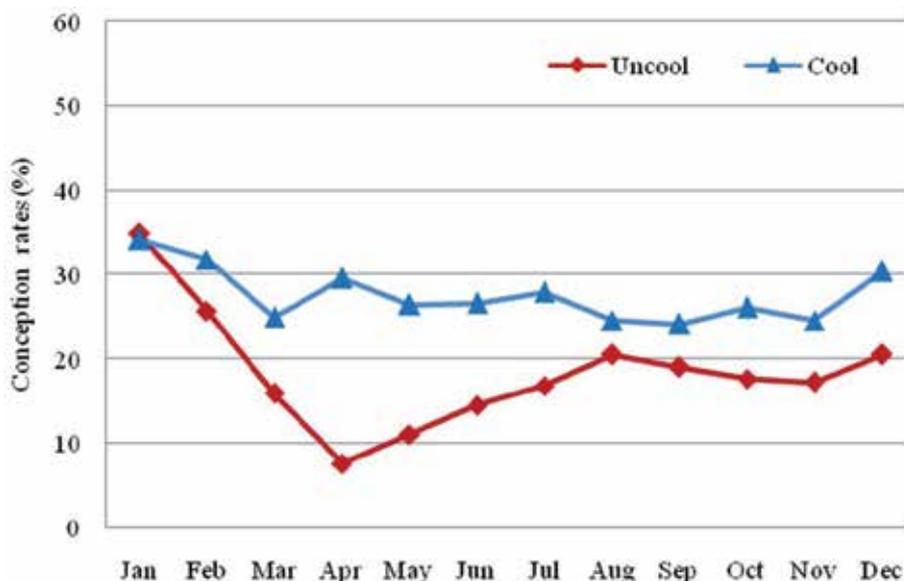
**Figure 7.** This chart show conception rates of dairy cows. It also illustrated that cow had cumulative hours of hyperthermia greater than 4-8 h per day that conception rate decline significantly ( $P<0.05$ ).



**Figure 8.** This chart show changes in mean vaginal temperature of cooled and uncooled cows throughout the day.

Infertility of dairy cows under tropical conditions is primarily caused by elevated body temperature. Therefore, cooling cows should improve conception rates. A variety of cooling

systems are available for heat-stressed cows. The intensive cooling cows with the combination of sprinklers and fans were used for improved reproductive performance. Cows were cooled in the holding area for a total 6-8 cooling periods and 4-6 cumulative h/day. Each cooling period combined cycles of sprinkling (0.5 min) and forced ventilation (4.5 min). Intensive cooling had significantly affected on decreased body temperature and allowed cows to maintain normal body temperature throughout the day [78]. In the same study, uncooled cows had high body temperature, daytime significant portion of the day and returned to normal body temperature during the late night and early morning (Figure 8).



**Figure 9.** This chart show conception rates obtained in uncooled cows during 2004-2007 and cooled cows during 2008-2011 on commercial dairy farm in the central part of Thailand.

For large scale survey was carried out during a 8 yr period (2004-2011) in commercial dairy farm. The conception rate of intensively cooled cows with sprinkler and forced ventilation was significantly higher than that of uncooled cows. Conception rates obtained in intensively cooled cows in this study were similar to those obtained in that same winter in uncooled cows (Figure 9). This current finding confirmed this adverse effect of heat stress. In hot climates there is a large decrease in the fertility of dairy cows during summer months. In addition, intensively cooling cows has the potential to eliminate the decline in conception rate of dairy cows under tropical conditions. Therefore, intensive cooling is essential in dairy cows under tropical conditions to prevent hyperthermia and its harmful effects on those cows.

## 11. Summary

The exposure of dairy cows to elevate temperatures have a variety of effects, including decreased fertility, depressed appetite, and decreased milk production, all of which contribute to the goal of decreasing the production of metabolic heat in order to maintain thermo-neutrality. This chapter showed significant advantages for the evaporative cooled

barn can be used to reduce heat stress of dairy cows housed in hot and humid climates. The combined effect of higher milk production and increasing lactation persistency, with minimal costs could improve the financial status of dairy operations. The benefit demonstrated increased income over costs.

In addition, respiration rates and rectal temperatures, which affect both milk production and reproduction, were reduced by this environmental modification. These findings suggest that the evaporative cooling and tunnel ventilation system has the potential to decrease the exposure to heat stress, alleviate the symptoms of heat stress and improve milk production and metabolic efficiency during early lactation. This modification of the barn environment can reduce some of the detrimental effects of heat stress on follicular development and can improve the response rate to the synchronization of ovulation in dairy cows in hot and humid climates.

The implementation of evaporative cooling systems for dairy cows in hot and humid climates increased the percentage of cows that initially established a pregnancy and increased successful pregnancy while decreasing early embryonic mortality, if these cows were sufficiently cooled after breeding. It appears that modification of environment need to be developed further to improve reproductive performance of dairy cows in hot and humid climates. The finding that the cooling of cows did not alleviate all the effects of heat stress on pregnancy rates suggests that the degree of cooling was not sufficient to prevent the adverse effects of heat stress. It is also possible that the cooling of dairy cows needs to be done not only in the housing unit but also in the holding and milking areas to improve pregnancy rates. Dairy cows in hot and humid climatic condition need to be intensively cooled to completely eliminate heat stress to improve production and fertility close to normal. Therefore, additional research is needed to determine the effects of environmental modification or improved cooling system on postpartum reproductive performance and production when compared to conventional methods of cow cooling in the tropical area. However, cooling intensification should be combined with reproductive management, hormonal application and nutritional management to minimize the decline in cow performances under hot and humid climatic conditions.

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## Acknowledgement

This study was supported by the Thailand Research Fund and Faculty of Veterinary Science, Chulalongkorn University.

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## Milk Production and Animal Health

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# Managing Mastitis in Heifers: An Initial Step in Improving Dairy Herd Health

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Stephen C. Nickerson

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50771>

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## 1. Introduction

Replacement heifers are critical to dairy herd productivity because they represent the future milking and breeding stock of all dairy operations. The goal should be to provide an environment for heifers to develop full lactation potential at the desired age with minimal expense. Animal health and well-being play vital roles in achieving this potential, and one disease that can influence future productivity is heifer mastitis caused by *Staphylococcus aureus*, the coagulase-negative staphylococci (CNS), and the environmental streptococci (*Streptococcus uberis*, *Streptococcus dysgalactiae*).

Unfortunately, most producers regard young dairy heifers as uninfected, and the presence of mastitis is not observed until freshening or until the first clinical flare-up in early lactation. Thus, animals may carry intramammary infections (IMI) for a year or more before they are diagnosed with mastitis (Boddie et al., 1987). The greatest development of milk-producing tissue in the udder occurs during the first pregnancy, so it is important to protect the heifer mammary gland from pathogenic microorganisms to ensure maximum milk production during the first and future lactations. In the USA, Louisiana researchers found that if bred heifers infected with *Staph. aureus* were left untreated, they produced 10% less milk in early lactation than those receiving therapy (Owens et al., 1991; Trinidad et al., 1990c). Likewise, research in New Zealand demonstrated that *Staph. aureus* mastitis in heifers resulted in significant production losses during the first lactation, which carried over into the subsequent lactation because of damage to milk-producing tissues of the udder (Woolford et al., 1983).

## 2. Prevalence of mastitis in unbred and primigravid heifers

A greater focus on heifer mastitis began in the mid 1980s after several dairy producers in Louisiana complained to university researchers that a large percentage of their heifers were

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freshening with clinical mastitis. Subsequent study of breeding age animals in a research herd revealed that IMI may be diagnosed as early as 6 months of age, and infections persisted throughout pregnancy and into lactation (Boddie et al., 1987). Other studies demonstrated that greater than 90% of breeding age and bred heifers (12 - 24 months of age) may be infected (Trinidad et al., 1990b). Most of the infections were shown to be caused by the CNS (*Staphylococcus chromogenes* and *Staphylococcus hyicus*) followed by *Staph. aureus* (20%). Mixed isolates of CNS and *Streptococcus* species were also found.

The prevalence of mastitis in 10 unbred Jersey heifers (10 - 12 months of age) in Louisiana was initially evaluated in 1985 and monitored over a 1-year period, which covered breeding age and gestation (Boddie et al., 1987). Samplings from the teat skin, teat canal, and mammary secretion were performed bimonthly and continued through time of calving. For bacteriologic analysis, a total of 388 samples from teat skin, 388 from teat canal keratin, and 216 from secretions were examined; not all quarters contained sufficient volumes of secretions.

For teat skin, staphylococci were isolated on mannitol salt agar, and the predominant colony type of a particular isolate based on color, rough/smooth, size, hemolytic pattern, and appearance was collected, sub-plated for culture on blood agar, and identified to the species level (Boddie et al., 1987). *Staphylococcus xylosum* (20.9%) and *Staph. chromogenes* (14.9%) were the predominant flora isolated from teat skin, followed by *Staph. warneri* (6.7%), *Staph. sciuri* (6.2%), *Staph. aureus* (2.8%), *Staph. hyicus* (1.3%), and *Staph. simulans* (1.3%). The most prevalent bacteria found in teat canals were *Staph. chromogenes* (41.0%), followed by *Staph. hyicus* (16.8%), *Staph. aureus* (10.0%), *Staph. xylosum* (1.0%), *Staph. warneri* (0.8%), and *Staph. sciuri* (0.5%).

In mammary secretions, *Staph. chromogenes* (49.5%) was the predominant organism, followed by *Staph. hyicus* (21.3%), *Staph. aureus* (13.0%), *Streptococcus uberis* (1.4), and *Staph. xylosum* (0.9%). The CNS species in teat canals and in secretions from a particular mammary quarter were present at the first sampling, and the same CNS species were isolated from teat canals and secretions from the same individual quarters with each successive sampling of the trial. Also, there appeared to be a correlation between colonization of the teat canal and IMI. For example, the major CNS species colonizing the teat canal (*Staph. chromogenes* (41.0%), *Staph. hyicus* (16.8%), *Staph. aureus* (10.0%), and *Staph. xylosum* (1.0%)) were also the predominant organisms causing IMI (*Staph. chromogenes* (49.5%), *Staph. hyicus* (21.3%), *Staph. aureus* (13.0%), and *Staph. xylosum* (0.9%)). Thus, teat canals infected with *Staph. chromogenes* (41.0%) were positively correlated with secretions (49.5%) infected with these bacteria. The overall correlation between teat canal colonization and IMI was 82.2% across these 4 CNS species.

This initial study (Boddie et al., 1987) indicated that teat skin, teat canals, and mammary secretions of heifers are colonized with CNS as well as *Staph. aureus* at an early age, and that infections may persist for up to 1 year. Species identification demonstrated that nearly all isolates from the same quarter throughout the study were the same biovariant based on the API Staph-Ident System as previously modified (Watts et al., 1984), which supports the

contention that isolates from each quarter over time were from persistent infections and not from new IMI occurring between sampling periods. Although the percentage of *Staph. aureus* isolates was lower than CNS, presence of this major pathogen demonstrated that it colonized teat canals at a much earlier age than documented previously (Rendel & Sundberg, 1962).

In a subsequent herd survey (Trinidad et al., 1990b), the prevalence of mastitis in breeding age and pregnant heifers was determined in 4 commercial dairies. Teat canal keratin and secretion samples were collected from 116 Jersey heifers, and results revealed that teat canal colonizations were present in 93.1% of heifers and 70.7% of quarters. *Staph. aureus* was isolated from teat canal keratin samples of 31% of heifers and 12.3% of quarters. Other organisms isolated from keratin and percentage frequencies were *Staph. chromogenes* (42.9%), *Staph. hyicus* (25.2%), other staphylococcal species (5.7%), *Strep. dysgalactiae* (0.6%), *Strep. species* (3.1%), and mixed isolates containing staphylococci and streptococci (5.7%). In this 4-herd survey, IMI were found in 96.9% of heifers and in 74.6% of quarters. Twenty-nine percent of heifers and 15.1% of quarters showed clinical symptoms of mastitis as evidenced by clots, flakes, and blood. *Staph. aureus* was isolated from 14.7% of quarters. This microorganism was also isolated from 25% of quarters with clinical symptoms. *Staph. aureus* causes severe damage to mammary tissue (Trinidad et al., 1990a), and infections are very difficult to eliminate in lactating cows. Other organisms isolated from secretions and percentage frequencies were *Staph. chromogenes* (43.1%), *Staph. hyicus* (24.3%), other staphylococcal spp. (3.6%), *Strep. dysgalactiae* (0.4%), *Strep. spp.* (3.3%), *Nocardia species* (0.4%), and mixed isolates containing staphylococci and streptococci (5.1%).

These initial studies on heifer mastitis were performed in Louisiana, USA, where a warm and humid climate with a long fly season may be conducive to IMI in these young dairy animals. Thus, a subsequent national trial was carried out using 1583 breeding age heifers from 28 herds to determine the prevalence of heifer mastitis in the states of California, Washington, and Vermont as well as in Louisiana (Fox et al., 1995). The majority of quarter infections were caused by the CNS (mean = 27.1%). The mean prevalence of *Staph. aureus* among the 4 sites was 2.9%, but was highest in Louisiana at 10.1% during the spring season. The overall IMI prevalence was greatest in Louisiana, and the highest frequency was during the 3<sup>rd</sup> trimester of gestation just prior to parturition, which was due to an increase in IMI caused by *Staph. aureus*, CNS, and environmental streptococci. Likewise, following parturition, the greatest prevalence of IMI was in Louisiana, which had the greatest percentage of heifers with IMI caused by the environmental streptococci and *Staph. aureus*, and the 2<sup>nd</sup> greatest prevalence of CNS among the 4 sites. For breeding age and bred heifers, the stage of pregnancy significantly affected IMI prevalence, which was highest for heifers in the last trimester of pregnancy, especially CNS IMI. This study by Fox et al. (1995) showed that site location had a significant effect on prevalence of mastitis, and that Louisiana had the greatest prevalence of IMI. This was postulated to be due to the warm and humid climate, as well as to a prolonged horn fly season in Louisiana that exposed udders and teats to an increased bacterial load that management practices failed to control.

### 3. Mammary leukocyte response to intramammary infection

In lactating cows, the milk somatic cell count (SCC) composed of leukocytes (macrophages, lymphocytes, neutrophils) and a small percentage of mammary epithelial cells, is considered an important parameter for assessing mammary health status (e.g. inflammation); milk yield decreases as SCC and incidence of mastitis increase. Thus, SCC in breeding age and pregnant heifer mammary gland secretions have been analyzed to measure the degree of inflammation and potential reductions in future milk yield. In a study by Boddie et al. (1987), the mean arithmetic SCC of quarters from unbred heifers infected with *Staph. chromogenes*, *Staph. hyicus*, and *Staph. aureus* were  $7.8$ ,  $8.5$ , and  $9.2 \times 10^6/\text{ml}$ , respectively, whereas the mean SCC of uninfected quarters was  $3.5 \times 10^6$ . The mean SCC of heifer secretions collected on the day of freshening were  $3.2 \times 10^6/\text{ml}$  and  $1.6 \times 10^6/\text{ml}$  for quarters infected by staphylococci and uninfected quarters, respectively. The mean SCC during the first 3 months of lactation in quarters infected with *Staph. chromogenes*, *Staph. hyicus*, and *Staph. aureus* were  $168$ ,  $193$ , and  $578 \times 10^3/\text{ml}$ , respectively, while the SCC of uninfected quarters was  $39 \times 10^3/\text{ml}$ . Thus, this study found SCC approaching  $200 \times 10^3$  for quarters infected with CNS during the first 3 months of lactation, and, based on previous studies (Jones et al., 1984; Kirk, 1984), SCC in this range are associated with milk loss. Approximately 13% of quarter secretions sampled prepartum contained *Staph. aureus*, and after freshening, the SCC of these quarters averaged  $578 \times 10^3/\text{ml}$ , a cell count that has been associated with a loss of  $>4.4$  lb (2.0 kg) of milk/day (Kirk, 1984).

Other studies have also demonstrated elevated SCC in heifer mammary glands infected with mastitis-causing bacteria. For example, in a study by Trinidad et al. (1990b), average arithmetic SCC in secretions from 325 quarter samples regardless of infection status was  $11.7 \times 10^6/\text{ml}$ . Infected ( $n = 240$ ) and uninfected ( $n = 85$ ) quarters had secretion SCC of  $13.6 \times 10^6/\text{ml}$  and  $5.7 \times 10^6/\text{ml}$ , respectively. Of the staphylococci, *Staph. aureus*-infected quarters had the highest secretion SCC/ml ( $17.3 \times 10^6$ ), followed by *Staph. chromogenes* ( $12.8 \times 10^6$ ), and *Staph. hyicus* ( $12.4 \times 10^6$ ). The mean SCC for non-agalactiae streptococci was  $15.5 \times 10^6/\text{ml}$ .

The volume of mammary secretion is very low in breeding-age animals; thus, SCC become concentrated, resulting in high SCC even in uninfected quarters. However, SCC approach  $20 \times 10^6/\text{ml}$  in quarters infected with *Staph. aureus*, and over  $13.6 \times 10^6/\text{ml}$  in those infected with the CNS and *Strep.* species. Such elevated SCC over a long period of time suggests that mammary tissue in affected quarters are in a state of chronic inflammation, which could adversely affect development of milk-producing tissues and negatively affect future milk yield. In response to infection, neutrophils become the major leukocyte type that infiltrates mammary tissue from the vascular system. The function of the neutrophil influx via chemotaxis is to phagocytose and kill mastitis-causing bacteria. But there is also evidence that this defense response can impair and disrupt mammary function (Akers & Thompson 1987; Capuco et al., 1986; Zhao & Lacasse, 2008). For example, in addition to the damage caused by bacterial toxins, the migration of leukocytes, namely neutrophils, across the mammary epithelial surfaces is thought to cause mechanical damage and/or chemical

damage (via release of reactive oxygen species) to the milk secretory cells as well as to the ductal cells of the mammary gland.

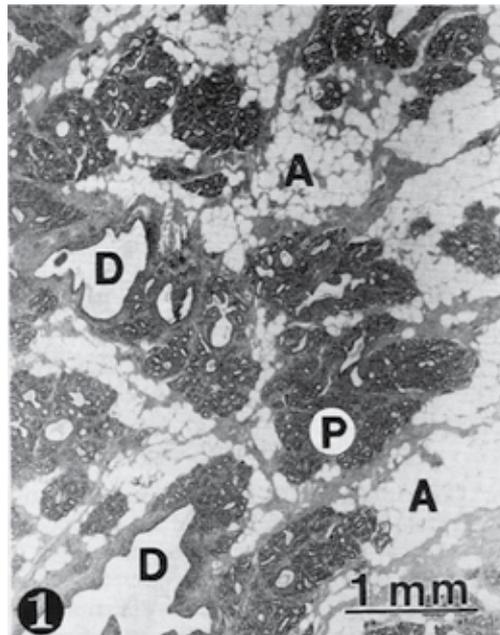
#### 4. Histological response of the mammary gland to IMI

Initially, the mammary glands of dairy heifers were studied to determine histological responses to teat canal colonization with *Staph. chromogenes* and *Staph. hyicus* (Boddie et al. (1987). Two unbred heifers were slaughtered, one at 8 months and the other at 18 months of age, and examination of mammary tissues from both heifers demonstrated a leukocyte reaction to the colonization of the teat canal. Cross-sections through the mid-teat canal demonstrated cocci colonizing keratinized cells of the canal lumen, and sections of distal teat cisternal tissues demonstrated heavy leukocyte infiltration with lymphocytes and plasma cells at Fürstenberg's rosette compared with uninfected tissues.

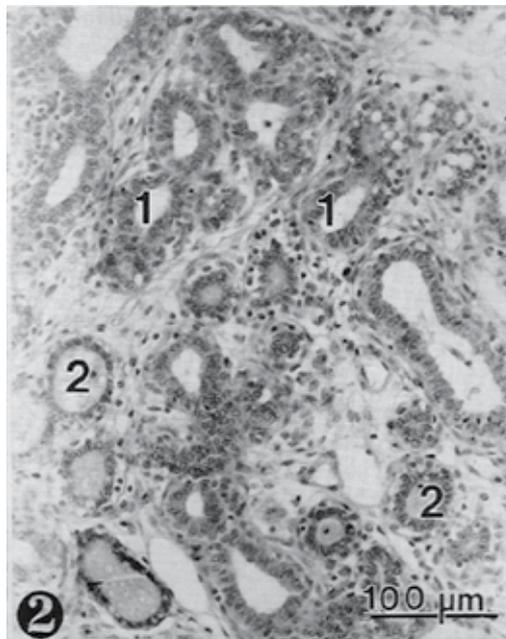
Subsequently, 7 unbred heifers, 14 - 26 months of age, were studied to evaluate the effects of IMI on leukocyte infiltration and characteristics of secretory tissue in developing mammary glands (Trinidad et al., 1990a). Histologic observations of tissue samples from lobes of mammary parenchyma of uninfected quarters showed that alveoli were small; the epithelial lining was composed of a single layer of cuboidal cells surrounding a small luminal space with little or no stained secretory product (See Figures 1 through 3). Inter-alveolar connective tissue area composed approximately half of the observed lobes, and a few infiltrating leukocytes, mainly lymphocytes, were observed.

Infected tissues, particularly those with *Staph. aureus* IMI, exhibited large amounts of inter-alveolar connective tissues and reductions in epithelial and luminal areas (See Figures 4-8). Such areas also exhibited leukocytic infiltration, particularly lymphocytes and neutrophils, into stromal and luminal areas. Hyperplasia of ducts and cisterns as a result of infection was also observed, and macro- and microscopic abscesses were found in the parenchyma of one quarter infected with *Staph. aureus*. Abscesses were tubercule-like with a circular, stratified fibrosis containing numerous lymphocytes, neutrophils, plasma cells, and multinucleated giant cells.

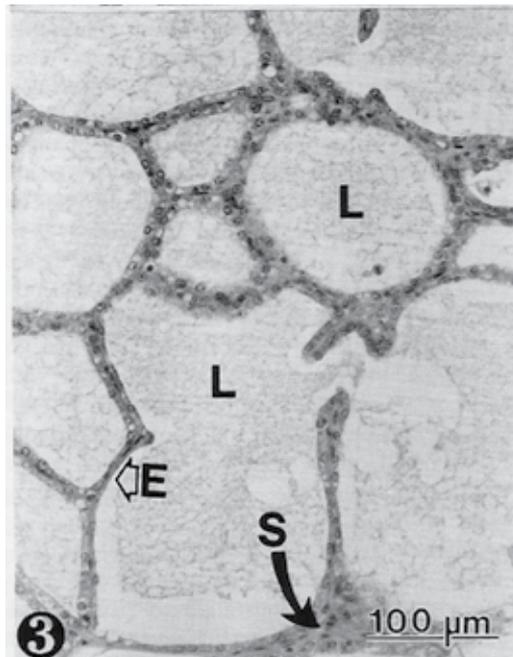
Results of morphometric analysis on parenchymal tissue components showed that percentages of each component in uninfected quarters were very similar to percentages from quarters infected with CNS, although quarters infected with CNS exhibited significantly more stromal area. Percentages of alveolar epithelium and lumen in quarters infected with *Staph. aureus* were significantly lower ( $P < .05$ ) than those in uninfected quarters and in quarters infected with CNS. Quarters infected with *Staph. aureus* also showed a greater percentage ( $P < .05$ ) of inter-alveolar stroma than did uninfected quarters and quarters infected with CNS. Thus, *Staph. aureus*-infected tissue demonstrated reduced secretory activity. The greatest development of secretory tissue in young heifers occurs during the first pregnancy, and developing secretory tissues may be affected adversely by bacterial infection and inflammation, leading to deposition of connective tissue stroma instead of milk secretory tissue and a subsequent deleterious effect on future milk yield.



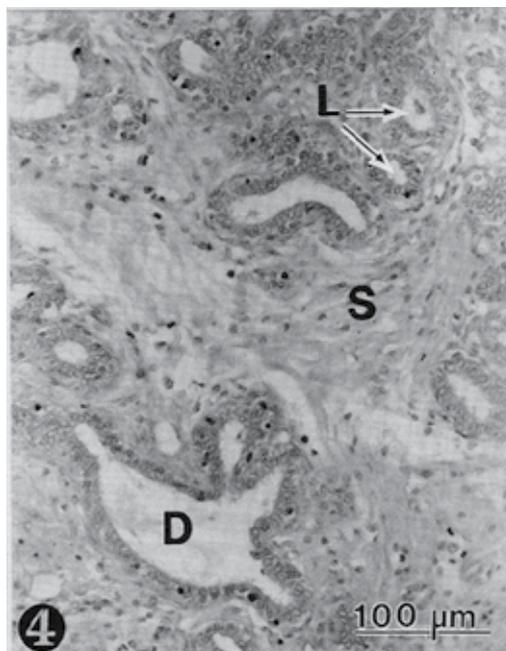
**Figure 1.** General view of a cross-section of a lobe of mammary tissue from an uninfected quarter exhibiting large ducts (D) and undeveloped lobules of parenchyma (P) among adipose tissue stroma. (A). x18.



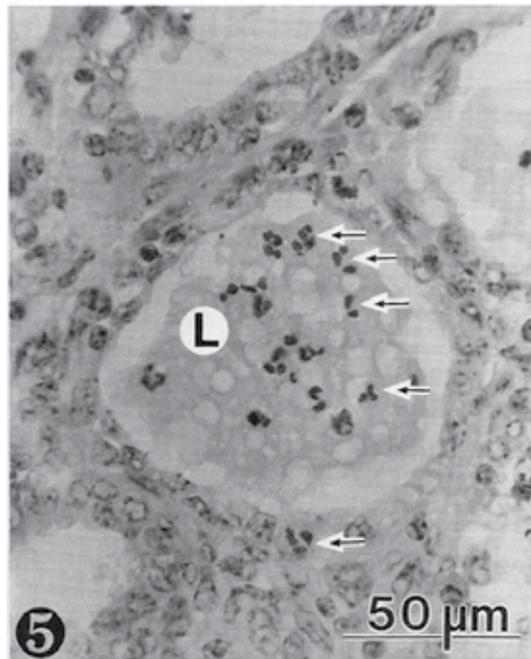
**Figure 2.** Portion of mammary parenchymal tissue typical of that obtained from uninfected quarters and those infected with coagulase-negative staphylococci exhibiting small alveoli with empty, ovoid lumens (1) and those with some secretions (2). x180.



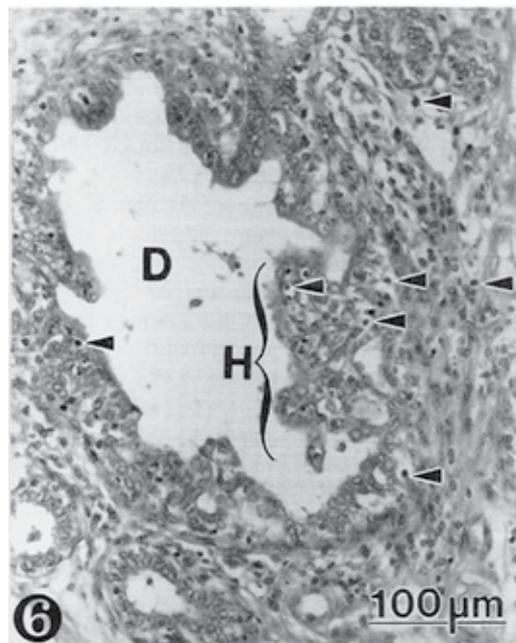
**Figure 3.** Portion of uninfected parenchymal tissue revealing limited stroma (S), flattened epithelium (E), and distended luminal areas (L) engorged with flocculent matter suggested active secretion. x180.



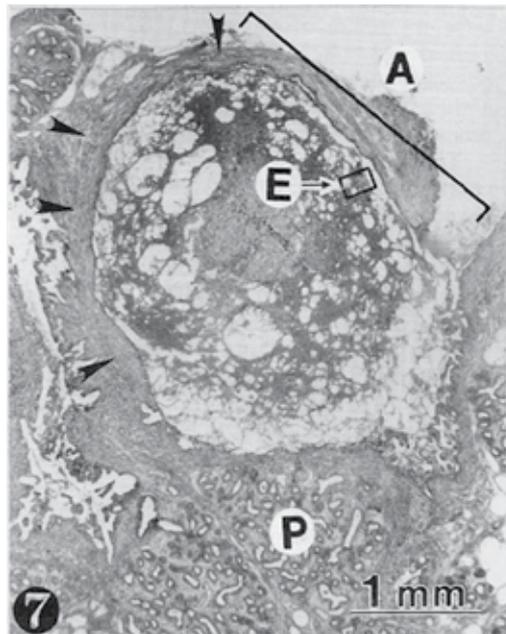
**Figure 4.** Parenchymal tissue from a quarter infected with *Staph. aureus* exhibiting a large interalveolar connective tissue stroma (S) and limited alveolar luminal areas (L). D = Duct. x180. Trinidad et al., 1990a.



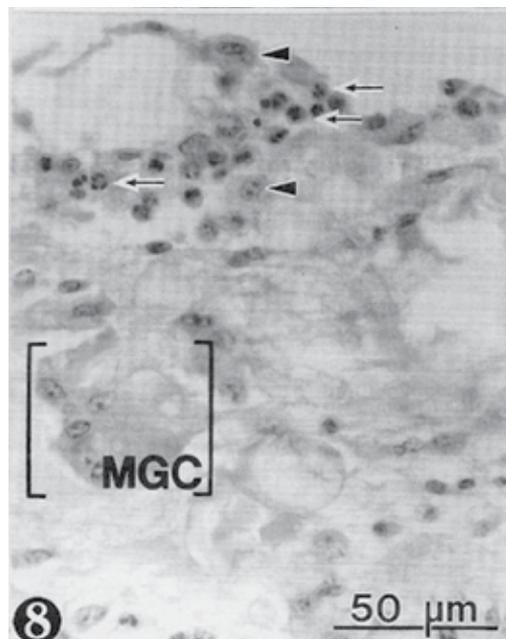
**Figure 5.** Parenchymal tissue from a quarter infected with *Staphylococcus aureus* showing numerous neutrophils (arrows) infiltrating a luminal area (L) of one alveolus. x500.



**Figure 6.** Extensive epithelial hyperplasia (H) was observed in ductal linings in the parenchyma from one quarter infected with *Staphylococcus aureus*. Lymphoid cells (arrowheads) were numerous in the epithelium as well as in the underlining connective tissue. D = duct. x180.



**Figure 7.** Abscess (A) from one quarter infected with *Staphylococcus aureus* exhibiting tubercle-like morphology with circular stratified fibrosis (arrows) and marked cellular infiltration. E = Portion of enlarged in Figure 8. P = Parenchyma. x18.



**Figure 8.** Magnification of an edge of the abscess shown in Figure 7. Neutrophils (arrows), macrophages (arrowheads), and multinucleated giant cells (MGC) were present in this area of the abscess. x500. Trinidad et al., 1990a.

Leukocyte infiltration into cisternal and parenchymal mammary tissues was also evaluated. Quarters infected with *Staph. aureus* exhibited the greatest tissue leukocytosis, followed by quarters infected with CNS and uninfected quarters. Leukocyte infiltration in gland cistern and secretory tissue for infected quarters was significantly higher ( $P < .05$ ) than that for uninfected quarters. Leukocytosis into teat cistern tissue was similar for uninfected quarters and those infected with CNS, but significantly lower ( $P < .05$ ) than quarters with *Staph. aureus* IMI. None of the uninfected quarters or quarters infected with CNS demonstrated marked leukocyte infiltration. However, marked leukocyte infiltration, particularly lymphocytes, into cisternal and parenchymal areas was commonly observed in quarters that were infected with *Staph. aureus*. The majority of leukocytes observed within alveolar lumina were neutrophils.

## 5. Efficacy of nonlactating cow antibiotic therapy

Because of the high level of infection commonly found in breeding age and pregnant heifers, especially mastitis caused by *Staph. aureus*, as well as the associated elevated SCC, antimicrobial therapy should be considered. The testing of various staphylococcal isolates obtained from heifers for susceptibility to antibiotics commonly incorporated into mastitis infusion tubes has shown that antibiotic resistance is usually low (Watts et al., 1995). Greater than 90% of mastitis-causing staphylococci are generally killed by the drug preparations used, based on in vitro sensitivity testing using zone diffusion analysis (Watts et al., 1995). From a practical standpoint, the administration of antibiotics by a parenteral route would be preferred; however, in the author's experience, neither subcutaneous nor intramuscular injections of drugs have been found to cure IMI in heifers because sufficient antibiotic does not pass into the mammary gland to be bactericidal. Thus, intramammary infusion is the route of choice.

In an initial study to evaluate the effectiveness of treatment, several heifers from each of the 4 commercial herds studied by Trinidad et al. (1990c) were randomly selected to receive a single intramammary treatment of a penicillin and dihydrostreptomycin product. Antimicrobial susceptibility testing demonstrated that 97% of the *Staph. aureus* isolates were sensitive to 12 antibiotics, including the product selected for treatment (Trinidad et al., 1990d). Teat ends were sanitized, and a dry cow antibiotic containing 1,000,000 units of penicillin and 1 gram of dihydrostreptomycin was infused into all quarters of 35 heifers using the partial insertion technique; 38 heifers served as untreated controls. Treatments were made at approximately 60 days prior to the calculated calving date.

Results showed that 97.1% of treated heifers (73.2% of quarters) were infected at the time of treatment, but, at calving, infected heifers and quarters in the treatment group were reduced to 40 and 34%, respectively. Antibiotic residues were limited to two heifers that were treated within 3 weeks of calving because estimated dates of parturition were miscalculated, but all quarters were free of antibiotic residue after 5 days; 2.9% of treated quarters had antibiotic residues at time of calving. Of the 38 untreated control heifers, 100% (71.2% of quarters) were infected at initial sampling, and at calving, mastitis in control heifers was reduced only

slightly to 97.4%; percentage of infected quarters increased slightly to 77.8%. The mean SCC at calving was also reduced as a result of therapy. For treated heifers, SCC decreased significantly ( $P < .001$ ) from  $11,825 \times 10^3/\text{ml}$  at time of treatment to  $3,439 \times 10^3/\text{ml}$  at calving. In the control group, SCC decreased from  $11,047 \times 10^3/\text{ml}$  to  $5,594 \times 10^3/\text{ml}$  ( $P > .05$ , not significant).

In the same study, *Staph. aureus* was isolated from 11 quarters of 6 treated heifers before antibiotic infusion (45.8%), but, at calving, this organism was isolated from only 1 quarter of 1 heifer (4.2%). In the control group, 18 quarters of 10 heifers were infected with *Staph. aureus* at time of treatment (45%). At calving, 6 of the control heifers still had *Staph. aureus* mastitis in 11 quarters (55%). Thus, the overall incidence of IMI was reduced 60% and that caused by *Staph. aureus* was reduced over 90%. From 20 to 26% of quarters with subclinical and clinical *Staph. aureus* mastitis are typically cured after antibiotic therapy (Dodd, 1992). In this study, 90.9% of quarters were cured; thus, therapy in heifers was highly effective in eliminating *S. aureus* mastitis compared with that for lactating cows.

In this study, an economic analysis was performed to justify use of the heifer treatment program (Trinidad et al., 1990c). Production data collected over the first 2 months of lactation demonstrated that *Staph. aureus*-infected heifers receiving nonlactating cow therapy during pregnancy produced an average of 5.5 pounds (2.5 kg) more milk per day than *Staph. aureus*-infected herd mates that did not receive treatment. At the milk price received at that time, the greater milk yield translated to a \$42.00 increase for treated heifers, which was well worth the \$5.00 cost of treatment. Other advantages include a longer productive life and higher income due to quality milk premiums.

Subsequent studies (Owens et al., 1991, 1994) with pregnant and nonpregnant heifers using a cephalosporin-based product formulated for nonlactating cows was also successful. Heifers that were either experimentally or naturally infected with *Staph. aureus* were infused 8 -12 weeks prepartum with one dose of 300 mg of a cephalosporin benzathine product and were compared with untreated controls infected with *Staph. aureus*. Results demonstrated that 100% of experimentally induced IMI and 87% of naturally occurring *Staph. aureus* IMI were eliminated in treated heifers at calving, and cured quarters remained negative at biweekly samples collected 2 months into lactation. Quarters remaining infected at calving with *Staph. aureus* were treated with a lactating cow product containing 200 mg of cephalosporin benzathine, but the cure rate was lower (50 - 56%). Thus, cure rates were greater when a nonlactating product was administered 8 -12 weeks prepartum than when a lactating cow product was given at time of calving.

After antibiotic infusion, SCC in infected quarters that cured decreased from  $15 \times 10^6/\text{ml}$  to  $4 \times 10^6/\text{ml}$  1 week later, and to  $700 \times 10^3/\text{ml}$  at calving. In contrast, none of the untreated control quarters infected with *Staph. aureus* cured spontaneously by the time heifers calved, and SCC at calving were  $5 \times 10^6/\text{ml}$ . Treated heifers in which *Staph. aureus* IMI were cured yielded a mean of 36.1 lb (16.4 kg) milk/day, and untreated controls that retained *Staph. aureus* IMI yielded 31.9 lb (14.5 kg)/day or 11% less during the first 2 months of lactation.

Generally, spontaneous cure rates for major mastitis pathogens are low. For example, in a subsequent study on heifer mastitis (Owens et al., 1994), spontaneous cure rates for *Staph. aureus* and the environmental streptococci were 9 and 6%, respectively. Thus, treatment is required to cure such infected quarters in these young dairy heifers. New IMI rates in uninfected quarters receiving no therapy over the period from 8 - 10 weeks prepartum were very low for most species of staphylococci. However, new environmental streptococcal IMI were common in previously uninfected, untreated heifers. So, as part of this study (Owens et al., 1994), a trial was initiated to treat quarters found to be negative at 8 – 10 weeks prepartum with cephapirin benzathine to determine if establishment of new environmental streptococcal IMI could be prevented during this 8 – 12 week prepartum period. Results showed that prophylactic treatment of such quarters prepartum reduced new environmental streptococcal IMI at calving by 93%. Thus, in this trial, use of nonlactating cow therapy was effective in preventing new IMI as well as curing existing infections. Reasons for this high cure rate are unclear, but the relatively small secretory tissue area of heifer mammary glands compared with mature cows might allow for greater drug concentrations in the udder of the heifer. Similarly, histological studies have demonstrated less scar tissue and abscess formation in the mammary glands of heifers compared with older cows (Trinidad et al., 1990a), a condition which would allow for better drug distribution and greater contact with colonized bacteria.

## **6. The optimum treatment schedule for maximizing efficacy of nonlactating cow therapy**

The question arises as to when is the best time to treat bred heifers for optimizing cures against *Staph. aureus* mastitis. A 2-year study involving 233 Jersey heifers was designed to answer this question (Owens et al., 2001). In this trial, heifers were quarter sampled shortly after they were confirmed pregnant and at 4-week intervals thereafter. At the initial sampling, 56.5% of quarters were infected with some type of organism, and 15.4% of quarters were infected with *Staph. aureus*. After the initial sampling, animals were treated with a one-time infusion of 1 of 5 nonlactating cow infusion products during the first (0 - 90 days), second (91 - 180 days), or third (181 - 270 days) trimester of pregnancy. Products evaluated were: 1) a combination of 1 million units of penicillin and 1 gm dihydrostreptomycin; 2) 300 mg cephapirin benzathine; 3) a combination of 400 mg novobiocin and 200,000 units of penicillin G; 4) 300 mg tilmicosin; and 5) 250 mg cephalonium (this last product was only infused during the first trimester of gestation). Results showed that cure rates for the 5 products were high, ranging from 67 to 100%, and significantly higher than the spontaneous cure rate (25%) observed in untreated control quarters. No differences were observed among the three treatment time periods during gestation. However, fewer new *Staph. aureus* infections occurred after treatment in the group infused during the 3<sup>rd</sup> trimester, indicating that treatment during this time will reduce incidence of new IMI after infusion and continuing to calving. Because therapy during the first, second, or third trimester of gestation had no effect on treatment efficacy, the timing of treatment is best determined by what is most convenient for the management practices of a

particular dairy. For example, heifers could be treated: 1) at time of artificial insemination; 2) during routine rectal palpation to determine pregnancy status; or 3) when moved to a new location in preparation for calving. Treatment should be administered no less than 45 days prior to expected calving date to prevent antibiotic residues at calving.

Sampimon & Sol (2005) studied the efficacy of nonlactating cow therapy (600 mg cloxacillin) administered intramammarily 8-10 weeks prepartum in 1) 5 low prevalence farms in which less than 15% of heifers had SCC of >150,000/ml at the beginning of the trial, and 2) 8 high prevalence farms in which greater than 15% of heifers had SCC of >150,000/ml at the beginning of the trial. Results showed that in high prevalence farms, treated heifers produced significantly more milk during the first 100 days of lactation compared with untreated controls; however, in low prevalence farms, treatment had no effect on production. In both groups of farms, SCC and incidence of clinical mastitis were significantly lower in heifers receiving antibiotic therapy. Authors concluded that nonlactating cow therapy was beneficial in high prevalence farms, but not in low prevalence farms.

The treatment of heifers during pregnancy with a nonlactating cow product is advantageous because: 1) the cure rate is higher than during lactation, especially against *Staph. aureus*; 2) there are no milk losses during therapy; 3) the risk of antibiotic residues is minimal; 4) SCC at calving is reduced; 5) new IMI with environmental streptococci is prevented; and 6) milk production is increased by approximately 10% in successfully treated animals in some herds.

## **7. Efficacy of parenteral antibiotic treatment and an infusible teat seal in curing and preventing IMI**

In one study involving 1,067 pregnant heifers in 30 New Zealand herds (McDougal et al., 2005), mammary quarters were treated 1 month prepartum with 1) an infusible teat seal composed of bismuth subnitrate; 2) parenteral administration of antibiotic via injection of 5 gm of tylosin i.m. daily for 3 days; 3) teat seal plus tylosin; or 4) no treatment in order to determine if treatment reduced the prevalence of IMI and incidence of clinical mastitis postpartum. Results demonstrated that heifers treated prepartum with teat seal exhibited a reduced prevalence of IMI as well as reduced incidence of clinical mastitis. However, treatment with tylosin prepartum did not reduce prevalence of mastitis or incidence of clinical mastitis postpartum.

## **8. Efficacy of lactating cow products in curing IMI**

Lactating cow products have been used successfully in heifers when treating infections caused by the environmental streptococci and CNS immediately prior to calving. Studies on this subject are typically performed on heifers in late gestation 2 - 3 weeks before calving. In an initial study conducted at the University of Tennessee, quarters of 115 pregnant Jersey heifers were infused one time at approximately 1 week prepartum with either 200 mg

sodium cloxacillin, 200 mg cephalirin sodium, or left untreated (Oliver et al., 1992). At the time of infusion, approximately 90% of heifers were infected in one or more quarters. For heifers left untreated, 78% of animals (44.5% of quarters) remained infected at time of calving. However, only 17.6% of the heifers (4.5% of quarters) remained infected at calving if they were treated prepartum, regardless of the product used. Results demonstrated that significantly fewer antibiotic treated heifers and quarters were infected at calving compared with untreated controls. This study also examined the influence of prepartum antibiotic treatment on subsequent lactational performance, and demonstrated that heifers receiving treatment produced approximately 1,000 lb (455 kg) more milk per lactation than untreated controls.

Mastitis pathogens were isolated from 76% of untreated control quarters at 7 days before calving, from 47% of samples at 3 days after calving, and from 29% of samples at 10 days postpartum. Throughout the remainder of lactation, pathogens were isolated from 30% of control quarters. A similar percentage of samples (70%) was positive for mastitis pathogens at 7 days before calving in antibiotic-treated quarters; however, only 8% of samples obtained at 3 days after calving and 4% of samples obtained 10 days postpartum contained pathogens; throughout the remainder of lactation, mastitis pathogens were isolated from only 11% of quarters. *Strep. uberis*, *Strep. dysgalactiae*, and CNS species were isolated most frequently in both untreated controls and antibiotic-treated heifer mammary glands.

In a subsequent study reported by Oliver et al. (1997b), mastitis pathogens were isolated from 67% of samples obtained from control mammary glands (quarters to be left untreated) 14 days prior to expected calving, from 56% of samples obtained 3 days after calving, and from 36% of samples obtained 30 days postpartum; throughout the remainder of lactation, mastitis pathogens were isolated from 45% of quarters. In quarters to be treated with 200 mg cephalirin sodium, 64% were positive for mastitis pathogens prior to antibiotic treatment; however, only 16% of samples obtained at 3 days after calving and 8% of samples obtained 30 days postpartum contained pathogens. Throughout the remainder of lactation, mastitis pathogens were isolated from an average of 12% of quarters. Coagulase-negative staphylococci were isolated most frequently followed by environmental mastitis pathogens.

A follow-up study was conducted to determine if prepartum therapy with penicillin-novobiocin or pirlimycin hydrochloride was effective in reducing the percentage of infection with mastitis pathogens during early lactation (Oliver et al., 2004). Almost 73% of Holstein heifers (34.3% of quarters) were infected 14 days before expected calving. Of the quarters infected 14 days before parturition, 76% were uninfected following treatment with penicillin-novobiocin, 59% were uninfected following treatment with pirlimycin, and 26% were uninfected in the untreated control group. The majority of IMI were due to CNS (44%) and *Staph. aureus* (30%).

Among the Jersey heifers, 96% of animals and 71.3% of quarters were infected 14 days before calving. Of the quarters infected at 14 days before parturition, 75% were uninfected following treatment with penicillin-novobiocin, 87% were uninfected following treatment with pirlimycin, and 56% were uninfected in the untreated control group. The majority of

IMI were due to CNS (61%), *Strep. spp.* (19%), and *Staph. aureus* (8%). Thus, prepartum therapy of heifer mammary glands with penicillin-novobiocin or pirlimycin hydrochloride was effective in reducing the percentage of heifers and quarters infected with mastitis pathogens during early lactation.

As a part of the University of Tennessee studies, milk production and somatic cell score data from 82 control heifers and 111 heifers treated with antibiotics before calving were evaluated (Oliver et al., 2003). Milk production (actual and 305-day) was significantly higher in heifers treated prepartum with antibiotics. Additionally, treated heifers had a significantly lower somatic cell scores than control heifers (2.04 vs. 2.63). Thus, prepartum antibiotic treatment to reduce the rate of mastitis in heifers during early lactation was economically beneficial. Treated heifers produced 1,168 lb (531kg) more actual milk than the untreated controls. Based on a milk price of \$18.50/cwt this resulted in \$216.24 per heifer increase in gross revenue. Considering treatment costs of \$15.60 per heifer including teat hygiene (\$0.10), antibiotic (\$10.00), labor (\$2.50), and residue testing (\$3.00), the net revenue amounted to \$200.64 per heifer. The researchers concluded that it would be profitable to treat heifers before calving as long as the milk price was above \$0.013 per pound and as long as the increase in milk production was greater than 84 pounds (Oliver et al., 2003).

Middleton et al. (2005) also evaluated the prepartum treatment of heifers with pirlimycin hydrochloride at 10-14 days before parturition on the prevalence of IMI at calving in 2 dairy farms. Postpartum sampling revealed that heifers receiving treatment in herd A had a higher overall cure rate and higher cure rates for IMI caused by CNS and *Staph. aureus* as well as lower SCC and lower prevalence of chronic IMI compared with untreated controls. The cure rate for *Staph. aureus* of 78% was significantly greater than that for untreated controls (8%). Treated heifers in herd B had a higher overall cure rate and higher cure rate for IMI caused by CNS compared with untreated controls, but CMT (SCC) scores and prevalence of chronic IMI were not different. In contrast to previous results of using lactating cow therapy, (Oliver et al., 2003), milk production did not differ between treatment groups. Authors concluded that routine prepartum therapy of heifers may not benefit all herds.

Subsequently, Borm et al. (2006) evaluated the efficacy of lactating cow intramammary therapy (cephapirin) administered to heifers 10-21 days prior to expected calving date in a 9-herd study involving 561 animals. Results demonstrated a 59.5% cure rate among mammary quarters that were infected prepartum and treated with antibiotic vs. untreated controls (31.7%). However, treatment did not significantly affect SCC or milk yield during the first 200 days of lactation. This observation is similar to that found by Middleton et al. (2005) who observed that although successful, treatment did not necessary reduce SCC or result in greater milk production. Authors concluded that prepartum treatment of heifers with lactating cow antibiotics may not be warranted as a universal strategy for mastitis management.

The studies on prepartum treatment with lactating cow therapy administered 7 - 21 days before calving (Borm et al., 2006; Oliver et al., 1992, 1997b, 2003, 2004) have shown treatment

to be effective for quarters infected with CNS but waiting until this time to treat chronic *Staph. aureus* mastitis might be too late. A mammary gland that has been infected with *Staph. aureus* for several months to a year will not develop normally, and treatment during the immediate prepartum period would most likely be of little benefit in curing infections or salvaging mammary tissue. At this point, the tissue damage would have already been done, and affected quarters should have been treated earlier in gestation to: 1) cure existing infections; 2) reduce chronic inflammation; and 3) allow mammary tissue to develop normally during the later stages of pregnancy.

Results of these trials demonstrated that nonlactating and lactating cow antimicrobial treatment of heifers known to be at risk for developing IMI is advantageous because the cure rate is much higher than that obtained when treating infections during lactation. In addition, most studies showed that SCC are lower, there is no milk loss due to therapy, risk of antibiotic residue at calving is minimal, and future milk production is increased in heifers cured of IMI.

## **9. Antibiotic residues in milk following prepartum lactating cow treatment**

A disadvantage of prepartum lactating cow antibiotic therapy for controlling mastitis in heifers is the potential for antibiotic residues, especially if heifers calve sooner than expected. In one study (Oliver et al., 1992), it was shown that 17% of colostrum samples from heifer mammary glands infused with cloxacillin were positive for antibiotic residues by the *Bacillus stearothermophilus* disc assay, the majority of which were from heifers that calved within 5 days of treatment. Only 4.5% of samples obtained at the first milking after parturition were positive for antibiotic residues if intramammary infusion of cloxacillin occurred  $\geq 7$  days before parturition. All samples obtained 3 days after parturition, the time when milk would likely be marketed for human consumption, were negative for antibiotic residues. In contrast, 85% of colostrum samples and 28.2% of samples obtained 3 days after parturition were positive for cephalosporin residues, and marked variability between time of antibiotic treatment and parturition with persistence of antibiotic residues was observed. Thus, antibiotic treatment of heifer mammary glands earlier in gestation may be advantageous from a residue standpoint, but the timing of antibiotic treatment and subsequent persistence in mammary secretions could impact efficacy.

Another study was conducted to determine if antibiotic treatment of heifer mammary glands earlier in the prepartum period reduced occurrence of residues in milk (Oliver et al., 1997a). A total of 82 Jersey heifers was used. Approximately half served as negative controls ( $n = 42$ ) and half received an intramammary infusion of 200 mg cephalosporin sodium ( $n = 40$ ) 14 days before calving. Forty percent of samples from cephalosporin-treated quarters were positive at the first milking after calving, but only 3.1% of samples obtained from antibiotic-treated quarters at the sixth milking (3 days) after calving were positive; 3 of the 4 positive samples were from a heifer that calved early and within 3 days of treatment.

Thus, the interval between prepartum antibiotic treatment and calving was related to persistence of residues during early lactation, and infusion of antibiotics 14 days prepartum (Oliver et al., 1992) compared with 7 days prepartum (Oliver et al., 1997a) reduced occurrence of residues in milk during early lactation. Similarly, Middleton et al. (2005) found that after heifers were treated 10-14 days prior to expected calving date with a pirlimycin lactating cow product, prevalence of IMI in early lactation was decreased without causing pirlimycin residues in milk at 3 days postpartum, even when a heifer was treated 1 day before calving (heifer calved early).

## 10. Role of vaccination in mastitis control

Although antimicrobial therapy is successful, the goal from a herd management perspective is to prevent new infections from occurring, and vaccination has been attempted as a prophylactic measure. Recent research has demonstrated that several experimental *Staph. aureus* vaccines, as well as one commercial vaccine, can increase antistaphylococcal antibody titers and reduce the new infection rate in heifers. A *Staph. aureus* vaccine formulated to stimulate pseudocapsule and alpha toxin antibodies was evaluated in heifers in New York (Sears et al., 1990). At 4 and 2 weeks prior to calving, heifers were given subcutaneous injections into the supramammary lymph node, and after calving, heifers were challenged with *Staph. aureus*. Vaccinates demonstrated a 52% reduction in new IMI. In addition, 64% of intramammary infections in control cows became chronic compared with only 12% in vaccinates.

A field study in Norway evaluated a *Staph. aureus* vaccine that contained two strains of whole, formalin-inactivated bacteria with pseudocapsule, alpha and beta toxoids, and mineral oil as an adjuvant (Nordhaug et al., 1994). A total of 108 pregnant heifers on 16 farms with an average *Staph. aureus* prevalence of 19.2% was used. Vaccinates were injected subcutaneously in the area of the supramammary lymph node with a dose of 2.5 ml at 8 and 2 weeks before calving. Results showed a 46% reduction in new IMI during the subsequent lactation. Antibody titers to *Staph. aureus* pseudocapsule and alpha toxin were markedly elevated in the serum of vaccinates, and these titers remained significantly higher in serum and milk during the entire lactation compared with those of unvaccinated controls.

In Argentina, a vaccine was developed based on an inactivated, encapsulated *Staph. aureus* strain, a crude extract of *Staph. aureus* exopolysaccharides, and inactivated, unencapsulated *Staph. aureus* and *Streptococcus* species in an aluminum hydroxide adjuvant (Giraudo et al., 1997). This formulation was evaluated in three groups of ten 24- to 26-month-old heifers each in a 7-month trial. The first group received an intramuscular injection of the vaccine in the neck at 8 and 4 weeks prepartum, the second group was vaccinated similarly at 1 and 5 weeks postpartum, and a third group (control) received placebo injections at 8 and 4 weeks prepartum. The research herd from which the heifers were selected had a bulk tank SCC ranging from 480,000 to 730,000, and 19% of quarters were infected with *Staphylococcus aureus*. This immunization program showed that the frequency of new *Staph. aureus* infection was reduced from 18.8% in controls to 6.7 and 6.0% for heifers vaccinated

prepartum and postpartum, respectively; the protective effect was maintained for at least 6 months.

In view of more recent studies showing success of vaccines in heifers, researchers in Louisiana evaluated a commercially available *Staph. aureus* vaccine in young dairy animals (Nickerson et al., 1999). The vaccine was a lysed culture of polyvalent *Staph. aureus* somatic antigens representing 5 phage types in an aluminum hydroxide adjuvant base, including serotypes 5, 8, and 336, the most common *Staph. aureus* serotypes associated with clinical mastitis (Lysigin<sup>®</sup>, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA). At 6 months of age, 35 Jersey heifers were vaccinated using a 5-ml dose intramuscularly in the semimembranosus muscle of the rear leg, and 14 days later, vaccinates received a booster dose, which was repeated at 6-month intervals. Another 35 heifers served as unvaccinated controls. Results demonstrated that: (1) the number of quarters exhibiting chronic intramammary infection during pregnancy was reduced 43.1% in vaccinates compared with controls; (2) rate of new intramammary infection during pregnancy was reduced 44.8%; (3) rate of new intramammary infection at freshening was reduced 44.7%; and (4) the SCC was reduced by 50% in vaccinates compared with controls.

In a subsequent, more in depth study using the same vaccine (Lysigin<sup>®</sup>), 106 Holstein heifers from the James River Correctional Center dairy herd Goochland, VA, USA were evaluated (Nickerson et al., 2009). This herd had a 9,979-kg rolling herd average milk production with an average SCC of ~200,000/ml. Previous microbiological culture of heifer mammary secretions indicated that approximately 35% of animals were infected with *Staph. aureus*.

At approximately 6 to 18 months of age, heifers were processed through a restraining chute to collect aseptic quarter mammary secretion samples for microbiological. Fifty-three heifers were vaccinated using a dose of 5 ml intramuscularly that was administered as above, and the other 53 heifers served as unvaccinated controls. Fourteen days after the initial processing, the vaccinated group was again processed through the chute and boosted with Lysigin<sup>®</sup>. All animals were maintained on pasture and rotated by age group through calving. At 6-month intervals after the initiation of the trial and through time of calving, the vaccinated group was again processed through the chute for boosting.

At 2-month intervals after the trial initiation and through calving, mammary secretion samples were collected for bacteriological culture and for the determination of electronic SCC (A/SN Foss, Hillerod, Denmark). Microbiological examination of quarter samples collected from bred heifers over gestation demonstrated that 19.8% of heifers (9.4% of quarters) were infected with *Staph. aureus*, 68.9% of heifers (34.3% of quarters) were infected with CNS, 6.6% of heifers (2.3% of quarters) were infected with environmental streptococci, and 1% of heifers (0.3% of quarters) were infected with coliforms.

At time of calving, heifers were enrolled in the Dairy Herd Improvement Program (DHIA) and data were recorded for milk yield, percentages and actual pounds fat and protein, days in milk, and SCC. Data on vaccine efficacy were examined in terms of mean percentage reduction in rate of new *Staph. aureus* or CNS intramammary infections achieved among immunized heifers compared with the rate among unimmunized controls at the time of

calving; differences between the percentage of heifers becoming infected among treatments was tested with the standard normal approximation.

Immunization with Lysigin® did not cause any adverse reactions at the injection site or systemically. Minimal swelling (<2.5 cm) was occasionally observed, which disappeared within 48 hours of administration. Vaccine efficacy data showed that the percentage of heifers with *Staph. aureus* intramammary infections at freshening was lower in vaccinates (13.3%) compared with controls (34.0%); a reduction of 60.9% ( $P < 0.01$ ). Likewise, an examination of health records showed that the percentage of heifers that were culled or died during the trial was reduced by approximately one-third by vaccination: 16.9% in vaccinates and 24.5% in controls ( $P > 0.05$ ). The vaccinated group also experienced a slight, insignificant reduction in mastitis caused by CNS. At freshening, intramammary infections with CNS were lower in vaccinates (64.2%) compared with controls (69.8%); a reduction of 8.1%.

Somatic cell counts in samples collected during first week of lactation irrespective of infection status were 45% lower in vaccinates compared with controls (287,317 vs. 522,345/ml). Somatic cell counts in samples collected during first week of lactation from uninfected heifers for vaccinates and controls were 66,095 and 132,754/ml, respectively; a 50.2% reduction; and for infected heifers, SCC were 441,764 and 892,176/ml, respectively; a 50.5% reduction. Somatic cell counts in samples collected during the prepartum period were highest for *Staph. aureus* ( $6,730 \times 10^3$ ), followed by the environmental streptococci ( $3,850 \times 10^3$ ), and CNS ( $3,510 \times 10^3$ ).

An examination of the 305-day lactation milk yield for the 1st lactation of both vaccinated and unvaccinated control heifers demonstrated an approximate 8.6% increase in vaccinates vs. controls (11,217 vs. 10,332 kg, respectively) or a difference of 886 kg. On a complete lactation basis, vaccinated animals produced 839 kg more milk than controls (12,537 vs. 11,698 kg, respectively); an increase of 7.3%.

The percentage of 305-day lactation fat was higher in vaccinates than controls (3.64 vs. 3.27%, respectively); however, the percentage of 305-day lactation protein was slightly higher in controls than vaccinates (3.06 vs. 2.95, respectively). Actual 305-day kilograms of both fat and protein were higher in vaccinates than controls (fat: 408 vs. 339 kg, respectively; protein: 330 vs. 315 kg, respectively). Likewise, on a complete lactation basis, actual kilograms of both fat and protein were higher in vaccinates than controls (fat: 460 vs. 393, respectively; protein: 370 vs. 353, respectively).

An examination of the number of days in milk for the first lactation demonstrated that vaccinates persisted 13 days longer than the unvaccinated controls (349 vs. 336 days). In addition, average first lactation SCC were 11,000 cells/ml lower in vaccinates compared with controls (49,000 vs. 60,000/ml).

Results of this Virginia investigation demonstrated that vaccinating dairy heifers according to the prescribed protocol with a commercial USDA licensed *Staph. aureus* bacterin, Lysigin®, reduced the number of new *Staph. aureus* intramammary infections at time of calving by 60.9%, lowered the SCC by 50%, and decreased the culling rate by approximately one-third.

In addition, overall milk yield, production of fat and protein, and number of days in milk were greater in vaccinated heifers compared with controls.

The decrease in frequency of new *Staph. aureus* intramammary infections at calving (60.9%) in vaccinates using Holstein heifers is higher than the 44.7% reduction observed in a Louisiana trial using the same vaccine in Jersey heifers (Nickerson et al., 1999). In both trials, SCC at calving were reduced by approximately 50%. The 60.9% efficacy found in the present trial is also higher than the 40.2% efficacy observed by Giraud et al. (1989), the 46% efficacy observed by Nordhaug et al. (1994), and the 52% efficacy observed by Sears et al. (1990). However, it is difficult to compare among the latter three trials as the vaccine formulations were all quite different.

The question becomes: Is it economically feasible to use this commercial vaccination protocol on young dairy heifers? Based on an average of 1,864 more lb milk per vaccinated heifer, which translates to 18.64 hundredweights (cwt) of milk (1,864/100), at the current (2008) price of \$25.00/cwt, an increased income of \$466.00/heifer would be realized (18.64 cwt x \$25.00/cwt = \$466.00). If each heifer was vaccinated beginning at 6 months of age until calving, this would entail vaccinations at 1) 6 months, 2) a booster 2-weeks later, and subsequently at 3) 12 months, 4) 18 months, and 5) 24 months, or a total of 5 immunizations through calving. At \$1.50/dose, this cost would total \$7.50, which when subtracted from the increased income from milk production, would yield a net income of \$458.50 per heifer (\$466.00 - \$7.50). This figure does not include the costs of labor involved in the immunization process; however, it is evident that vaccination is well worth the cost of the vaccine. Not only does it reduce new infections in first calf heifers at parturition, it may also reduce the introduction of *Staph. aureus* to the milking herd.

It is obvious that use of experimental and commercially available *Staph. aureus* vaccines can be used to prevent new infections, especially when used in heifers. Efficacy has been shown to range between 44 to 61% with reductions in SCC of 50%. This prevention strategy may represent a major control mechanism for managing *Staph. aureus* in the future, especially as new antigens and adjuvants are added to vaccine preparations.

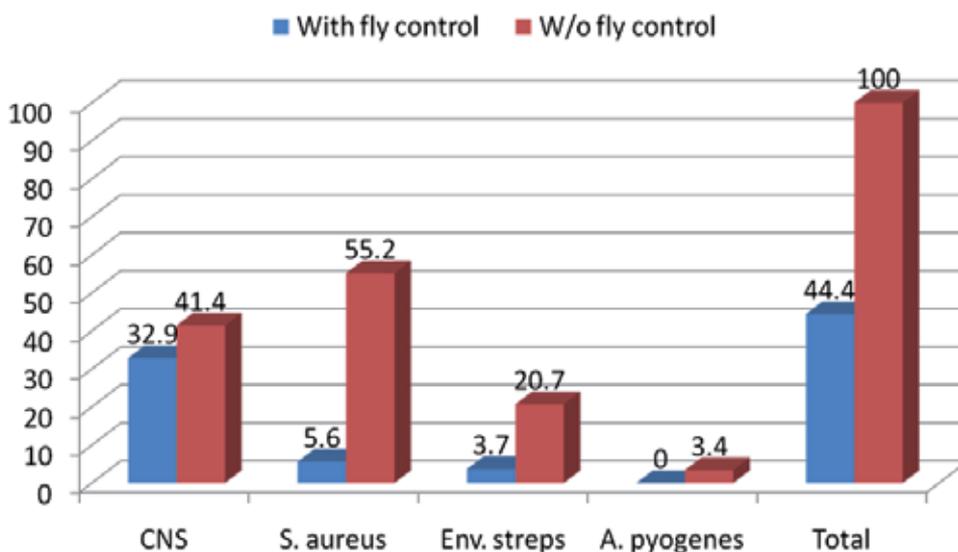
## 11. Use of fly control to manage heifer mastitis

Historically, the major association between flies and intramammary infections has been with the development of summer mastitis, in which the biting fly, *Hydrotoea irritans*, is the proven vector (Chirico et al., 1997). Summer mastitis is an isolated seasonal problem primarily in July, August, and September in heifers and dry cows of northern Europe, and may be controlled by insecticidal sprays. In the US, fly control is used to reduce these insect pests on farm premises, and subsequently reduce animal stress, but its application as an adjunct management practice for preventing new cases of mastitis and reducing SCC has not really been considered or embraced by producers.

Surprisingly, very little research has been conducted on the relationship between mastitis in adult cows and fly control; most studies in this regard have been carried out in dairy heifers.

An initial survey performed at Louisiana State University showed that prevalence of mastitis in bred heifers was significantly lower in dairy herds that used some form of fly control for their lactating cows, dry cows, and heifers compared with herds applying no fly control (Figure 9) (Nickerson et al., 1995). The greatest reductions were in numbers of *Staph. aureus* and the environmental streptococci, both major mastitis pathogens in adult cows associated with elevations in SCC.

Results of this survey also demonstrated that bred heifers having teats with bite lesions and scabs caused by the blood-sucking horn fly (*Haematobia irritans*), exhibited a 70% frequency of intramammary infection compared with a 40% frequency in heifers with normal teats. Such infections are always associated with elevated SCC in excess of 5 million/ml in these young animals. See Figure 10 below illustrating horn flies and lesions on heifer teats.



**Figure 9.** Prevalence of mastitis in Louisiana dairy herds with and without a fly control program.

Since that first survey, researchers have proven through DNA studies that the horn fly is not only responsible for teat lesions on heifers, but is indeed a vector in the transmission of mastitis-causing bacteria, such as *Staph. aureus*, from heifer to heifer (Owens et al., 1998). Such mastitic heifers serve as sources of IMI for transmission to the entire lactating and nonlactating herds.

Once it was established that the horn fly was a vector in the transmission of mastitis-causing bacteria, the next step was to develop management practices to reduce flies and lower the prevalence of intramammary infections. Insecticide-impregnated tags placed on the tail switch in close proximity to the udder during the spring and summer months were successful in reducing horn fly populations by 60% as well as the incidence of mastitis during the first 2 months after placement (Nickerson et al, 1997). In heifers with tail tags, mastitis incidence increased from 8.6 to 15% (1.7-fold increase), while in controls, incidence

increased from 17.1 to 52.4% (3.1-fold increase). As observed above, infections were associated with marked elevations in SCC. However, after 2 months, tags fell off and replacing them was impractical from a management standpoint.



**Figure 10.** Udder of a 10-month-old heifer illustrating horn flies and lesions on teat ends.

In a subsequent trial, the daily dietary supplementation of an insect growth regulator helped to suppress fly populations but not enough to prevent new cases of mastitis in dairy heifers (Owens et al., 2000).

Lastly, the use of an insecticidal pour-on every 2 wk for 6 wk followed by treatment with insecticidal ear tags reduced fly populations and decreased the incidence of new *Staph. aureus* by 83% during a 6-mo trial in heifers during the warm season in Louisiana (Owens et al., 2002). Mastitis in heifers caused by *Staph. aureus* was associated with SCC in excess of 10 million/ml.

These studies demonstrate that, during the warm and humid months of the year, horn flies do serve as vectors in the transmission of heifer mastitis, which is associated with elevated SCC in these young dairy animals. Although research has not been conducted to show this same association in lactating and dry adult cows, it is assumed that fly populations play some role in the elevation of mastitis and SCC observed in the hot summer months. And, with the proposed reduction in the SCC legal limit to 400,000/ml in the USA, and in light of the fact that milk buyers are imposing their own limits, some as low as 250,000/ml, it is imperative that dairymen utilize all possible means to prevent new cases of mastitis and their associated SCC. A simple fly control program can serve as an important adjunct to the basic 5-point plan of mastitis control and assist dairymen in lowering their bulk tank SCC and earn quality premiums for their product.

## 12. Influence of dietary supplementation on mammary health

Another management tool to reduce the level of infection and SCC when heifers calve as well as throughout lactation is through dietary supplementation. Diet plays a role in udder resistance to infection because certain nutrients affect various mammary resistance mechanisms, namely: (1) leukocyte function, (2) antibody transport, and (3) mammary tissue integrity. In one study, heifers received selenium (0.3 ppm/day) and vitamin E (50 to 100 ppm/day) supplementation starting 60 days prepartum (Hogan et al., 1993). A selenium booster injection (50 mg) was administered 21 days prior to freshening, and the dietary supplementation was continued throughout lactation. Dietary supplementation reduced staphylococcal and coliform infections at calving by 42%. Although rate of new infection during lactation did not differ from unsupplemented controls, the duration of infection caused by organisms other than *Corynebacterium bovis* was reduced 40 to 50% in supplemented heifers. Clinical mastitis in supplemented heifers was reduced 57% in early lactation and 3.2% throughout lactation, and the mean SCC was lower. Thus, vitamin E and selenium improved udder health of heifers, and the effect of dietary supplementation was most evident at calving and in early lactation.

In a more recent study, dairy heifers were fed a daily supplement beginning at 5 months of age containing an immunostimulant composed of B-complex vitamins and yeast extract (Eubanks et al., 2011). Compared with unsupplemented control animals, those supplemented with the immunostimulant exhibited greater leukocyte expression of L-selectin and interleukin-8 cell surface receptors, suggesting the capability for a greater immune response to bacterial infection. Preliminary results also showed a lower incidence of *Staph. aureus* mastitis in supplemented heifers.

## 13. Conclusions

Prevalence of mastitis in unbred, breeding age, and pregnant dairy heifers is higher than formerly realized. Infected mammary quarters, especially those with *Staph. aureus* IMI exhibit reduced secretory potential and marked leukocyte infiltration and accompanying inflammation. Both nonlactating and lactating commercial antibiotic infusion products have been used successfully to cure existing infections and reduce SCC, and nonlactating therapy prevents new IMI with environmental streptococci. However, the goal is to prevent new infections from occurring in these young dairy animals through management strategies aimed at vaccination, fly control, and dietary supplementation. Results of experimental and commercial vaccine trials illustrate that immunization will reduce *Staph. aureus* mastitis in heifers at calving between approximately 45 and 60%, with reductions in SCC of 50%. Likewise, a fly control program for heifers will decrease incidence of *Staph. aureus* mastitis by up to 83%. Lastly, dietary supplementation to boost the immune systems of heifers has been shown to reduce incidence of mastitis at calving as well as to lower SCC. As global milk quality standards become more stringent, management practices based on curing existing infections and preventing new IMI in heifers will ensure that these young dairy animals enter the milking herd free of mastitis with low SCC. Such practices should be considered for incorporation into dairy herd health programs in herds suffering from a high prevalence of heifer mastitis, especially that caused by *Staph. aureus*.

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# **Bovine Mastitis Pathogens: Prevalence and Effects on Somatic Cell Count**

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/51032>

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## **1. Introduction**

Bovine mastitis is the most prevalent and costly disease, affecting dairy farms worldwide. Economic losses associated with mastitis derive mainly from a decrease in milk production and to a lesser extent, from the culling of chronically infected cows, cost of veterinary treatment, and penalties on milk quality (Seegers *et al.*, 2003). Mastitis is caused by a wide spectrum of pathogenic agents that penetrate the teat canal and multiply in the udder cistern. The majority of mastitis cases are produced by a relatively small group of bacteria, including *Staphylococcus aureus*, *Streptococcus uberis*, *Mycoplasma spp* and *Escherichia coli* (Calvinho & Tirante, 2005). Bovine mastitis is characterized by inflammation of the mammary gland. The inflammation severity depends on the causative agent and the host response (Bannerman *et al.*, 2004; Barkema *et al.*, 2006; Burvenich *et al.*, 2003; Petzl *et al.*, 2008). Resident and recruited cells together play an essential role in immediate defense against local infection (Rainard & Riollet, 2006). Extensive neutrophil recruitment from the circulation to the lumen of the mammary gland is a hallmark of the early immune response to mammary infection (Thomas *et al.*, 1994; Sordillo & Streicher, 2002; Oviedo-Boyso *et al.*, 2007). When designing mastitis-prevention and control programs, it is worthy to take on account the adoption level of mastitis-prevention management practices and control programs as well as the etiology of the intramammary infections (IMI), the herd-level prevalence of contagious mastitis pathogens, and the general factors that influence milk production.

## **2. Mastitis pathogens agents**

### ***Staphylococcus aureus***

*Staphylococcus aureus* (*S. aureus*) colonize the nipple skin, advance through the mammary gland canal into the gland. The IMI with *S. aureus* predominantly cause subclinical mastitis resulting in a chronic infection lingering lifelong (Bannerman *et al.*, 2004; Riollet *et al.*, 2000; Yang *et al.*,

2008). During the infection's early stages, the mild damage may be reverted but *S. aureus* infections, in its peracute mastitis presentation generates gangrene and severe tissue damage. In comparison with *Streptococcus agalactiae*, *S. aureus* is more difficult to be eradicated. *S. aureus* infections cause a 45% decrease on milk production per quarter, reflected as a 15% per infected animal (NMC, 1999). The chronic, subclinical infections account for approximately 80% of mastitis related costs, due to reduced milk yield and product quality (Shim *et al.*, 2004). In practice, an elevated somatic cell count (SCC), over 300, 000 to 500, 000 cells/ml, indicates high prevalence of infected glands with *S. aureus* in a herd (NMC, 1999).

### ***Streptococcus agalactiae***

*Streptococcus agalactiae* (*S. agalactiae*) causes contagious mastitis, an obligated pathogen of the mammary gland, which is transmitted directly among cows during milking (NMC, 1999). *S. agalactiae* infects the gland cistern and ducts of the mammary gland causing irritation, swelling and subclinical mastitis. The infected cow shows mere clinical signs without abnormalities drawn in milk. However, low production rates and high SCC are usually registered. *S. agalactiae* infections are related to Bulk-tank milk figures around a 1,000, 000 cells/ml on SCC or higher. Currently, these figures are rarely seen because the control measures and milking management had been improving along with better antibiotic treatment (Hillerton & Berry, 2003).

Globally *S. agalactiae* is a low prevalence pathogen. In Canadian bulk milk, its prevalence ranged between 6% in Alberta (Schoonderwoerd *et al.*, 1993), and 43% in Québec (Guillemette *et al.*, 1992). In the Prince Edward Island, Keefe *et al.*, (1997) determined a herd prevalence of 18%. Furthermore, a study recently performed in Canada (Richard G.M. *et al.*, 2010) demonstrated the low prevalence of *S. agalactiae* at 4.4% and in Argentina, in the last 25 years, the mastitis prevalence due to *S. agalactiae* has been 0.3% in the four quarters before-delivery (Calvinho *et al.*, 2001).

### ***Mycoplasma* spp**

*Mycoplasma* spp are highly contagious microorganisms, but less common than *S. agalactiae* and *S. aureus*. Nevertheless, *Mycoplasma* spp damage the secretory tissue, induce the gland fibrosis, abscesses and the lymphatic nodules fibrosis (NMC, 1999). Animals from all ages are susceptible, as well as at any time during lactation. Those in early lactation are more susceptible to *Mycoplasma* infection and it can be isolated from high production animals without signology.

Mycoplasmosis is frequently related to the mastitis outbreak onsets, to the introduction of new animals to a herd, to previous respiratory or articular disease, and to herds with unresponsive mastitis to antibiotic treatment. When at least the recurrent mastitis, a non-signs illness and an unresponsive treatment are observed, a mycoplasma infection is suspected.

*Mycoplasma* infection prevalence at the herd-level is estimated by *Mycoplasma* culture from Bulk Tank Milk (BTM) and has been suggested to be between 1 and 8% in the USA (Fox LK., *et al.*, 2005). These monitoring of mycoplasma-mastitis performed through BTM cultures assumes that the appearance of a *Mycoplasma* sp in it indicates that there is at least one cow in the herd affected with mycoplasma and that environmental contamination of the bulk tank by

mycoplasma is unlikely, hence a false positive result is discarded. The speciation of mycoplasma mastitis pathogens requires secondary tests, usually only carried out by specialized laboratories from colonies presumptively identified as *Mycoplasma* spp and with specific end point PCR for *M. alkalescens*, *M. bovigenitalium*, *M. bovirhinis*, *M. californicum*, *M. canadense* and *M. bovis* (Hirose *et al.*, 2001; Kirk JH. *et al.*, 1997) applied to determine the genus and specie prevalence from BTM samples collected monthly between 1989 and 1995 from 267 dairy herds. From these *M. bovis*, *M. canadense*, *M. californicum*, *M. bovigenitalium*, *M. alkalescens*, were retrieved from 209 (78.2%) dairies and they had been identified and reported as potentially pathogenic *Mycoplasma* organisms. Further studies, in the herd level such as, Fox *et al.*, (2003) and the Northwest Dairy Association (NDA), processed milk from 463 herds concluding 93 herds diagnosed as mycoplasma-positive from BTM. *Mycoplasma* was more likely to be present in samples from herds shipping higher milk amounts, therefore mycoplasma is indirectly related to the herd size and the larger the herds are, the higher mycoplasma caused mastitis prevalence will be. From the same study, a year later, *Mycoplasma* spp were not detected in any herd. These finding suggested that Mycoplasma caused mastitis can be controlled and eliminated from herds. This observation is supported by the studies done by, Brown *et al.* (1990), who reported that an outbreak of *Mycoplasma bovis* mastitis was controlled by an intensive identification scheme to find infected cows, culling the unproductive ones, and segregating and milking the left under a milking time hygiene procedure, also Bicknell *et al.* (1983) reported similar findings with intensive identification schemes to determine cows with *Mycoplasma bovis* mastitis and successfully managed with segregation and culling. Similar findings were reported by Mackie *et al.* (2000) specifically for *M. californicum* and *M. canadense*. The exception was reported by Jackson and Boughton (1991) who observed that segregation and culling were not necessarily required for controlling a *M. bovigenitalium* outbreak.

### **Coagulase-negative *Staphylococci* (CNS)**

Coagulase-negative *Staphylococci* (CNS) are considered opportunistic mastitis pathogens, resident colonizers on the teat skin, rarely causing clinical mastitis (Hogan *et al.*, 1987) and are frequently not reported in mastitis studies (Bramley & Dodd, 1984). However, CNS are isolated from cases of subclinical and clinical mastitis and as the cause of IMI in lactating cattle with subclinical prevalence of 31.1% at parturition and 27.9% postpartum (Hogan, 1997; Fox, 2009). Moreover, CNS are the most frequently isolated pathogens from mastitis in heifers. This bacteria group comprises more than 50 species and subspecies (Pyörälä S. *et al.*, 2008). Coagulase-negative *Staphylococcus* species differ from each other in antimicrobial susceptibility, virulence factors and host response to infection (Birgersson *et al.*, 1992; Devriese *et al.*, 2002; Taponen S. *et al.*, 2009). Thus, identification of species may be relevant for epidemiological surveys, the assessment of their pathogenic significance and for developing specific management practices to prevent mastitis. Perhaps it could be worthy to study them as individual species. There are many differences regarding the pathogenicity of different species of CNS that are studied with molecular diagnostic techniques (Zadoks & Schukken, 2006).

The most commonly isolated species of CNS from bovine mastitis are *Staphylococcus chromogenes*, *Staphylococcus epidermitis*, *Staphylococcus hyicus* and *Staphylococcus simulans*. Prevalence studies have demonstrated that CNS are the bacteria group most frequently isolated

from milk samples with high SCC (Pitkälä *et al.*, 2004; Bradley *et al.*, 2007; Piepers *et al.*, 2007; Sampimon *et al.*, 2009). In mammary quarter infection prevalence ranges between 28.9–74.6% prepartum, and 12.3–45.5% at calving. CNS are the most prevalent cause of subclinical IMI in heifers and coagulase-positive *Staphylococci* (CPS) are the second most prevalent pathogens, while in other studies the environmental mastitis pathogens are more prevalent. Generally, the pathogens that cause mastitis in heifers are the same as those that cause infections in older cows. The risk factors for subclinical mastitis appear to be dependent on the season, herd location, and trimester of pregnancy; all suggesting that management has great impact in the prepartum disease control. Regarding clinical mastitis, the most prevalent mastitis pathogen has been reported to be CNS as well as CPS and environmental mastitis pathogens. Heifers are at a higher risk for clinical mastitis during the periparturient period including those related to diet, intrinsic mammary gland factors such as swelling and milk leaking, and factors associated with management changes and the heifer's introduction to the milking herd (Fox, 2009).

The prevalence of IMI with CNS has been increasing in North America, Europe and Latin America (Calvinho *et al.*, 2001, Jánosi1 & Baltay, 2004; Sampimon *et al.*, 2009; Pantoja, *et al.*, 2009) (Table 1 and Table 2). CNS are the most frequently isolated pathogen group from IMI in The Netherlands, estimated as 10.8% at the quarter level and 34.4% at the cow level. Fourteen species of CNS were identified and the most relevant were *Staphylococcus chromogenes* (30.3%) *Staphylococcus epidermidis* (12.9%) and *Staphylococcus capitis* (11.0%) and prevalence of CNS IMI was higher in heifers than in older cows. Geometric mean quarter SCC of CNS-positive quarters was 109,000 cells/ml, which was approximately twice as high as culture-negative quarters. Quarters infected with *S. chromogenes*, *S. capitis* and *Staphylococcus xylosum* had a higher SCC ( $P < 0.05$ ) than culture negative quarters, while quarters that were culture-positive for *S. epidermidis* and *Staphylococcus hyicus* tended to higher SCC than culture-negative quarters. An increased prevalence of CNS-IMI is associated with the herd-level variables such as a taped source of drinking water, single dry-cows housing, monthly SCC measure, veterinary udder health monitoring, outdoors season pasturing, percentage of milk contaminated stalls, and bulk milk SCC (BMSCC) > 250,000 cells/ml. Currently the prevalence of CNS-IMI is already high in heifers around their first calving (Borm *et al.*, 2006), the lower prevalence of CNS in multiparous cows may be explained by the fact that in the 80% of the farms included in this study, the practice of antibiotic dry off and post-milking teat disinfection applied twice a day during lactation was used. Also pasturing during the outdoor season was associated with an increased prevalence of CNS-IMI, and the summer period is related to active flies, especially the horn fly *Haematobia irritans* which can transmit *S. aureus* (Owens *et al.*, 1998) and possibly transmits CNS.

Country	<i>Staphylococcus aureus</i>	<i>Streptococcus agalactiae</i>	<i>Mycoplasma spp</i>	Environmental <i>Streptococcus spp</i>	CNS*	Environmental pathogens	Reference
Iran	-	-	48.75%	-	-	-	Ghazaei, 2006
Mexico			9.92%				Infante., <i>et al.</i> , 1999

Country	<i>Staphylococcus aureus</i>	<i>Streptococcus agalactiae</i>	<i>Mycoplasma spp</i>	Environmental <i>Streptococcus spp</i>	CNS*	Environmental pathogens	Reference
Argentina	2.0%	0.3%	-	-	25.3%	-	Calvinho., et al., 2001
Hungria	32.5%	-	-	12.8%	41%	6.8%	Jánosil & Baltay, 2004
Netherlands	-	-	-	-	10.8%	-	Sampimon et al., 2009
Wisconsin	-	-	-	-	12.8%	-	Pantoja, 2009
Canada	74%	4.4%	SD	-	-	-	Richard., et al., 2010
Germany	5.01%	-	-	8.7%	17.17%	-	Schwarz., et al., 2010

(\*)Coagulase-negative *Staphylococci* (CNS)

**Table 1.** Pathogen prevalence in Bovine Milk from some productive regions

Country	<i>Staphylococcus aureus</i>	<i>Streptococcus agalactiae</i>	<i>Mycoplasma spp</i>	<i>Staphylococcus</i> Coagulase-Negative	Reference
Pennsylvania, USA	150 000 to 700 000 cells/ml				Erskine R.J. et al., 1987
Hungary	400 000 cells/ml				Jánosi & Baltay, 2004
Mexico			465 000 cells/ml		Miranda-Morales RE et al., 2008
Netherlands				109,000 cells/ml	Sampimon et al., 2009
Wisconsin, EEUU	600,000 cells/ml			190,000 to 519,000 cells/ml	Pantoja, 2009
Canada					Richard et al., 2010
Germany	>100 000 cells /ml				Schwarz D, et al., 2010

**Table 2.** Somatic cell count (SCC×1000 cells/ml) associated with the mastitis causing microorganism in different countries.

### Environmental mastitis pathogens

*Streptococcus* spp are among the outstanding environmental pathogens as well as *E. coli* and *Corynebacterium* spp. Environmental *Streptococcus* spp are present in dairy herds causing clinical and subclinical mastitis, its presence has been exacerbated due to the increasing implementation of control strategies against contagious pathogens such as *Staphylococcus aureus*. These programs had reduced the contagious mastitis incidence, however, they had

low effect on the mastitis caused by *Streptococcus* spp, catalase-negative cocci, and by environmental coliform bacteria which affect the udder. Among *Streptococcus* spp, *Streptococcus uberis* (*S. uberis*) is the most frequent as bovine udder pathogen (Olde Riekerink *et al.*, 2008). Moreover, the dairy environment is a determinant factor for mastitis development due to *S. uberis* and *Streptococcus dysgalactiae* subsp. *dysgalactiae* (*S. dysgalactiae*), and stabled dairies are in greater risk than those held in open pastures (NMC, 1999). Other *Streptococcus* spp related in lesser amount to bovine mastitis are *Streptococcus parauberis* (*S. parauberis*), *Streptococcus salivarius* (*S. salivarius*), and *Streptococcus sanguinis* (*S. sanguinis*) (Whitman, 2009). Some Enterococcus such as *Enterococcus faecium* (*E. faecium*), *Enterococcus faecalis* (*E. faecalis*), *Enterococcus saccharolyticus* (*E. saccharolyticus*) (Østerås, *et al.*, 2006). *Aerococcus viridans* (*A. viridans*) has been also related to mastitis but its role has not been elucidated yet (Devriese *et al.*, 1999; Zadoks *et al.*, 2004). In Hungary, Jánosi and Baltay, (2004) determined that the environmental caused mastitis by *Streptococcus* sp and *E. coli* had a prevalence of 12.8% and 6.8 % respectively.

The environmental pathogens, by themselves, are not enough frequent and persistent to cause mastitis or as a significant elevation of somatic cells counts (SCC) of bulk milk (values over 400,000 cells/ml). However, 66% of mastitis caused by environmental *Streptococci* and 85% of those caused by coliform bacteria, display clinical presentation. Therefore, losses due to this type of mastitis can reach substantial amounts even in herds with low SCC (<300,000 cells/ml), mainly due to a high incidence of clinical mastitis as it has been estimated around a 46% of clinical mastitis per year in herds with bulk milk SCC counts of less than 200,000 cells/ml

### 3. Somatic cell counts (SCC)

Throughout the world in the last ten years, udder health programs have been increasing (Godkin *et al.*, 1999; Østerås *et al.*, 1998; Plym 1996a; Plym *et al.*, 1996b; Sargeant *et al.*, 1998), and regarded as a critical production issue on dairy farms. In Europe, the European Economic Community (EEC) since 1998 does not recommend consumption of milk with SCC over 400, 000 cells/ml. In North America the limit has been established at 750, 000 (USA) and in Canada at 500, 000 cells (Sargeant *et al.*, 1998).

Somatic cells are, in great quantity, cells of the immune system (80% in uninfected quarters, and 99% in quarters with mastitis) (Sordillo *et al.*, 1997). They are part of the natural defense mechanisms, including lymphocytes, macrophages, polymorphonuclear and some epithelial cells (Pillai *et al.*, 2001). Somatic cells are therefore a reflection of the inflammatory response to an IMI. Somatic cell counts are often used to distinguish between infected and uninfected quarters according to the general agreement between infection status and the inflammatory response to infection reflected as an increased SCC. As with any diagnostic test, errors will occur when solely depending on a single test. To minimize error, diagnostic test parameters such as sensitivity & specificity are calculated at various cut-off values in the continuum SCC (Schepers *et al.*, 1997). In North America and Europe the SCC for an uninfected quarter is approximately 70, 000 cells. There is of course variation around this mean; its value can increase with age, decreasing milk production and days in milk period (Schepers *et al.*, 1997).

Hence, to be able to distinguish between infected and uninfected quarters a cut-off of approximately 200,000 to 250,000 cells is accepted (Dohoo *et al.*, 1991; Laevens *et al.*, 1997; Leslie *et al.*, 1997; Schepers *et al.*, 1997). At this cut-off value, diagnostic sensitivity is approximately 75%, and specificity approximately 90% (Schepers *et al.*, 1997). The 200,000 cells cut-off is not considered a physiological cell concentration in milk able to distinguish between healthy and unhealthy udders, but it is a practical value under field conditions (minimizing diagnostic error). Erskine *et al.* 1987, evaluated 32 dairy herds, 16 with low SCC less than or equal to 150,000 cells/ml and 16 with high SCC greater than or equal to 700,000 cells/ml. From the 16 herds with low SCC, *S. agalactiae* was isolated in two herds (12.5%), and *S. aureus* was isolated from seven herds (44%). Moreover both microorganisms were found in all of the herds with high SCC, a program of post-milking teat dipping and treatment of all cows at the beginning of the non-lactating period was practiced in the herds with low SCC. Whist *et al.* (2007) reported low SCC in milk from heifers having *Streptococcus dysgalactiae* IMI and in non-infected glands the results indicated that SCC were high (between 50,000 and 100,000 cells/ml) during the immediate postpartum period, within the next 5 days after calving.

#### 4. Bulk tank milk (BTM) SCC

BTM SCC is a general indicator of the udder health in a herd and it is also regarded as an indirect measure of milk quality (Schukken *et al.* 2003). Elevated SCC, are correlated with changes in milk composition, casein and more serum-derived whey proteins, as well as increased proteolytic and lipolytic activities (Auldist & Hubble, 1998). SCC may, however, vary greatly depending on factors such as number of lactations, stage of lactation, season and milking frequency (Harmon, 1994; Pyörälä, 2003). In BTM, where the total volume of milk will dilute effects from affected quarters, SCC appears to be less sensitive and specific as a biomarker for milk quality, e.g. suitability for cheese production (Leitner *et al.*, 2006).

Bulk tank milk SCC assist in directing milk quality control programs and assist with the identification of risk factors in herds. The production of milk with low bacterial counts starts at the farm and is influenced by many procedures related to farm management practices. At the farm level, microbial contamination of BTM occurs through three main sources; bacterial contamination from the external surface of the udder and teats, from the surface of the milking equipment, and from mastitis organisms within the udder (Murphy & Boor, 2000). The levels and types of microorganisms in BTM provide valuable information on the hygienic conditions during the steps of milk production. The microbiological count methods are used to monitor hygienic quality of raw milk including the total aerobic count (TAC). TAC is the most common method for the assessment of bacterial quality of raw milk, it estimates the total number of bacteria present at the farm's pickup time, providing an overall hygienic milk-quality measure; however, it is limited for the identification of the bacteria contamination source. An alternative has been the standard plate count (SPC) and the preliminary incubation count (PIC), a selective count is measuring psychotropic bacteria, which will grow and multiply under improper refrigeration conditions. These organisms can create undesirable odors and off-flavors. Many psychotropic bacteria can also produce heat-stable enzymes that will survive pasteurization degrading and reducing milk and milk

products during shelf-life (Hayes & Boor, 2001). The laboratory pasteurization count (LPC), another selective count, estimates the number of thermophilic bacteria, mainly from the surfaces of poorly cleaned farm equipment that will survive a laboratory-scale batch pasteurization process. Thermophilic bacteria have been associated with spoilage of pasteurized milk. The Coliform count (CC) measures the number of coliform bacteria in milk, organisms primarily coming from the cow's environment, therefore high CC will give an estimation of the production sanitary status and practices. Coliforms can also incubate on residual films of improperly cleaned milking equipment (Reinemann *et al.*, 2003).

The results from a case-control study indicated that TAC and PIC were mostly related to cow and stall hygiene, whereas LPC and CC were related to equipment hygiene (Elmoslemany *et al.*, 2009; Jayarao *et al.*, 2004), and included among the bacteria groups associated with bovine IMI are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma spp*, *Streptococcus spp*, *Escherichia coli*, and SCN.

## 5. Prevalence of mastitis pathogens and somatic cell counts

In Mexico, the prevalence of mastitis pathogens in BTM SCC from 224 milk samples of 112 herds was as follows; *Mycoplasma spp* were isolated from 62 herds (55%), *S. aureus* from 34 cattle barns, *S. uberis* and CNS were isolates from milk from 42 herds (37.5%) and from 43 (38.3%) bulk tank milk samples. The geometric mean of SCCs was 465, 000 cells/ml. No significant differences were observed in SCCs between *Mycoplasma spp*, *S. aureus* and *Streptococcus spp* positive and negative herds ( $P > 0.5$ ) (Miranda-Morales, *et al.*, 2008).

In Latin America, few data had been carried out regarding microorganism's prevalence and SCC in cases of clinical and subclinical mastitis. Nonetheless, regarding bovine mastitis, Calvinho *et al.*, (2005) assessed the primary pathogens prevalence, and its relation with the general udder health status in Argentina from 1983 to 2001. The subclinical mastitis showed a prevalence of 25.3% of *S. aureus* in the 80's and through the years it has been decreasing until a level of 13.9% in 2000. This situation was also observed regarding *S. agalactiae*, which has been reducing its prevalence from 8.8 to 1.6%; *Streptococcus spp* from 19.3 to 6.5% and coliforms from 2.7 to 2%. The prevalence observed for the same pathogens causing clinical mastitis, were low prevalence levels for *S. aureus*, *S. agalactiae* and coliforms respectively from 34.45 to 29.2%, 13 to 3.9%, and from 20 to 4.4%. In contrast the CNS, *S. dysgalactiae* and *Streptococcus spp* registered rising prevalence from 2.1 to 12.7%, 1.7 to 15.9%, and from 6.4 to 19.8% respectively. This situation was also seen among SCC registering levels of 400, 000 to 900, 000 cells/ml in the 80's, since after a sustained decrease in the SCC from BTM in recent years; in 2004 ranging around 300, 000 cells/ml, and in 2005, an average of 384, 000 cells/ml (SAGPyA, 2005). The producers have been implemented systematically control programs based on hygiene and antibiotic therapy, there has been a decrease in the prevalence of contagious pathogens and environmental relative increase, however it should be noted that the SCC values remain high compared with those of countries with high dairy development. In Perú, Ortiz, *et al.*, 2006, assessed the SCC in dairy herds of different technological levels in Arequipa, milk samples were collected twice in 2005. The stables were stratified according to

their technological level in high, medium and low. The general average of SCC were  $505 \times 10^3 \times 10^3 \pm 150$  cells/ml, and significant differences between technology levels were identified as SCC were  $353$ ,  $559$  and  $603 \times 10^3$  cells/ml for high, medium and low, respectively ( $p < 0.05$ ), feature explained by the dilution of somatic cells in a greater volume of milk and a more rational application of best practices to prevent and control mastitis in the most sophisticated stables. On the other hand, limited access to training adversely affects low-technology. In a study by Moraga *et al* (1994) in Chile, the prevalence of bovine mastitis in the years 1972 to 1992; subclinical mastitis in 1972 was 45.42%, and by 1992 the prevalence had reduced to 38.65%, traduced on a 14.90%. Regarding clinical mastitis a continuous prevalence reduction of 12.86% from 74.41% to 64.84% was determined during the same period. Furthermore, the SCC were reduced from 1,983,310 cells/ml to 1,055,240 cells/ml, in these 20 years. These decrements on the severity of subclinical mastitis obeys the current control measures spread in the early 70's, such as post-milking disinfection of teats and drying therapy used in the 66.7% of the farms studied as well as the general infrastructure improvement. Finally despite the progress, acceptable control mastitis levels have not yet been reached.

In Mexico, Infante, *et al.*, (1999), observed in a commercial dairy herd (282 cows) in lactation a sudden atypical clinical mastitis outbreak with 28 cases of severe purulent mastitis, hard swollen mammary glands and lacking systemic signs of illness. The treatment non- responsive cases (Table 1) suggested the spreading through the milking machine and other management practices, further cultures determined the presence of *Mycoplasma californicum* and *Mycoplasma canadense*. A second study performed by Miranda-Morales, *et al.*, (2008), revealed that *Mycoplasma* spp were present in the 55% of the 62 herds included, also that *S. aureus* was present in the 30% of cattle barns and that *S. uberis* and CNS were present in 42 herds (37.5%) and 43 herds (38.3%) according to the BTM samples, respectively. The geometric mean of SCCs was 465, 000 cells/ml and no significant differences were observed among *Mycoplasma* spp, *S. aureus* and *Streptococcus* spp positive and negative herds ( $P > 0.5$ ) (Table 2 and 5).

Overall, prevalence of mastitis is over 10%, in samples of direct milk *Staphylococcus aureus* has a prevalence  $>30\%$  in contrast to an  $<5\%$  prevalence of *Streptococcus agalactiae*, and a prevalence between 15 and 41% has been reported for CNS. *Mycoplasma* has been reported in a few prevalence studies and environmental mastitis pathogens have an average prevalence of  $>15\%$ . However in BTM *Staphylococcus aureus* have registered consistently high figures from 30% and up to 74%, followed by the prevalence values of *Streptococcus agalactiae* around 40%, and in BTM *Mycoplasma* spp had variable prevalence figures ranging from 50% to 85%. Regarding SCC, values of 100, 000 – 700, 000 cells/ml are associated to the presence of *Staphylococcus aureus* and *Streptococcus agalactiae*. For *Mycoplasma* spp, SCC values are  $> 200, 000$  cells/ml, and SCC of 100, 000 and up to 500, 000 cells/ml are associated to CNS infection. Currently, in America, BTM-SCC values are around  $> 200, 000$  cells/ml, therefore milk quality requirements are barely meet except for some regions that had achieved SCC levels of  $< 200, 000$  cells/ml, and low prevalence of mastitis associated pathogens. Therefore, herd overall studies are mandatory for mastitis control programs including duration of lactation, season, milk production and parity. But will also be guided by the prevalence of mastitis pathogens and by the, geographic region and production practice.

Country	BTM	SCC	Reference
Seattle, USA	93	533 000 cells/ml	Fox L.K. <i>et al.</i> , 2003
Argentina	7358	384 000 cells/ml	SAGPyA, 2005
Peru	15	500 000 cells/ml	Ortiz Z.C. <i>et al.</i> , 2006
Argentina	51	250 000 cells/ml	Vissio, C., <i>et al.</i> , 2007
Mexico	112	465 000 cells/ml	Miranda-Morales R.E., <i>et al.</i> , 2008

**Table 3.** SCC values of BTM milk samples associated with mastitis pathogens of some regions worldwide.

Reference	No. of Dairy herds	No. of bovine	Gland infected (%)
Zurita., <i>et al.</i> , 1972		1 137	48,81%
Moragay., <i>et al.</i> , 1993	30	2 321	41,10%
Chaves., <i>et al.</i> , 1996		19	37%
Calvinho., <i>et al.</i> , 2001		86	62,8%
Sampimon., <i>et al.</i> , 2009	49	1 960	10,8%
Castillo., <i>et al.</i> , 2009		2 116	72,61%

**Table 4.** General overview of mastitis prevalence.

Reference	Dairy herds studied	<i>Staphylococcus aureus</i>	<i>Streptococcus agalactiae</i>	<i>Mycoplasma</i> spp
Kunkel, 1985	2346	-	-	1.3%
Guillemette., <i>et al.</i> , 1992		-	6%	-
Schoonderwoerd., <i>et al.</i> , 1993		-	43%	-
Keefe., <i>et al.</i> , 1997		70%	18%	
Kirk., <i>et al.</i> , 1997	267	-	-	78.2%
Fox., <i>et al.</i> , 2003	664	-	-	14%
Sato, 2004	118	71.6%	-	-
Sato, 2004	40	27.55%	-	-
Riekerink., <i>et al.</i> , 2006	258	74 %	1.6 %	1.9%
Howard, 2006	7	57.1%	-	-
Ghazaei, 2006	48	-	-	85,25%
Miranda-Morales., <i>et al.</i> , 2008	112	30%	-	55%
Richard & Riekerink., <i>et al.</i> , 2010	226	74%	4%	-

**Table 5.** Prevalence of contagious mastitis pathogens in BTM.

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# Clostridial Spores in Animal Feeds and Milk

Pascal Drouin and Carole Lafrenière

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50775>

## 1. Introduction

Milk conservation and its organoleptic quality are greatly affected by different microbial contaminants (Table 1). These microorganisms are present either directly on the animal, in the farm environment or on the milking equipment. Industry requirements and country regulations require that the number of bacteria in raw milk be under a specific amount, often of 100 000 bacterial cells ml<sup>-1</sup>. Recent bacterial counts from a survey in Québec (Canada) found that most raw milk samples were under 50 000 bacterial cells ml<sup>-1</sup> (<http://www.lait.org/fichiers/RapportAnnuel/FPLQ-2010/controleQualite.pdf>).

Contamination of raw milk by *Clostridium* may cause important economic losses in specific type of cheese, mostly hard and semihard cheeses. Epidemiologic studies demonstrated that silage was in close relation with the raw milk contamination by *Clostridium* (Klijn et al., 1995).

Bacteria	<i>Lactococci, Lactobacilli, Leuconostoc, Pseudomonas fluorescens, Pseudomonas fragi, Bacillus spp., Clostridium, Corynebacterium, Arthrobacter, Microbacterium</i>
Pathogenic bacteria	<i>Bacillus cereus, Listeria monocytogenes, Yersinia enterocolitica, Salmonella spp., Escherichia coli, Campylobacter jejuni</i>
Fungi	<i>Aspergillus, Fusarium, Penicillium</i>

**Table 1.** Example of microbial contaminants of raw milk

## 2. The Clostridium

Bacteria from the genus *Clostridium* share specific characteristics. Their capacity to form heat resistant spores and their intolerance to oxygen being the principals. Isolated from many

environments, they are generally considered as ubiquitous. Different species still require specific growth conditions; some are psychrophilic while other are mesophilic or even thermophilic. The genus also contains pathogenic species, like *Clostridium tetani*, *Clostridium botulinum* and *Clostridium perfringens*. Some species are recognized as plant endophyte and could fix atmospheric nitrogen (Minamisawa et al., 2004).

Cells from the genus *Clostridium* are defined as Gram-positive, endospore-forming rods and most species are obligate anaerobes with varying tolerance to oxygen (Pahlow et al., 2003). More than 100 species had been described in this genus, but recent advances in genetic phylogeny allow more specific classification of these organisms.

A group of clostridia species had been recognized as important milk contaminant. Like most other *Clostridium*, even if these species are ubiquitous, they are responsible for a specific defect of some type of cheeses, called "late blowing" (see section below). Three species had been frequently detected in late blowing cheese samples: *Clostridium tyrobutyricum*, *Clostridium butyricum* and *Clostridium sporogenes* (Cocolin et al., 2004), with *C. tyrobutyricum* being the dominant specie. Together, these species are called "butyric acid spores". Silage, a forage conservation technique, is frequently pointed as the principal source of butyric acid spores of ruminant feed. The specie *C. tyrobutyricum* is one of the most frequently isolated clostridial species in silage samples (Pahlow et al., 2003). *Clostridium* species commonly found in silage could be separated in three groups: proteolytic clostridia (group 1), *Clostridium butyricum* group (group 2), and *Clostridium tyrobutyricum* (group 3) (Pahlow et al., 2003). Group 1 and 2 clostridia proliferate at pH generally over 5, while *C. tyrobutyricum* group will grow at lower pH, but rarely under pH of 4.5. The *C. butyricum* group includes *Clostridium beijerinckii* and *Clostridium acetobutylicum* and, like *C. tyrobutyricum*, ferment a wide range of carbohydrates to butyric acid and acetic acid.

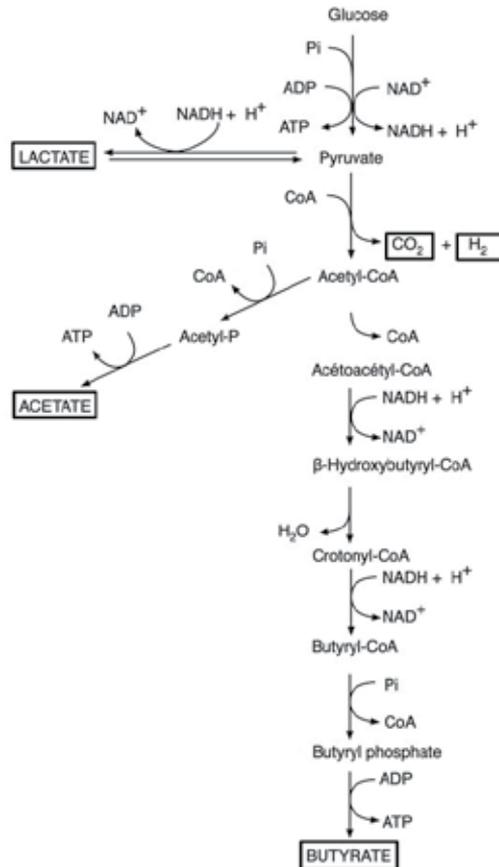
### 2.1. Physiology and ecology of the *Clostridium tyrobutyricum*

The different species of the genus *Clostridium* colonized a wide range of ecological niches but some species could be found only in very specific habitat. Soil is generally considered the habitat for most species, but since their metabolism is mostly related to organic matter degradation, soil mainly acts as a reservoir for the preservation of their spores. *C. tyrobutyricum* is present in agricultural soil but his habitat is not clearly defined (type strain ATCC 25755), even if it will proliferate under other conditions.

*C. tyrobutyricum* main fermentative metabolism is saccharolytic, allowing reduction of carbohydrates and lactate to butyrate. The butyric acid fermentation pathway is summarized by the following stoichiometric reactions:



Lactic acid could come directly from the environment of the organism. Those pathways involve condensation of two pyruvate molecules, derived either from glucose or lactate (Figure 1).



**Figure 1.** Metabolic pathway of glucose fermentation by *Clostridium tyrobutyricum* (adapted from Zhu and Yang (2004) and Rooke and Hatfield (2003)).

Soil and decaying plants, including older plant parts in close contact with soil is the natural habitat of *C. tyrobutyricum* (Ercolani, 1997). In forage stands, moist condition which favour microbial development and accumulation of dead leaves near the ground could produce conditions typical for the germination of clostridial spores. These conditions include low oxygen concentration, adequate humidity and presence of specific germination elicitors. For most clostridial species, molecules acting to promote germination include glucose, amino acids, organic acids and/or chelating molecule. For *C. tyrobutyricum*, acetate and ammonium are the principal germination compounds (Bergère, 1969). These two compounds are often present in decaying plant material because acetate is present following reduction of carbohydrates and deamination of amino acids. Recent work aim to understand clostridia development in cheese by scanning electron microscopy reported that L-alanine in conjunction with L-lactate was the most potent inducer of clostridial spore germination (Bassi et al., 2009).

When feed is contaminated by clostridial spores and ingested by the animal, spores could migrate through the rumen and concentrate in relation to total digesta volume. Digestion

processes contribute to concentrate the number of spores, which could be quite high in cow manure. However, the enteric environment does not generally provide an adequate environment for the germination of spores of these species. It only plays a role in population diversity and transport of the organism to other environments.

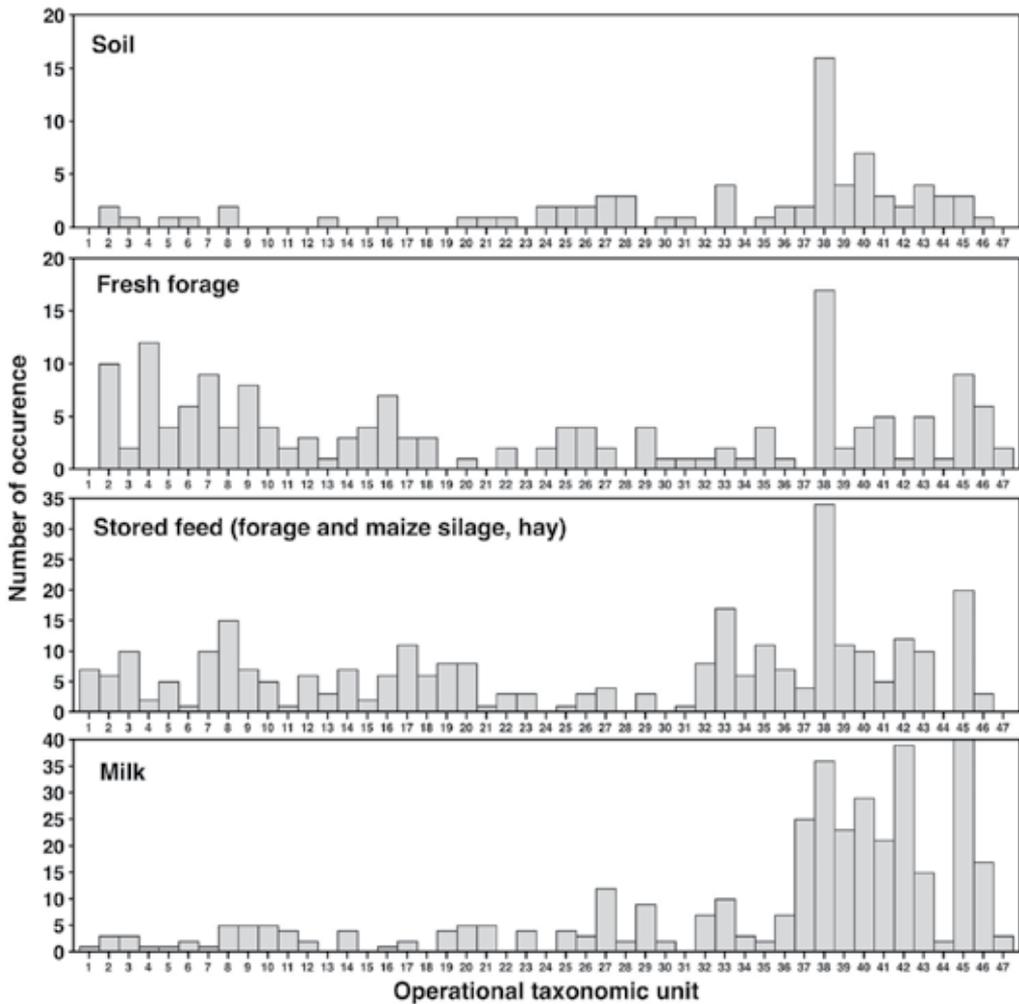
Clostridial cells usually stop growing in presence of O<sub>2</sub>, but growth resumes when O<sub>2</sub> concentration is under the physiological limit of the species. Oxygen sensibility depends on the metabolic level at time of exposure. Vegetative *Clostridium* cells could tolerate low oxygen concentration for short time period. Oxygen tolerance had not been measured for every specie, but some species could tolerate concentration as high as 3% O<sub>2</sub>. Acidic conditions as measured by pH and osmotic pressure as measured by A<sub>w</sub> are also physical conditions that limit growth of *Clostridium* species. In fermenters, *C. tyrobutyricum* was able to tolerate pH as low as 4.5 (Zhu & Yang, 2004) suggesting that it could grow in silage environment under a wide range of physico-chemical conditions. Other species of *Clostridium* could be present in the soil and decaying materials like small rodent or bird corpses and manure. *Clostridium tetanii* is a good example of clostridia species generally recognize as present in soil. *Clostridium sporogenes* could be found in soil and manure. Other highly pathogenic species could also be present in manure, *Clostridium perfringens* and *Clostridium botulinum* are some of them.

Many clostridial species are specialized and need specific conditions to grow. *Clostridium cellulolyticum* is a mesophilic (optimum growth temperature between 25 and 40°C) specie that is specialized in cellulose catabolism in composting process. Another example of a specialized specie is *Clostridium thermolyticum*, able to degrade cellulose to ethanol under thermophilic conditions (optimum growth temperature between 40 and 70°C) and so, use in industrial processes. *Clostridium difficile* is a specie mainly found in the intestinal tract of warm blood animal, like human, and may cause mortality after severe disturbance of intestinal microflora in hospital environment.

## 2.2. Diversity of *Clostridium* at the farm level

On farm, many different environments exist thus leading to diversity of clostridial species. Recent advances in molecular diversity techniques were applied to clostridial populations in order to follow distribution of different species. Diversity studies were performed in different environments of environment like soil, milk, plant surfaces, landfills, water treatment plants, and biogas fermenter (Herman et al., 1995; Julien et al., 2008; Klijn et al., 1995; Knabel et al., 1997; Van Dyke & McCarthy, 2002). For most of these studies, PCR primer sets designed specifically to amplify *Clostridium* species related to Cluster I (Collins et al., 1994) were used. One of these studies reported diversity of clostridia species in four different environments on four milk farms in Quebec (Canada) from soil to the raw milk (Figure 2) (Julien et al., 2008). Diversity patterns obtained following PCR-DGGE in fresh forage and stored feed samples are distinct from soil and milk samples. Operational taxonomic unit, (representing isolated band from the diversity pattern of a sample), related to *C. tyrobutyricum* (32, 33, 35 and 38) are present in all environments, but their number of

occurrence ratio is higher in stored feed. Soil diversity pattern shows less specificity in diversity, while milk clostridial diversity seem to be more related to contamination by feces since OTU related to *Clostridium disporicum* showed high occurrences. *C. disporicum* isolates are often present in swine manure and manure biofilm (Leung & Topp, 2001).

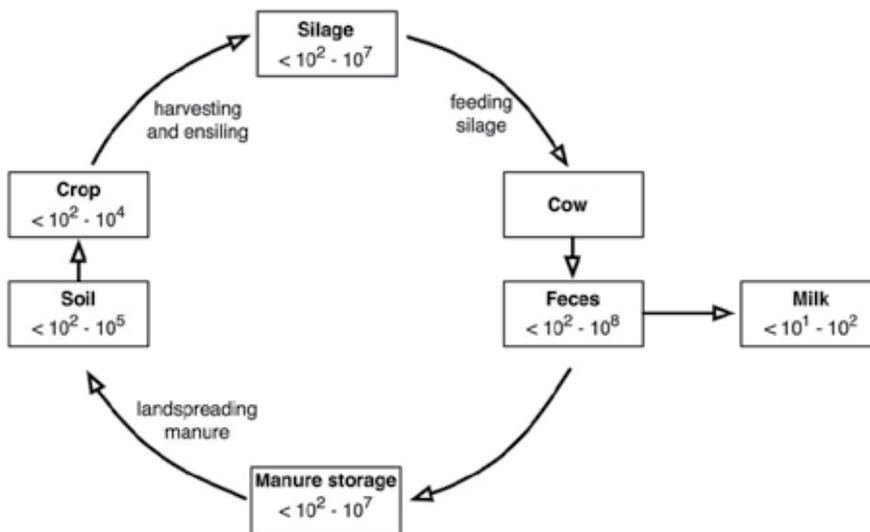


**Figure 2.** Diversity and frequency of occurrence of clostridia species from different farm environments determined by the molecular diversity technique PCR-denaturing gradient gel electrophoresis following amplification of Cluster 1 (Collins et al., 1994) associated species (Julien et al., 2008). Operational taxonomic unit 32, 33, 35 and 38 are similar to *C. tyrobutyricum* sequences in gene banks; OTU 38 is also closely related to *C. sporogenes*, a specie close to *C. tyrobutyricum*; OTU 42, 45, 46 and 47 are closely related to *Clostridium disporicum*

### 3. Contamination of animal feeds and raw milk

Depending on climatic conditions, season, and specific feeding requirements, ruminant diet could include fresh forage during summer months or all year long under favourable climate, or be fed with hay, silage, and/or grains (including corn) during winter season in northern regions. Butyric acid spores could contaminate all of these feed types, but at different level and thus be part of a contamination cycle. Among clostridial species at farm level, species related to Cluster 1 are a major concern in relation with cheese making. However, some other species also need consideration for their potential in health problem namely *C. botulinum*.

When silage is used as a feed source on a milk farm, silage conservation is of particular importance. Different events will take place that have relevance on the number of clostridial spores that could end up in raw milk in the bulk tank. During mowing and harvesting, contamination of crops by soil particules and manure aggregates will happen. During silage fermentation, butyric acid spores could germinate and grow if conditions are met. Animal will ingest the contaminated silage and spores will be released in feces. Clostridial spores will subsequently end up in manure, where population shift will happen before being applied again on crops. It could also contaminate teats, thus facilitating contamination of milk while milking the cows. These events can be considered as the spore cycle on a farm (Figure 3) (Pahlow et al., 2003).



**Figure 3.** Contamination cycle of clostridial spores from soil to raw milk at the farm level (adapted from Pahlow et al., (2003)).

#### 3.1. The soil, reservoir of *Clostridium* and organic fertilizers

Numbers of clostridial spores in the soil following plate counts on Reinforced Clostridial Agar (RCA) lead to mean counts around  $4 \log_{10}$  CFU  $g^{-1}$  soil (Julien et al., 2008). Considering

the low selective capacity of this medium in relation with the high diversity of clostridial species that could be present in the soil environment (Figure 2), this number greatly overestimate the number of strictly "butyric acid" spores present in soil. Even though, these different species could contaminate forage plant following harvesting and, cause hygienic problem in silage that could affect yield and health in some herds (see section 4. *Clostridium botulinum*). Number of butyric acid spores on plant surfaces is higher following plant harvesting since harvesting machineries, either mower, harvester or baller, contribute to the dispersion of soil dust leading to spores contamination on the plant surface. The type of mower used has a direct incidence on plant contamination. Disk mower will pick up soil if dry or ground surface is unlevelled. Speeds at which the disks rotate allow aspiration of soil. It is then very important to raise the cutting table to mow at least 7-8 cm above ground level. On a trial in an old grass stand, spore numbers was one order of magnitude lower when cutting height was set to 10 cm versus 7 cm (Drouin et al., unpublished results). This is particularly critical in a new stand or on a stand that received manure application, which could be up to 3 orders of magnitude higher (Drouin et al., unpublished results). Utilization of a windrower to accelerate forage wilting is also an operation that could contaminate the forage with dust soil.

The conservation method used to store forages has a direct impact on the subsequent number of spores. Hay will usually not allow germination of spores and subsequent development of the organism. Silage made from grasses or legumes could lead to important population of clostridial spores according to the ensilability of the crop. Lactic acid fermentation of whole corn silage is generally fast enough to ensure a good conservation. However, under aerobic instability environments, *Clostridium* development had been observed (Vissers et al., 2007a).

### **3.2. Manure and slurries as organic fertilizers and their role in butyric acid spores contamination of soil and plants**

Livestock manure (solid or liquid) is an important source of plant nutrients, and it can be valorized as fertilizers, especially with current high prices of mineral fertilizers. Concern has been expressed about the effect of manure application on soil properties and general hygienic quality of herbage over a number of years (Anderson & Christie, 1995). Contamination of herbage with manure (solid or liquid) by enterobacteria and clostridia may be important and represent a potential risk for silage making and health of the herd.

Manure contains different microorganisms, including enterobacteria such as *Escherichia coli*, *Salmonella* sp., *Campylobacter* sp., other bacteria such as *Listeria monocytogenes*, protozoa such as *Cryptosporidium parvum*, and viruses (Vanotti et al., 2005). Size of the population and species diversity depend on season, temperature, manure type (solid or liquid), and manure management (aerated laguna, compost, etc). Number of a specific microorganism could easily reach  $10^{10}$  cells  $g^{-1}$  slurry. Depending on the environmental conditions, it is generally recognized that size of population of pathogen species will decrease faster than generalist's species. Even so, some pathogens could survive for long period. Kudva et al. , (1998) in

USA showed that *E. coli* O157:H7 could survive 21 months in solid manure but if it was composted, survival would be four months. Similarly, *Cryptosporidium* and *Giardia* were eliminated by composting if temperature was maintained over 55°C during 15 days (Van Herk et al., 2004). This was reached during the fourth week of composting with windrow turnaround two times/week. In liquid manure, aerobic and anaerobic digestions are techniques used to decrease pathogen microorganisms.

Several clostridia species had been identified in manure. They were mainly proteolytic, because this environment contains high level of proteins and amino acids. Polysaccharides degraders Clostridia are also present because of high concentration of polysaccharides like cellulose and lignin (Table 2).

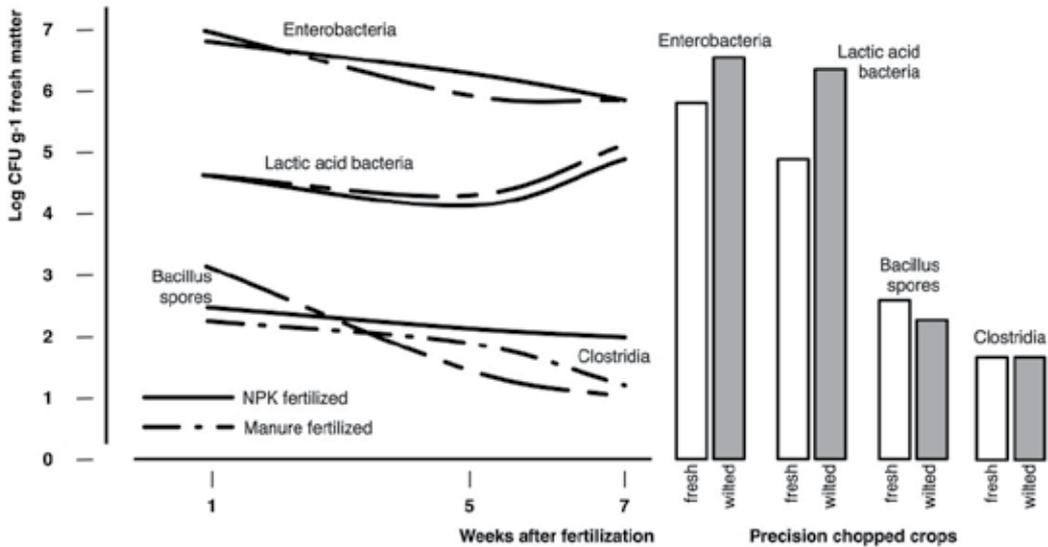
Clostridia group	Example of species
Proteolytic	<i>Clostridium sporogenes</i> <i>Clostridium disporicum</i> <i>Clostridium botulinum</i> <i>Clostridium propionicum</i>
Polysaccharides degrader	<i>Clostridium cellulolyticum</i> <i>Clostridium thermocellum</i> <i>Clostridium cellulovorans</i> <i>Clostridium phytofermentans</i>

**Table 2.** Clostridium species identified in manure (either liquid or solid)

Aeration of liquid manure may be performed on some farms before spreading on fields. Aeration has a positive effect on the number of different undesirable microorganisms by reducing number of enteric microbes like *Bacillus* sp., *Campylobacter*, coliphages, *L. monocytogenes*, *Yersinia enterocolitica* (Heinonen-Tanski et al., 1998). Different mechanisms could explain these results: presence of oxygen radicals, competition with aerobic microorganisms, increase in pH and production of nitrite (Heinonen-Tanski et al., 1998).

Fertilisation with aerated liquid manure act positively in overall quality of silage over un-aerated liquid manure (Heinonen-Tanski et al., 1998). However, clostridial spores do not seem to be affected by this treatment. Inhibition of clostridial population in silage will be more easily controlled by lactic acid fermentation (Langó & Heinonen-Tanski, 1995).

Time after spreading of livestock manures is important to allow stabilization of microbial population and thus reduction of undesirable microorganisms in silage environment. Ultraviolet radiation, water stress and competition with normal epiphytic population are the principal factors contributing to lower enterobacteria population after spreading. Östing & Lindgren (1991) and Davies et al. (1996) studied the impact of timing application on crops and silage harvesting prepared from manured forage stands. In their study, enterobacteria and coliform populations diminished after a seven weeks period following manure dressing (Figure 4). At that time, coliforms were below detection level. They also observed the impact of wilting on corresponding cell numbers (Figure 4).



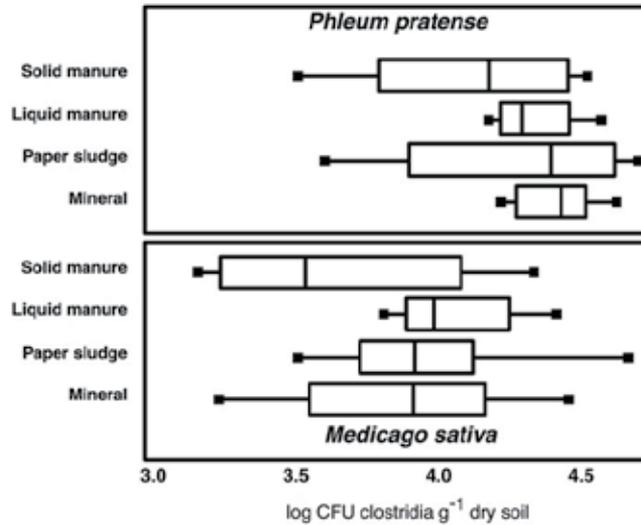
**Figure 4.** Relation of time on microbial populations on grasses fertilized with manure and mineral fertilizer and corresponding residual populations at time of harvest. Adapted from Östling and Lindgren (1991).

Clostridia spores in the soil seem to vary with forage stands following application of organic fertilizers. Figure 5 shows difference in number of soil clostridia from two different crops stands fertilized with the same four fertilizers: beef cow solid manure, beef cow liquid manure, paper sludge and mineral. Soil under alfalfa showed a clostridial population that was one order of magnitude inferior to the similar conditions under timothy.

Aggregates of farmyard manure present on the soil or on shoots could be picked up at harvesting (Rammer et al., 1994). In the silo, these aggregates create small pockets where fermentation is less efficient and thus lead to higher pH. Microorganisms developing in and around those pockets will differ from those growing only on nutrients from the crops. Enterobacteria and clostridia are present in higher number in these pockets.

Rapid development of the biogas industry will lead to high volume of digestates that will eventually be applied on crops as organic fertilizers. Clostridia are responsible for part of the microbial processes taking place during that kind of anaerobic fermentation and, at present, few studies had been conducted to study their diversity.

Regulatory offices will also require evaluation of pathogen microorganisms and coliforms before allowing spreading permits. Moreover, milk quality agencies will need to conduct investigations on the bacterial spores in relation with animal feed quality, either in pasture or silage.



**Figure 5.** Boxplot of clostridial soil population four weeks following application with four different fertilizers on two different set of plots, timothy (*Phleum pratense*) and alfalfa (*Medicago sativa*) (Lafrenière et al., 2005).

Even if application of organic fertilizers do not seem to affect soil microbial population (Anderson & Christie, 1998; Rammer et al., 1994), incorporation of organic matters will positively influence soil microbial population (Janvier et al., 2007).

In the short term, application of animal manure will increase the number of clostridial spores on the crop (Pahlow et al., 2003). Care should be taken to uniformly apply organic fertilizers to avoid entry of manure aggregates with forages while harvesting and subsequently contaminate silage. Interval between application and harvest should also be determined to minimize entry of aggregates in the silo via the harvesting machineries.

### 3.3. Contamination of the plant

Plants are in contact with clostridial spores at different moments during their growth. After seed germination, the fragile root and shoot epidermal cells are directly in contact with soil and could easily collect spores, which could then bind to microsites along surfaces and subsequently be incorporated inside cells layers as the plant grow. Clostridial spores could also penetrate root following breakage of the outside layer by mechanical action or when ruminant are grazing. Above grounds structures could also be contaminated with clostridial spores by action of raindrops on the soil and by wind dispersion of soil.

Natural plant contamination by butyric acid spores is generally below detection level when using plate counts techniques ( $\log_{10} < 2$ ). The presence of dead and decaying tissue near ground level could lead to higher counts, around 10 to 100 spores g<sup>-1</sup> plant tissue. Counts on

corn stalk, leaves, silk and maturing kernel differ by several order of magnitude (Table 3) following plate counts on RCA medium using the technique develop by Jonsson, (Jonsson, 1990). These organisms do not constitute a true epiphytic bacterial population because they develop on decaying matter, not directly from plant exudates.

Corn structure	Spore counts ( $\log_{10}$ CFU $\text{g}^{-1}$ fresh weight)
Stalk	2
Leaves	2 to 3
Silk	2.3 to 3.6
Kernel	3.4 to 3.6

Drouin, et al. unpublished data

**Table 3.** Butyric acid spore counts on different part of maize

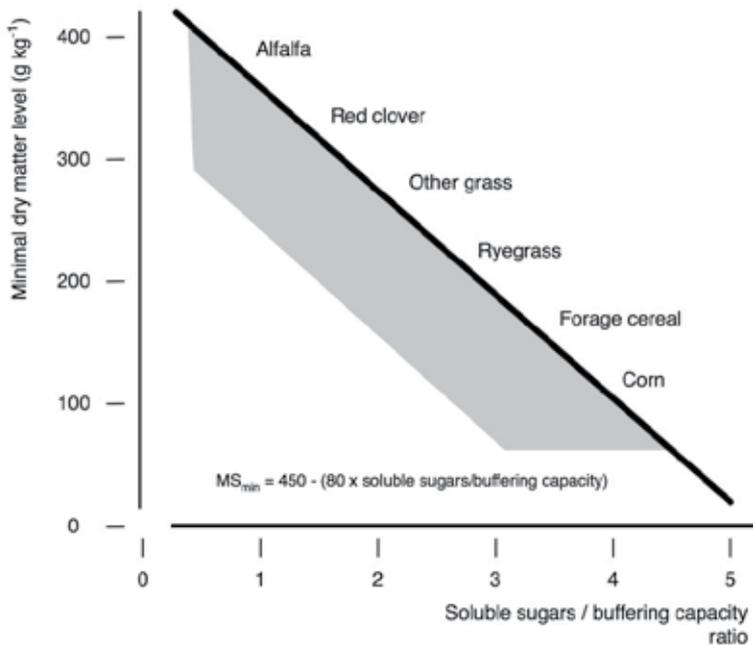
### 3.4. Silage

Silage is a conservation method that gained in popularity in the last half century all over the world (Wilkinson et al., 2003). This technique is use to store forage or other fermentable crops to feed ruminants. In livestock production, forage silage as a feed varied from 40 to 100% of the diet. The goal of silage making is to preserve the nutritional value of the forage while limiting lost of dry matter. The ensiling process to make silage is well described elsewhere and won't be presented in details here (McDonald et al., 1991; Pahlow et al., 2003; Rooke & Hatfield, 2003). From a microbiological point of view, *Clostridium* development must be avoided in the ensiling process because it may affect palatability, lower energy of the feed and may cause metabolic disorder.

Making silage is an ecological microbial process, and control of *Clostridium* development is principally made by the interaction of pH and dry matter content (Wieringa, 1969). Lowering the pH is done by lactic acid bacteria which ferment sugars released from the plant. The amount of lactic acid to lower the pH to inhibit *Clostridium* development is related to plant sugars, buffering capacity and dry matter content. The relation between these biochemical parameters had been modeled by Weissbach et al. (1974):

$$\text{DM} = 450 - 80 \times (\text{water soluble carbohydrates} / \text{buffering capacity})$$

According to this relation, sugars and buffering capacity determine dry matter content needed to ensure a good conservation. Sugars and buffering capacity vary with many factors but still fairly related to species as presented in Figure 6. Simulation model (Leibensperger & Pitt, 1987) showed that clostridial silages are favoured by lower sugar-buffering capacity ratios, lower dry matter content, lower initial population of lactic acid bacteria, and high initial temperatures, and high initial pH.



**Figure 6.** Parameters above the line should be sufficient to allow a good lactic fermentation to reach anaerobic stability. Parameters in the gray zone are at risk for a good conservation. High risk of bad conservation increases below the gray zone. (adapted from Weissbach, 1996).

Conditions in the silo could favour germination of butyric acid spores and development of vegetative cells of those organisms. Population as high as  $9 \log_{10}$  CFU g<sup>-1</sup> silage could be observed, but generally their number averaged between 3 to 5  $\log_{10}$  CFU g<sup>-1</sup> silage. Counts of clostridial spores in the silo could be highly variable. Two grass silage ensiled in clamp silo showed differences in number of clostridial spores in relation with depth. High spore concentration was observed in the first 50 cm (Spoelstra, 1990). Until recently, high concentration of butyric bacteria spores was associated with anaerobic instability of silage due to a lack of lactic acid to lower the pH during the primary fermentation phase or to a low concentration of nitrate (Kaiser et al., 2002). Visser et al., (2007a) reported high concentration of butyric bacteria spores in association with aerobic instability. These observations were also observed by Borreani and Tabacco (2008, 2010).

Butyric silage must be avoided as silage ingestion by the animal could be reduced and hygienic status might be a problem. Lactic acid molecule used for the production of butyric acid by clostridia contributes to raise the pH of the silage, as lactic acid (pKa= 3.86) is a stronger acid than butyric acid (pKa= 4.82). Consequently, this environment is less inhibitory for many other microorganisms. Moreover, as the pH raises, proteolytic clostridia species establish themselves and will degrade protein to amino acids and to ammonia (silage clostridia of group 1). These biochemical modifications will reduce the nutritional value of the feed.

Clostridia are strongly inhibited by the presence of nitrite (Spoelstra, 1985). Nitrite is an intermediate in the reduction of nitrate to ammonia and nitric oxide. High concentration of nitrate in grass forage may result from intensive grassland fertilization with nitrogen. Value could reach 1 to 8 g kg<sup>-1</sup> DM (up to 30 g kg DM<sup>-1</sup>) (Spoelstra, 1985). Even if high N fertilization contribute to lower the amounts of fermentable carbohydrates in relation with protein and thus the buffering capacity, high nitrite concentration might be beneficial. Within few hours after ensiling, bacteria and plant start to reduce nitrates leading to nitrite and nitric oxide accumulation during the first week. This period is crucial for the silage quality as it corresponds to time where germination conditions are optimal for clostridial spores, because pH has not reach anaerobic stability level.

Interestingly, clostridia are also able to reduce nitrate to ammonium. Experimentation with irradiate grass inoculate with *C. tyrobutyricum* had shown that self-inhibition by nitrite had not occurred, even though nitrate was completely reduced (Bousset-Fatianoff et al., 1971). In clostridia, nitrate reduction is coupled with dissimilating nitrate mechanisms, not with electron transport phosphorylation. Ammonia acts as an electron sink during fermentation processes. So, when clostridia use ammonium as electron acceptor, less butyrate and acetate are produce since these compounds are also electron acceptor for their metabolic pathways (Spoelstra, 1985). Inhibition of clostridia by nitrite act synergistically with the acidic pH of the environment. In *C. sporogenes*, the phosphoroclastic system in which pyruvate is oxidized to acetate and responsible for ATP production is inhibited by NO, a product of nitrate decomposition under acidic conditions (Woods et al., 1981).

Enterobacteria could have positive effect on *Clostridium* development. Enterobacteria are able to grow under anaerobic condition and are facultative anaerobes, catalase positive. They can reduce nitrate and strictly depend on fermentable carbohydrates (McDonald et al., 1991). On the other hand, some enterobacteria species could decarboxylate and deaminate amino acids and utilize nitrogenous constituents of organic compounds as energy-yielding compounds is their respiration chain. Even if their presence in generally undesirable in silage because they compete with lactic acid bacteria for nutrients, they may also reduce nitrate to ammonium, thus increasing the buffering capacity of the silage and delay the rapid decline of pH.

### 3.5. Contamination of milk

High number of butyric spores has an indirect role in cheese quality as their presence in some type of cheese may cause a defect called "late blowing". Chemical composition of raw milk does not present a favourable environment for clostridial spore germination, so acting as a transition medium for these spores. Cheese fermentation by lactic acid bacteria allows accumulation of lactic acid that will induce germination of spores. Favorable conditions for late blowing are mainly observed in hard and semi-hard cheeses, including Emmentaler, Gouda, Edamer, Comté, generally shortly aged and of high pH. Dryer cheese, like Parmesan and Cheddar are generally less affected.

Late blowing symptoms present appearance and gustative changes in the cheese, following fermentation of glucose and xylose to butyric acid and gas, mainly hydrogen and CO<sub>2</sub>. Gas production will show balloon-like expansion (Figure 7) and a rancid distasteful consistence is obtained (Innocente & Corradini, 1996). Four clostridial species are frequently observed in late blowing cheese: *C. butyricum*, *C. sporogenes*, and *Clostridium beijerinckii*, with *C. tyrobutyricum* being the principal culprit (Cocolin et al., 2004).



**Figure 7.** Example of late blowing defect following gas production.

Level of contamination by butyric acid spores in milk could be controlled before and after milking. As mentioned in previous sections, high contamination level of butyric acid spores in silage could be an important factor and several cheese producers required that their milk suppliers feed no silage to their milking cows. This should also apply for corn silage as high counts of clostridial spores were observed in aerobic deteriorated silage (Vissers et al., 2007a). Good management of silage constitute the first step in controlling subsequent contamination steps. In order to prevent development of butyric acid spores in silage, care should be taken to reach an excellent conservation of the silage in order to reduce contamination of butyric acid spores in raw milk via dirty teats (Table 4).

Animal behaviour must also be considered as part hygiene program on the farm. Some cows prefer to lie down on dirty patches. Teats of these cows are generally more heavily contaminated with feces conducting to contamination of milk (Visser et al., 2007b).

After milk being collected on farms, milk could be process differently according to different regulations dictated by countries. Pasteurisation has no effect on clostridia, as their spores are heat tolerant. Other techniques should be use to remove butyric spores.

In Europe,, most countries allow the utilization of lysozyme or nisin. Lysozymes are acid hydrolase enzymes produced by animal cells, like lymphocyte or egg white cells that hydrolyse bacterial cell wall. Lysozymes from egg white (0.5 % dry weight) are able to kill most vegetative clostridial cells, but have no direct action on the spores (Wasserfall & Teuber, 1979). Lysozymes are classified within the Class I enzyme and are therefore

considered acceptable as food additive. Adding egg white lysozymes in cheese is allowed in several countries (European Communities Directive no. 95/2/EC).

Nisin are antibacterial peptides and classified as lantibiotic, produced by different species of the Order Lactobacilliales. This family of bioactive peptides have the capacity to kill bacteria via pore formation in cell-wall following binding with Lipid II (Hsu et al., 2004) in different pathogen, like listeria and clostridia. Similar to lysozymes, nisin mode of action also target vegetative cells and has no effect on their spores. Addition of Nisin E234 (from *Streptococcus lactis*) in different foods was approved by the Food and Agriculture Organisation of the United Nations.

New filtration and centrifugation technique now allow removal of bacteria and bacterial spores in milk (Su & Ingham, 2000). Bactofugation use high-speed centrifugation technique that could often be performed at pasteurization temperature. Bactofugation will remove 86 to 92 % or aerobic spores and between 91 and 97 % of anaerobic spores.

Strategies	Actions
Lower butyric acid spores contamination at ensiling	<ul style="list-style-type: none"> <li>• Set mowing cutting height above 7 cm to minimize soil entry in the silo.</li> <li>• Limit manure aggregates in the silo.</li> <li>• Silage additive may help under sub-optimal conditions.</li> </ul>
Develop an efficient lactic fermentation	<ul style="list-style-type: none"> <li>• Wilt to recommended level in accordance to the silo type.</li> <li>• Fill and close rapidly the silo to restrict infiltration of air in the silo.</li> <li>• Adding a lactic inoculant will contribute to faster the lactic fermentation.</li> </ul>
Ensure good aerobic stability	<ul style="list-style-type: none"> <li>• All previous actions to minimize development of butyric bacteria will help.</li> <li>• Ensure high compaction and well sealed silo.</li> <li>• Never wilt over 500 g kg<sup>-1</sup>DM</li> <li>• Silage inoculants may help.</li> <li>• Silage removal at feed out should be adapted to the type of silo, the herd size and the season.</li> </ul>
Good milking hygiene	<ul style="list-style-type: none"> <li>• Routinely practice good hygiene in milking parlours with excellent teat cleaning before milking the cows</li> </ul>

**Table 4.** Strategies to reduce butyric acid spores contamination in Silage (Lafrenière et al., 2008)

Microfiltration technique use ceramic microfiltration membranes that are able to retain suspended particles, microorganisms and fat. Protein, carbohydrates, minerals and acids contain in the milk will pass through. Microfiltration will remove more than 99 % of the aerobic and anaerobic spores of milk. Both centrifugation and microfiltration will modify milk composition and are mainly use for commercial cheese manufacturing, since only skim milk could be use and organoleptic characteristics of raw milk are modified.

#### 4. *Clostridium botulinum*

Considering other clostridia species in silage, *Clostridium botulinum* needs to be introduced in relation to potential health risk for the herd. Like butyric acid spores, *C. botulinum* is group within Cluster 1 (Collins et al., 1994). This specie is normally found in soil and frequently present in the animal gastrointestinal tract (Holdeman, 1970). *C. botulinum* produces heat-labile neurotoxin that could be lethal to man and other animals. Five different toxins have been characterized and identified as A, B, C, D, E, and F. By convention, strain that produces toxin D is identified as *C. botulinum* type D. Disease induced following ingestion of these toxins is called botulism.

Types C and D are generally associated with animal botulism. Five factors are required for an animal to suffer from botulism: presence of viable organism, an environment that will support growth and toxin production, ingestion of toxin, absorption of toxin, and susceptibility of the host (Holdeman, 1970). The toxin acts by inhibiting production of acetylcholine in nerve cells connected to muscles, leading to paralysis of the lung or other locomotor muscles. Most cases are related to ingestion of contaminated foods.

Important outbreaks of botulism in cattle had increased over the past decades (Lindström et al., 2010). This trend may be explained by the growing use of plastic-packaged forage silage, allowing the growth and toxin production from *C. botulinum*. These outbreaks are often large, affecting hundreds of animal (Steinman et al., 2007), causing enormous economic losses due to death of intoxicated animals and reduction in milk production (Yeruham et al., 2003). Prevalence of botulism is often seasonal and linked to feed consumed by the animals.

In the vast majority of cattle botulism outbreaks, the source of the neurotoxin is the feed. Contamination routes differ according to *C. botulinum* types. A frequently reported source of type C spores is poultry litter. Distribution of poultry litter as manure on grass stand could lead to subsequent silage contamination. Silage presenting fermentation problems will not restrict growth of botulinum spores thus, risk of botulism exists when feeding these silages to cattles (Lindström et al., 2004). Presence of small animal carrions, like mouse, in silage is a source of type C and D botulism. Spores present inside gastrointestinal tract of carrions will germinate and subsequently will produce toxins that subsequently contaminate surrounding silage. Type B botulism in cattle seems to be more frequently associated with rye, barley, brewer's grain and maize (Lindström et al., 2010). Spore cycle is a source of recontamination in cattle herds since botulism spores are fairly persistent in feces.

Cattle environment introduce important risk of contamination of milk by botulism spores, but studies on the prevalence and contamination level of raw milk are scarce. Milk counts showed that numbers were generally under detection level (2040 spores L<sup>-1</sup>). Milk products, like cheese, may have contamination level around 10 spores g<sup>-1</sup> cheese (Franciosa et al., 1999). Cheeses that may be contaminated are the same as the ones with butyric acid spores.

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*Edited by Narongsak Chaiyabutr*

Book “Milk Production - An Up-to-Date Overview of Animal Nutrition, Management and Health” is organized into three main sections, and is concerned with the animal nutrition, animal management and, breeding and animal health. This book permits the reader’s exposure to the expert’s experience and scientific style of interpreting and integrating available data into his own views. This book will be useful for students, researchers, teaching staff, practicing professionals connected with dairy science, animal science, food science, nutrition, physiology, biochemistry, veterinary medicine and other related fields. Each chapter in this book has an extensive bibliography which can future aid the reader in keeping abreast of the developments in this field.

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