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Animal Welfare

*Edited by Muhammad Abubakar
and Shumaila Manzoor*



ANIMAL WELFARE

Edited by **Muhammad Abubakar**
and **Shumaila Manzoor**

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Meet the editors



Dr. Muhammad Abubakar, a scientist from the National Veterinary Laboratory, Islamabad, Pakistan, has over 15 years' experience in various areas of veterinary sciences. His expertise on transboundary animal diseases (TADs) at national and international levels is well known. He has established various diagnostic laboratories for the diagnosis of TADs. Dr. Abubakar has been involved in the organization and conduct of training for field and laboratory staff. He has published numerous research papers, review articles, and book chapters on different areas of veterinary sciences. He has also worked in academia in teaching and research supervision at graduate and undergraduate levels. He is currently supervising two journals in the area of veterinary sciences (*Research Journal for Veterinary Practitioners and Veterinary Sciences* and *Research and Reviews*) as an editor-in-chief.



Dr. Shumaila Manzoor, a scientist and laboratory worker from the National Veterinary Laboratory, Islamabad, Pakistan, has over 6 years' experience in various areas of veterinary disease diagnosis. Her expertise on transboundary animal diseases (TADs) is well recognized and she has worked with FAO control programs of TADs in Pakistan. Dr. Manzoor has been involved in training for field as well as laboratory staff. She has published research papers, review articles, and book chapters on different areas of veterinary disease diagnosis and control.

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Oscar Madzingira

Preface

It is essential to understand the recent advances in animal welfare and its implication in changing global scenarios.

Open accessed and clearly offered, *Animal Welfare* provides a concise introductory chapter and details three aspects of animal welfare. This is the first edition of the book so it covers the introductory level of topics that are written specifically for veterinary and animal health students and scientists. These topics provide an excellent introduction to animal welfare and its application to emerging scenarios. The book is self-contained, with everything needed to understand the importance of animal welfare and its perspectives.

In the first section on international scenarios, the focus is on the global perspectives of animal welfare. In this introductory section, there is a detailed description of the importance of animal welfare for changing global perspectives. The way forward and future concepts are also covered under this section. Another important area covered in this section is the welfare indicators linked to resistance to disease.

In the second section, the book provides emerging areas related to animal health and welfare in which three different chapters are included. The first chapter covers health assessments in sea turtles. The second looks at the impact of lameness in dairy cattle welfare. The third chapter analyzes the welfare of pet animals and zoonosis.

The third section links animal welfare to husbandry practices. Two key chapters are included: one is bovine rational taming and its relationship to welfare and the other examines animal welfare considerations in food producing animals.

Examples are drawn from the field of veterinary and dairy science throughout the book, and a clear account is provided of the importance of different areas of animal welfare. The various concepts are clearly and concisely expressed. The writing is clear, realistic, and quite positive on various aspects for easy understanding.

The book covers the following main areas:

- Global scenarios
- Animal health and welfare
- Husbandry and welfare

Each section comprises chapter(s) from renowned experts from each area and gives readers a unique opportunity to explore the topics.

I would like to express my gratitude to all the contributors of this book, including the authors of the accepted chapters. My special thanks go to the Author Service Manager, Ms.

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Global Scenario

Introductory Chapter: Animal Welfare—Global Perspective

Muhammad Abubakar, Shumaila Manzoor and
Abdullah Iqbal

Additional information is available at the end of the chapter

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1. Introduction

Animal welfare is gradually becoming a critical issue in the world. No religion of the world promotes malicious treatment to animals; all of them advocate to treat animals with kindness [1]. There is no recognized definition of animal welfare rather animal welfare definition changes from country to country and sometimes even different regions of the same country [2]. We lack any specific criteria to measure animal welfare. Humans treat animals depending upon their socioeconomic status and surroundings [3].

Many researchers use five freedoms concept to define animal welfare. According to this five freedom concept, animals should be “free from hunger and thirst, free from discomfort, freedom from pain and disease, freedom from fear and distress, free to express normal behavior” [4]. According to OIE statement, animal welfare means how an animal is surviving in an environment. The animal is either healthy, relaxed, well fed, and expressing innate behavior or suffering from pain and disease [5].

Two basic reasons for treating animals badly are poverty and the dearth of knowledge. In most of the countries, pack and draught animals are regularly overburdened and abused [6]. In modern, intensive farming animals are given better feed, better disease control, and climate-regulated facilities, but on the other hand, with subsistence system, animals have more likelihoods of expressing natural behavior and move around [7].

Animal welfare was added to the Organization for Animal Health (OIE) Strategic Plan for the period 2001–2005. Development of explicit animal welfare standards by OIE has diverted the global attention toward farm animal welfare. Animal welfare standards give the option to

member countries to exert international pressure OIE [8]. For example, if a member country violates the standards, other countries and agencies can ask for corrective action. Trade of animals and their products is mostly based on bilateral or multilateral agreements between contracting countries, so the role of World Trade Organization is very limited. In such contracts, generally countries overlook the animal welfare standards to lure new markets [9].

2. Animal welfare and international trade

Establishment of OIE guidelines provides a real advancement in the area of animal welfare that can be applied to global scale. The OIE guidelines do not force the member countries for national legislation. Enforcement of the guidelines is beyond the mandate of OIE. These guidelines provide a basic outline for countries to adopt them according to their own resources, political policies, and social structure just like other international agreements, for example, child education and climate change [10].

These guidelines can influence the international trade within the trade rules of the World Trade Organization (WTO). The agreement on the Application of Sanitary and Phytosanitary Measures allows that the importing country can ban the supply if it is harmful to the health of humans, plants, or animals. If any supply is banned due to animal welfare issues on the basis of two points that can be challenged [9]. First, it is a clear fact that animal welfare is linked with the incidence of diseases and injuries, but this relationship is not opined as the point on which importing country can ban on imports of animals from violating country or company. Second, according to WTO rules, two similar products cannot be differentiated on the basis of how these are produced [11].

On the other hand, consumer demand has compelled many multinational companies to consider animal welfare as an issue [12]. This trend has forced many multinational food companies to market products that meet animal welfare standards. NGOs working on animal welfare are putting pressure on food companies to label the animal welfare status of their products. Many exporters improved the animal welfare practices to meet the requirement of their trade partners [13].

3. Welfare of farm animals

Farming trends have been totally changed during the last century. The world has shifted to intensive farming that has increased the production of animals and decreased the expenses. This system seems very good from economic point, but animal welfare is mostly neglected in this system [14]. In intensive system, animals are considered as product manufacturing machines not as living creatures. Animals are kept inside the shed for most of the day and cannot show normal behavior. Concrete floors of housing increase the chances of hoof problems in cows [15].

Veal crate was another example of brutality to young calves. The EU has banned the veal crate from 2007. The EU has also banned sow stalls from 2013 [16]. Wild pigs do rooting and foraging for 75% of their daylight hours [17]. Such activities are not possible in intensive farming systems where pigs are kept in overcrowded pens. These frustrated creatures start biting tails of each other and farmers performed tail docking to control these vices [18].

4. Welfare of poultry birds

Cage system is used for rearing laying hens for commercial purpose all around the world. The European Union has banned the battery cage system for rearing laying hen from 2012 due to health issue and animal welfare. But other developed countries like New Zealand, Australia, and US have no such regulations for the layer. India is the third largest egg producing country, but has no rules and regulations regarding the spacing of laying hens in cages [16]. Actually, in India, per hen space in battery system is less than anywhere in the world. Just like India, in other Asian and African countries, welfare of laying hens is ignored by the community and government [19].

In contrast to layers, broilers are kept on litter. Two major problems that broilers face is overcrowding and rapid growth [20]. In 2010, the European Union has set the regulations for spacing of broilers depending upon the mortality rate of the farm and environment control of shed [21]. Other large poultry meat producing countries such as Brazil and Thailand kg/m² spacing is lower than EU law because of high temperature, humidity, and cheaper land [22]. In Asia, there is no such defined bird spacing rule is present. There is no regulation regarding increased leg deformities in broilers due to the rapid growth anywhere in the world.

5. Welfare of farmed fish

Fish farming is very ancient business in many countries of the world. In this century, fish farming shows rapid growth and adopts new husbandry systems. Asian countries dominate this industry by having more than 70% share in fish and fish product exports, whereas European countries have only 8% share [23]. Regardless of that much production, Asian countries lack any rules and regulations for transport and killing of fish. EU follows the OIE standards for transport, stunning, and killing of fish [24].

6. Welfare of wildlife

Wildlife species living near the human population are most affected by human acts. Increase in human population is also putting pressure on the wildlife species. For safety reasons, humans kill many predators that enter in civic areas, for example, wolf encounters in Central Europe. Stray dogs are killed very brutally in many countries. For example, in Kenya, stray

dogs are killed by strychnine. Wild boars are killed frequently in many countries because they damage gardens and crops [25].

Overpopulation of elephants in Kruger National Park, South Africa has been affecting other animal's habitats too. For population control of elephants, many methods are suggested by the South African Ministry of Environmental Affairs and Tourism such as culling, fertility control, translocation, and capture. Many NGOs working for the elephants' preservation opposed these steps and opined that overcrowding of elephants is mainly due to the increase fencing of the park [26]. In Yellowstone Park, around 3500 bison were killed due to fear of possible outbreak of brucellosis [27]. When we talk about wildlife welfare, there are two concepts: first, when only welfare of one species is under consideration and second, when we focus on ecoethics. Humans are responsible for whole ecosystem welfare [28].

7. Conclusion and future prospect

Animal welfare has become a topic of very serious discussion now a day. There are chances with increased public awareness that animal welfare can become the cornerstone for trade of animals and poultry products to developed countries. Multinational companies can act as a role model for other companies by adopting animal welfare standards. Global trade is also a bright opportunity for less developing countries. If these countries adopt OIE animal welfare standards and produce products according to those standards, they can compete with developed countries. There would be more profit for developing countries because they have inexpensive land and labor. On the other side, better welfare standards also result in better health of animals. Humane transport of poultry would decrease mortality and better carcass quality. Good feeding and housing of dairy animals would sequentially increase the milk production. It is the need of the hour that the WTO should also consider animal welfare as an issue and amend its rules.

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Welfare Indicators Correlated with Resistance to Disease: The Romanian Experience

Silvana Popescu, Eva Diugan,
Carmen Dana Sandru and Marina Spinu

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Abstract

The chapter aims to reveal the importance of indicators in defining equine welfare and their relationship with the immune system and subsequent resistance to diseases. The sharp economic changes after the World War II changed the role played by horses in the society. The improvement of modern breeds, their involvement in different equestrian activities, the development of sports horse shows and industry, the increase of the economic value of the individuals on the basis of their performances, as well as the emergence of modern “natural” training theories implicitly led, in developed countries, to a change of public attitude toward this animal. According to World Organization for Animal Health (OIE) principles, animal welfare and animal health are closely linked. The correlated use of the welfare assessment by direct indicators with the investigation of the immune status proved to be a powerful tool in the interdisciplinary research on how the horses cope with different management conditions and the health outcome of different raising technologies. The dimensions of this approach have not been exhausted by far in this study which is merely a first step taken in Romania to a better understanding on protection and use of these animals.

Keywords: horses, welfare indicators, indicator ranking, seasonal variability, raising system

1. Introduction

The World Organization for Animal Health (OIE) defines animal welfare as a multifaceted public policy issue that includes scientific, ethical, economic and political dimensions Belton [1]. Concerns about animal welfare and the knowledge available in this area nowadays represent the sum-up of the results that emerged and accumulated from numerous studies conducted over the years. The principles on animal welfare underlying OIE very well synthesize

the level where this area finds itself currently as follows: (1) animal welfare and animal health are closely linked; (2) the animal's five freedoms lie at the basis of well-being; (3) animal welfare standards are scientifically well-founded; (4) ensuring animal welfare safeguards the well-being of mankind; (5) the use of animals must be carried out with ethical responsibility in order to ensure their well-being; (6) a high level of animal welfare would increase animal productivity and would provide economic benefits; (7) equivalent results based on performance criteria are the basis for comparing animal welfare standards [1].

Although the expansion of scientific research and the number of experimental researches in all sectors where horses are used is very high, it seems that so far no systematization and/or synthesis of the results have been achieved to provide neither practical assessment tools nor methods for influencing equine status. There is a high inconsistency of the nature and content of legal documents referring to horses, of the descriptions of activity fields in which horses are used, and of the ways these animals are perceived by owners/caretakers in different countries of the world.

During the twentieth century, the massive reduction in military and agricultural uses of horses in the developed countries occurred simultaneously with the increase in the numbers of animals used for sports, competitions and leisure [2]. The improvement of modern breeds, their involvement in different equestrian activities, the development of sports horse shows and industry, the increase of the economic value of the individuals on the basis of their performances, as well as the emergence of modern "natural" training theories implicitly led, in developed countries, to a change of public attitude toward these animals. From the economic point of view, there are countries that receive considerable income from equestrian activities. According to the International Equestrian Federation (EIF), Europe's equine industry is a growing sector, with the number of riders rising annually by about 5% [3].

Equine, as any other mammalian well-being status, is connected with resistance to disease. Due to their versatility, horses, when compared to other farmed species, are even more exposed to various types and levels of stress, thus showing a high variability of immune responses and subsequent susceptibility to microbial infections. Technologies include immunological prevention of microbial diseases by vaccination, but welfare indicators are also very important in measuring the outcome of these operations.

2. Welfare indicators in Romanian horses

2.1. The welfare assessment of the studied horses: working protocol

Horses under two different raising systems, private and state stud, were subjected to the evaluation [4]. The animals were assessed in two different seasons: privately owned working horses (171 animals in the winter and 168 in the summer) and breeding horses (breeding stallions: 62 heads in the winter and 66 in the summer; broodmares and young horses: 137 animals in the winter and 146 in the summer; reproduction stallions: 14 heads in the winter and the same 14 during the summer). The keeping and housing conditions were highly

variable in the privately owned horses, mostly in barns along with other domestic animals of the household. The breeding horses as the property of a state stud were kept under standard conditions regulated by the national law.

This chapter describes the methodology of development and initial testing of the horse welfare assessment protocol, than presents its final version [5–9]. The protocol was constructed using 30 indicators (selected based on their relevance in a previous field-testing). Out of all the indicators, only four were indirect, resource based measures. The indicators were grouped based on the five freedoms of animal welfare, each of these investigating specific aspects of one of the freedoms. Being a practical tool, the protocol contains the detailed methodology for the assessment of each indicator (specifying the source in case of adaptations from other authors), the possible scores and their assignment methods, providing also exemplifying pictures for the majority of the measures. A specific feature of this protocol was the assignment of minimal scores for the negative variants of the indicators and of the maximal scores where the animal showed good welfare status. In addition to the evaluation based on the assessment protocol, supplementary data were recorded, based on the responses of the owners to a questionnaire.

The individual welfare score was calculated by summing up the scores for each assessed indicator; the classification of the horses in qualitative welfare categories was based on the use of a grid with numerical limits. All the animals included in the study were assessed using this protocol.

In order to evaluate the freedom from hunger and thirst, the body condition score was employed and data were recorded regarding the frequency and ways of watering for each horse (owners' questionnaires based answers). For the investigation of the freedom from discomfort, scores were assigned depending on the fecal soiling of the hindquarters and of the lateroventral abdomen and for the presence or absence of skin lesions on the hip point. During the assessment of freedom from pain, injury and disease an inspection was made concerning the quality of the horses' hair coat, mane and tail, presence of skin lesions (on the body, on the lower part of the legs, on lip corners and in the harness contact points), swellings of tendons/joints, hoof horn quality, the shape of the hoof, the quality of the sole, the length of the hooves, the aspect of the shoes and shoeing quality, the presence or absence of hampered respiration, secretions from the nostrils or eyes, coughing, diarrhea; also the presence of sight was tested and the owners were asked about checking of the horses' teeth. For this part of the assessment, the gait of the horses was also inspected. The freedom to express normal behaviors consisted of asking the owners about the social company (of their conspecifics) of the horses and their access to free exercise. For the investigation of the freedom from fear and distress, the general alertness of the animals was observed and three behavioral tests were employed to explore the quality of the human-horse relation.

The results were centralized and processed for the analysis of the welfare indicators. The prevalence of each possible variant for each indicator was calculated; these were afterward compared among categories and seasons. To offer an overview of the most important results, only those indicating welfare problems of the assessed horses were presented here (**Tables 1–5**).

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
I. FREEDOM FROM HUNGER AND THIRST									
<i>Body Condition Score (BCS)</i>									
BCS unacceptable	23.98 ^a	18.45 ^{ab}	1.61 ^c	0 ^c	15.34 ^{abc}	8.22 ^{bc}	0 ^{bc}	0 ^{bc}	P<0.05
<i>Watering Pattern and frequency</i>									
1.2 times/day	5.26	4.76	0	0	0	0	0	0	P>0.05

Table 1. The prevalence of welfare problems identified in the horses in different keeping conditions and seasons, within the investigation of the freedom from hunger and thirst. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant ($P > 0.05$); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different ($P < 0.05$).

2.2. Results and discussion

The prevalence of unacceptable body condition scores in horses was lower than that indicated in reports of the studies assessing working horses in the developing countries. Except for the working horse category evaluated during winter, the majority (60.12–100%) of the animals had good body condition. Almost a quarter of the working horses were found in unacceptable body condition in the winter (**Table 1**) and such cases were also observed in other horse categories, at a lower extent. The inadequately low watering frequency (once or twice per day) was recorded exclusively in the working horses (**Table 1**). Nevertheless, from all the categories, only the reproduction stallions had unlimited access to drinking water. Together with other factors (insufficient feeding in the first place), the reduced water consumption lead to body weight losses in horses, by limiting the feed ingestion and lowering the efficiency of the nutrient usage.

The assessment of barn hygiene based on the body hygiene of the animals is frequently used in dairy cows [10–13], but not in horses, since it is considered that the owners clean their horses whenever they leave the stable. The high prevalence of horses having their hindquarters and lateroventral abdomen soiled by dejections proved that this indicator was fit to the conditions of the present study, but also demonstrated the ignorance of the owners regarding the body hygiene of their animals. The prevalence of horses with dirty rumps showed significant differences among the studied categories, but also between the seasons (**Table 2**). The horses kept on pasture or housed in stables with a decent amount of bedding and mechani-

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
II. FREEDOM FROM DISCOMFORT									
<i>Dirty hindquarters and lateroventral abdomen</i>									
Dirty	54.97 ^a	45.24 ^a	0 ^{bc}	0 ^{bc}	16.79 ^b	0 ^c	0 ^{bc}	0 ^{bc}	P<0.05
<i>Lesions on the external points of the ileum</i>									
Wounds	15.79 ^a	11.4 ^a	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	P<0.05

Table 2. The prevalence of welfare problems identified in the horses in different management conditions and seasons, within the investigation of the freedom from discomfort. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant ($P > 0.05$); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different ($P < 0.05$).

cal cleaning in place did not show fecal soiling of their bodies, in spite of not being groomed daily. These results confirmed the importance of stable cleaning and bedding usage for the horses' comfort of rest [14].

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
III. FREEDOM FROM PAIN, INJURY AND DISEASES									
<i>Hair coat quality</i>									
Abnormal	30.4 ^a	27.98 ^{ab}	9.67 ^{bc}	16.67 ^{abc}	16.06 ^{bc}	10.27 ^c	0 ^{abc}	0 ^{bc}	P<0.05
<i>Quality of the mane and tail</i>									
Abnormal	20.47 ^a	20.84 ^a	11.29 ^a	7.58 ^a	16.06 ^a	6.85 ^a	35.71 ^a	21.43	P<0.001
<i>Body lesions</i>									
Superficial	29.82 ^{ab}	33.93 ^a	11.29 ^c	15.15 ^{bc}	10.22 ^c	7.53 ^c	7.14 ^{abc}	0 ^{bc}	P<0.05
<i>Lesions on the lower parts of the legs</i>									
Severe	6.44 ^{ab}	10.71 ^a	8.06 ^{ab}	6.06 ^{ab}	0.73 ^b	0 ^b	0 ^{ab}	0 ^{ab}	P<0.01
<i>Lip corner lesions</i>									
Present	22.23 ^a	24.4 ^a	9.68 ^{ab}	6.06 ^b	0 ^b	0 ^b	0 ^{ab}	0 ^{ab}	P<0.05
<i>Harness contact point lesions</i>									
Wounds	21.64 ^a	19.05 ^a	0 ^b	0 ^b	0 ^b	0 ^b	0 ^{ab}	0 ^{ab}	P<0.001
<i>Swellings of tendons/joints</i>									
Tendon and joint	17.54 ^a	21.43 ^a	9.68 ^{ab}	12.12 ^{ab}	0 ^b	0 ^b	0 ^{ab}	0 ^{ab}	P<0.001
<i>Hoof horn quality</i>									
Abnormal	40.94 ^a	39.91 ^a	27.42 ^{ab}	21.82 ^{ab}	5.11 ^c	0 ^c	0 ^{bc}	0 ^{bc}	P<0.05
<i>Shape of the hoof</i>									
Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
Abnormal	33.33 ^a	25 ^{ab}	8.06 ^{bc}	6.06 ^c	10.95 ^c	7.53 ^c	0 ^{bc}	0 ^{bc}	P<0.05
<i>Sole surface quality</i>									
Abnormal	24.56 ^a	23.22 ^a	6.45 ^b	7.58 ^b	5.11 ^b	0 ^b	0 ^{ab}	0 ^{ab}	P<0.05
<i>Too long/too short hooves</i>									
Yes	52.63 ^a	52.38 ^a	32.26 ^{ab}	24.24 ^b	13.87 ^b	12.33 ^b	14.28 ^{ab}	21.43 ^{ab}	P<0.01
<i>Improper shoes</i>									
Yes	61.98 ^b	71.43 ^b	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^b	P<0.001
<i>Abnormal gait</i>									
Abnormal gait	13.45 ^a	17.26 ^a	8.06 ^{ab}	9.05 ^{ab}	2.92 ^b	2.05 ^b	0 ^{ab}	0 ^{ab}	P<0.05
<i>Dispnea (hampered respiration)</i>									
Present	33.34 ^a	22.62 ^a	14.52 ^{ab}	15.15 ^{ab}	4.38 ^b	2.05 ^b	0 ^b	0	P<0.01
<i>Coughing</i>									
Present	19.3 ^a	13.1 ^a	6.45 ^{ab}	10.6 ^{ab}	0 ^b	0 ^b	0 ^{ab}	0 ^{ab}	P<0.01
<i>Nasal secretions</i>									
Present	17.54 ^a	11.31 ^{ab}	8.06 ^{ab}	0 ^b	3.65 ^b	2.05 ^b	0 ^{ab}	0 ^{ab}	P<0.001
<i>Eye secretions</i>									
Present	10.53 ^a	5.96 ^{ab}	6.45 ^{ab}	4.54 ^{ab}	1.46 ^b	0 ^b	0 ^{ab}	0 ^{ab}	P<0.01
<i>Blindness</i>									
Both eyes	1.75	4.17	4.84	0	0	0	0	0	P>0.05
<i>Diarrhea</i>									
Present	9.36 ^{ab}	10.71 ^a	0 ^{bc}	4.54 ^{abc}	0 ^c	0 ^c	0 ^{bc}	0 ^{bc}	P<0.05
<i>Checking of teeth</i>									
Never	88.31 ^{ab}	92.26 ^a	93.55 ^a	86.36 ^{ab}	93.43 ^a	92.46 ^a	71.43 ^{ab}	64.28 ^b	P<0.05

Table 3. The prevalence of welfare problems identified in the horses in different management conditions and seasons, within the investigation of the freedom from pain, injury and disease. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant (P > 0.05); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different (P < 0.05).

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
IV. FREEDOM TO EXPRESS NORMAL BEHAVIORS									
<i>Social company</i>									
None	26.31 ^{abc}	31.55 ^a	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c	P<0.05
<i>Access to free exercise</i>									
None	88.89 ^a	60.12	100 ^a	100 ^a	0 ^c	0 ^c	0 ^c	0 ^c	P<0.001

Table 4. The prevalence of welfare problems identified in the horses in different keeping conditions and seasons, within the investigation of the freedom to express normal behavior. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; Table 4 legend: BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSs = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant ($P > 0.05$); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different ($P < 0.05$).

The presence of the hip point (ileum) lesions was used as an indicator of the comfort around resting only in Romania. The preexistent individual conditions accelerating the development of decubital lesions on the bony parts of the animals' body include the loss of the natural padding in thin horses but also the precarious hygiene of the skin. Yet, it seems that the main factor triggering these injuries is the inappropriate quality of the resting surface, as these lesions were found even in obese horses or those not having any other body lesions. The statistically nonsignificantly higher ($P > 0.05$) prevalence of the hip wounds and scars in the working horses during the winter compared to summer, confirm identical causality in both seasons; during the summer part of the animals were housed outside the barns, where they could find softer surfaces on the ground to rest.

Table 3 presents the differences between the horse categories assessed for the prevalence of welfare problem indicators in the frame of freedom from pain, injury or disease.

According to recent studies, the good quality of the hair coat could be a valuable indicator of good welfare. In the present study, the prevalence of normal hair coat was significantly lower in the working horses ($P < 0.05$) (**Table 3**), compared to stallions, broodmares and young horses during the warm season.

The injuries, on both the horse's body and legs, were more frequent in working horses than in those used for reproduction (**Table 3**). However, the risks for specific welfare problems are different, according to the way the horse is used. Among all the breeding horses, the highest exposure to the risk of injury was in the category of breeding stallions, because of their fights in the case of accidentally untying in the stables [15].

The lip corner lesions produced by the use of the bit and the injuries on the harness contact points were considered "marker" lesions for the improper use of the horses, their prevalence being significantly higher ($P < 0.05$) in this study within the working horses' group than in the animals used for breeding (**Table 3**). Some risk factors contributing to the development of the lesions at harness contact points are the thin body condition by losing the protection of the subcutaneous fat layer, the omission of brushing out the dirt from the horses' hair coat before harnessing and the usage of improper harnesses. Even though the working horses in

Indicators	% in each season and category								P
	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs	
V. FREEDOM FROM FEAR AND DISTRESS									
<i>General attitude</i>									
Apathy	2.34	1.78	3.23	3.03	0	0	0	0	P>0.05
<i>Response of the horse in the human approach test</i>									
Aggressiveness	1.74	2.38	0	0	5.11	4.11	0	0	P>0.05
Fear/avoidance	22.23	20.84	22.58	25.76	22.62	25.34	0	0	P>0.05
<i>Response of the horse in the human walk besides test</i>									
Aggressiveness	4.68	5.37	0	0	5.84	6.16	0	0	P>0.05
Fear/avoidance	45.03 ^a	38.69 ^{ab}	30.65 ^{ab}	31.81 ^{ab}	35.77 ^{ab}	38.36 ^{ab}	0 ^a	0 ^a	P<0.05
<i>Response of the horse in the human physical contact test</i>									
Aggressiveness	5.26	8.34	0	0	8.76	8.22	0	0	P>0.05
Fear/avoidance	51.46 ^a	51.78 ^a	38.71 ^{ab}	43.94 ^{ab}	55.47 ^b	54.8 ^b	0 ^a	0 ^a	P<0.01

Table 5. The prevalence of welfare problems identified in the horses in different keeping conditions and seasons, within the investigation of the freedom from fear and distress. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; ns = difference not statistically significant ($P > 0.05$); P = significance of difference between categories and seasons; abc Values in a row with no common superscript are significantly different ($P < 0.05$).

developing countries present much more frequently all types of lesions comparing with the Romanian horses, these problems could be reduced further by the involvement of the horse owners in adopting better practices when working with the animals.

All the leg and feet problems showed higher prevalence in the working horses than the breeding animals, the differences being significant in several cases (**Table 3**). Besides the risks that are inherent for using the horses for work, part of these problems (such as the improper length and shape of the hooves) were produced by disregarding the good horse management practices and neglect, these problems being found also in the breeding horses (**Table 3**).

Understanding the importance of proper care for the hooves of the horses, even when the animals are not used for work, is most important as the occurrence of the disorders at this level can affect the quality of the gait, producing permanent abnormalities [16].

The respiratory disorders of the working horses were more frequent during the winter compared to the summer and were observed significantly more often in this category than in the other groups (**Table 3**). The influence of the air quality and microclimate conditions could not be overlooked, at least for the etiology of such diseases as recurrent airway obstruction or pulmonary emphysema (heaves) [17].

The prevalence of the situations when the assessed horses were restricted from displaying normal behaviors is presented in **Table 4**. In keeping conditions similar to the natural ones, both the access to social company of the conspecifics and to free exercise were ensured simultaneously. In this study, even if the majority of the working horses did not have the company of other horses, yet more than half of them were in the company of other mammals, probably the only advantage of mixed housing of several farmed species. The lack of possibilities to fulfill the need for free exercise in breeding stallions and many of the working horses was the aspect of highest concern (**Table 4**). It was observed, also, that those involved in taking care

for the horses (owners and employed caretakers) were not aware of the fact that neither exercise during draft work nor training cannot replace the free exercise for the animals.

The general apathetic attitude, the lack of response to the environmental stimuli can indicate severe disease or extreme physical or mental fatigue [18–19]. This behavior was noted only in a small percentage of the horses, without significant differences between the categories or the assessment season (**Table 5**). The frequency of the indifference or friendly response to the assessor is not presented here, even if overt indifference, along with depressed mental state, can indicate extremely severe welfare problems, such as learned helplessness [20].

It was observed that for each behavioral response type (aggressiveness, fear, indifference or friendliness), even if statistically significant differences were found between the assessed horse categories, the season and repetition of the assessment, in the majority of the cases on the same animals, did not have significant influence on the variability of the response in the behavioral tests. This suggested the existence of a temperamental trait, named by the researchers “reactivity to humans,” stable in time and across situations. The study of these behavioral responses, in the light of present knowledge regarding human-animal relation, allowed the estimate of the behavior of humans during their interactions with the studied horses, as these animals respond to human proximity according the way they perceived previously the inter-specific interaction. Knowing this aspect and manipulating horses appropriately by all those involved in keeping and caring for these animals is important, not only for contributing to the animals’ positive mental experiences but also for human safety during work [21–22].

Figure 1 presents the distribution of the assessed horse population depending on the individual welfare scores and **Figure 2** shows the prevalence of the animals after their classification into the qualitative welfare categories, based on the obtained individual scores.

The working animals had the lowest individual scores, especially during the winter. About a quarter (25.6%) of these horses were classified as having acceptable welfare when assessed in summer, even though their number was lower in winter (18.13%) (**Figure 2**). The most concerning situation was that of the horses being in the unacceptable welfare category, as the improvement of their life quality represented an emergency.

The overall situation of the breeding stallions (**Figure 3**) was more problematic than that of the broodmares and young horses, the breeding stallions having higher prevalence of minimal welfare situations (17.75% in winter and 9.09% in summer), when compared to the broodmares and young horses (4.38% in winter and only 1.37% in summer).

Considering that the individual welfare scores are composed by the partial scores assigned for all the indicators assessing the five freedoms of animal welfare, the prevalence of the higher scores showed an overall better situation, regarding all the aspects of horse welfare.

Maximal scores (between 46 and 50) were recorded only for the broodmares and young horses (23.35% in winter and 31.1% in summer) (**Figure 1**) and for more than 90% of the reproduction

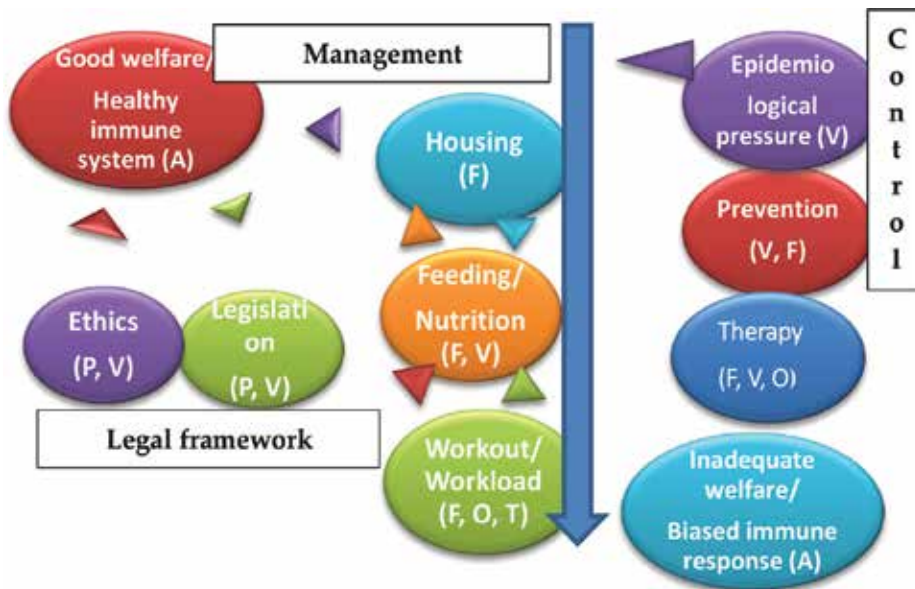


Figure 1. Some of the factors/interventions influencing the progression from good to poor welfare of the animal (A-animal, P-political decisions, V-veterinarian intervention, F-farmer conduct, O-owner conduct).

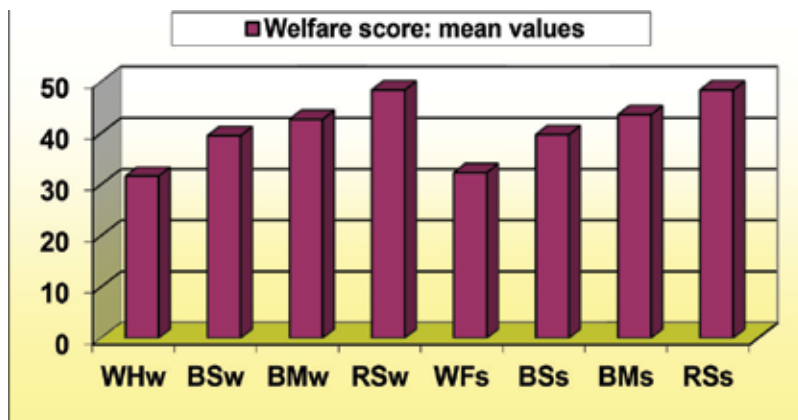


Figure 2. Mean values for individual welfare scores in horses. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer.

stallions, in both seasons (**Figure 2**). For further increase, the prevalence of good welfare scores is important to provide good housing and management conditions to horses, but also the possibility to meet species-specific needs [23].

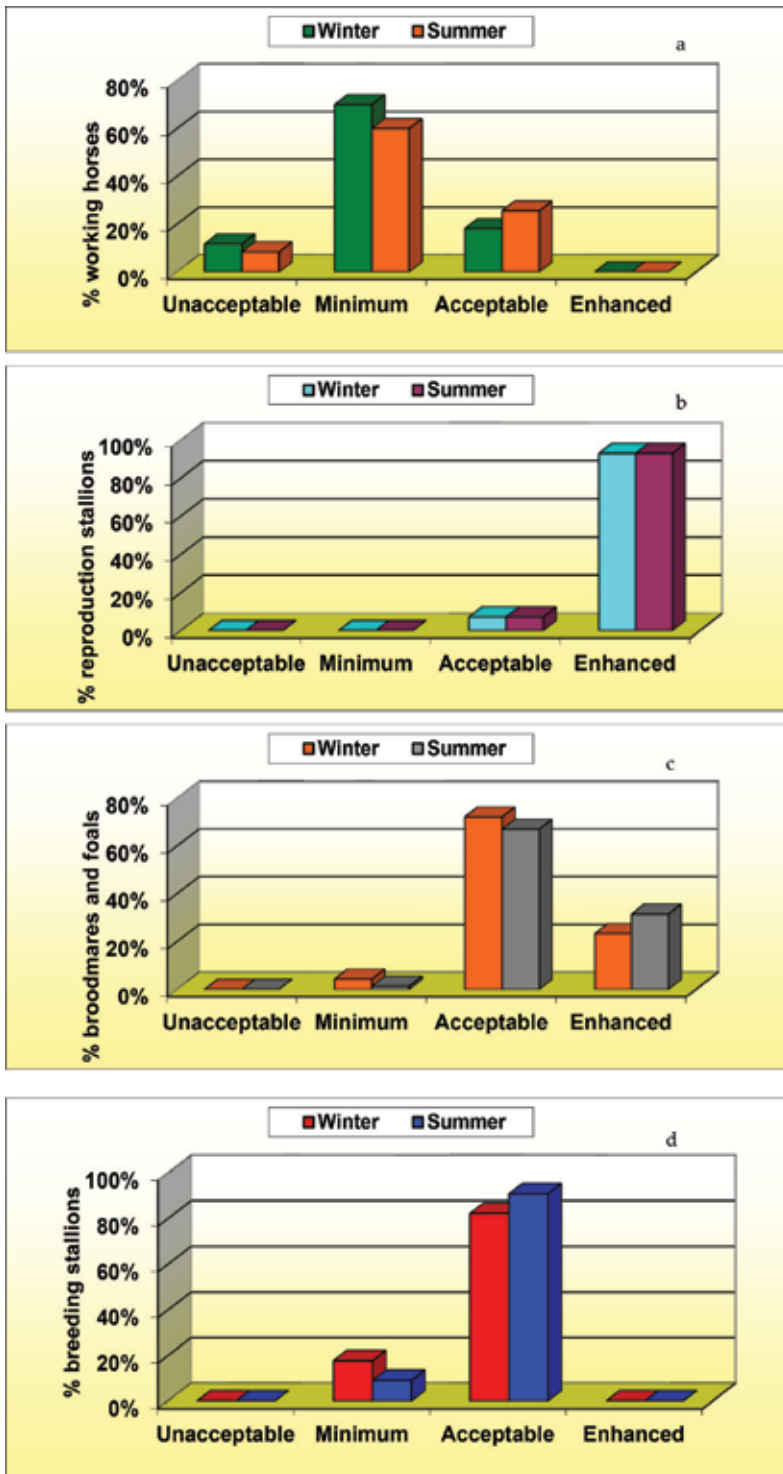


Figure 3. The distribution of the individual welfare scores according to the four qualitative classes, season and horse categories (a) working horses, (b) reproduction stallions, (c) broodmares and foals, (d) breeding stallions.

3. Assessment of immunological parameters involved in resistance to microbial diseases in horses

3.1. Methods

In order to assess the immune status and the defense potential against infectious diseases of the animals in experimental groups, differential white blood cell counts, total serum immunoglobulins and circulating immune complexes (CIC), the bactericidal capacity of their serum as well as *in vitro* phagocytic activity and *in vitro* blast transformation ability of leukocytes were tested [24–25]. Furthermore, the N/L ratios were calculated as indicators of stress.

The differential white blood cell count and the N:L ratio were performed for all of the studied horses; for the other tests in both evaluations (in the winter and in summer) 15 samples were randomly selected from each horse category, and 5 from the group of reproduction stallions, respectively.

3.2. Results and discussion

The mean results for the N:L ratio (**Table 6**) varied, showing significant differences in several cases, depending on the category of the tested horses and on the season.

The higher values of the N:L ratio in the majority of the working horses might indicate their constant exposure to acute bouts of stress and, most probably, also to chronic stress. In similar manner, although of lower intensity compared to working horses, the N:L ratio suggested significantly higher stress levels in the breeding stallions as opposed to the reproduction stallions. The fact that the improper housing and management, along with deprivation of the basic needs can lead to stress that can become chronic in animals, with all the negative consequences, has already been proven. In the opinion of some authors, a N:L ratio around 3 indicates an impairment of the health status which cannot be restored without substantial changes in the working schedule or management system.

The immunoglobulins had low mean values (**Table 7**) in all horse categories, in both seasons.

From the point of view of health status and disease resistance of an animal, the decrease of the adaptive immune system's functionality can be disastrous, exposing the host to a variety of opportunistic infections. The persistent lowering in the antibody production may occur in chronic stress (because of the endogenous production and release of corticoids) or if long-term/high doses of exogenous corticosteroids are given; also in immunodeficiency syndromes, incurable diseases or severe malnutrition. The variations in the total serum immunoglobulin levels which were observed in horses with no clinical illness signs proved the usefulness of this indicator in detecting subclinical disorders [27–28]. Nonetheless, it has to be considered that the serum concentration of total immunoglobulins depended, besides the antibody production rate, on their complexation rate with obtainment of circulating immune complexes and the clearance of the latter. For this reason, the results need to be corroborated for these two parameters.

The values for the concentration of circulating immune complexes (CIC) were relatively similar in the breeding horses and higher in the working animals, but did not exceed the normal

Parameter	Normal values*	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs
WBC	5.6 – 12.1	10.71 ^{ab}	11.03 ^a	9.09 ^{ab}	8.49 ^{ab}	8.63 ^{ab}	9.05 ^{ab}	8.31 ^b	8.43 ^b
Neutrophils	2.9 – 8.5	7.42 ^{ab}	7.83 ^a	5.82 ^{ab}	5.26 ^{ab}	4.97 ^{ab}	5.22 ^{ab}	4.81 ^b	4.64 ^b
Lymphocyte	1.2 – 5.1	2.39 ^{ab}	2.30 ^a	2.77 ^{ab}	2.63 ^{ab}	2.76 ^{ab}	2.74 ^{ab}	3.0 ^b	3.1 ^b
Monocytes	0 – 0.7	0.6	0.6	0.3	0.1	0.3	0.2	0.1	0.1
Eosinophils	0 – 0.8	0.3	0.2	0.2	0.3	0.6	0.6	0.4	0.4
Bazophils	0 – 0.3	0	0.1	0	0.2	0	0.1	0	0.1
N:L ratio	** 0.8 – 2.8	3.1 ^a	3.4 ^a	2.1 ^b	2.0 ^b	1.8 ^{bc}	1.9 ^{bc}	1.6 ^{bc}	1.5 ^c

Table 6. Mean values of the white blood cell counts ($\times 10^9/L$), including the N:L ratio, in the studied horses [25–26]. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; abc Values in a row with no common superscript are significantly different ($P < 0.05$).

limits in none of the horse categories. The results in the winter testing were higher than in summer, excepting the broodmares and young horses. This tendency could be the outcome of prolonged housing during the winter, in the presence of higher antigenic stimulation, compared to the periods of the year when the horses spent more time outdoors. Even in the breeding stallions, that were permanently housed, the lower ventilation rates during the winter might have increased the antigenic load in the barns' microclimate. Higher CIC values in the working horses might be due to the housing of the horses, usually along with other animal species. This housing type could lead to increased antigenic stimulation, even if part of the microorganisms was specific for a single animal species. In the same time, the management of barn cleaning, probably better in the organized conditions of a farming system, could strongly influence the microbial load and presence of abiotic antigens in the barns.

The phagocytic index was the highest for the first reading at 45 min, especially in the working horses evaluated during winter and in the broodmares and young horses during the summer. In all horse categories, the phagocytic activity decreased subsequently. This tendency was not observed in breeding stallions, with a more efficient overall phagocytic activity [29]. Attempts to monitor the *in vitro* efficacy of an alcoholic milk thistle (*Silybum marianum*) extract as an phagocytosis enhancing agent did not deliver a positive outcome (Table 8) (Figure 4).

Parameter	WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs
Sample size	15	15	15	15	15	15	5	5
Minimum	0.264	0.218	0.247	0.236	0.242	0.228	0.258	0.258
Maximum	0.468	0.378	0.374	0.314	0.345	0.342	0.338	0.338
Mean	0.342 ^a	0.296 ^b	0.314 ^{ab}	0.283 ^b	0.292 ^b	0.287 ^b	0.292 ^{ab}	0.297 ^{ab}
Median	0.327	0.299	0.314	0.286	0.287	0.284	0.272	0.301
Standard deviation	0.059	0.047	0.038	0.022	0.031	0.034	0.034	0.033
Standard error of the mean	0.015	0.012	0.009	0.006	0.008	0.009	0.015	0.015

Table 7. Descriptive statistical parameters of the total serum immunoglobulin concentrations (expressed in optical density units ODU) in the studied horses. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer; abc Values in a row with no common superscript are significantly different ($P < 0.05$).

Variant		CMI	CMv	AMPi	AMPv	IMi	IMv	APi	APv
		WHw	WHs	BSw	BSs	BMw	BMs	RSw	RSs
Control	ln45-ln0/45	0,006	0,011	0,001	-0,003	0,014	0,011	0,007	0,006
	ln60-ln45/15	0,004	-0,001	-0,004	-0,002	0,0001	0,001	0,003	0,001
Alcohol	ln45-ln0/45	0,002	0,008	-0,001	0,001	0,031	0,033	0,011	0,001
	ln60-ln45/15	0,003	0,0001	-0,002	-0,005	0,005	0,006	0,002	0,003
<i>Sylibum marianum</i>	ln45-ln0/45	0,0003	0,003	-0,001	-0,001	-0,001	0,001	0,001	0,001
	ln60-ln45/15	0,002	0,002	0,001	0,007	0,002	0,004	0,002	0,004

Table 8. Mean values of the phagocytic index in the studied horses. WHw = working horses, in winter; WHs = working horses, in summer; BSw = breeding stallions, in winter; BSs = breeding stallions, in summer; BMw = broodmares and young horses, in winter; BMs = broodmares and young horses, in summer; RSw = reproduction stallions, in winter; RSs = reproduction stallions, in summer.

The *in vitro* blast transformation of leukocytes aimed at quantifying the functional ability of the cells as a measure of potential antimicrobial reactivity, but also at investigating the effects of several mitogens and plant extracts on the immune response to select compound able to alleviate the stress effects on the immune system. For that, 12 variants were performed for each tested sample. The control culture (spontaneous blast transformation, no addition) had the lowest growth index in the broodmares and young horses, while the highest values were recorded for the breeding stallions, during both seasons. The oat (*Avena sativa*) extract had most frequently an inhibitory effect on growth, while the shepherd’s purse (*Capsella bursa-pastoris*) extract showed stimulating effects, especially during winter. The enhanced stimulating effects of different plant extracts were associated with keeping conditions closer to the natural ones.

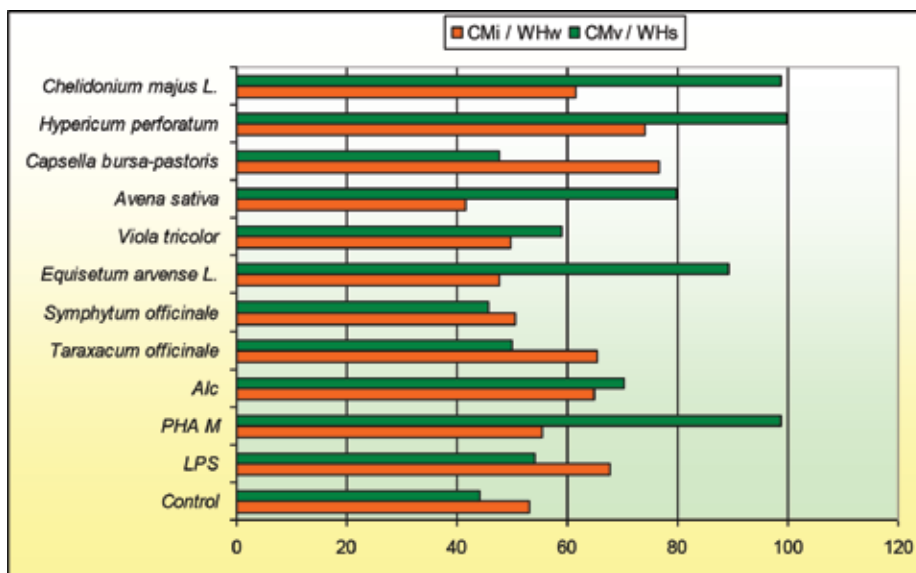


Figure 4. Mean values of the blast transformation indices (%) in the working horses during summer and winter (WHw = working horses, in winter; WHs = working horses, in summer; Alc = alcohol; PHA M = phytohemagglutinin M; LPS = lipopolysaccharide from *E. coli* cell wall).

The bactericidal capacity of the serum in the studied horses was superior (both as efficiency and as duration) against the gram positive bacteria (*Streptococcus spp.*), compared to that against the gram negatives (*Escherichia coli*). Even if in the stallions (breeding and reproduction), the mean values of the results were higher and the bactericidal activity was observed more in the successive dilutions, versus the other horse categories, yet this nonspecific immune defense mechanism was not influenced by the keeping conditions and management of the animals.

4. Investigation of the relationships between the welfare degree and immune reactivity in the assessed horses

To investigate the relationship between well-being and the immune status of the horses, the data were analyzed using the SPSS statistical program (version 17). Spearman rank (rs) correlation coefficients were calculated for the study of the relationship between the variables, since the obtained data did not show a normal distribution. Correlations were considered significant at the significance threshold $P < 0.05$, distinctly significant at the threshold $P < 0.01$ and very significant at $P < 0.001$.

Category	N/L ratio	Total Ig	CIC	Blast transformation
Working horses, in winter				
Individual welfare score	-0,41**	-0,54*	-0,48**	-0,27*
Stimulation index PHA M	-0,31*		0,37*	
Total immune globulins	-0,38*			
Working horses, in summer				
Individual welfare score	-0,46**			-0,21*
Stimulation index		-0,26*		
Breeding stallions, in winter				
Individual welfare score	-0,38*	0,64*	0,36*	0,2*
Individual welfare score, summer	-0,22*			
CIC breeding stallions, winter	0,61*	0,43*		
Breeding stallions, in summer				
Individual welfare score	-0,39*			0,32*
Individual welfare score, winter	-0,21*			
CIC breeding stallions	0,22*	0,38*		
Total immune globulins	-0,38**			
Broodmares, young horses, in winter				
Individual welfare score	-0,27**	0,21*		
Total immune globulins	-0,32**			
Stimulation index control culture	-0,28*			
CIC		-0,52**		
Broodmares, young horses, summer				
Individual welfare score	-0,33**		0,27*	0,32*
Individual welfare score, winter	-0,23**			
CIC		0,64**		
Stimulation index control culture		0,72**		
Stimulation index <i>H.perforatum</i>		0,36**		

Table 9. Significant correlations found between individual welfare scores and several immunological indicators in the studied horses.

Of all the indicators, the N:L ratio correlated most frequently with the welfare score of the assessed horses. The increase of this ratio showed the acute stress response in the first place, but through negative correlations, it showed also the presence of chronic stress in horses.

The increased concentration of the immunoglobulins was associated with those keeping conditions where the antigenic pressure was high, indicating a stronger adaptive response as the animals' welfare degree was higher. In those horses kept in near-natural conditions, the concentration of CIC indicated the functionality of the normal processes of antibody synthesis and clearance by immune complexation. Interpreting the correlations between CIC concentrations and the individual welfare score needed the additional consideration of total serum immunoglobulins, to differentiate between the chronic disease status and lack of antigenic stimulation. The increase of phagocytic indices in the horses with low individual welfare scores could indicate the participation of hypoxia producing chronic diseases in the enhancement of phagocytosis. The few correlations obtained between the indices of blast transformation and the individual welfare scores showed the concomitant variation of these two parameters. In the case of the working horses in winter, a drop in the immune reactivity was possible on the background of a general hyporesponsiveness, involving the chronic stress caused by the environment.

According to the results, the bactericidal capacity of the serum proved to be more intense in those situations where the antigenic pressure was increased, and its efficiency was improved as the animals had a higher degree of welfare (**Table 9**).

5. Conclusions

This study, including various horse categories from Romanian raising systems indicated that there was a variation of welfare drawbacks depending on the workout category. In working horses, there was the highest prevalence of welfare problems, such as the lack of constant access to drinking water, the lack of comfortable resting surface, the harness-caused lesions, the health problems of the legs, the inadequacy of shoeing practices, the lack of conspecific company and lack of access to free exercise. The major welfare problems of the reproduction horses and breeding stallions were the lack of permanent access to drinking water and the lack of free exercise possibility, respectively. The human-horse relationship represented a welfare problem, whose suitability was difficult to assess in all of the studied horse categories.

Neutrophil:lymphocyte ratios, total serum immunoglobulins and the circulating immune complexes' levels helped identifying situations which impose an intense adaptive response and immune defense, triggered either by chronic or acute stress or an increased antigenic pressure in the habitat.

The correlated use of the welfare assessment by direct indicators with the investigation of the immune status proved to be a powerful tool in the interdisciplinary research on ways the horses cope with the demands imposed by different management conditions. The dimensions

of this approach have not been exhausted by far in this study, which is merely a first step taken in our country to a better understanding of how these animals could be simultaneously used and protected.

Conflict of interest

None of the authors have any existing or possible conflict of interest, including financial, personal or any other relationship which could influence their scientific work.

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Animal Health and Welfare

Health Assessments in Free-Ranging Sea Turtles: Perspective of Animal Welfare in Wildlife

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Abstract

Sea turtle health is an important component of conservation since these taxa is susceptible to infectious diseases that can cause illness increasing its mortality. Threats to health, survival, and reproduction of sea turtles are increasingly documented; however, prevention and control has not yet been successfully achieved. Thus, the need to develop conservation strategies on an ecosystem scale is a growing concern. Information about health indicators of sea turtles is a useful tool to achieve the best possible conservation measures. The objective of this study was to establish a baseline of health assessments of free-ranging Eastern Pacific green turtles (*Chelonia mydas*) from developmental habitats in Baja California Sur. Here we contribute with a clinical exam for sea turtles and reference interval values of vital signs (Corporal temperature: subadults, 21.22 ± 3.43 ; juveniles, 22.2 ± 2.95 . Heart rate: subadults, 37.41 ± 2.95 ; juveniles, 38.27 ± 4.09 . Pulse rate: subadults, 15.07 ± 3.88 ; juveniles, 19.97 ± 0.82), that in conjunction with the complete blood count and plasma blood biochemistry, 100% of the turtles were classified as "healthy." Development of site-specific health indicators for wild, healthy sea turtle populations is an important factor in creating effective management protocols and thus enhances our ability to understand the effects of anthropogenic and environmental changes on sea turtle health.

Keywords: semiology, vital signs, hematology, blood chemistry, sea turtles welfare

1. Introduction

Sea turtle health is an important conservation component and is often overlooked. These species are susceptible to infectious diseases that can cause severe illness increasing its mortality [1]. In the case of the Eastern Pacific green turtle (EPGT) *Chelonia mydas* population, it is gradually recovering, yet much remains unknown about its health status due to its complex

life cycle, especially its ethology, migratory conditions, and sometimes the inaccessibility to the study areas (places for study them). However, threats to health, survival, and reproduction of free-ranging sea turtles are being increasingly documented, and their prevention and control measures have not yet been successfully achieved [2]. That is why there is a growing concern about the need to develop conservation strategies to operate at the ecosystem scale [3] and, therefore, develop health assessments for marine turtles. It becomes a useful tool that can lead to the best possible conservation measures.

Oceanographic conditions of marine waters and coastal lagoons in Baja California Sur (BCS) provide diverse habitats and optimal conditions to carry out different stages of the life cycle for five of the seven species of sea turtles that exist in the world: the hawksbill turtle (*Eretmochelys imbricata*), green and Eastern Pacific green turtles (known locally as black turtle) (*Chelonia mydas*), loggerhead (*Caretta caretta*), olive ridley (*Lepidochelys olivacea*), and leatherback turtle (*Dermochelys coriacea*) [4], which are exposed to a wide range of factors specific to their environment and anthropogenic factors that can cause diseases and death. In the Baja California Peninsula, since 2016, health assessment programs have been applied and have been an integral part of management efforts to enhance the recovery of particularly the loggerhead turtle and the EPGT. These strategies include (1) on-site protection with health evaluations, (2) determination of their condition and distribution, (3) determination of the diet, (4) characterization of the population of origin and confirmation of species, (5) morphometric data collection, (6) individual sea turtle tagging, and (7) release. Initially, the monitoring efforts were intended to collect morphometric data and individual sea turtle tagging because in the past decades EPGT survival at BCS declined severely due to consumption [5]. Actually these efforts were notably successful, and these stocks of EPGT appear to be in the process of recovery [6]. However, in 2014 and 2016 there are disease reports and mortality associated with illness [7, 8]. Therefore, the idea of carrying out a program of health assessments to complement conservation efforts was proposed in order to minimize the threat of any potentially catastrophic disease outbreaks. Minimizing the risk of diseases and/or introducing diseases to a population can be accomplished by using the best available diagnostics to test turtles for evidence of infection or disease and taking a proactive approach to disease management (i.e., treatment or immunization as technology becomes available). The current impact on declining survival and the role of disease in the EPGT population are limited and in some cases unknown. Therefore, veterinarian research is needed to improve or provide animal welfare efforts. Internationally the information about sea turtle health is principally based on hematological values [9, 10, 11]. Through these methods, various aspects of health status of an individual are obtained.

The results can be related and linked to anatomical, morphological, and functional changes as well as to nutritional and reproductive status [12]. However, descriptive hematological values and morphological characteristics of blood cells in sea turtles are regionalized and have been inconstant [13]. It is important to consider that blood elements can be affected by a number of factors such as age, sex, season, stress, diet, hormonal processes, oxygen pressure, body hydration [9], geographic area, and water temperature [11]. In order to document local health indices, health assessments, hematological values, and vital signs should be generated at baseline. In Mexico, there are several studies related to sea turtles hematology [14, 15, 16, 17];

however, regionally, in BCS, there are still few published works [18, 7, 13, 19]. Thus, there is an urgent need to clearly indicate the health status and rate of diseases in regional sea turtle populations [8]. The present study was aimed at obtaining baseline information on selected health and disease indicators from the EPGT. The objectives of the study were to (1) assess the health of sea turtles via physical examination to document clinical signs, (2) generate their values of vital signs, (3) determine hematological values of EPGT from BCS, and (4) evaluate the results obtained to generate conservation strategies together with local authorities.

2. Materials and methods

During 2016, monthly field trips were carried out with captures of sea turtles in the pacific coast of Baja California Sur, particularly in coastal lagoons of feeding and development of *Chelonia mydas*. All captures were made using monofilament nylon gillnets. This method consists in the extraction of organisms from a 100-m-long net (locally known as “chinchorro”), with a drop of 5 m and a mesh size of 60 cm; this net is pulled from a small boat and left in periods of 6–8 h, covering day lapses in the areas considered transit of sea turtles and channels with constant flow of currents in the lagoons. The “chinchorro” net is reviewed in periods of an hour or an hour and a half to minimize the risk of death by asphyxiation or damage to organisms.

2.1. Clinical exam

Once the turtles were loaded onto the boat, identification of the species was carried out, each animal was allowed to rest for a few minutes alone and in a clear space; during this time, we observed the turtle in detail paying special attention to the behavior and movements, as well as the type and level of activity of each animal. The physical evaluation was carried out with the participation of two people to manipulate the animal and a third to record the observed changes and the data [20]. This process was carried out as gently and quietly as possible. First, one examiner held the animal on the back (placing his hands on the front flippers) to avoid excessive movement and reduce stress by struggle; the other performed a detailed systematic inspection with cranial-caudal and dorsal-ventral orientation. We carefully checked the skin and tegument for signs of excessive desquamation, abscesses, scars and wounds, the presence of injuries from hooks or nets, and the presence of leeches, barnacles, etc. Next, we also examined the position, shape, size, symmetry, color, and proportions of the head and skull as a whole with the intention of determining injuries, traumas, or pain. The visible structures of the eyes such as the conjunctiva, the sclera, the cornea, the iris, the pupil, and the ductal openings of the lacrimal sac were then checked, using a pocket medical scanning lamp, with which also we observe the trophism and symmetry of the eyes and their associated structures. Is important to note that in sea turtles, the eye is protected by the dorsal and ventral eyelids, which are keratinized and mobile; there is also a secondary eyelid on the lateral canthus that is keratinized but does not move [22]. In this step we put special attention in this area, since it is known that one of the main diseases in marine turtles (fibropapillomatosis) manifests initially in this zone [10]. In the nostrils it was checked if there was nasal discharge or congestion, traumatic injuries, and presence of foreign bodies. The ranphoteca was reviewed by the

presence of injuries and traumas mainly. Afterwards, we checked the position and continuity of the maxilla and mandible to discard or confirm fractures or dislocations; it is important to emphasize that the maxilla and mandible vary according to each species; nevertheless, they maintain similar anatomical and functional characteristics [22]. We put special attention in the area between the maxilla and the mandible because it is a place of development of fibropapillomatosis [10]; also the interior of the mouth and lumen of the esophagus were carefully reviewed to evaluate the color of the mucous membranes and identify abnormalities such as oral plaques, ulcerations, and abnormal odor and the presence of foreign bodies such as hooks or ropes. Afterwards, the entire neck was inspected, exploring its shape (short, long, and normal), volume (wide, thin, and normal), position (central, with lateral deviation, in flexion, and in extension), its mobility, and the presence or absence of injuries and neoplasms, as well as the presence of foreign bodies (ropes and hooks) and epibionts, leeches, and ectoparasites. Likewise, the musculoskeletal structures and the mobility of the neck were explored, as well as the parotid, submaxillary, and sublingual regions and the region close to the structures of the shoulders and neck. Then the glands were inspected by palpation. After that, the flippers were reviewed in detail (skin, muscles, bones, and joints) looking for lesions, the presence of epibionts, inflammations, fractures, etc. [20]. When evaluating the carapace we look for evidence of abnormal keratinization, changes in firmness and flexibility, injuries caused by boats and propellers, ulcerations, vesicles, osteomyelitis, shark bites, deformities, fibropapillomas, fractures, or the presence of epibionts, leeches, algae, and ectoparasites [20], and finally all the anatomical, morphological and functional changes observed were recorded in a health format. During this review, the registration of vital signs (carapace temperature, plastron, inguinal area, cloaca, and heart rate record) and the exploration of sensitivity and reflections of each animal were included. To generate vital signs, the carapace temperature was measured three times with a digital infrared thermometer type gun Steren® HER-425, placing it at a distance of 10–20 cm from the carapace and pointing in the central surface of the carapace (using a scute index as a reference). Subsequently, an average of the three temperatures was calculated and recorded in a database. The organism was reviewed in detail ventrally, with special attention to the plastron and cloaca following the same dorsal revision methodology. The body temperature was taken three times in the center of the plastron and in the left inguinal area; an average of these was calculated following the same methodology. During the physical examination, in the ventral review, the turtles were auscultated with an IUMED® model 400 stethoscope, which was placed on the skin between the neck and the proximal frontal flipper. Then, deep pressure was made to locate the resonance of the heartbeat; once identified, the heart rate (HR) was recorded for 30 s and multiplied by 2. Next, the deep cloacal temperature was measured with an electronic thermometer model SureTemp® Plus 690 (Veterinary) Welch Allyn; this was introduced gently (approximately 1.5 cm) into the cloaca of the turtles, and then, the probe was tilted so that the tip of it was in contact with the tissue and when the final temperature was reached and appeared on the thermometer screen, it was registered. After completing the temperature measurement, the probe was gently removed from the cloaca of the turtle. The next vital sign measured was pulse rate, in marine turtles it is very difficult to measure the pulse in a practical way for its anatomical and morphological characteristics; therefore, the pulse rate in relation to their body weight was measured using the following equation: $X * (Y^{-0.25})$, where X = beats per minute (lat/min) and Y = weight in kilograms (kg) [21].

After the examination, turtles were sexed according to the criteria of sexual dimorphism described by [22] (classified as females, males, and undefined based on the size of the tail in relation with the size of the turtle). Then, to estimate the size of sexual maturity of the animals, the morphometric data was collected using a flexible measuring tape [23]. The morphometric data collected were curved carapace length and curved carapace width; immediately after, the turtles were tagged with metallic marks of monel 400/inconel 625 in the back flippers following the methodology described by Balazs and finally were released. All values obtained during the clinical examination of turtles evaluated were recorded in **Table 1**.

2.2. Sample collection

2.2.1. Blood samples

At the end of the physical examination, the individuals were positioned in ventral recumbency on a flat and fixed surface; the eyes of the turtles were covered with a wet cloth to avoid stress by manipulation. The head of the turtle was placed below the body and protruding from the flat surface on which the turtle was placed. Approximately 10 ml of blood was collected from the dorsal cervical sinuses, which are located on both sides of the midline of the neck between 1/3 and 1/2 of the distance between the back of the head and the anterior edge of the carapace, approximately 2.5 cm lateral to the midline, 2–3 cm below the surface of the skin. Once located, the needle was inserted perpendicular to the dorsal surface of the neck at 1–3 cm depth into the vein [24], using double pointed needles (1.5 inches, 32 mm caliber) connected to a Vacutainer© holder; finally, the needle was removed from the vein and the venipuncture site was covered with the index finger by pressing to avoid the formation of

	EPGT (<i>Chelonia mydas</i>) subadult (n = 59)	EPGT (<i>Chelonia mydas</i>) juveniles (n = 20)
	Mean ± SD	Mean ± SD
CCL	75.61 ± 4.48	51.37 ± 3.71
Weight	40.94 ± 18.92	18.39 ± 1.50
Temperature (°C)		
Carapace	23.22 ± 3.97	22.76 ± 3.91
Plastron	22.61 ± 3.40	23.37 ± 4.10
Inguinal	21.22 ± 3.43	22.2 ± 2.95
Cloacae	19.85 ± 2.25	22.44 ± 3.13
HR	37.41 ± 2.95	38.27 ± 4.09
Pulse	15.07 ± 3.88	19.97 ± 0.82
Sex	34(U) 23(F) 2(M)	19(U) 1(F)

CCL: curved carapace length; cm: centimeters; SD: standard deviation; °C: degree celsius; HR: heart rate; F: female; M: male; U: undefined.

Table 1. Mean and standard deviation of the curved carapace length, weight, vital signs (carapace temperature, plastron, groin and cloaca, heart rate, and pulse) and subadult and juvenile EPGT sex.

bruises until it was sure that no more blood came out. Blood samples were stored in two 7 ml Vacutainer® tubes (Becton Dickinson, Franklin Lakes, New Jersey). The first tube lacked an anticoagulant in order to recover serum later and perform a blood chemistry panel [25], and the second tube included lithium heparin (He/Li) as an anticoagulant to determine complete blood count [10, 26]. Blood samples were transported in a cooler at 4°C to the marine Botany Lab and Oceanography Lab at the Autonomous University of Baja California Sur (UABCS) where they were immediately processed.

2.3. Hematology

2.3.1. Complete blood count and blood chemistry

Hematocrit (HCT) was manually measured filling a capillary of the micro-hematocrit, supporting one of the ends on one blood drop; subsequently, the end closest to the blood was covered with plasticine and the capillary was introduced into a microcentrifuge for 5 min at 15,000 rpm in an automatic microcentrifuge model ECOspin III® (medical ECONET®); with a metric ruler the length of the column formed by sedimented red blood cells was measured in the capillary and was referenced in percentage to the total length occupied by the blood that fills the capillary with the next equation—hematocrit (%): $A/B(100)$ with (A) total length of blood in the capillary and (B) length of the cell fraction (100). The total red blood cell count (erythrocytes) and white blood cell count (leukocytes) was made in a Neubauer chamber, using Natt and Herrick methodology [27]; 10 µL of blood was drawn from the anticoagulant tube and used to obtain a 1:100 dilution with 990 µL of Natt-Herrick diluent [27]. This was incubated for 3 min and then a drop was deposited in a contrast Neubauer chamber (Improved Neubauer, BOECO®, West Germany) of 0.1 mm depth and 0.0025 mm². Next, the camera was placed in an optical microscope (Olympus® CX31) for 2 min until the solution stabilized. Observation under a minor objective (10×) was started to locate the primary quadrant of leukocyte count. Hemoglobin assay (Hb) was determined by commercial Labtest® kit. Erythrocyte indices (MCV, means cell volume; MCH, corpuscular hemoglobin; MCHC, corpuscular hemoglobin concentration) were calculated from the total erythrocyte count, hematocrit, and hemoglobin. In the case of thrombocytes, the number of these cells per 1000 erythrocytes was counted. Leukocyte count was carried out through the blood smears made at the time of blood sample collection (two blood smears were prepared, air dried, and then fixed with methanol). Later on, they were stained with a rapid blood staining kit Hemacolor® (Merck Millipore®) at the Marine Botany Laboratory in the Autonomous University of Baja California Sur (UABCS). Blood smear slides were reviewed for leukocytes and classified based on the morphological features [26, 28] using a microscope Olympus® CX31 with 40× and 100× lens and a Neubauer hemocytometer, according to Natt and Herrick method [27]. For cell identification, we followed the criteria established by [26]. Plasma was separated by an automatic centrifuge model ECOspin III® (medical ECONET®) at 3000 rpm for 15 min and pipetted into 2 ml sterile vials and stored in a freezer at -4°C until transfer for further chemical analysis. Blood plasma values were processed at Fidelis labs with the routine technique; these samples were analyzed using a chemistry analyzer and commercial kits. Selected blood values from biochemical analysis were albumin, globulin, urea, creatinine, aspartate aminotransferase (AST), alkaline phosphatase (ALP) (except in juveniles), blood urea nitrogen (BUN), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and total protein. In addition,

Variable	Mean ± SD	Range	Mean ± SD	Range
	Subadults (n = 59)		Juvenile (n = 20)	
HGB	11.07 ± 0.80	10–13.5	8.72 ± 0.69	7.5–9.9
HCT	31.75 ± 1.39	28–34.7	39.6 ± 1.67	37.4–42.25
MCV	62.84 ± 2.79	50.1–73.1	61.91 ± 2.38	53–70
MCH	19.04 ± 1.60	150–218.6	19.10 ± 1.90	16.9–23
MCHC	17.07 ± 2.08	12–19.3	11.82 ± 1.06	10.1–13.2
E	1.17 ± 0.48	0.4–4.62	0.48 ± 0.04	0.42–0.54
TH	16.11 ± 3.75	13.3–24.9	18.76 ± 0.72	17–20
LEU	8.48 ± 0.50	7.1–11.1	2.13 ± 0.19	1.8–2.4
LYM	22.81 ± 0.80	20–27	31.38 ± 2.75	28–36
MOs	1.9 ± 0.56	1–6	2.23 ± 1.30	1–6
EOS	1.43 ± 0.23	1–2	0.23 ± 0.43	0–1
Het	66.61 ± 2.42	59–73	62.38 ± 1.98	60–65
BA	0	0	0	0

HGB: hemoglobin (g/dl); HCT: hematocrit (%); MCV: mean corpuscular volume (fl); MCH: mean corpuscular hemoglobin (pg); MCHC: mean corpuscular hemoglobin concentration (gr/dl); E: erythrocytes (1,000,000/mm³); TH: thrombocytes (1000 /mm³); LEU: leukocytes (1000/mm³); LYM: lymphocytes (%); MOs: monocytes (%); EOS: eosinophil (%); BA: basophils (%); Het: heterophil (%); g/dl: gram/dilution; fl: femtoliter; pg: picogram.

Table 2. Complete blood count for sub adult and juvenile EPGT (*C. mydas*).

Variable	Mean ± SD	Range	Mean ± SD	Range
	Subadult (n=59)		Juvenile (n=29)	
Urea	31.03 ± 1.32	19–38	24.30 ± 2.13	21–28
BUN	12.69 ± 1	9–17	10.5 ± 1.12	9–12
Creatinine	0.62 ± 0.12	0.5–0.8	0.62 ± 0.13	0.4–0.9
AST	242.14 ± 2.75	202–255	250.15 ± 19.72	212–278
ALT	33.44 ± 0.52	30–39	34.15 ± 2.79	28–39
ALP	56.16 ± 7.59	40–80	—	—
LDH	142.1 ± 40.63	70–284	267.76 ± 32.15	202–299
TP	7.07 ± 0.59	4–8.6	5.03 ± 0.53	4.2–6.1
Albumin	2.67 ± 0.54	1.2–3.7	1.53 ± 0.27	1.2–1.9
Globulin	3.89 ± 0.41	2.9–4.6	3.46 ± 0.31	3.1–3.9
A/G ratio	0.52 ± 0.06	0.2–0.8	0.26 ± 0.07	0.1–0.4

Urea (mg/dl), BUN: blood urea nitrogen (mg/dl); creatinine (mg/dl); AST: aspartate aminotransferase (U/L); ALT: alanine aminotransferase (U/L); ALP: alkaline phosphatase (U/L); LDH: lactate dehydrogenase (U/L); TP: total protein (g/dl); albumin (g/dl); globulin (g/dl).

Table 3. Blood chemistry values for subadult and juvenile EP green turtles (*C. mydas*).

we calculated albumin/globulin (A/G) values according to [9]. Finally, the values obtained from the blood biometrics of the turtles were recorded in **Table 2** and the blood chemistry values are shown in **Table 3**.

3. Results

During 2016, a total of 79 *C. mydas* were captured in two coastal lagoons in BCS. The organisms were classified as subadults (59) and (20) were classified as juveniles according to their size and weight; these results are shown in **Table 1**.

In the case of the blood values, the values obtained in the complete blood count are reported in **Table 2** and the values of blood chemistry are shown in **Table 3**.

4. Discussion

4.1. Height, weight, and sex distribution

According to the average of CCL (75.61 ± 4.48) and weight (40.94 ± 18.92), 59 turtles were classified as subadults and 20 turtles (CCL, 51.37 ± 3.71 ; weight, 18.39 ± 1.50) were classified as juveniles [29, 30]. The gender of 34 subadult turtles and 19 juvenile turtles could not be determined and were registered as unidentified gender; 23 subadult turtles were classified as female, 2 as males, and finally 1 juvenile as female which were classified based on the criteria proposed by [22].

The physical exams did not show evidence or clinical signs of neoplasms and diseases nor lesions that could compromise organ function or life.

4.1.1. Vital signs

Vital signs results (corporal temperature, heart rate, and pulse rate according to the weight) help us to determine immediate alterations in the basic functions of marine turtles, monitor health problems, and indicate the physiological state of core organs (brain, heart, lungs, etc.) [31]. These values also indicate immediate functional changes in organisms that otherwise could not be qualified nor quantified [32]. The principal advantage of this type of study is that the values can be registered anywhere and do not require complex material to be measured.

4.1.2. Temperature

Marine turtles are exposed to a wide diversity of environmental changes during their migration and ontogeny, which is why we place such importance on health monitoring. Sea surface temperature (SST) variation can affect immune system function, subsequently influencing threats to infectious disease. Reports suggest a high frequency in fibropapillomas (FPs) with the increase of water temperature in summer [33]; on the other hand, [34] suggests that when

there is a decrease in temperature, marine turtles undergo stress, also leading to an incidence of FP. According to the average SST during sampling and the turtles' average temperature, a phenomenon among green turtle species, cold stunning (a form of hypothermia) [35, 36], and FP were eliminated as threats. On the other hand, when environmental temperature decreases sharply, the speed of the metabolic chemical reactions decreases as well as the quantity of energy that individuals can exert in their activities [31]. If the corporal temperature increases too much, the biochemical reactions are unbalanced and the protein synthesis involved in physiological functions is disrupted or even reduced [32]. The advantage of the regulatory system of marine turtles lies in their ability to save energy by maintaining their corporal temperature. In addition, the low nutritional needs of this species allow them to survive in different environments during migration [37]. The disadvantage is that their core activities depend on environmental temperature, and thus, they cannot stand long periods of time (more than 24 h) in low temperatures (less than 9°C).

Under this temperature, they become lethargic and affected by cold stunning possibly causing its death [35, 38]. Nevertheless, they use strategies as they enter into a turbid state, a kind of hibernation that helps them to tolerate low temperatures [39]. Deep cloacae temperature is representative of the actual environmental temperature [40]. By acquiring a baseline value threshold, this allows wildlife rehabilitators to act quickly to address cold-stunned turtles, which have a recovery chance [36, 41]. Due to a similar inguinal and cloacae temperature, the use of a digital infrared laser thermometer without contact was effective in order to monitor this vital sign. This type of thermometer is recommended instead of the cloacae one because it is less invasive and less stressful for the turtle. When using the laser thermometer, it is recommended to record the temperature as soon as the turtle is captured and before the physical examination in order to collect the most real-time values. If vital signs are recorded long after capture, the environment can affect the temperature of the turtle and the recorded values then will have a large error margin.

4.1.3. Heart rate (HR)

Cardiac auscultation in sea turtles is complicated due to the presence of the carapace, plastron, and muscular structures [41]. However, there are several ways (Doppler probe or ultrasound) to evaluate heart rate. In this study the HR was measured and recorded using a stethoscope and depended based upon the expertise and experience of the examiner. For subadult and juvenile turtles, the resting heart rate matched with valued proposed by [42, 43] who suggest that normal heart rate in green turtles at a temperature of 24°C (75°F) varies from 30 to 60 beats per minute. Due to the fact that the HR obtained from all the turtles analyzed did not present considerable variations, physiological alterations that can lead to tachycardia, such as fatigue, excitation, digestive processes, or gravid females [32] were eliminated. Hyperthermia, hydremias, septicemia, and pericarditis in the case of the pathological disorders [44] were eliminated. HR values did not indicate bradycardia, which may be associated with physiological processes such as starvation or lethargy [31] and pathological processes such as cerebral compression, vagus nerve excitation, intoxication, or others [44].

4.1.4. Pulse rate

The pulse rate of individuals in relation to their body weight was calculated using the equation cited by [21]. During vital signs analysis, the pulse rate should be comparable with the heart rate. In marine turtles, this is complex due to their anatomy and morphology; however, it is known that in certain pathologies, such as hemodynamically inefficient systems of atrial or ventricular premature complexes or atrial fibrillation with high cardiac frequency, etc. [44], a pulse rate frequency lower than the HR (pulse deficit) can be evident. The pulse rate can also decrease (bradisfigmia, bradisphyllia, or *pulsus rarus*) or increase (tachyphygmia, tachyphyxia, or *pulsus frequens*) [32, 45]. In marine turtles, as in other species, the autonomic nervous system is the main determinant of pulse rate; thereby, in response to vagotonic stimuli, a bradysfixia will be presented and to sympatheticotonic stimuli, a tachysphyxia [31].

In this case, it was not possible to evaluate if the pulse rate presented irregularities in a practical way nor the succession of pauses that separate the pulsating waves (in a physical way). However, it was considered that subadult and juvenile turtle pulse rates were regular. The data suggest that the succession of diastolic pauses was stable and continuous (the duration between them is similar). Therefore, pathological causes such as arrhythmias due to variable blocks, polyextrasyses, or atrial fibrillation were dismissed. It is also true that intermittent pulse rate due to sinoatrial or atrioventricular blockages, false intermittent pulse rate by premature ventricular systoles, and hemodynamically inefficient extrasystoles [44] were eliminated.

4.2. Hematological values

Hematological values for subadult and juvenile organisms are similar to the previous ones reported internationally for healthy green turtles [11] and probably are associated to age, reproductive stage, and food availability as [25] suggested. These values can be related to sea turtle's home range; therefore, there are no "optimal" values. When integrating the physical examination results and vital signs, no clinical symptoms were observed (anemia, dehydration, bleeding, and malnutrition) [46], nor were the presence of possible respiratory, renal, gastrointestinal, inflammatory, and infectious chronic diseases or neoplasms [10].

4.3. Complete blood count (CBC)

CBC is a diagnostic orientation tool for diverse causal agents of diseases. It is only diagnostic when the agent or damage directly affects the blood cells [47], for example: the presence of hemoparasites and cases of lymphocytosis, heterophilia, leukocytosis, red blood cells with intracytoplasmic inclusions, remains of organelles, precipitated hemoglobin, and others [48]. Those mentioned above are reported in the literature as changes associated with chronic and active infectious processes [7, 49]. Morphology of blood cells in this study was similar to the previously reported cases by [26, 28, 50]; thus, the presence of chronic active infectious processes and the presence of hemoparasites [7] were dismissed.

4.3.1. Hematocrit (HCT)

HCT values were similar to those reported for healthy *C. mydas* in Hawaii [9] and Peru [51] and those reported by [13] from the same region in BCS. Then, anemia, dehydration,

hemorrhages, destruction of red blood cells, and malnutrition (iron deficiency and vitamins) were dismissed. Cardiac diseases, dehydration, hypoxia, pulmonary fibrosis, erythrocytosis, etc. were also excluded because there were not increased values [52, 53, 54].

4.3.2. Total proteins (TP)

The values for TP obtained in this work were found within normal ranges for *C. mydas* [12, 13, 25], which show that there is no hyperproteinemia, hypoproteinemia, or chronic inflammatory diseases, malabsorption, protein-losing enteropathies (e.g., by parasites), renal failures, or hepatic problems [54].

4.3.3. Erythrocytes

There were no erythrocytosis observed according to Reséndiz [13] criteria and the values reported by [55], so we confirm that there were no dehydration, renal diseases, oxygenation problems or drowning threats, heart disease or neuropathies, and polycythemia [56, 57]. Low values were not found either and autoimmune diseases, blood loss, bone marrow insufficiency, infections or neoplasms, hemolysis, prolonged infections, and nutritional deficiencies were dismissed [56, 58].

4.3.4. Leukocytes

Leukocyte values obtained were similar to those reported by [10] and were within the ranges indicated by [51] for healthy *C. mydas*. Our values suggested that there was no leukocytosis; therefore, there was no presence of infectious diseases, inflammatory diseases, severe physical stress, or tissue damage [59]. Likewise, leukopenia was dismissed due to the absence of autoimmune diseases, bone marrow failure due to neoplasms, fibrosis and renal diseases [60], as well as pathologies in the liver and spleen [49].

4.3.5. Hemoglobin (HGB)

HGB values were within the ranges reported for healthy *C. mydas* by [9, 51]; therefore, hemolytic anemia, bone marrow deficiency, renal diseases, poor nutrition, and bleeding in the digestive tract [56] were eliminated. No elevated levels were found so there is no presence of hypoxia, dehydration, or pulmonary lesions and diseases [61].

4.3.6. Mean corpuscular volume (MCV)

MCV values were normal according to what was reported by [55, 51], for which microcytic anemia caused by low values and macrocytic anemia caused by increased values [52, 56] were dismissed.

4.3.7. Mean corpuscular hemoglobin (MCH)

There were no values below those proposed by [9] so that the evaluated turtles did not present hypochromic anemia. There were no higher values than the ranges proposed by [51], dismissing hyperchromic anemia [62].

4.3.8. Mean corpuscular hemoglobin concentration (MCHC)

MCHC values obtained were similar to those reported by [9], suggesting that there were no iron anemia deficiency, blood loss, neoplasms in the digestive tract, macrocytic anemia, or hepatic problems [54, 63].

4.3.9. Thrombocytes

Thrombocyte counts were similar to those reported by [55], for which thrombocytopenia, thrombocytosis, autoimmune disorders, and anemia [64] were dismissed.

4.4. Lymphocytes

The number of lymphocytes that were obtained was similar to those reported by [10, 25] for healthy *C. mydas* and does not indicate lymphocytosis; therefore, struggle and lymphocytosis in young animals by physiological reasons were eliminated [63]; lymphopenia was not observed as there are no severe stress, hyperadrenocorticism (endocrine diseases), viral infections [60], lymphangiectasia, and quilotorax [54].

4.4.1. Eosinophils

Eosinophilia was not observed, indicating no stress and all possible cases of parasitosis, hypersensitivity, tissue degradation, hypoadrenocorticism, hypereosinophilic syndrome, and leukemia [60] were dismissed.

4.4.2. Basophils

Basophilia was not identified; thus, there was no response to disease- or antigen-specific production; therefore, hypersensitivity and mastocytemia were dismissed [54].

4.4.3. Heterophiles

The values for heterophiles obtained were similar to those reported by [9] and by [10] for which heterofilia and any pathological process caused by bacteria and fungi were dismissed, as well as severe stress, hyperadrenocorticism, inflammation, and leukemia [54, 63]. Heterophilia was not observed and there were no severe inflammations, excessive food intake, bacteriological infection (gram negative), and myeloid hypoplasia [60].

4.5. Blood chemistry (BC)

Measurement of the chemical elements that compound the blood with other lab procedures and clinical exams help to diagnose, emit a prognosis, and if appropriate, evaluate the efficiency of a treatment [65]. BC is a diagnostic tool, so the metabolites that are tested do not specify tissue or organ damage [59]; therefore, it is not an effective method to correctly or completely diagnose the health status of any species [7, 53].

Collectively, plasma proteins perform a nutritive function, exert colloid osmotic pressure, and help maintain acid-base balance. Individually they work as enzymes, coagulation factors, hormones,

and transport substances [66]. Hyperproteinemia was not observed, eliminating the possible cases for dehydration, hyperglobulinemias associated with chronic inflammatory diseases, hyperalbuminemias, or hemolysis [61]. Hypoproteinemia was also not observed; thus, possible cases of chronic malnutrition, protein malabsorption, poor digestion, protein-losing enteropathies (parasitism), blood loss, chronic hepatitis, or renal diseases were all dismissed [65].

4.5.1. Albumin

Albumin values were in the normal range according to [67, 18], eliminating the possible cases of dehydration. No alterations in protein synthesis (starvation, malabsorption of the small intestine, hepatic processes, and severe trauma) were observed, nor a decrease in protein synthesis from kidney, intestine, hemorrhage, and sepsis [65].

4.5.2. Globulins

Globulin values were within the normal range indicated by [3, 11, 18]; in BCS, therefore, possible cases of hyperglobulinemia and hypoglobulinemia were dismissed [65].

4.5.3. Albumin/globulin (A/G)

Values obtained were similar to [18] in a nearby area, whereby possible cases of renal proteinuria or increased production of immunoglobulins by antigenic stimulation were dismissed. Likewise, there were no increase in A/G values, so possible cases of immunoglobulin production were dismissed too. Hemorrhages and dehydration were also dismissed as possible cases [65].

4.5.4. Urea

Urea values rise due to increased protein degradation, which is caused by intestinal hemorrhage, necrosis, hyperthyroidism, and others. This can also be increased by a reduction of renal perfusion (dehydration, shock, and hypoalbuminemia), for acute or chronic renal insufficiency, and for obstruction of urinary flow. While values decrease if there is abnormal hepatic function or by reduced protein intake [61, 68], in this study, urea values were similar to those reported by [3, 51], therefore suggesting normal, healthy ranges when compared to the above situations.

4.5.5. Creatinine

Creatinine values were similar to the ones proposed by [3, 69] for healthy *C. mydas*; therefore, dehydration, acute and chronic renal failure, and obstruction of the primary urinary flow and rupture of the bladder were dismissed [61, 68].

4.5.6. Aspartate aminotransferase (AST)

AST values are within the limits reported by [11] for healthy *C. mydas* and were similar to those reported by [18]. Thus, these levels were considered normal for the region and this species. Therefore, liver damage, skeletal and cardiac muscle damage (ischemia), septicemia, toxemia, and hemolysis [70] were dismissed.

4.5.7. Alanine aminotransferase (ALT)

ALT values were similar to those proposed by [12] for healthy *C. mydas*; therefore, hepatic problems such as damage, failure, etc., were dismissed.

4.5.8. Alkaline phosphatase (ALP)

ALP values were similar to those obtained by [11] in Puerto Rico and to those described by [18] in BCS. Therefore we concluded that there were no biliary obstruction, hepatic damage, extensive or generalized bone disease, neoplasms, septicemia, starvation, and hepatic regeneration [61, 70].

4.5.9. Blood urea nitrogen (BUN)

BUN levels obtained were within the ranges indicated by [51, 11] for healthy *C. mydas*. Thus, heart failure, excessive levels of protein in the digestive tract, dehydration or renal diseases, as well as malnutrition and hepatic failure [68] were dismissed as possible cases.

4.6. Lactate dehydrogenase (LDH)

Similar values of LDH were observed to those reported by [3, 51]. Thus, any blood flow deficiency, hemolytic anemia, hepatic diseases, muscle injury or muscle weakness [70], and abnormal formation of new tissues (neoplasms) [9] were dismissed as possible cases.

5. Conclusion

A baseline of the health assessments and vital signs for turtles in BCS was established, which can be used as a reference for future research; also, hematological values of these organisms were generated. These results indicate that sampled turtles were healthy.

CBC and BC values in sea turtles may vary with species, sex, age, weight, diet, and stage of development, reproductive phase, whether they are in migration or whether they are resident, and dependence on environment. CBC levels allow researchers to diagnose diseases when the presence of a causative agent or damage directly affects the blood cells. BC analysis is a diagnostic tool; however, the metabolites do not specify tissue or organ damage. The hematological results obtained should not be taken as parameters or as prevalence or incidence data since they do not calculate the disease rate because the statistical characteristics of spatial epidemiology were not achieved. Rather, these values work as reference indicators of health status for Eastern Pacific green sea turtles in BCS.

The establishment of a baseline of health assessments and hematology profiles for healthy wild sea turtles is a priority for their conservation and management. Since normal hematology values have not been established for most free-ranging marine turtle populations, this type of research could be included as part of the monitoring protocol for sea turtles in foraging areas. Such information can provide a foundation for conservation strategies such as the early detection of threats, risks, and diseases. Sea turtle health studies should be integral and sequential, as they must follow a rigorous medical order. Regular health monitoring allows

the diagnosis of infectious disease threats, prevents diseases, and can estimate future spreads leading to the development of strategically relevant conservation programs. These studies act as indicators for sea turtle health status at the ecosystem and population levels.

It is necessary to continue collecting data surrounding vital signs and hematological values, as well as develop a diagnostic interpretation to generate health parameters for comparison with other sea turtle populations. By improving the health status monitoring of sea turtles in BCS, researchers can effectively prevent population species declines, potentially related to diseases.

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Conflict of interest

The authors declare that there is no conflict of interest; this manuscript is original, and it has not been published or submitted to another journal or editorial.

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The Impact of Lameness on Dairy Cattle Welfare: Growing Need for Objective Methods of Detecting Lame Cows and Assessment of Associated Pain

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Abstract

Dairy cows are the major animals reared for milk production worldwide. Lameness is a manifestation of painful condition due to injury or disease in the foot, regarded as a major welfare problem in dairy cows. An effective lameness management requires prompt identification of lame cows. The objectives of this systematic review were to discuss the various techniques of detecting lameness, assessment of the associated pain, and the impact of lameness on dairy cow welfare. Results from the literature search yielded 534 papers, with 102 papers meeting the inclusion criteria. The eligible studies were discussed in two sections which were; (1) lameness detection systems and their application in pain assessment using four methods: gait and behavioral variables, physiological parameters, pressure nociceptive threshold and blood biomarkers; (2) impact of lameness on animal-based welfare measures. Despite the limitations in the use of automated locomotion scoring systems, results showed the technique remains a promising tool for the prompt detection of lame cows compared with manual systems (MLSS). More investigation of such systems could aid the validation of pain in cows with various degree of lameness. Further studies are required for early lameness detection and minimizing the welfare implications in dairy herds.

Keywords: lameness, locomotion, pain, claw lesions, animal welfare, behavior, dairy cows

1. Introduction

The primary aims of dairy husbandry include efficient productive life cycle and good animal welfare. Nevertheless, the continuous selection of cows for high production under intensively managed systems remains a challenge in achieving optimal animal welfare [1]. Aside from being a production limiting condition, lameness is regarded as one of the most important welfare problems in dairy cows. The five freedoms described as the fundamentals of good welfare requires an animal to be provided with adequate food and water, comfort, environment to express normal behavior, the absence of pain and disease, and freedom from fear and distress [2]. On this note, lameness has been shown to impact negatively either directly or indirectly on the aforementioned basics of animal welfare [3].

The factors contributing to the welfare consequences of lameness are multifaceted. Lameness arising from foot or claw lesions is one of the most painful conditions in dairy cattle [3]. To relieve such pain, alteration in gait and posture is evident during locomotion and standing. These events often affect other behavioral activities including feeding, lying pattern, rumination, and social ranking, thus contributing to why lame cows lag behind their sound herd mates in productivity [3]. These behavioral changes have been reported not only in severely lame cows, but also in moderate lameness events [4]. Another aspect is farmers' perception of the importance of lameness and the expertise required in providing adequate care to lame cows. Farmers have been reported to underestimate the prevalence of lameness in their herds compared to trained personnel [5] while regarding only severely lame cows as those requiring treatment [6]. These events contribute to cows becoming chronically lame and reducing their chance of recovery following treatment [7]. Hence, measures to detect slight changes in locomotion remains pertinent to reduce the welfare implications of lameness.

Currently, the manual locomotion scoring system (MLSS) remains the gold standard for the detection of lameness in dairy herds. Though, simple to use, constraints such as their subjectivity and low sensitivity in detecting cows with claw lesions are key issues [8], thereby limiting the assessment of pain associated with lameness. These constraints supported the development of automated lameness detection systems, where sensors and pressure weighing platforms are employed to monitor locomotion. The validation of both manual and automated locomotion scoring systems (ALSS) has been attempted using pain assessment. Recently, researchers have employed techniques entailing the measure of pressure nociceptive method (PNT), physiological parameters [9, 10], biomarkers and expression of genes associated with inflammation in clinically lame cows or based on the presence of claw lesions [11]. These emerging methods have been shown to be more sensitive in detecting lame cows and for better understanding of the etiology and the related pain.

This review discusses recent findings on the welfare implications of lameness in dairy cows based on the associated pain and vital behaviors. Also, the techniques used in the assessment of pain in lame cows either based on clinical diagnosis or detection of specific claw lesions are highlighted. Particularly, the strength and weakness of the locomotion scoring systems are highlighted with emphasis on objective methods for detecting lameness.

2. Materials and methods

This review covers peer reviewed articles, published in English between 2005 and 2017, reporting on the detection of lameness and assessment of the related pain, and their impact on dairy cow welfare. To achieve our stated objective, a narrative integrative review was carried out from October, 2017 to February, 2018 and bias was attempted to be reduced by exploring aspects of systematic review. Three databases namely PubMed, Google scholar and Web of Science were used for the literature search. Where relevant, publications cited in the retrieved articles were also reviewed. The search terms used included "Lameness", "Locomotion", "Claw lesions", "Behavior", "Dairy cows", "Pain", "Welfare", and "Body condition". A total of 576 and 37 records were retrieved from database and other sources respectively. For the study selection process, the PRISMA checklist was employed (**Figure 1**).

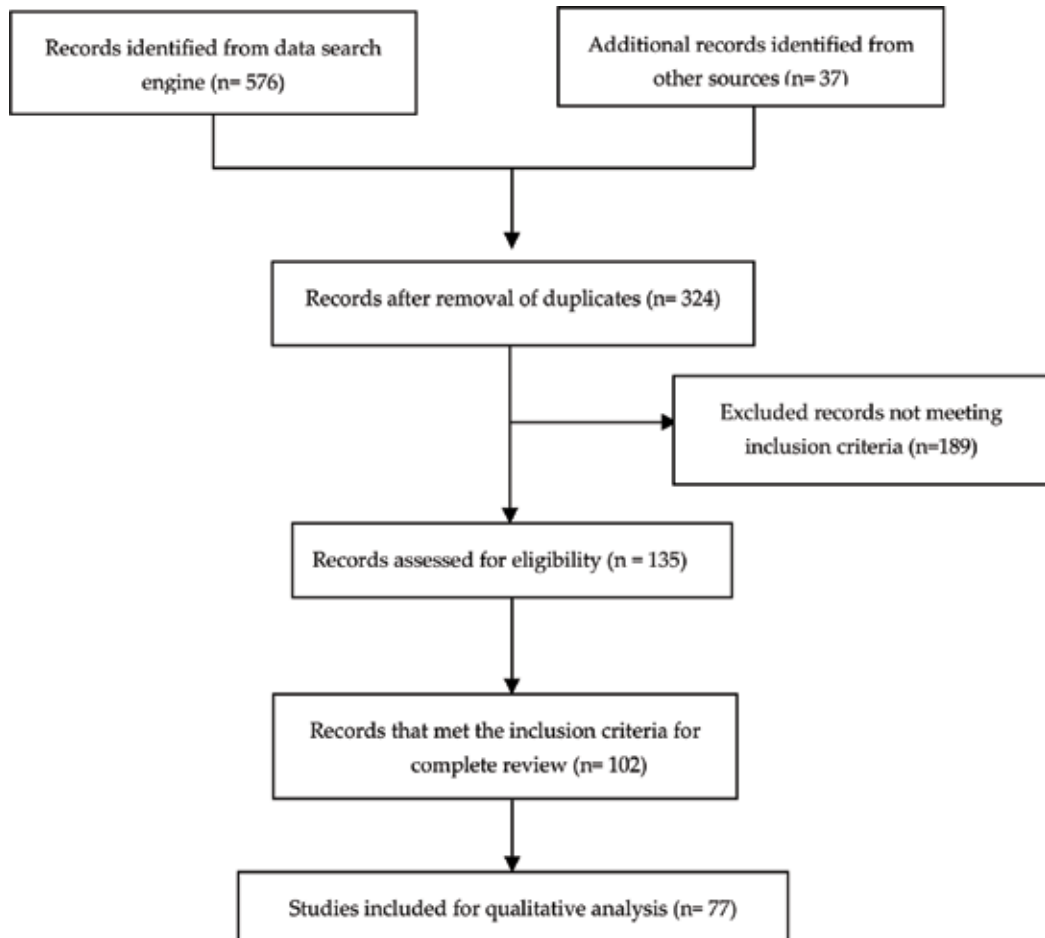


Figure 1. Flow diagram of study selection process using PRISMA procedure.

3. Claw lesions causing lameness

About 90% of lameness disorders in dairy cows are attributed to claw lesions [12]. Claw lesions have been broadly categorized into infectious and non-infectious causes [13]. Non-infectious causes also described as claw horn disruptive lesions (CHDLs), are pathological alterations arising from the internal capsule or claw horn tissues. Sole ulcers (SU), sole hemorrhages (SH) and white line disease (WLD) are the major lesions in this category (**Figure 2**). These conditions are multifactorial, as supporting evidence suggest the interplay between rumen acidosis laminitis complex, prepartum metabolic, and hormonal changes affecting the stability of the pedal bone and suspensory apparatus [14, 15], as well as biomechanical reaction at the claw-floor interface [16]. In addition, animal-based measures such as body condition loss, reduced thickness of the digital cushion (DC) [17], injured hock, and overgrown claw have been associated with increased odds of CHDLs [18, 19].

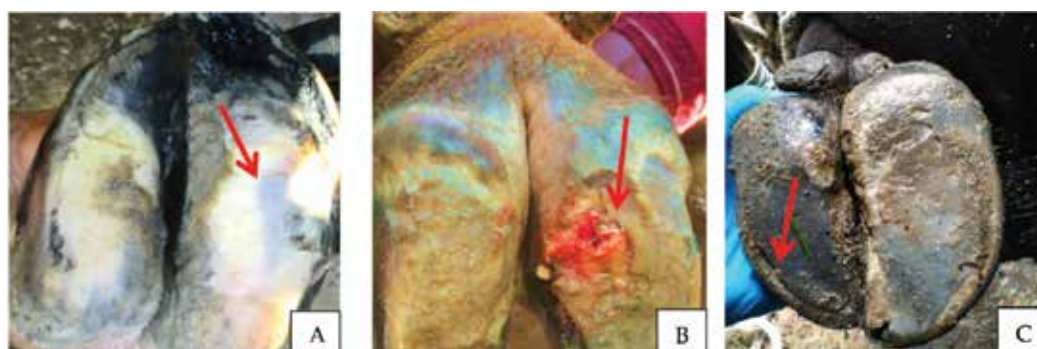


Figure 2. (A) Severe sole hemorrhage (red arrow) and toe necrosis (B) sole ulcer (red arrow lateral claw) (C) white line fissure (red arrow medial claw).



Figure 3. (A) Digital dermatitis at plantar surface of the foot (red arrow) (B) suspected case of foot rot with characteristic swollen coronet, interdigital lesion and purulent discharge.

Digital dermatitis (DD) and foot rot are important claw diseases classified under the infectious etiology. DD lesion is manifested as a circumscribed moist ulcerative mass on the plantar surface of the foot and may extend to the interdigital space [20]. They often result from bacterial pathogens capable of invading a broken digital skin and presence of infected cows in a herd is a vital risk factor [20]. Foot rot is a sub-acute or acute necrotic (decaying) infectious disease of cattle, causing swelling (especially around the coronary band) and lameness in at least one foot (**Figure 3**). The bacterium often isolated is *Fusobacterium necrophorum*. The infectious agents normally gain entry through mechanical injury and softening of the interdigital skin due to prolonged standing, exposure to contaminated manure, and walking on abrasive and rough surfaces. Also, environmental conditions such as high temperature and humidity could affect the integrity of the digital skin [21].

4. Pain associated with lameness

Lameness is a welfare problem manifested in response to pain and discomfort. Specifically, painful sensations from CHDLs are presumably due to the inflammation and compression of the corium in the affected area [21, 22], whereas infectious claw lesions such as DD could be painful both at active and healing stages [23]. According to the International Association for the Study of Pain, pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage” [24]. The definition highlighted two major components comprising of nociception and experiencing pain. Nevertheless, in animals, the concept of the emotional experience of pain is often excluded as described by Zimmerman [25], “it is an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance and may modify species-specific behaviors including social behavior.” Such protective and modified behaviors were suggested as requirements for an animal in pain to reduce and prevent the relapse of injury, stimulate healing and enhance their survival in various habitats [26]. An aspect of this concept was suggested in dairy cattle as they could hide signs of noxious stimuli, perceived as an adaptive behavior to evade predators. Such event might contribute to the presence of painful claw lesions in cows without eliciting clinical lameness [27].

Another aspect is the significance of acute and chronic pain in lame cows. Generally, chronic pain is perceived as a sensation different from acute pain in animals. It is believed that certain stimuli or unceasing nociceptive events enhance the development of chronic pain [28]. Lameness is often categorized as acute or chronic based on the duration of the insult. Chronic cases were defined by higher locomotion score (LS) persisting for more than two weeks [7]. Nevertheless, prompt detection of lame cows is often lacking and contributes to chronic lameness events and hyperalgesia (abnormal increased sensitivity to pain). This is suggestive that such cases either cause more pain or cattle find it difficult to conceal them. Hence, an effective pain management during therapeutic claw trimming (CT) is vital [29].

5. Pain assessment in lame cows

In humans, pain can be directly assessed by a rating scale scored by a patient. However, the situation is different in animals, where physiological and behavioral alterations need to be gleaned for the same purpose [30]. Since pain is the cause of the altered gait in lame cows, the assessment of gait variables is considered as an indicator of the severity using locomotion scoring systems.

5.1. Manual or visual locomotion scoring systems (MLSS)

Locomotion scoring systems (LSS) are widely used for the detection of lame cows and monitoring lameness prevalence in dairy herds [31]. This system entails trained personnel scoring the cow on a numerical scale by observing certain gait and postural variables during locomotion. A nine-point scoring scale described by Manson and Leaver [32] was one of the first detailed LSS where parameters such as asymmetric gait, abduction, and reluctance to bear weight, and difficulty in turning and rising were used in categorizing lame cows. Thereafter, postural traits including arched back, head bobs, and stride length (gait measure) were introduced in classifying cows into five categories ranging from sound to severe lameness [33]. The five scale LS developed by Sprecher [33], which remains the most widely used in lameness related research [8]. Later, head bobs as a postural indicator of lameness using a four-level scoring scale was introduced [34], whereas tracking up and joint flexion was included by Flower and Weary [35]. Attempts to design standardized MLSS include the system developed by the Welfare Quality® Assessment Protocol for Cattle [36] and DairyCo. [37]. The presence of numerous MLSS depicts the lack of consensus and constraints on standardized measures of classifying lameness. The constraints in the application of MLSS include subjectivity and low inter-rater agreement [8], low sensitivity in detecting claw lesions as found during CT [38], influence of animal-based measures and environmental factors [39], and need for training and expertise [8].

5.2. Automated lameness detection methods

Based on the limitations in the use of MLSS, advances have been made in developing automated lameness detection systems or locomotion scoring systems (ALSS). The analysis of gait and postural pattern in ALSS is based on either one or combination of kinematic, kinetic, and indirect approaches. Kinematic principle involves the assessment of changes in specific body segment at a given time interval using automated systems such as accelerometers and image processing techniques [40, 41]. Parameters such as step length, step height and back curvature are measured in such systems to detect the degree of impaired locomotion. Also, techniques involving the attachment of accelerometers to the limbs to measure the accelerations during locomotion [42] and sensitive walkways containing pressure sensors [43] are related to kinematic principle.

On the other hand, kinetic gait analysis deduces information from the force applied to the limbs as seen in ground reaction force systems such as force plates or weight recording platforms [43]. Impaired locomotion is evaluated by measuring the force exerted on the floor by the hooves

when a cow walks on the force plates, or the weight distributed when standing on the platform [44]. Behavioral and production variables are used as indicators in the indirect approach. The alterations in daily lying behavior (duration and bouts) and standing time monitored by accelerometers were used in detecting lame cows [45] and specifically, those likely to develop claw horn lesions [22]. Likewise, live weight gain and milk meters were measured as production indicators of impaired locomotion using combined sensors [46]. A non-invasive tool for prompt diagnosis of claw lesions using infra-red thermography has also been demonstrated in few studies [47, 48]. Although the technique was not used in categorizing lame cows, increase in the surface temperature of the lame hind limb was consistent with the presence of claw lesion.

Overall, ALSS that present results positively correlated with MLSS are beneficial in prompt detection of lameness. For instance, the gait variables in the Gaitwise system [43] and improved images processing using 3D cameras [49] when compared to MLSS could detect mildly lame cows. Garcia et al. [50] also reported Sp and Se above 80% using milking robot and leg-mounted accelerometers. Recently, the extracted gait cycle variables (cow pedogram data) using high-frequency accelerometers were able to accurately detect hind limb lameness and foot pathologies [51]. The latter technique has practical advantage based on the little space required for their set-up and data availability. Nevertheless, there are certain constraints in application of ALSS. This include the use of MLSS as gold standard in validating various ALSS, high cost of installations [8], on-farm practical usage [43], and recently, farmers' preference or perception for lameness detection systems [52]. Additionally, there is need for improved methods of securing the cows' hoof on weight measuring platforms and capacity of detecting mild and severe claw lesions.

5.3. Assessing pain in lame cows using MLSS and ALSS

Researchers have considered the validation of pain in lame cows by investigating their response to various management protocols and comparing the findings between MLSS and ALSS.

5.3.1. Gait and behavioral variables

Gait attributes such as the use of visual LS and weight shifting between rear legs has been reported in studies relating to pain assessment in lame dairy cows. Clinically lame cows that were treated by the application of foot blocks and CT showed significant improvement in mean LS [53]. Similar results were reported following oligofructose induction of lameness [54] and lame cows injected with ketoprofen [55]. The administration of flunixin meglumine produced significantly less weight shifting between the rear limbs in lame cows indicative of pain relief [56], whereas combining the same drug with CT showed no improvement in LS [44]. Again, the weight borne on the rear limbs in lame cows improved following ketoprofen injection [57].

Regarding specific claw lesions, a positive association was reported between higher LS and increasing severity of SH [11]. In another study, a mean reduction of LS (1.5 points on 5 point scoring scale) was recorded in dairy cows affected with CHLs and infectious claw lesions one week after CT [10]. By using a pain scoring scale, DD affected cows that were treated

with salicylic acid had significantly lower pain score after 2 weeks compared with the control group [58]. These findings indicate management routine targeted at reducing pain in lame cows could be assessed by their response to gait variables.

Certain behavioral alterations have been investigated as measures of pain and stress in lame cows. In a clinical trial, an equal number ($n = 42$) of acutely lame cows with or without CHLs were observed during CT [59]. Lame cows showed significantly higher leg movement than the sound cows, indicating a greater response to pain. Similarly, rumination, self-grooming and feeding time were observed in 16 dairy cows divided into two equal groups (lame and non-lame) [60]. Results showed that all the behaviors were significantly less displayed in lame cows compared to non-lame herd mates. However, despite the improvement in weight distribution amongst the rear limbs in lame cows after CT, lying duration was not different between lame and sound cows [57].

5.3.2. Pressure or mechanical nociceptive threshold (MNT)

Primary nociceptors are stimulated in the manifestation of pain in lame cows. For instance, tissue damage at the site of injury results in impulse transmission to the central nervous system and subsequently interpreted as pain. The higher sensation to pain in lame cows is the mechanism interpreted in pain assessment when measuring the pressure or mechanical nociceptive threshold (MNT). Claw or foot pain is assumed to have a strong impact on limb locomotion. To objectively determine the associated pain, the amount of pressure required to initiate the withdrawal of the limb upon compression with hoof tester or algometer was proposed by Dyer et al. [61]. Less pressure was required for cows with higher LS to induce such limb retraction. As such, cows with higher LS could be experiencing more pain compared to those with lower LS. Also, by pressing the metatarsus (dorsal area) with a mechanical pin, the pressure at which the cow reacts is a good estimate of the threshold for pain sensation [62].

By comparing the association between MNT and LS, a lower MNT was found in cows with moderate to severe lameness ($LS \geq 3$) compared with sound cows [63].

Leg withdrawal time has been employed as a parameter for MNT in lameness detection. For instance, hyperalgesia persisted in lame cows despite treatment with a non-steroidal and anti-inflammatory drug [53]. Another study reported no significant difference in the MNT of cows affected with active and healing DD lesions, which was suggestive of painful sensations at both stages [23]. Recently, MNT was measured after CT of cows affected with claw lesions [10]. The result showed that the MNT of cows with CHDLs increased significantly after trimming, but only tended in those with DD. The finding supports the use of CT in the management of pain associated with CHDLs. Also, the overall outcomes are signs of the multimodal approach required in addressing specific causes of lameness and the associated pain.

5.3.3. Physiological parameters and biomarkers

In response to pain, the physiological activity of the hypothalamus-pituitary-adrenal axis and the autonomous nervous system is activated resulting in the release of cortisol; the main glucocorticoid hormone [64]. Accordingly, a significant increase in the plasma concentration

of cortisol was reported in clinically lame cows compared to non-lame groups [65, 66]. Similar results were observed in lame cows compared to the untreated group after inducing lameness using oligofructose approach [54] and evaluation of cortisol level in the milk of lame cows [66]. However, in the assessment of cows affected with various degree of SH (mild, moderate, and severe), plasma cortisol level was not significantly different [11]. More research is needed to assess the pain associated with specific claw lesions causing lameness.

Based on the rumen acidosis laminitis complex in the development of CHLs, recent studies have investigated the role of haptoglobin (an acute phase protein elevated in response to inflammation) in lameness events. As such, increased concentration of haptoglobin was found in clinically lame cows and those affected with sole ulcers [54, 66], but only tended to occur in cows (not clinically lame) with severe SH compared with mild and moderate cases [11]. This shows that cows with claw lesions experience pain and discomfort even without being clinically lame.

Chronic pain and stress is another important aspect in lameness. Clinical parameters relating to cardiovascular function such as heart rate (HR) and variability parameters were reportedly increased in chronically lame cows [9]. Contrarily, the increment in measures such as HR, respiratory rate, and rectal temperature occurred only in severely lame cows, whereas mildly lame cows had significantly increased plasma level of cortisol and Hp [63]. One could infer that the latter biomarkers (Hp and cortisol) as more sensitive indicators of pain compared with clinical parameters. However, other conditions not associated with pain and periodic fluctuations might influence plasma cortisol concentrations [67]. Also, reliable changes in plasma cortisol level can only be ascertained following series of measurements before and after treatment. Factors such as behavioral changes, animal restraint, and tissue sampling might initiate stress and pain, thereby affecting the results.

Measurement of the coronary band temperature (CBT) and skin temperature (ST) around the limb using infrared thermography (IT) in lame cows has also been reported [47, 48]. Although significantly higher CBT was observed in cows with CHLs and DD compared with healthy cows, increased LS was only recorded in those with SU [47]. The authors concluded that CBT was a promising tool for the detection of SU. In addition, the higher lameness prevalence obtained using digital IT compared with MLSS was suggestive of better objective quality.

5.3.4. Blood biomarkers and gene expression

Recently, gene expression profiling relating to hematological parameters and physiological responses have been attempted for a better understanding of the mechanisms and associated pain in lameness events [11, 68]. Pro-inflammatory cytokines and acute-phase proteins (APPs) have been shown as potential biomarkers of bovine lameness [69]. A major function of pro-inflammatory cytokines (interleukins) is to stimulate the productions of APPs such as Hp and serum amyloid A (SAA) [69]. In a randomized control trial, gene expression coding for peripheral blood mononuclear cells (PBMCs), glucocorticoid receptor (GR- α), interleukin (IL-1B), and metalloproteinase (MMP-9) were not significantly different between lame and non-lame cows, despite indication of pain and stress by higher plasma cortisol in the former group [60]. In another study, a fold increase in the gene coding for interleukin (IL-2 and IL-10), MMP-13, and chemokine C-C motif receptor-5 (CCR5) were observed in lame cows

compared with non-lame herd mates [70]. In order to identify potential diagnostic biomarkers in transition dairy cows with the likelihood of developing lameness, Zhang et al. [62] found significant changes in serum concentrations of certain metabolites, pro-inflammatory cytokines and APPs in several weeks prior to when the cows became lame. Most importantly, in lame cows, serum lactate concentrations increased from 8 to 4 weeks prepartum and became more evident the week clinical lameness ensued.

Based on specific claw lesions, despite higher concentration of Hp was found in cows with severe SH compared with mild and moderate cases, no difference was recorded in the expression of genes associated with lameness amongst the various categories [59]. However, lame cows affected with sole ulcers compared to sound cows had higher or tendency of relative expression of the gene coding for cytokines (IL-1 α , IL-1 β , IL-10), MMP-13, GR- α , and Hp [11].

6. Impact of lameness on animal-based welfare measures

6.1. Feeding behavior and rumination

Feeding pattern is an essential behavior that influences the nutrition and welfare of the cow. The measures for feeding behavior are related to the duration and frequency expended in eating at feed bunk and number of visits after feed delivery. Cows with higher LS [71] or clinically lame [42] had significantly reduced feeding time, feeding frequency, and higher feeding rate compared with sound cows. Another study indicated that lower silage intake and less time spent feeding occurred in cows with mild gait alterations prior to when they became severely lame [72]. The prolonged lying duration in lame cows after feed delivery was suggestive of lower time spent standing to eat compared to sound cows [73]. These events could be more evident during peak production level when there is higher risk of lameness incidence. As shown by Palmer et al. [71], the significant reduction in feed intake (dry matter) was only present at early days in milk in cows with higher LS. These findings support the aftereffects of lameness on body condition loss. Nevertheless, periparturient feeding behaviors could influence the rate of body condition loss at early lactation, thus supporting the development of CHDLs from the negative impact on the digital cushion. This process could be triggered around calving as changes in standing duration could increase the pressure of the pedal bone on the corium.

Rumination is an innate behavioral need in cattle and, therefore, considered as a vital measure in evaluating their well-being [74]. Cows spend an average of 8 hours daily ruminating with the physiological benefits similar to sleep in humans [75]. Few studies have indicated negative associations between lameness and rumination [60, 76], whereas the results of other authors [51, 77] depicted no relationship. Recently, Beer et al. [78] developed a model for automated lameness detection via data obtained from leg-mounted accelerometers and nose-banded sensors. Although the best predictor for lameness was the model comprising of number of standing bouts and walking speed, significantly lower eating and ruminating time was observed in lame cows compared with non-lame group. More importantly, lame cows had fewer ruminating chews and boluses. Weigele et al. [4] using the same ALSS found similar outcomes in feeding behavior (eating time and jaw movements) between non-lame and moderately lame cows after corrective CT. These findings suggest that the impact of lameness on rumination could be dependent on the specific foot pathologies causing lameness.

However, two studies investigated the impact of specific claw lesions on feeding and postural behavior after CT [79, 80]. DD-affected cows ruminated more when standing, whereas such behavior only tended in those with SU compared with the healthy group [79]. Cows affected with infectious claw lesions spent less and more time in lying down and standing up, respectively and higher feeding rate compared to those with CHDLs [80]. Additionally, lying duration and LS decreased significantly after CT with no difference in ingestive behavior. Therefore, changes in postural and feeding behavior in lame cows could vary depending on the specific claw lesion and degree of severity. More research is required to quantify rumination efficiency and feeding behavior as related to claw lesions causing lameness.

6.2. Lying behavior

Lying behavior is an important activity in dairy cows with benefits such as conditions to rest after milking, effective rumination, greater space for other herd mates' movement, and increased perfusion of the mammary gland [81]. Conditions influencing the lying down duration could affect the budgeted time for other activities [82]. For instance, prolonged standing on hard floors and unhygienic resting surfaces could affect claw health and subsequent development of claw lesions. Evidence has shown that lame cows lie down for longer durations compared to healthy herd mates [22]. They also display frequent lying bouts as signs of the on-going discomfort [22].

The impact of specific claw lesions on lying behavior has been demonstrated in few studies. Cows affected with DD were found to have spent longer time lying down on concrete and abrasive floors [83]. Another author reported higher lying down duration in cows affected with DD compared to those with sole ulcers [84]. Lame cows with one or more CHDLs laid down significantly longer than sound herd mates [22]. Overall, the direction of the association between lameness and lying behavior requires more investigation as such changes might be consequences of lameness, or the other way around. Also, since other conditions aside lameness might affect lying behavior, the assessment of the behavior could proffer the need for further examination of the limb.

6.3. Social interaction with herd mates and estrus behavior

One natural herd interaction is social activity manifested in form of self-grooming and caudal licking. Since lame cows are in pain and distress, they are less likely to express such behavior compared to non-lame herd mates. Almeida et al. [60] in a randomized control trial reported significant reduction in self-grooming in lame cows compared to sound herd mates. Other researchers investigated the impact of flooring systems (rubber mats and concrete floors) on the aforementioned behaviors in lame and non-lame cows [85, 86]. Overall, self-grooming and caudal licking were displayed effectively in non-lame cows and preferably on soft cushioning floors than concrete floors. Cows find it easier to groom and lick other herd mates on rubberized floorings due to better comfort and greater slip resistance.

Estrus behavior is another important trait affected in lame cows. Events such as delayed cyclicity and low oestrus expression arising from the disturbance in hormonal function and follicular development were reported in lame cows compared to sound herd mates [87]. Claw lesions cause pain, increased plasma concentration of cortisol, and delayed activity of the

luteinizing hormone, thereby leading to impaired follicular growth [88]. Such disturbance in ovarian activity might delay estrus expression and detection necessary for successful inseminations. Other authors have linked the reduced conception rates in lame cows to lower standing duration to be mounted [88]. However, production losses in lame dairy cows are interlinked as feeding behaviors might lead to reduced intake of dry matter vital and energy balance needed for folliculogenesis and ovulation.

6.4. Hock condition

Hock conditions have been increasingly assessed as an indicator of dairy cattle welfare. Most especially, the lateral aspect of the hock is composed of little fatty tissues and muscles, thus making the area prone to traumatic injuries. Such injuries are generally referred to as “hock lesions” and they appear in form of hair loss, broken skin, visible wounds, localized or extensive swelling [89]. The hock condition score (HCS) is used in grading the severity of hock lesions. An example is the 4 point scoring scale developed by Gibbons et al. [90], which simplifies the manifestation of poor hock condition by measuring the area affected by hair loss, swelling or ulceration. Hock lesions have been reported as important causes of lameness in dairy herds [91]. As reviewed by Sadiq et al. [92], positive associations were reported between lameness and hock injuries in several cross-sectional studies. Cows with injured hocks were more likely (OR = 1.4) to be lame in a large Canadian study [18], while significant correlation was reported between lameness and hock lesions prevalence at animal and herd level [93]. The study designs presumed that lameness and hock injuries occur within the same time frame and the direction of the events need to be elucidated. However, monitoring the prevalence of both conditions could enhance the provision of better welfare, since factors influencing their occurrence are similar [94]. For instance, the severity of hock lesions and the risk of lameness might be related to the comfort of the lying surface. The increased lying duration in lame cows on hard and abrasive surfaces or beddings might precipitate hock injuries. Lameness could also result from severe hock injuries, possibly connected to slips and falls when cows are housed on highly slippery floors. Hence, preventive measures for hock lesions could potentially reduce lameness occurrence, thus improving dairy cow welfare.

6.5. Leg hygiene

Good animal welfare requires freedom from discomfort and disease. Maintaining herd cleanliness is a key approach to ensure adequate animal health. However, such practice remains a major challenge in intensively managed dairy facilities due to persistent contamination of stalls and resting surfaces with manure and urine. At cow level, leg hygiene is used as an estimate to overall herd cleanliness. Cook [95] developed a subjective leg hygiene scoring system by measuring the level of manure contamination of the lateral aspect of the rear limbs. The scoring system has been used to assess the relationship between leg hygiene and lameness occurrence. Accordingly, increased odds of clinical lameness [18] and prevalence of DD [96, 97] were reported in cows with poor leg hygiene. The study designs implied that dirty legs could either be a factor for lameness, or the other way round. Mechanical injury or softening of the digital skin and other sensitive areas of the claw might occur in conditions where the foot is exposed to manure slurry, thus aiding the occurrence of DD and other claw lesions.

Factors suggestive to influence such poor leg hygiene include inappropriate concrete floor designs for efficient drainage [98], usage of rubber mats [99], and cleaning frequency [97]. Nevertheless, changes in the budgeted time for essential activities in lame cows could be a factor. For instance, the prolonged lying down time, less activity and self-grooming in lame cows compared to sound herd mates could increase the exposure of the udder and limbs to manure contamination. Farmers might also accord little importance to lame cows, thus limiting the care for affected cows during routine cleaning.

6.6. Body condition score

Based on the manifested pain, reduced frequency of visits to feed bunk, lower feeding duration, and reduced capacity to compete for feeding space, it is expected for lame cows to lose body condition score (BCS). Nevertheless, the event could be the other way round, with cows in low BCS likely to develop CHDLs causing lameness [100]. The relationship between BCS and CHDLs stemmed from understanding the composition of the digital cushion (fatty pad) as an adipose tissue that annuls the contusive forces directed unto the pedal bone [101]. Hence, thinning of the DC from the mobilization of fats during negative energy balance could predispose to CHDLs incidence [102]. Recently, the reduced thickness of the DC and body condition loss was described as mechanisms for the development of CHDLs [15]. This complex relationship further depicts the multifactorial nature of lameness. Other theorized mechanisms such as ruminal acidosis laminitis complex, activation of metalloproteinase, and periparturient hormonal changes might come into play within the same time frame.

Considering the welfare implications of lameness, the freedom from hunger and thirst is one of the fundamental provisions of good animal welfare [2]. According to Whay and Shearer [3], BCS could be seen as an alternative indicator of hunger and body condition loss through reduced feeding duration, lowered competitiveness for feed, and negative energy balance as defensive mechanisms against on-going clinical lameness. If these events could precipitate suboptimal nutrition, then an association is present between hunger and lameness. Whilst there are sparse studies investigating the impact of lameness on drinking behavior, the alterations in budgeted time seen in lame cows could affect such behavior.

7. Conclusion

Lameness remains a condition with significant impact and burden to optimal welfare in dairy cows. With the majority of lameness events attributed to foot or claw lesions, assessment of the painful condition necessitates objective techniques for early detection of cows in discomfort prior to the development of obvious signs of lameness. ALSS are promising tools for the detection of lame cows. However, their applications in the assessment of pain associated with lameness require further evaluation. As such, the validation of various lameness detection systems could be more explored through the assessment of gait and behavioral variables, nociceptive threshold, physiological parameters, and blood biomarkers. In addition, consideration of specific claw lesions causing lameness is important to further understand the association between variables assessed in various lameness detection systems and validation

of the associated pain. Further works are needed to investigate the impact of specific claw lesions on dairy cow welfare. Such result could enhance the adoption of definite preventive measures and management practices to reduce the occurrence of lameness in dairy herds.

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Conflict of interest

The authors declare no conflict of interest.

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Welfare of Pet Birds and Potential Zoonoses

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Abstract

The human-animal interaction had long been established and currently emerged in multiple aspects including housing of animals for food and as pets. The “pet birds” are the wild or exotic birds having high genetic value and are housed under captivity as companions or for ornamental purposes. The commonly housed pet birds are either passeriformes or psittaciformes. These birds are housed under conditions to meet standard requirements for welfare of pet birds. Besides the pet birds and human relationship, these birds are potential carriers or transmitters of several pathogens considered responsible for zoonotic diseases. The range of the zoonotic diseases consisted of bacterial, viral, parasitic and fungal diseases. The mode of transmission is also an important entity for understanding the spread mechanism of zoonotic diseases. The transmission and spread is predominantly through the direct contact and in the few conditions through the vectors; termed as vector-borne transmission. Altogether, in this chapter, the authors have discussed different aspects of welfare of pet birds, categories of zoonotic diseases along with mode of transmission and spread of zoonoses. At the last, few aspects of welfare of pet birds and prevention and control guidelines of zoonoses are suggested for the personal biosafety and public health.

Keywords: welfare of pet birds, Zoonosis, exotic birds, biosafety, public health

1. Introduction

The term “pet birds” employs on every bird which feeds on different plant seeds or grains and could be kept legally under captivity which could spent all or some time in cages. The increased

popularity of “pet birds” particularly during last few decades has highlighted the significance of pet bird zoonoses and pet bird medicine throughout the world [1]. Further, the relationship among humans and birds is diverse that existed since centuries. Humans are using wild birds for different purposes i.e. meat, eggs, skins and feathers as food or for ornamental purposes. The relation has emerged from historical sport hunting to companion or pet birds which are currently considered as “family members” in several regions of the world [2]. The majority of pet birds are originated from either passeriformes (canaries and finches etc.) or psittaciformes (budgerigars, parrots and parakeets etc.) [3]. These birds particularly the psittaciformes are naturally wild inhabitants and are now domesticated to be kept as companions or for ornamental purposes, among all the birds, parrots are the best pets which have been domesticated since the times of Alexander the Great and ancient Egyptians [4]. Further, the pigeons are among the first domesticated birds which are being reared for meat, racing and historically were used as courier pigeons particularly during wartimes [2]. Similarly, there are many factors which influence the owners to select an appropriate species of a bird to be housed as pet bird i.e. size of the birds, color, average age, sound and interaction of birds with humans. The strong relationship of owners with their pet birds increases liabilities to provide sufficient housing conditions, clean food and water as well as this relation also poses the risk of transmission and spread of zoonotic diseases. The main zoonotic diseases include bacterial (chlamydia, salmonellosis and tuberculosis), viral (influenza virus infection, West Nile fever and new castle disease), parasitic (*Cryptosporidium* infection) and fungal (aspergillosis) diseases [5]. The main transmission and spread is through direct contact with carrier or sick pet birds, however the indirect transmission is also important for some viral infections, for example, the transmission of West Nile Fever virus (WNV virus) through mosquitoes [6]. With an increased trend in pet bird housing, remarkable increase in veterinary diagnostic was noticed throughout the world [7]. Collectively, in this chapter, the authors have discussed different categories of zoonotic diseases of pet birds, the transmission and spread of zoonoses along with few prevention and control guidelines.

2. Welfare of pet birds

The urge to have exotic pets or pet birds for ornamental purposes is prehistoric which must be governed by some of the regulations. For example, in East Asian or American regions, the pet bird keeping is quite popular [8, 9]. To assure certain welfare rules for pets, the Convention on International Trade in Endangered Species (CITES), is an international agreement that deals as licensing and governing system for the trade of animals. Exotic pet birds are obtained from the wild, born in captivity from wild parents and then reared and bred in captivity [10]. While keeping the exotic or wild birds under captivity, certain standards must be followed which includes

- Proper housing having sufficient space according to the breeding requirements
- Free access to clean water and food

- Disease control by following regular vaccinations and proper treatment strategies
- Freedom from discomfort and distress etc.

3. Zoonotic diseases of pet birds

The zoonotic diseases of pet birds are mainly divided into bacterial, viral, parasitic and fungal diseases as outlined in **Figure 1**. These zoonotic diseases have significant impact on biosafety and human health [3].

3.1. Bacterial diseases

The important bacterial diseases are:

- Chlamydophilosis (also termed as ornithosis, psittacosis or parrot fever) is most threatening zoonotic disease caused by a bacterium *Chlamydoiphila psittaci*. Psittaciformes birds are highly susceptible to *Chlamydoiphila* in comparison to passeriformes birds [11–13]. The clinical signs and symptoms in humans ranged from mild respiratory signs to severe pneumonia along with diarrhea, conjunctivitis, arthritis and genital organ infection. Veterinarian and pet birds breeders are first susceptible hosts for chlamydophilosis [14–16]. The zoonotic *Chlamydoiphila* cloud also contribute to antibiotic resistant in humans [16].
- Salmonella species are frequently isolated from many pet birds including both passeriformes and psittaciformes. The disease cloud be classified as asymptomatic carriers to birds having severe clinical symptoms including diarrhea, septicaemia, osteomyelitis, depression, crop stasis, dehydration, anorexia [17]. Salmonella is potential zoonotic pathogen

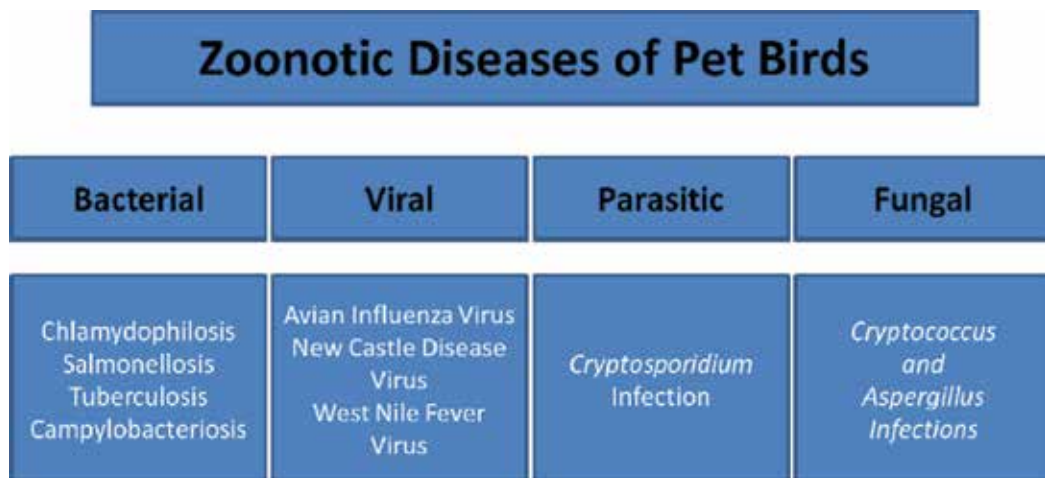


Figure 1. Categories of zoonotic diseases of pet birds.

associated with human transmission particularly from passeriformes birds [18]. The incidence of human salmonellosis could be severe along with severe clinical signs including vomiting and diarrhea [19].

- iii. Tuberculosis caused by *Mycobacterium* species is also believed to be transmitted by pet birds particularly psittaciformes. The commonly isolated species are *Mycobacterium genavense* and *Mycobacterium avium*, respectively [17]. However, the major human pathogen *Mycobacterium tuberculosis* is rarely reported in birds as potentially zoonotic agent.
- iv. Campylobacteriosis caused by *Campylobacter jejuni* is primarily responsible for food borne infections including gastro enteritis (diarrhea, vomiting), headaches, and depression, leading sometimes to death. The condition was reported as potentially zoonotic [20, 21].
- v. Some other bacterial pathogens associated with pet birds which are considered as zoonotic include Pasteurella, Klebsiella, Yersinia, Pseudomonas and *Escherichia coli* [22]. Highly pathogenic *E. coli* O157:H7 is most fatal strains transmitted from wild passeriformes to cattles followed by its introduction into the food chain which has been reported in several studies [22, 23]. Even the toxigenic strains of *E. coli* are reported in pet birds which are responsible for zoonoses [24]. Collectively, the bacterial pathogens are important for owners and breeders of pet birds.

3.2. Viral diseases

The viral pathogens associated with pet birds zoonoses includes Avian Influenza virus (AI virus), West Nile Fever virus (WNF virus) and New Castle Disease virus (ND virus). Among these AI virus is a major risk to breeders and owners of the pet birds, particularly avian influenza A is global public health threat. In one of the recent studies AI virus (H9N2) was characterized in samples originated from pet birds and the potential role of pet birds was observed in transmission and spread [25]. International pet trading also increases the risk of transmission and spread of highly pathogenic zoonotic AI viruses, for example the highly pathogenic H9N2 strain was identified from parakeets which were imported to Japan from Pakistan [26]. The significant morbidity and mortality rates are reported recently in humans due to influenza virus infections [27]. Further, the role of wild migratory birds is considered important for transmitting the pathogen in several regions of the world. The virus also spread from endemic countries to other locations through international trade of exotic and pet birds [28]. However, the commercial markets of pet birds are still a greater risk for zoonotic transmission and spread of highly pathogenic AI viruses [29]. Secondly, the zoonotic transmission and spread of different arboviruses (*Flaviviruses*) could not be neglected including WNF virus which is an emerging vector-borne zoonotic disease [30]. The pet birds are also involved in transmission and spread of WNF virus as described in recent study, further, the international routes of wild migratory birds also served as potential reservoirs for zoonotic transmission and spread as described in recent cross sectional study [6]. Mostly, the birds are infected exhibiting sub clinical course of the disease, however, birds may develop a clinical form of the disease with ocular and neurological symptoms. At the last, Newcastle disease which is caused by Avian *Paramyxovirus* is also described in pet birds and transmission to humans is possible with mild

clinical signs and symptoms which includes mild conjunctivitis [17]. Concludingly, AI and WNF viruses are potentially zoonotic having significant impact on public health whereas ND virus is less important zoonotic pathogen.

3.3. Parasitic diseases

The parasitic disease are rarely contracted and are considered as least zoonotic as compared to bacterial or viral pathogens. However, one important disease in birds is caused by *Cryptosporidium* which is characterized by intestinal and nephrotic symptoms which leads to sever diarrhea in humans [30]. The condition is usually caused by either *C. meleagridis* or *C. baylei* which are considered as zoonotic [30–32]. The prevalence of zoonotic *C. ubiquitum* in different birds (Alexandrine parakeets, Atlantic canary, budgerigar, cockatiel, crested myna, rock dove, and silky fowl, Fischer's lovebird and rosy-faced lovebird) is considered significantly important for public health [32]. Similarly, Toxoplasmosis caused by *Toxoplasma gondii* is reported in 3 out of 140 pet birds in Fujian, China [33]. *Toxoplasma* was also isolated in love birds [34]. However, the main role of pet birds in transmission and spread is not well characterized. Finally, the risk of transmission and spread of parasitic infection is only important among young children who are closely associated with pet birds and are unaware of hygienic handling [17].

3.4. Fungal diseases

The pet birds are continuous source of different fungal infections which are transmissible to human, the main pathogens include *Cryptococcus* and *Aspergillus*, the former is considered as an opportunistic pathogen for humans [35], whereas later is more frequently isolated fungi from pet birds [17]. In both of the fungal pathogens, the zoonoses is considered as less important as the humans could contract the clinical disease or few clinical symptoms from environmental or other sources. In one of the previous studies, cryptococcal meningitis was reported in a female with exposure to a pet magpie that was confirmed genetically by identifying the isolates from the cerebrospinal fluid of the patient and fecal material of the bird [36]. Unfortunately, there is dearth of data with regard to aspergillosis in pet birds. *Aspergillus* spp. are isolated from pet birds from acute as well as chronic cases which is considered as less zoonotic threat [7]. Collectively, among young children and immunocompromised patients the risk of transmission of fungal pathogens from pet birds could not be neglected.

4. Route of transmission

The possible route for transmission and spread of zoonoses among breeders or owners of pet birds includes direct or vector-borne transmission.

4.1. Direct transmission

Passeriformes and psittaciformes are commonly housed pet birds as companion or for ornamental purposes. This is also quite natural that the owners or breeders of pet birds have very

close contact with their birds, thereby probability of disease contraction increases. The direct contact also involves the shedding of zoonotic pathogen in drinking water that normally comes in contact with breeders or owners of pet birds [25]. The range of direct transmission varies from allergic alveolitis to severe chlamydia. The respiratory or inhalation route may result in psittacosis, allergic alveolitis or asthma among owners or breeders of pet birds [37, 38]. The contact with drinking water and foods of pet birds is also associated with transmission and spread of zoonotic diseases [37]. There is an increased numbers of different pet birds particularly at shop facilities or markets which indirectly increases the risk of transmission and spread of different diseases particularly salmonellosis and chlamydia [39]. One of the another aspects is “kitchen-housing” of pet birds that also has an impact on zoonotic transmission and spread of several infections to the owners of the pet birds. Different meetings or gatherings of pet birds by the owners in different pet bird exhibitions or pet bird shows also increases the risk of transmission and spread of various pathogens. Therefore, unusual close contact with pet birds should be avoided to control direct transmission of infectious pathogens.

4.2. Vector-borne transmission

The vector-borne zoonotic diseases are also a major problem for breeders and owners of pet birds. For example, different species of mosquitoes are responsible for transmission of *Flaviviruses* like WNF virus [40]. Mosquitoes (*Culex*, *Anopheles* and *Culiseta*) play an important role in transmission and spread of WNF virus, therefore, it is important to control the mosquitoes population in the surroundings of pet birds [41, 42]. Normally, the mosquitoes acquire infection while feeding on infected pet birds or other animals, therefore mosquito-borne infections also resulted in an outbreak form in a particular region [43]. Similarly, ticks (particularly genus *Ixodes*) also play role in transmission and spread of several pathogens which include *Borrelia burgdorferi* which is causative agent of Lyme disease [44]. The role of tick infestation was found among European songbirds and further transmission to humans [45]. The presence of ticks was also demonstrated on migratory birds in Iceland [46]. Even the large birds or zoo birds also have an incidence of tick infestation as recently observed in captive Emu at Healesville sanctuary, Victoria, Australia [47]. Some species of mites are also reported to suck the blood of pet birds including *Dermanyssus gallinae*, this is a nocturnal ectoparasite and found on pet birds as well as on the surroundings which may involve in the transmission and spread of *Coxiella burnetii*, *Salmonella* and *Listeria monocytogenes* infections [48–50]. Altogether, the transmission and spread of zoonotic diseases which are caused by either mosquitoes, ticks or mites could be well controlled by regular cleanliness of pet birds and surroundings of pet birds [48, 51].

5. Guidelines for welfare of pet birds and for prevention of potential zoonotic diseases

To control the transmission and spread of potential zoonoses, at least the below mentioned precautionary measure should be adopted [10, 11]:

- Free access to water and food for good health of pet birds
- Good housing facility which protects from harsh environment conditions
- Sufficient space to maintain the natural behavior
- Careful handling
- Prevention and control of diseases of pet birds
- Cleanliness of clothing and shoes following contact with other birds
- Washing of hands before and after handling birds
- Regular cleanliness of cages, food and water
- Discard the remaining food (fruits and vegetables) regularly
- Regular washing and cleanliness of cages at least once a week
- Storage of food in clean and sealed containers
- Cleanliness and disinfection of cages before use
- Quarantine of the sick or newly introduced pet birds
- Restricted access of persons having their own pet birds
- Follow the rules for import or export of pet birds
- It is also recommended that breeders or pet shop owners should control the transmission and spread of zoonotic diseases [39].

Usually, sellers or breeders of pet bird are well aware of the precautions; however, the risk of zoonoses is higher in the cases of housing the pet birds for the first time particularly when children or their parents are not informed and guided properly.

6. Conclusion

In conclusion the pet birds must have free access to clean food and water, freedom from discomfort, distress, pain, injury and several diseases. At the end, the relation of pet birds and humans is an interesting entity which cannot be neglected as potential source of zoonoses. A good strategy could be isolation of new pet birds for first few days from the existing pet birds for control of potential diseases. The understanding of natural behavior of pet birds, zoonotic disease and different routes of transmission and spread along with basic information with regard to potential zoonotic disease could minimize the risk of zoonoses to the owners and breeders of pet birds, ultimately providing good care to pet birds.

Conflict of Interest

The authors declare no 'conflict of interest'.

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Husbandry and Welfare

Bovine Natural Taming

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Abstract

Animal behavior is a function of species biology, interaction with the environment and experience previously acquired. Natural taming is a set of techniques used to tame the animals without stress and with patience and respect. They are conditioned to follow man's commands in a quiet way, resulting in more reliable and productive animals. Cattle learning process has enabled the development of management techniques, such as the interactions between them and humans. These techniques aim to use the cattle natural behavior during the management operations, allowing gathering and moving the animals to other areas of the pasture, corrals or even outside the farm. These techniques are mainly important when cattle are raised on pasture, with less contact with people, resulting in less herd control and greater reactivity of animals to management. Many repetitive exercises are used, conditioning the animal in a gentle and progressive manner, without the use of force and pain, to obtain the desired commands. It is an activity that requires patience, bringing good results, better than those achieved through traditional taming, which can cause many traumas.

Keywords: beef cattle, behavior, dairy cattle, gentle, management, welfare

1. Introduction

Humans and animals have interacted for thousands of years, and from 6000 B.C., this relationship has intensified [1]. In twentieth century, as world population increased a lot, intensive

breeding systems have emerged and so considerably altering the environment available to animals. New production systems require physiological and behavioral adaptations of the animals and the men to the new management used.

Taming is a domination and submission process that men teach animals to do what men wish [2]. Unfortunately, there still have cruelty and punishment with physical pain in some cases. Management that does not respect the animal and does not promote animal welfare cannot be accepted anymore. With the strengthening of the concept of animal welfare, we have sought ways to realize the taming and the desired management through non-aversive methods that do not leave undesirable behavioral effects in the herd. Thus, the natural taming stands out in the animal routine management in the farms, for presenting fast and efficient results.

Natural taming is a set of techniques used to tame the animals without stress and with patience and respect. They are conditioned to follow man's commands in a quiet way, resulting in more reliable and productive animals [3].

2. Natural taming

2.1. Bovine ethology

Ethology is the study of animal behavior in natural environments. The main interest of ethologists is instinctive or innate behavior [4]. Ethologists believe that the secrets of animal behavior are in the genes and in the way they were modified during evolution to deal with particular environments. Mammals innate behavior patterns can be replaced by learning. However, in some situations, animals tend to revert to patterns of innate behavior, generating conflict between conditioned and instinctive behaviors [5].

The temperament presents low to medium heritability and repeatability [6]. In cattle, the heritability was estimated from 0.40 [7] to 0.53 [8].

The cattle are gregarious animals and, by their nature, are reluctant to separate from their herd companions and to join with strange animals. When subjected to situations that cause pain, social isolation, sudden noise, fear, cattle present stress and react to these aversive situations by modifying their behavior, which may increase movement or attempt to escape [9, 10].

The animal temperament determines how it will react during handling. There is genetic effect, but animal previous experience influences very much too [11]. Animals handled with care and quietly tend to have a lower flight zone, being easier to work than those ruthlessly managed [12].

2.2. Behavior of cattle

Cattle are herbivores that live in groups, being considered prey in the nature. To stay alive, these animals need to be on constant alert to escape from predators. This behavior aims at perpetuating the species [13].

Usually cattle behavior can be determined by the way these animals react to other same species animals, other living organisms, the environment and the overall reactions to adapt or adjust to the various conditions encountered [14]. On the other hand, in pastures and corral animal behavior is a result of the instinct and the response of learning from the environment [15].

According to Bertenshaw and Rowlinson [16], cattle learning process has enabled the development of management techniques, such as the interactions between them and humans. These techniques aim to use the cattle natural behavior during the management operations, allowing gathering and moving the animals to other areas of the pasture, corrals or even outside the farm. These techniques are mainly important when cattle are raised on pasture, with less contact with people, resulting in less herd control and greater reactivity of animals to management. Such conditions increase the risk of accidents while handling animals.

Cattle learning process, such as the interactions between them and humans, has also enabled the development of management techniques. These techniques aim to use the cattle natural behavior during the management operations, allowing to gather and move the animals to other areas of the pasture, corrals or even outside the farm. These techniques are mainly important when cattle are raised on pasture, with less contact with people, resulting in less herd control and greater reactivity of animals to management. Such conditions increase the risk of accidents while handling animals.

2.3. Group social life

Animals live in group to protect themselves from predators. Living in groups it is easier to escape. Still today, cattle should always be conducted in groups and handled in groups. It is quite stressful for the animal to be isolated from the herd, which can lead to behavior of sudden changes such as aggression and agitation.

The cattle recognize other animals in the group, and the regrouping can alter the normal animal behavior [17] and normally sets out the hierarchy. Grouping of unfamiliar animals is found to increase aggression and social stress. The animals become less efficient to produce due to the effects on feed intake [18]. After regrouping cows have inferior milk yield and increase the somatic cell count in the milk [19].

Social dominance behavior is a major component of social behavior in cattle, as groups of cattle will establish social hierarchies, which can reduce the level of aggression in the herd [20].

2.4. Bovine sight, flight zone and balance point

For taming and natural handling, it is necessary to know and understand animal behavior, how they sense environment and how they express their feelings. In this way, it is possible to analyze and predict their reactions, getting an efficient, appropriate and less stress management. Animal behavior is a function of species biology, interaction with the environment and experience previously acquired. Man must provide the necessary resources for cattle adaptation to production systems, thus avoiding damages to animal welfare and economic return.

Because they are a natural prey, cattle have an important characteristic, which is the lateral location of the eyes. This location allows for binocular and monocular vision, with a 345° view field [21]. Therefore, the animal has the side view without turning your head. However, there is a zone behind the animals, called the blind zone, in which they cannot see [22].

Figure 1 shows a bovine vision.

According to eyes location, bovines move in circles and always keep a safe distance from the handler, from where they can see him. That is why we must be careful when handling the animal, because if the animal does not see the human approach, it may become scared and has sudden reactions. Shadows, strong reflections and bright colors could be seen as threats to animal, which could generate difficulties and management problems. Cattle can perceive details and objects that go unnoticed by the man's vision, which can make management difficult or even impossible. The flight zone is the minimum distance allowed by the animal to approach humans before starting to move (escape), and their size depends on the animal. Different animals will have different flight zones, depending on the domestication degree and the interaction with the handlers [23].

Very quiet animals have a small or no flight zone, so people can touch them. However, if an animal walks away with the approach of a person, it is because it has entered its zone of escape [24]. Balance point is an imaginary line at the animal's shoulder, forming an 90° angle with its body. Bovine animals move forward when people are behind that point, and backward if people are before the break-even point. **Figure 2** shows flight zone and where handler must be for the animal to move.

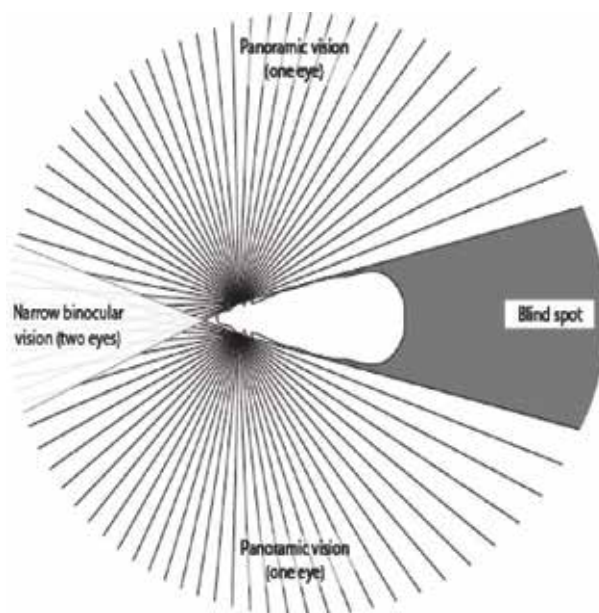


Figure 1. Vision field. Adapted from Krohn et al. [2].

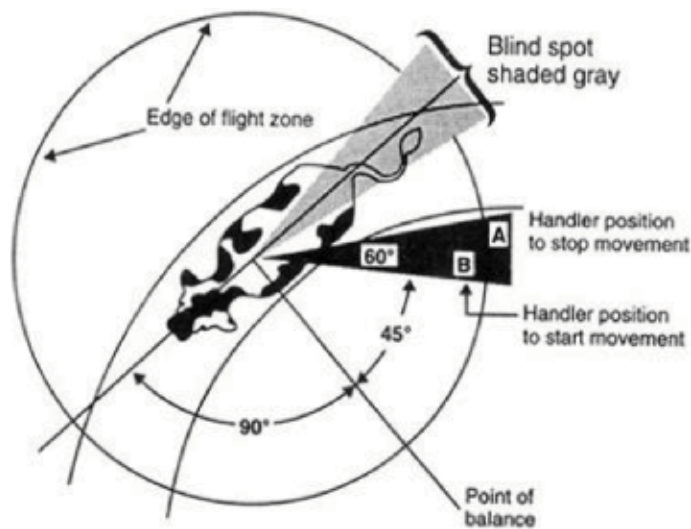


Figure 2. Bovine flight zone and balance point. Adapted from Grandin [25].

According to Grandin [25], handlers must work stay at the edge of the flight zone to move animals calmly and easily. When the handler wants the animal to move forward, it is necessary to position himself within the flight zone and in a caudal position from the balance point to an angle of 45° (point B). The positioning even more caudal, between 45 and 60° in relation to the balance point (point A), usually results in the displacement stoppage. This would occur because the handler would be approaching the cattle blind zone, causing the animal to turn its head to keep it in its visual field, stopping walking. If the animal does not stop, it will walk in circles. When the handler positioning is more frontal to the balance point, animal usually moves backward. However, it is worth mentioning that the flight zone and the balance point are dynamic and always change with the movement of the handler and the animal.

Thus, natural taming is a method that the handler decreases the animal flight zone and allows minimal stress cattle management. However, the natural taming aim is not to eliminate the flight zone, as it is indispensable for the animal movement in an adequate management.

2.5. Traditional taming X natural taming

Cattle are treated as wild animals in traditional taming. The pain is used to obtain cattle responses and allows them to impose their will on the trainer. In addition, this method intended to modify behavior through intimidation and fatigue and employs mechanical procedures based on repetition without reflection.

The cattle present stress and react to aversive situations modifying their behavior when subjected to isolation, pain, sudden noise or fear. This fear can generate an aggressive attitude and an attempt to escape. Fear is a highly stressful factor and can elevate stress-related hormones, such as noradrenaline, adrenaline, and cortisol, to levels higher than many adverse physical factors such as animal's housing [26].

The cattle natural taming is based on the behavior observation and the animal relation with the environment that they are inserted. It is an interaction method with cattle based on respect and communication in a language that the animal can understand, instead of using fear, intimidation and pain. This technique consists of a long, but useful, process of teaching the cattle by means of handler confidence. Therefore, animal makes the movements by his will and not because he is obliged. Many repetitive exercises are used, conditioning the animal in a gentle and progressive manner, without the use of force and pain, to obtain the desired commands. It is an activity that requires patience, bringing good results, better than those achieved through traditional taming, which can cause many traumas.

The difference between taming methods is not just to give reward or not, but to do it when it is deserved. It is essential to be calm as possible, but powerful when necessary. In traditional or natural taming, the movements and responses that we expect from cattle are the same, but the difference is in the way we ask for the movements.

2.6. The cattle learning process during the natural taming

It is necessary to learn how to communicate with cattle to taming and manage adequately these animals, listening and respecting them to establish leadership and teach to follow our commands. For this, it is indispensable to understand how bovines learn, and, in this way, the method can be applied in all types of management and situations. This still prevents the learning and practice of bad habits that are not proper to the animal behavior.

According to Frazer and Broom [27], the learning is defined as the acquisition of knowledge through practical experiences after a situation and its repetition. Learning results in a permanent change in animal behavior in order to respond adequately to the command proposed. The cattle learning during the process of natural taming is established when we create a stimulus and, if the animal chooses the correct answer, we immediately interrupt that stimulus.

Then, learning is reinforced with repeated situations that make the animal to understand the command that must be accomplished. It is not a tireless sequence of irrational repetitions that will determine learning, but a sequence of repetitions that will make the animal be able to reflect and choose the best action. The learning can result in a creation of habits and automatic responses. The habits formation can be accelerated with repetitions during the training, being very effective the schedules with repetitions of a task and interposed with moments of relax.

It is important to reward all cattle efforts when they do what we ask for. With positive reinforcement, the attitude is followed with a nice reward soon after the desired response. Reinforcement is a word widely used in learning theory and taming. It is characterized by increasing or decreasing the possibility of behavior associated with a given situation. Delayed reward is not effective because the animal is not able to make the association between the reward and the attitude. A good example is to caress the back, belly, and perineum region and walking away quietly, indicating that you do not cause damage and so will be emphasizing the correct behavior. It is the commitment to serve us that generates retribution, not the perfect execution of the movement. How greater is the positive reinforcement, more quickly is the adaptation to the habit.

But, in taming, part of the training is performed with negative reinforcement, which should not be confused with punishment, since this type of reinforcement is not associated with violence, punishment or pain, as in traditional taming [28]. Negative reinforcement is defined as the removal of something that bothers the animal to produce the expected response at the time the desired task is performed. This reinforcement occurs during the undesirable behavior and not after it and must stop at the exact moment when the wrong answer ends and the right answer starts. An example of a negative reinforcement would be to maintain the pressure on the halter rope until the animal takes a step forward, when we relieve the rope pressure, demonstrating to the animal that it has made the right decision.

The retribution with the takedown of the stimulus from each advance, no matter how small the advance is, is an incentive for cattle to commitment more and more to seek the right response and thus comfort.

Punishment is a painful stimulus after an unwanted attitude, resulting in pain. When a punishment is performed, the correct task or response will not be tied to the animal's memory and the fear will be present, which does not allow establishing a trust relationship between man and animal. Therefore, punishment should be avoided in taming [29].

2.7. The relationship between man and animal: desensitization

A very important practice in natural taming is the animal desensitization, in which various stimulus and objects are used to learn and create habits [30]. This makes sounds, objects, animal's housing and situations that, at first, seem to bother cattle become routine.

First, the flight zone must be established for the animals that are training, and the delimitation of this area allows the adoption of methods that allow the approach or the distance, depending on the reactivity of each animal. The reduction of the flight zone should be carried out with the approximation, using ropes and swabs, generating gain of animal confidence and performing the desensitization (**Figure 3**).

With gradual desensitization, the animal realizes that the man's presence is not negative and allows the approach and the physical contact. The physical contact allows to please the animals with caresses and brushing. These desensitization actions allow contact with the udder and hind limbs (**Figure 4**), which facilitates the management of cows and heifers and facilitates the process of adaptation of these animals to the milking parlor. These desensitization actions allow contact with the udder and hind limbs (**Figure 4**), which facilitates the management of cows and heifers during the process of adaptation of these animals to the milking parlor.

Positive interaction between men and cattle results in benefits for both, since establishing confidence reduces the risk of accidents, promotes animal welfare and may increase milk and meat production.

After the approach and direct contact with the animals, we must move on to the next training phase. At this stage, the animals received halters and learn to be guided by the handler, they also learn how to stand in the right way and receive daily operations such as bathing and brushing (**Figure 5**).



Figure 3. Reduction of flight zone and gradual approach during the process of natural taming using ropes and swabs. Source: Nilson Dornellas de Oliveira.



Figure 4. Desensitization of the udder and hind limbs. Source: Nilson Dornellas de Oliveira.



Figure 5. Confidence established. Source: Nilson Dornellas de Oliveira.

3. Conclusions

Natural taming is gentle for bovine, and its principle is based on no violence. The animal is dominated by patience, by caring, by cautious approach, by progressive and by repetitive lessons. In taming process, we always create favorable and unfavorable situations for the cattle to seek their comfort. If it makes the right choice, we usually suspend the stimulus. We should never forget to positively reward the right choices with resources such as rest, caress and food. The training must have good quality, not necessarily be long and time-consuming, prioritize the quality of the work, not the quantity.

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Animal Welfare Considerations in Food-Producing Animals

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Abstract

Animals play many important roles in humans' lives. They are a source of food, companionship and wealth. Increasing global demand for food has resulted in the intensification of livestock production. Intensive production systems are aimed at maximising profits by rearing more animals on smaller pieces of land in order to produce more food. The other systems of animal production are semi-intensive and extensive production systems. Of the three systems, the semi-intensive system offers the best option for enhancing animal welfare in all species. Animal welfare can be defined in many ways depending on people's views of animals. The underlying theme of animal welfare is to enhance physical and mental health of animals. Despite improvements in livestock farming techniques over the years, the welfare of farm animals remains a major concern. The major welfare concerns in the livestock industry relate to the rearing and management of dairy calves; the rearing of sows in gestation and farrowing crates; the housing of layer chickens in battery cages and the restriction of feed in broiler parent stock. Good animal husbandry is a basis for promoting the welfare of animals.

Keywords: farm animal welfare, cattle, poultry, pigs

1. Introduction

Over the years, animal production has intensified in order to meet the escalating global demand for food. As a result, many food animals are now reared in relatively small spaces where little attention is paid to their well-being. To maximise on profits, breeding schemes often place emphasis on production traits and pay little attention to other traits that are

necessary for the physical and mental well-being of animals. For example, the breeding of dairy cows over the years has produced a cow that can produce large volumes of milk but with a large pendulous udder that is prone to mastitis [1, 2].

In the modern era, the human-animal bond has become much stronger, with animals forming an important part of humans' lives. This and animal welfare training and research at universities and colleges around the world have helped to increase awareness of animal welfare in modern-day societies. Available research on animal welfare is biased towards farmed animal species due to the large populations of these animals and the high degree of public concern they raise due to their role in human lives [3].

Animal welfare is a difficult concept to define because it has no single definition. It can have multiple meanings and interpretations depending on the background and moral view of the person concerned. There are many perspectives and ethical positions on animal welfare which arise as a result of differences in values and experiences within society. Animal welfare can be defined as 'how well an animal is coping with the conditions in which it lives' [4, 5]. This is the generally accepted definition of animal welfare. In its simplified form, animal welfare is about ensuring that animals are not cruelly treated or caused unnecessary pain and suffering. It describes how well an animal is coping mentally and physically with the conditions in which it lives. In situations where animals manage with little expenditure of resources and effort, the animal's welfare status is said to be satisfactory. In other situations, animals may fail to cope with conditions in their environment resulting in a poor, negative or bad welfare status [6]. In other words, animal welfare refers to the physical and emotional state that is produced in animals by human attitudes and practices, the amount and quality of resources available to an animal and by the environment in which the animal lives. Psychological well-being is particularly important because farm animals are sentient beings, that is, they have feelings and emotions [7] and can therefore suffer psychological trauma. Good and bad experiences are an acceptable part of an animal's life as long as animals are able to adapt. However, physical and mental suffering that exceeds an animal's ability to cope should as far as possible be kept to a minimum.

The five freedoms of animal welfare [8] can be used as a framework for defining and assessing animal welfare. The five freedoms are applicable to farm animals, but require prior knowledge of species-specific wants for successful implementation. The focus of four of the five freedoms is on relieving suffering or doing away with the negative components that have the potential to impair the well-being of animals. However, one of the 'five freedoms' seeks to promote the positive aspects by advocating for animals to be provided with conditions that enable them to express normal behaviour. The 'five freedoms' are essential as a basis for preventing animal suffering and poor welfare. They have gained widespread acceptance by regulatory bodies, industry, animal advocacy groups and the World Organisation for Animal Health (OIE) and have been incorporated into many codes of practice around the world.

There is increasing recognition and awareness that animals need to experience positive emotions to have good welfare and a good quality of life. To promote positive emotions, some authors have proposed a 'sixth freedom' titled 'Freedom to undergo positive experiences' (by providing the conditions necessary to experience positive emotions such as feeling contented, pleasure, relaxed, excited) [9]. Current research is focussed at finding ways of enhancing the well-being of animals by promoting positive states rather than removing or preventing the

negative aspects. Concepts similar to the five freedoms have also been developed and amplified into a five-domain model for promoting animal welfare. The five domains include nutrition, environment, health and behaviour, as well as an overarching mental component [10].

The OIE has produced guidelines on animal welfare aimed at promoting international trade between World Trade Organisation members [5]. Although not mandatory, these standards promote and smoothen trade between OIE member states.

1.1. The five freedoms

1. Freedom from hunger and thirst by providing ready access to freshwater and an appropriate diet to maintain full health and vigour.
2. Freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from pain, injury or disease by preventing, rapid diagnosis and treatment of diseases.
4. Freedom to express natural behaviour by providing sufficient space, proper facilities and company of the animal's own kind for social interactions.
5. Freedom from fear and distress by ensuring conditions and care which avoid mental suffering (appropriate treatment and surroundings).

The welfare status of animals is not constant. It is ever changing due to the fluctuation of the factors responsible for good or bad welfare. Therefore, the welfare status of an animal can be good, bad or somewhere in between [11] and varies with time. In general, an animal is in a good state of welfare if it is healthy, comfortable, well-nourished, free from pain, fear and distress and is able to express innate behaviour [5]. Good animal welfare requires good husbandry including disease prevention and treatment, humane handling and slaughter, and the provision of suitable nutrition and shelter [12]. Evidence that an animal has a good state of welfare includes having low levels of disease, displaying of innate behaviour, normal reproduction and living longer.

Ensuring animal welfare is a human and collective responsibility that includes consideration for all aspects of animal well-being, including proper management, housing, nutrition, disease prevention and treatment, animal care, humane handling, and, when necessary, humane euthanasia. Domesticated species have retained some of the adaptations of their ancestors, and so they need an environment which allows them to express their unique natural behaviour. Whilst not all natural processes are good for animal welfare, positive natural behaviours that enhance well-being should be promoted [9]. Poor animal welfare can manifest as high mortality rates, poor reproduction, increased incidence of disease, body damage, behaviour anomalies, heavy internal parasite and tick burdens and severe malnutrition. Human concern for animal welfare is based on the awareness that animals are sentient and that they have a valuable role in human lives. All vertebrates (mammals, birds, reptiles, amphibians and fish) are sentient in that they have the capacity to experience pain, distress, suffering, positive and negative feelings [12]. Farm animals are sentient beings, which mean they have feelings and emotions. As a result, they can suffer fear and pain, but also experience pleasure and

happiness. Animals as sentient beings have the ability to evaluate the actions of other animals in relation to themselves and to remember some of their own actions and consequences and to assess risks and benefits [7]. There is still a lack of convergence on which animals are sentient or not among researchers. Sentience is important to welfare because the animal's level of awareness and cognitive abilities influence people's attitudes and therefore their treatment of them. Sentient or not, all living organisms should be handled and treated with the utmost care until scientific evidence proves otherwise. Examples of complex abilities that highlight sentience in some farm animals are given below.

Pigs are capable of using deception [13] and knowledge held by other individuals to search for food [14].

Cattle value social interactions with other individuals [15] and have been reported to remember up to 50–70 other individuals [16].

Chickens can exercise self-control [17] and can show signs of emotional frustration [18].

1.2. Different views regarding animal welfare

Society's views of animal welfare are influenced to varying degrees by cultural beliefs and economic viewpoints. Two divergent views stand out among many—the animal welfare and animal rights standpoints. The animal welfare viewpoint advocates for the judicious use of animals by humans as long as their welfare status is satisfactory and unnecessary pain and suffering is not inflicted. Some in society disagree with the use of animals by humans. They believe that animals should be afforded basic rights so as not to be misused by humans. These are animal rights advocates who view animals as equal to humans and are against the exploitation of animals in any form. An animal rights view is an ethical position in which non-human animals are recognised as having rights that go beyond the basic animal welfare considerations. Some of these rights equate to the rights afforded to humans [19]. Although the two views are often divergent, there is some convergence in that both views seek to improve animal welfare [20]. Some of the views of animal welfare and animal rights advocates are presented in **Table 1**.

1.3. Why are we concerned about animal welfare?

Domesticated animals provide humans with benefits such as meat, milk and draught power. Just as humans expect some personal benefit from a long day of hard work, animals deserve support for the benefits they accord to humans. Therefore, humans have a moral obligation to ensure that animals have a good quality of life [11]. In food production systems, attention to animal welfare can improve productivity, quality, food safety and economic returns [21] and therefore contribute to food security and economic prosperity.

Interest in animal welfare is on the rise around the world. More attention is now placed on animal welfare in research areas, the media and in politics. Animal welfare has been on the agenda of the OIE for over a decade because of its link to animal health and food safety which are in turn linked to human health. A reduction in animal diseases is directly linked to a reduction in zoonotic diseases in humans. In some parts of the world, animal welfare now has great influence on local and international trade of animal products. A link between animal welfare and productivity is well documented. Lamé dairy cows have a reduced milk

Animal welfare advocates	Animal rights advocates
Judicious use of animals for human benefit is morally right	Using animals for human benefit is morally wrong
Human interests always come first before animal interests.	Humans interests should not overrule animal interests
Humans should not cause animals unnecessary pain or death	Humans should not cause pain or death at all in animals
Treat animals as humanely as conveniently possible	Treat animals humanely always and eliminate human-made causes of animal suffering
Humane euthanasia/killing of animals	No killing of animals
Objects to cruel practices such as dog fighting, confinement of veal calves, and pregnant sows	Abolish the use of animals in any form, it is exploitation.
	Animals have the same rights as humans

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Table 1. A comparison of different views relating to animal welfare.

production and reproductive capacity [22] which reduces farm profits. The health and well-being of animals can have a direct impact on growth, reproduction or meat quality, and is therefore important to producers, food retailers, customers and others in the supply chain. For the producer in particular, adherence to animal welfare guidelines and standards can increase business profitability by improving productivity and efficiency, as well as by reducing losses [23]. In some countries, it is a legal requirement to provide for the needs of animals. The economic benefits of good animal welfare in the meat industry include [21]:

- Reduced carcass damage due to bruises and injuries.
- Lowered incidence of dark firm dry (DFD) and pale soft exudative (PSE) meat which are candidates for condemnation.
- A reduction in employee accidents during handling and stunning.
- A reduction in labour costs due to the smooth and easy movement of animals through the pens, races and restrainer
- Improved public perception of the meat industry which can serve as a marketing tool.

In this chapter, welfare concerns in farm animals specifically cattle (dairy and beef), pigs and poultry shall be discussed in light of current scientific knowledge. Due to the broad nature of the subject, only the major welfare concerns are highlighted.

2. Farm animal welfare

2.1. Animal welfare concerns on dairy cattle farms

A typical commercial dairy farm herd structure comprises of calves, heifers, cows and bulls that are reared on pastures, indoors or both. In housed systems, cattle depend entirely on

humans for feed, water and shelter. In most cases, mobility and exercise are restricted and there is limited opportunity for animals to select their diet [5]. Pasture systems provide dairy cows with some degree of autonomy over the selection of their diet, and cows have the opportunity to exercise and express some normal behaviours in the space provided. The welfare of housed cattle can generally be considered to be lower than that of animals that are kept outdoors, but this will depend on the overall quality of farm and animal management. A combination rearing system made up of both housing and pasture systems can be the best option for good animal welfare if it is combined with good farm management [5].

Good stockmanship is important for dairy cattle welfare. The stockman's knowledge, skills and attitude determine the standard of welfare on a dairy farm. Good stockmanship is the ability to identify and respond promptly to animals' needs with an attitude of empathy towards animals [23, 24]. Good animal management skills are crucial to providing an acceptable level of animal welfare. Stockman, who is knowledgeable, skilled and experienced in dairy cattle behaviour and husbandry, is an asset to the dairy farm because they can detect and correct welfare issues early [25].

There are a number of activities that are carried out on dairy farms which if not properly managed can negatively impact animal welfare. There have been improvements in some areas of the dairy cow welfare including the productive lifespan of animals, but issues related to lameness, mastitis, calf management, animal handling housing management, pain relief during surgical procedures, employee training, culling, mortality and management of downer cows remain as areas of concern [26]. According to the International Dairy Federation [25], these practices can be grouped into five action areas for the improvement of animal welfare namely stockmanship, feed and water, physical environment, husbandry practices and health management. Good dairy welfare depends on appropriate facilities design, environmental and animal management factors which include the provision of appropriate care and husbandry. Lactating cows and dairy calves are the animals most at risk of welfare problems on a dairy.

2.1.1. Environmental factors and dairy cattle welfare

Depending on climatic conditions, dairy breed and management, heat and cold stress can be a cause of poor welfare on dairy farms. Indigenous breeds are better adapted to withstand the prevailing climatic conditions and are therefore unlikely to suffer from heat or cold stress compared to exotic breeds of cattle. Environmental factors that promote heat stress are high ambient temperatures, high relative humidity, poor ventilation, high stocking rates and a lack of shade. Susceptibility to heat stress is influenced by animal-related factors such as age, breed, body condition, coat colour and density [5]. Heat stress is more of a problem in the tropical and subtropical climates. Affected cattle show a reduced feed and water intake; milk production declines; respiration rate rises; dehydration occurs and their milk production declines. Mortalities may occur in cases of exposure to prolonged high temperatures. With good management, heat stress can be anticipated or detected early. Measures for mitigating heat stress include the provision of fresh water (*ad libitum*), cooling fans, shade and reducing the stocking density especially in housed animals. Neonates and young animals are more prone to cold stress and may die as a result. Shivering and huddling are some of the indicators of cold stress [5].

Good quality air is essential for the health and welfare of confined dairy cattle. Poor ventilation and air quality due to dust particles or gases such as ammonia or hydrogen sulphide may cause respiratory discomfort and infections [5]. The design of facilities, ventilation systems and the type and management of bedding determine the quality of air in the surroundings of animals. Ammonia tends to accumulate in poorly ventilated facilities. It is produced from manure, wet bedding or straw. According to the OIE [5], ammonia levels in the animal environment should not exceed 25 ppm. The rule of thumb is that if the air quality in the environment of cows is not pleasant for humans, then it is most likely to cause respiratory discomfort in cattle as well [5].

Although dairy cattle can withstand different types and levels of noise, exposure to hissing and sudden unexpected noises can trigger stress and fear reactions. Startled animals may panic and injure themselves as they try to escape. The major sources of noise on a dairy farm are ventilation fans, alarms, feeding and other machinery. Equipment operated on a dairy farm should be sited, operated and maintained in a manner that minimises nuisance of noise to the animals. Dairy cattle also require adequate light especially those that are reared indoors. Sufficient light is necessary for animals to feed and express their natural behaviour. Adequate light also permits the inspection and detection of animals that require attention such as injured animals [5].

Resting places for dairy cows need to be well drained to permit comfortable resting. Muddy and hard concrete surfaces do not allow animals to rest comfortably. Due to the risk of pressure sores associated with concrete surfaces, bedding made from straw or other suitable soft material is appropriate. Muddy grounds expose cattle to the risk of foot rot, lameness and mastitis, factors which negatively affect cow welfare. Surfaces of raceways and grounds should be made of non-slip material to prevent claw injuries and fractures due to slipping. Uneven raceway floor surfaces discourage forward movement of cattle in races.

Poor facility and equipment design and maintenance is one of the causes of poor welfare on dairy farms. Poor operation of milking equipment especially with regard to the pressure in the milking machine can cause teat injuries. Improperly designed feeding systems can promote agonistic behaviour resulting in some cows being bullied and underfed. Milking parlours, free stalls, standings, cubicles, races, chutes and pens should be free from sharp edges and protrusions that can injure cattle. The loading facilities including the slope of ramps should permit loading with minimal stress and injury to animals [5].

2.1.2. Animal health management on a dairy farm

Disease burden is a major cause of poor welfare in animals including dairy cattle. The implementation of biosecurity measures that are commensurate with disease risk prevents the entry and spread of diseases on a dairy herd farm promoting good welfare. Biosecurity measures include fencing, controls at the entrances, disinfection procedures, quarantine and testing of new animals before they are introduced into the herd.

For effective welfare, an animal health management program which covers the diseases prevalent in a particular area including internal and external parasites is mandatory. Vaccination of dairy cattle as a preventative measure against disease should be based on expert advice taking into consideration diseases that are prevalent in an area.

Personnel responsible for cattle should be trained and skilled to detect signs of disease in a large dairy herd. Lameness, a painful condition of the legs and feet, is one of the conditions that need to be detected and treated early because of its negative effects on welfare and milk production. Lameness is a multifactorial condition that can be caused by infectious or mechanical causes. Hard surfaces including concrete floors, zero grazing and muddy surfaces are risk factors for lameness [27]. Very lame cows should be taken off hard surfaces and placed on soft surfaces such as bedded pens. A large number of lame cows in a herd are a sign of poor welfare standards on a farm [28].

Non-ambulatory cattle must be identified early and given prompt treatment to prevent further suffering. Management of non-ambulatory animals includes frequent turning of the animal to prevent pressure sores and irreversible muscle damage; the provision of water at all times and feed at least once a day; regular milking to prevent pain from engorged udders. Protection of downer cows from heat and predators is important for their survival. Non-ambulatory cattle are not to be moved except if it is absolutely necessary. If they are to be moved, utmost care should be practised using methods that avoid dragging or lifting the animal in a way that might exacerbate injuries. In cases where treatment has failed or recovery from injuries is unlikely, non-ambulatory animals should be killed on site using humane methods [5].

2.1.3. Nutrition and animal welfare

To produce large volumes of milk, dairy cows require a balanced ration that meets their physiological needs. Special attention should be paid to the quality and quantity of nutrition in the last month of pregnancy with regard to energy balance, roughage and micronutrients. If a balanced ration is not given during this period, the cows may lose body condition and succumb to periparturient diseases. Grain-based and new diets should be introduced slowly to prevent excessive engorgement which can lead to disease. Cows need to have access to palatable roughages such as silage, grass and hay *ad libitum* to promote digestion and normal rumen function. Body condition scoring can be used as tool to monitor the nutritional status of a dairy herd. Any deviation from the acceptable range of body condition score should be promptly corrected [5].

Colostrum is the first milk produced by a cow after giving birth. It contains essential nutrients and antibodies for the protection of newborn calves against diseases. Neonates have a weak immune system. Colostrum serves to provide passive protection of the calf during the period when its immune system is still developing. Management of a dairy farm should be such that all neonates receive adequate colostrum within the first 6 h of birth and not beyond 24 h because the absorption of antibodies from the gastrointestinal tract is negligible after this period. Calves that do not receive colostrum show stunted growth and are prone to respiratory, gastrointestinal and other diseases [5].

Although milk replacer is essential for the healthy growth and welfare of calves, feeding it as the sole source of nutrition after 4–6 weeks of age limits the physiological development of the rumen. Calves over 2-weeks old should have a sufficient daily ration of fibrous feed to stimulate rumen development [5].

2.1.4. The effect of the social environment on animal welfare

Cattle are social animals that show dominance hierarchies. Animal welfare issues may arise from mixing incompatible animals such as bulls. Bulls will naturally fight and injure each other and vulnerable animals such as the sick or injured, very young and very old animals. Horned and non-horned cattle should not be mixed because horned animals can inflict injuries on other animals.

2.1.5. Effect of genetic selection on animal welfare

Selection of animals during breeding should not be based on production traits alone, but also on health- and welfare-related traits [5]. The development and conservation of genetic lines of dairy cattle that reduce animal welfare problems should be encouraged during breeding programs. Examples of such traits include nutritional maintenance requirements, ease of calving, body conformation, temperament, disease resistance and heat tolerance [5]. Dystocia is a welfare risk to dairy cattle. Heifers should not be bred before they reach the stage of physical maturity sufficient to ensure the health and welfare of both dam and calf at birth. Animals observed to be having difficulty in calving should be assisted by a competent handler as soon as possible. When a caesarean section is required, it must be carried out by a competent veterinarian.

2.1.6. The welfare of dairy calves

Newborn calves are susceptible to hypothermia under cold conditions. Therefore, they should be kept warm depending on ambient temperatures. Soft, dry bedding and supplemental heat can be given to prevent cold stress.

Many strategies are used to separate or wean the calf from the cow in the dairy industry. Early separation is done within 48 h of birth, while in gradual weaning, a calf is left with the dam for a longer period. Weaning is stressful to both the calf and the cow especially when a strong bond has been developed. The stress of weaning has a negative effect on the immune system and predisposes calves to pneumonia [29]. Weaning should be made only when the ruminant digestive system has sufficiently developed to enable a calf to maintain growth, health and good welfare. On commercial dairy farms, calves are weaned from their mothers within hours of birth. This causes severe distress to both the calf and the cow, and studies have reported that this may cause long-term negative effects on the calf's physical and social development. However, it can be argued that at early separation, the cow-calf bond is not as strong as it can be.

Most bull calves have little value to a dairy farm unlike female calves that can be raised as replacement heifers. They are slaughtered or sold for veal or beef and are commonly transported over long distances at an early age exposing them to severe stress and hunger [30]. Young calves are at particular risk of thermal stress. Special attention should be paid to management of the thermal environment (e.g. provision of additional bedding, nutrition or protection to maintain warmth and appropriate growth). Individual calf-housing may

facilitate monitoring of health of very young calves and minimise the risk of disease spread. There is now a shift towards rearing calves in groups for better welfare. Calves that are reared in groups should be of similar age and physical size to prevent aggression and competition for resources such as feed. Whether they are reared individually or in groups, calves need space to turn around, rest, stand up and groom [5]. Individual housing in hutches prevents social contact, limits opportunities for movement especially if the calves are tethered. Some housing designs do not permit visual contact with other calves. In other types of housing, the calf can exit the hutch and see other calves especially where the tethering chain is longer. However, group housing can increase the incidence of infectious diseases and aggression among calves [31].

2.1.7. The welfare of dairy cows at the time of milking

Milking, whether by hand or machine, should be carried out in a calm and considerate manner in order to avoid pain and distress. Special attention should be paid to the hygiene of personnel, the udder and milking equipment. The gathering of cows from the paddocks, movement along the race, holding in pens and entering and exiting the milking shed should be carried out calmly. All cows should be checked for mastitis at milking. Milking machines, especially automated milking systems, can cause teat and udder injuries if they are not maintained and operated according to the manufacturer's recommendations. A regular milking routine should be established relevant to the stage of lactation and the capacity of the system. Special care should be paid to animals being milked for the first time. They should be familiarised with the milking facility prior to giving birth. Long waiting times before and after milking can lead to health and welfare problems (e.g. lameness, reduced time to eat). Management should ensure that waiting times are minimised [5]. Under or overmilking should be avoided as milk engorgement and overmilking are both painful [32, 33].

2.1.8. Painful husbandry procedures that can affect animal welfare

Husbandry practices such as dehorning and castration are routinely carried out in cattle for reasons of management, animal welfare and human safety. Practices that have the potential to cause pain should be performed in such a way as to minimise pain and stress to the animal. Such procedures can be performed at an early age or using anaesthesia or analgesia under the recommendation or supervision of a veterinarian. Alternatively, the painful procedure can be replaced with a non-surgical alternative or a decision can be made to use the least painful procedures [34].

Horned dairy cattle are commonly disbudded or dehorned in order to reduce animal injuries and hide damage, improve human safety, reduce damage to facilities and facilitate transport and handling. The selection of polled cattle is a preferable alternative to dehorning. Performing disbudding at an early age is preferred, rather than dehorning older cattle. Thermal cautery of the horn bud by a trained operator with proper equipment is the recommended method in order to minimise post-operative pain and promote wound healing. Disbudding should be done before the horn bud has attached to the skull to reduce pain. The use of anaesthesia and analgesia is strongly recommended when performing disbudding, and should always be used during dehorning. Other methods of disbudding include: removal of the horn buds with

a knife and the application of chemical paste to cauterise the horn buds. Where chemical paste is used, special attention should be paid to avoid chemical burns to other parts of the calf or to other calves. This method is not recommended for calves older than 2 weeks. Operators should be trained and competent in the procedure used, and be able to recognise the signs of pain and complications that may include excessive bleeding or sinus infection.

Ear-tagging, ear-notching, tattooing, branding and radio frequency identification devices (RFID) are methods of permanently identifying dairy cattle. The least invasive approach should be adopted whichever method is chosen. It should be accomplished quickly, expertly and with proper equipment. Freeze branding and hot iron branding systems should be avoided where alternative identification methods exist (e.g. ear-tags). When branding is used, the operator should be competent in procedures used and be able to recognise signs of complications.

2.1.9. Disaster preparedness and management

Plans should be in place to minimise and mitigate the effect of disasters (e.g. earthquake, fire, drought, flooding, blizzard, and hurricane). Such plans may include evacuation procedures, identifying high ground, maintaining emergency feed and water stores, destocking and humane killing when necessary.

In times of drought, animal management decisions should be made as early as possible and these should include a consideration of reducing cattle numbers. Humane killing procedures for sick or injured cattle should be part of the disaster management plan.

2.2. Animal welfare concerns on beef cattle farms

Commercial beef cattle production systems fall into one of extensive, intensive and semi-intensive systems. Animal welfare concerns of beef cattle in different production systems are similar to those of dairy cattle discussed above.

In extensive production systems, cattle are raised outdoors on pasture. Outdoor-reared cattle have the ability to choose their own diet, water, shelter and have ample space for exercise and for exhibiting their natural behaviours, which is good for their well-being. Therefore, extensive systems of production offer many advantages because they are the system that is closest to an animal's natural environment. However, the welfare status of cattle raised in extensive systems also depends on the quality and quantity of available grazing and on how farms are managed. With good management, extensive systems represent the best opportunity to provide cattle with the right conditions for their well-being. Often times however, little effort is put into the management of extensive cattle production systems, resulting in poor cattle welfare. During periods of drought, supplementary feed is required to sustain the health of animals. Due to the size of the farms, it is difficult to inspect the condition of all animals regularly. As a result, some animals may suffer or even die from injuries or dystocia because some of the farms are large. Cattle raised on pastures tend to carry heavy tick and internal parasite burdens. Often, predators have the opportunity to prey on calves and younger animals and cause distress in older animals. Other animal welfare concerns on extensive beef cattle production farms include:

- Heat stress due to lack of shade.
- Mixing of incompatible animals such as bulls which results in constant fights.
- Inadequate monitoring of calves especially for colostrum intake.
- Painful husbandry procedures such as dehorning, castration, animal identification and hot iron branding that are carried out without analgesia.

In intensive beef production systems, cattle are confined and depend entirely on humans for basic needs such as shelter, food and water. Therefore, their welfare is dependent on the quality of management on the farms. High stocking densities can reduce welfare in intensive production systems as disease risk and competition for feed are high. Due to limited space, animals are not able to exhibit some of their natural behaviours. Semi-intensive production systems are a combination of both intensive and extensive production systems. They have the advantages of both systems, and theoretically, semi-intensive systems offer the best welfare compared to the other two.

2.3. Animal welfare concerns on commercial pig production farms

Pigs are one of the first farm animal species in which the negative consequences of intensive production systems on animal welfare were described. Research indicates that pigs have high cognitive and emotional capacities [35, 36]. In the natural environment, pigs are social animals which explore their environment in search of food. In the wild, it is common to see a social group of young and adult pigs. Domesticated pigs have retained most of their ancestor's natural behaviour despite the process of domestication [37]. This means that domestic pigs have a natural urge to display these behaviours but are restricted by conditions in the environment. Many of the welfare concerns identified in the earlier years of intensification such as high stocking densities, poor ventilation, tail biting, tail docking, the use of fully slatted floors and solitary confinement of sows remain a challenge to date [36].

The primary requirement for acceptable pig welfare is the maintenance of good health, provision of adequate space and a conducive environment for animals to express their natural behaviour [36]. To promote good welfare in intensive production systems, space allowance in housing should permit animals to lie down, rest and stand up with no difficulty. Housed pigs must be able to see and have physical interaction with other pigs [36, 47]. Lack of social interaction can result in boredom and can manifest as vices such as sham-chewing and bar-biting. Intensive pig production systems prevent pigs from exhibiting behaviours such as wallowing in mud and escaping from aggressors. It has been reported that the performance of natural behaviours is associated with positive feelings in pigs. More specifically, the presence of spontaneous play behaviour in pigs has been reported to indicate a state of feeling good. Therefore, play behaviour can be used as an indicator of positive or good welfare [36]. In intensive production systems, pregnant sows are commonly confined to gestation crates for most of the 16-week gestation period. Many sows are subjected to restricted feeding regimes [36]. By nature, pigs spend a considerable time of the day foraging for a variety of different foodstuffs. By contrast, in intensive pig farming, the animals get uniform feed only once or twice a day, which limits eating behaviour to a bare minimum. Due to barren

housing conditions, including hard, often wet and slippery slatted floors, pigs generally have no opportunity to comfortably lie down or nest and are hindered in their locomotion. Poor air quality (notably high levels of ammonia) often results in ocular, olfactory and respiratory discomfort. When compared to sows kept in group housing, sows kept in stalls have been shown to spend less time resting, foraging or exploring and more time standing, drinking and engaging in repetitive abnormal behaviours such as sham-chewing and bar-biting which are indicators of poor welfare [35, 38, 39]. Pig houses should be adequately lit to stimulate normal behaviour and must have good ventilation to prevent the build-up of ammonia and heat. Noises around pig houses have been reported to cause fear and distress [36]. Gestation crates are small and narrow with bare slatted floors which expose sows to cold stress, injuries and lameness. In a gestation crate, a sow can only stand up, make a few steps forwards or backwards, but cannot turn around for the duration of its stay. This restriction of movement and the lack of exercise can lead to a reduction in muscle mass and osteoporosis which may result in frequent bone fractures. Higher rates of urinary tract infections have also been reported in confined sows, due to decreased water consumption. Although sows in stalls can see and smell adjacent sows, they suffer from psychological problems due to confinement and a lack of social interaction. As a result, the sows exhibit stereotypic behaviour such as bar-biting and sham-chewing (with nothing in their mouth). Gestation crates have been banned in many countries such as Sweden, United Kingdom and New Zealand. Other countries have reduced the time that sows spend in gestation crates or have introduced group housing to alleviate the suffering of sows. The welfare of sows in group-housing systems is highly dependent on management of the social group and on the feeding system used. Although group housing offers better welfare conditions, it has some drawbacks. For example, it is associated with a much greater risk of aggression and injury, and makes the management of individual feeding difficult. In competitive feeding systems, lower ranking sows in a group may lose body condition due to limited access to feed, while higher ranking sows may dominate the group and overfeed as a result [38, 39]. To overcome these problems, techniques for mixing and managing the nature and sizes of social groups, individual electronic sow feeders (ESFs) and individual feeding stalls that protect sows from one another during feeding have been used with success. The productivity of well-managed group-housed sows is comparable to that of sows kept in individual stalls [39, 40].

Sows are moved to farrowing crates 1 week before farrowing and stay in these crates until piglets are weaned at 3–4 months. Farrowing crates are even narrower than gestation crates, but have additional space for piglets. Bars separate a sow from the piglet area to prevent the sow from crushing the piglets. The small and narrow space of a farrowing crate makes it difficult for a sow to stand up and lie down. As a result, sows spend most of their time lying down. Although these crates protect piglets from being crushed, they seriously compromise the well-being of sows. Due to the limitation of space and other resources, sows are unable to display their natural nesting behaviour and are unable to escape from injury, pain, wet or cold floor and insects. In farrowing crates, piglets face the possibility of starvation if the sow fails to produce milk [39]. The other major welfare issues affecting piglets relate to the painful procedures that are carried out within the first week of life. Teeth clipping, tail docking and castration are routinely performed without anaesthesia and the pain seriously affects piglets as evidenced by the vocalisation.

Tail biting is one of the serious welfare problems associated with intensive pig production systems and can occur at any stage of pig growth in group-housed pigs. Pigs bite each other's tails causing damage which can result in infection which travels up the spinal cord causing spinal abscesses and abscesses in many organs including the lungs. Producers often dock the tails of piglets to reduce the incidence of tail biting. Although intended as a corrective measure, tail docking is a painful procedure that is carried out in many countries around the world. It has been reported that piglets squeal, grunt and attempt to escape during tail docking—indicating that it is a painful and uncomfortable procedure. Lack of enrichment in the pig house (and boredom) can lead to aggression and tail biting. Other risk factors for tail biting include high stocking density, compact floors with no bedding and high levels of ammonia. Many studies have reported that providing pigs with a combination of straw daily, good ventilation and mixing of sex groups can reduce the incidence of tail biting [40–42].

2.4. Animal welfare concerns on commercial broiler chicken farms

Broiler chickens are the most numerous of any farmed land animal in the world. They are raised on intensive, semi-intensive or extensive systems of production. In intensive systems, chickens are confined to a poultry house, while in extensive systems, birds are permanently confined outdoors. In semi-intensive production systems, birds are kept in a poultry house with access to an outdoor area.

One of the welfare concerns in broiler parent stock is the routine, severe feed restriction that starts at early age. If parent stocks are fed *ad libitum*, they would show health and reproductive problems due to their rapid growth rate and size. Diet restriction happens throughout the lifespan of birds, but is most severe during the rearing phase, when birds are fed about 25% of their voluntary intake. Birds that are feed restricted show signs of chronic hunger, boredom, nutritional deficiencies and abnormal behaviour such as persistent pecking at non-feed objects, pacing and aggression [43].

Broiler chickens are bred to grow fast and reach market weight in as short a time as possible. On average, the daily growth rate of broilers has doubled over the years to 59 g per day [44]. Selection for rapid growth has brought about other problems that impair the well-being of birds such as poor bone health, leg deformities, lameness, ruptured tendons and diseases such as ascites and sudden death syndrome. Due in part to genetic selection for faster growth, the rate of muscle deposition for meat in broiler chickens far exceeds the rate of development of the skeleton and cardiovascular system leading to leg deformities, lameness, heart failure locomotion problems footpad dermatitis, breast blisters, joint and skeletal disorders and lung failure [44–46]. The high muscle mass and reduced cardiovascular function in broilers also makes them less tolerant to heat stress. Broilers with leg problems are common in intensive production systems than in other systems. They often spend a lot of time lying on the floor or struggling to walk. It has also been reported that the selection for rapid growth has been at the expense of the bird's immune system, making birds more susceptible to diseases [43].

Overcrowding has the potential to cause welfare problems in chickens that are raised on litter. In overcrowded houses, birds may not reach feed and water and leg problems are prevalent due to the lack of exercise. It has also been shown that increasing chicken densities reduces the duration of preening sessions. In crowded houses, heat stress can be a problem because

more heat is dissipated by the birds than that which is lost from the house. Research has demonstrated that the health and welfare of broilers is compromised at stocking densities above the range of 34–38 kg/m² [47]. Reducing the stocking density is a reasonable means of overcoming this problem, but this may reduce profits. Heat stress can be prevented by providing fresh water *ad libitum*, improving litter quality and ventilation, and the monitoring of humidity in the house.

In crowded houses, the litter may be wet, the air humid and polluted with dust, bacteria, fungal spores and ammonia. Therefore, poor quality litter may negatively affect welfare by exposing chickens to respiratory diseases, contact dermatitis of the feet and hocks and by reducing growth rates. Good quality litter supports the legs and promotes natural behaviours like ground scratching and dust bathing which are indicators of good bird welfare [44].

Broiler chickens are reared in large groups. Any disease that arises is likely to spread within and affect the whole flock. Therefore, disease prevention and management on broiler farms is important for their welfare. The implementation of strict biosecurity measures and health management is an important part of disease prevention. Vaccination of birds against diseases prevalent in a specific geographical area is mandatory to protect the birds. Persons working with chickens need to be trained to recognise diseased birds in a flock so that treatment can be effected before the disease spreads widely [5].

A wide variety of artificial lighting regimes are used to raise broilers. A lighting schedule of 23 h of light and 1 h of darkness has been used to quicken growth. In some systems, the chickens are raised under continuous lighting. Reduced periods of darkness are detrimental to the welfare of birds as they can cause abnormal eye development [48], leg disorders, sudden death syndrome, higher mortality and ascites syndrome. A 4-h period of uninterrupted darkness per day is recommended as a minimum requirement for good welfare of chickens [49]. On the other hand, a lack of brighter lighting may result in uncomfortable, painful changes in the eye, reduced feeding activity and lower growth rates.

Free-range systems offer the potential for higher welfare than intensive systems. They offer more space, an enriched environment but slower growth rates. Birds can express their natural behaviours such as ground pecking, perching, scratching, dust-bathing and foraging [49]. Birds in free-range systems have stronger bones and less problems with pain and lameness, but are prone to predation, internal and external parasites. Semi-intensive systems offer the best of both intensive and extensive broiler management systems.

When broiler chickens have reached market weight, usually between 4 and 6 weeks of age, they are caught and placed into crates for transport to the abattoir. Birds are typically caught by the legs, inverted and carried in groups of 3–4 birds per hand to transport crates. During this process, in addition to fear and stress, leg and wing fractures may occur [50].

2.5. Animal welfare concerns on commercial egg production farms

Commercial laying hens are moved to laying farms just before they start laying eggs, between 16 and 20 weeks of age. They are typically kept until 72-weeks old, when egg production and quality decline. Most commercial laying hens are raised either in battery cages, on deep litter or on free-range systems. The traditional housing of egg-type chickens in conventional cages,

long perceived as the most efficient method of housing laying hens, is now widely considered to have a negative effect on the welfare of hens due to restrictions on bird behaviours such as wing flapping, perching, scratching, dust-bathing and nesting. Laying hens in conventional cages are generally healthy and productive, but the lack of space and barrenness of the environment significantly constrain locomotion and other motivated behaviours such as nesting and perching and leads to osteoporosis [51]. Barren battery cages were banned throughout Europe and replaced with 'furnished' battery cages that offer better welfare conditions for birds, but still restrict some natural behaviours. In addition to feeding and drinking space, battery cages now offer more useable space per bird (750 cm²), litter and facilities for perching, nesting and scratching [52]. Overcrowding in cages can lead to frustration and stress which is manifested as feather pecking and cannibalism. To prevent these vices, the birds are beak trimmed. Beak trimming is a painful procedure that hinders a hen's natural pecking behaviour and the sensory capability of the beak [48]. As a result, Sweden has banned beak trimming and many countries are expected to follow [44]. However, despite many years of research, there are no reliable methods for preventing feather pecking and cannibalism, although risk factors have been identified [53]. Due to overcrowding and the wire cage surface, caged birds are prone claw overgrowth and injuries. The lack of exercise in the cages combined with the constant demand for calcium for egg production can result in weak bones due to osteoporosis. The weak bones are painful and easily fractured during handling or when hens are startled and flap their wings. However, laying hens housed in non-cage and free-range systems have a greater risk of infectious diseases, internal and external parasites, and generally have higher rates of mortality due to disease and cannibalism than hens housed in conventional or enriched cages [54].

Deep litter systems provide the same welfare conditions as has been described for broilers. Hens have freedom and space to move around within a building. Litter is provided by using wood shavings or straw. The house is provided with perches for roosting, nest boxes, foraging and material for dust-bathing. Welfare conditions for free-range systems are as described for broiler chickens raised on the same system. Free-range systems have the potential to provide the best welfare. In these systems, hens are reared under a shed with exit holes allowing birds to access the open-air spaces covered in vegetation. The shed provides the same welfare conditions as in housed birds, while the welfare conditions in the outdoor areas are similar to those for broilers on under an extensive management system as described above. In all systems of hen production, feather pecking can be a major welfare problem.

The means of disposal of unwanted male chicks is one of the major animal welfare concerns in the commercial egg production industry. Millions of male chicks from selectively bred egg-laying strains are not suitable for meat production and so are disposed of by killing at 1–3 days old. Some of the methods used to kill the chicks are not humane. These methods include the use of mechanical apparatus that macerate the live chicks, dislocation of the neck, decapitation, suffocation or exposure to gas mixtures [44]. In the USA, maceration and gas killing are methods that are approved by the American Veterinary Medical Association [55], but the former is a method that is not aesthetically acceptable to many in society. The killing of male chicks has ethical connotations because of people's views on animal life [56].

2.6. Animal handling and the role of the stockman

The handling of animals confines and brings them much closer to humans than they would under natural conditions. This is a stressful period especially for animals that are not used to being handled. Young stock need to come into regular contact with the stockman so that they will not be too frightened when they are handled [57]. Some animals may show evidence of excitement and agitation, while in others, the mental suffering may not be evident. Dairy cattle are used to being closer to people and equipment, due to the regular milking sessions. Any infringement on dairy cattle welfare is likely to be from the poor design of the handling facilities or equipment and from inappropriate actions of stockman. Beef cattle that are reared extensively on pastures with little handling and human contact may suffer mentally during handling. Only properly trained dogs shall be used as an aid for herding animals. Animal handlers should be aware that the presence of dogs can stress and cause fear in animals and should therefore keep dogs under control at all times. The use of dogs in housed systems, collection yards, or other small enclosures where animals cannot freely escape is not recommended.

Stockman need to be well trained, competent, show empathy and have the capacity to detect animal welfare problems early and institute remedial actions in the shortest possible time. Animals in any production system need to be inspected regularly to identify any problems and situations that may compromise welfare. For example, lactating cows should be inspected at least once a day. Some animals may need to be inspected more frequently, for example, neonatal calves, cows in late gestation, newly weaned calves, cattle experiencing environmental stress and those that have undergone painful husbandry procedures or veterinary treatment [23].

A good stockman needs to be acquainted with behavioural changes that reflect poor welfare. The attitude towards animals also matters. Even trained stockman can contribute to poor animal welfare if they have negative attitudes towards animals. Welfare issues that can arise during animal handling relate to injuries such as bruises, damage to the nasal septum and bone fractures. Poorly designed handling facilities can inflict injuries to animals from sharp protrusions, fractures due to trapped legs and from slippery surfaces. Animal handling and restraint should be firm but effective. The following practices and principles help to ensure safe, humane and effective handling and restraint of all animal species on the farm:

- Design facilities as appropriate for each species—solid sides, well lit, non-slip floors and no protrusions that can cause injuries.
- Use only trained and skilled personnel to handle animals.
- Take advantage of animal behaviour by making use of the flight zone and the point of balance to move animals.
- Use an effective restraint method appropriate for each species to prevent injuries.
- Avoid shouting or making noises that excite animals.

- Move animals at their normal walking pace.
- Ensure that handling does not cause stress, pain or injury.
- Use electric prodders when necessary and only if there is space ahead for animals to move.
- Use nose rings with caution as they can damage the nasal septum of cattle.
- Do not pull animals using their appendages such as testicles, tail, horns, ears, legs and the head.
- Twisting the tail to move an animal inflicts unnecessary pain.
- Whips inflict pain and cause injuries on animals. It is advisable to use very light plastic tubing and other plastic material to move animals.

2.7. Assessment of animal welfare

Animal welfare is a science. Therefore, the assessment of the welfare status of animals should be based on objective measures and not emotions. According to Forkman and Lund [58], measures of animal welfare can be broadly categorised into management-based, resource-based and animal-based measures. Management-based measures include management routines such as the use of analgesics at dehorning, treatment with antibiotics, age at weaning or number of inspections of the herd per day. However, these measures can be difficult to measure, and in most cases, they contribute a small part to the overall animal welfare assessment protocol. Resource-based measures include the size of housing pens, provision of perching material and cleanliness of drinking water. Resource-based measures are repeatable, but are risk factors rather than actual measures of animal welfare. Animal-based measures include measures of animal behaviour, disease or injuries. These measures reflect the true experience of an animal more than the other measures. However, they require prior extensive training and at times have to be measured over a long time. The 'Five Freedoms' are an embodiment of what animals need to have a good state of welfare. They are a useful minimum checklist that can be used to assess animal welfare. Most protocols for assessing animal welfare incorporate the five freedoms. The overall goal of animal welfare assessment protocols is to aggregate information that can be used to give an overall impression of the welfare status of animals.

3. Conclusions

Commercial livestock farming is profit orientated, and as a result, it will always be associated with animal welfare infringements because it cannot replicate the natural environment. The objective of animal welfare is to promote the physical and mental health of animals and positive effects that promote well-being. It is now widely recognised that animals are sentient beings that are aware of their surroundings and can remember and suffer painful experiences. It is therefore imperative that their welfare is promoted to prevent suffering. The welfare of farm animals can be measured using the 'five freedoms' and other criteria. The welfare

status of animals can be good, poor and or be in between these two extremes depending on the management conditions to which an animal is exposed. Good welfare can be enhanced primarily by providing good management especially those aspects that facilitate the expression of innate behaviours. Good stockmanship is crucial to the success of animal welfare programmes on livestock farms. Poor animal welfare can manifest as high mortality rates, reduced reproduction, increased disease incidence, body damage, behaviour anomalies, heavy tick and internal parasite burdens. Despite advances in technology and a reasonable increase in awareness of animal welfare, the major animal welfare concerns remain in various livestock production systems. Increasing global demand for food and decreasing land area will always put pressure on the livestock industry to intensify production. The well-being of animals remains under threat.

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Conflict of interest

There are no conflicts of interest associated with this chapter.

Notes/thanks/other declarations

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It is vital to understand the recent perceptions in animal welfare and its implications in changing global scenarios. The introductory chapter to Animal Welfare provides concise details of the application of emerging these scenarios. The book is self-contained with everything needed to understand the importance of animal welfare and its perspectives. This is the first edition of the book so it covers the introductory level of topics, which are written specifically for veterinary students, classroom use, and practitioners who require more knowledge of animal welfare. The book contains sections on global perspectives, animal health and welfare, and husbandry and welfare. Each section comprises chapters from renowned experts in each area and gives readers a unique opportunity to explore the topic.

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