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New Advances in the Dairy Industry

Edited by Muhammad Subhan Qureshi



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Meet the editor



Prof. M. Subhan Qureshi obtained his DVM, MSc (Hons), and Ph.D. from the University of Agriculture Faisalabad (UAF), Pakistan. After working 22 years with the provincial livestock department of KP, Pakistan he joined the University of Agriculture, Peshawar, Pakistan, where he was engaged in researching animal health, reproduction, entrepreneurship, and bio-risk management. Dr. Qureshi has published sixty-five journal articles, three book chapters, and fifteen books. His research interests include stress physiology and animal welfare. He has worked with faculty members and students to win research grants to respond to local problems and explore business and industrial aspects of livestock and poultry production. He worked on the Triple Helix Model of Good Governance based on academia-industry-government linkages as the founder of the Dairy Science Park, Pakistan.

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Preface

This book provides a comprehensive overview of the dairy industry. It is divided into four sections.

Section 1, “Dairy Technology,” includes one chapter on the bio-molecular characteristics of whey proteins in relation to inflammation. Whey protein is categorized to contain a plethora of healthy components conferring anti-inflammatory and immunostimulatory roles related to all metabolic syndromes.

Section 2, “Reproductive Biotechnology,” includes one chapter on folliculogenesis, fertility and biotechnology in dairy cattle. The chapter has attempted to describe the most important aspects of this process. Moreover, not all follicles reach the preovulatory phase and can be fertilized, thus the author of the chapter has discussed how reproductive biotechniques can positively influence the fertility of bovine females. The chapter also describes the influence of antral follicle count on reproductive performance and the correlation to biotechniques was described. Finally, the chapter presents alternatives on how to improve fertility and productive efficiency in dairy herds.

Section 3, “Entrepreneurship Development,” includes three chapters.

The first chapter discusses the fact that vermi-products have a good livelihood and entrepreneurship-generating opportunities

The second chapter on “green gold” refers to the major bottleneck of dairy effluent treatment plant (ETP) operation. The generation of nutrient-rich wastewater from processed milk results in the annual production of 7.93 tons of carbon dioxide gas during treatment in a seven to eight-step process. The chapter proposes a carefully selected, tailor-made bacterial consortium in a biofilm reactor with a four- hours incubation in a single-step operation, transforming to transform the total volume of wastewater into ammonia-rich liquid biofertilizer. This technique helps to solve one of the major concerns for sustaining the expansion of the dairy industry, hence making the EETPffluent Treatment Plant (ETP) operation an eco-friendly operation.

The final chapter in this section provides information on low-cost and user-friendly dairy farming innovations suitable for all kinds of farms maintained under rural conditions existing in different tropical countries.

Section 4, “Sustainable Development,” includes four chapters.

The first is on regional breeds and examines the situation of cattle and buffalo in Europe that supply milk to the processing industry. It provides a brief description of the origin and cognition of different regional and local dairy breeds, the presentation of morphological and productive characters, as well as the perspective of rearing or conservation.

The second chapter in this section, the chapter on minerals nutrition reports its interaction with the Health and Reproductive performance of Dairy Cows. This chapter discusses the linkages of several mineral elements into the health and reproductive performance that affect the dairy industry of dairy cows.

The third chapter regards the prediction of future milk yield. A time series analysis of data from a dairy farm used both descriptive and inferential statistics, covering a total of 179 days of milk production. The results reveal that the milk yield of cows is declining, and that milk output is time-dependent and that the model is ARIMA.

Finally, the section ends with a chapter that presents the research methodology that generates an integrated database of the mind of a dairy consumer, regarding nine different dairy products. The set of studies deals with a variety of end products, presenting alternative messages about each product. The chapter demonstrates how the science of Mind Genomics is further applied through a typing tool, known as PVI (personal viewpoint identifier). The PVI is able to identify the mind-set of any individual that provides a binary response to six short questions. The chapter concludes with a vision for the future of the Mind Genomics research methodology in the fields of science and business.

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Section 1

Dairy Technology

Bio-Molecular Characteristics of Whey Proteins with Relation to Inflammation

Anwar Ali, Quratul Ain, Ayesha Saeed, Waseem Khalid, Munir Ahmed and Ahmed Bostani

Abstract

Whey proteins in bovine milk are a mixture of globular proteins manufactured from whey which is a byproduct of cheese industry. Whey protein is categorized to contain plethora of healthy components due to wide range of pH, promising nutritional profile with cost effective and diverse functionality. Reportedly there are three categories of whey protein, whey protein concentrate (WPC) (29–89%); whey protein isolate (WPI) 90% and whey protein hydrolysate (WPH) on the basis of proteins present in them. Whey proteins is composed of β -lactoglobulin (45–57%), immunoglobulins (10–15%) α -lactalbumin (15–25%), glycomacropptide (10–15%), lactoperoxidase (<1%) and lactoferrin nearly (1%). Whey protein plays an important role and is validated to confer anti-inflammatory and immunostimulatory roles related to all metabolic syndromes. According to molecular point of view whey proteins decrease inflammatory cytokines (IL-1 α , IL-1 β , IL-10 and TNF- α); inhibits ACE and NF- κ B expression; promotes Fas signaling and caspase-3 expression; elevates GLP-1, PYY, CCK, GIP and leptin; chelate and binds Fe⁺³, Mn⁺³ and Zn⁺². In this chapter we will discuss significant biological role of whey proteins related to inflammatory health issues.

Keywords: Whey Proteins, Inflammatory Cytokines, Molecular Signaling

1. Introduction

The protein system in bovine milk is made up of two main protein families: casein (insoluble) and whey proteins (soluble). Casein is the major portion 80% of total protein content weight by weight (w/w) and is easy to separate from non-fat milk through isoelectric precipitation (by adding acid or producing it in situ) or rennet-driven coagulation, and the leftover is whey. Whey proteins consists of spherical molecules as well as amino acids either acidic/basic or hydrophilic/hydrophobic in composition of their polypeptide chains that are evenly distributed to form a helical shape [1]. Profiling of whey protein including its chemical and physiological properties like β -lactoglobulin (β -LG), α -lactalbumin (α -LA), immunoglobulins (IG), bovine serum albumin (BSA), bovine lactoferrin (BLF), and lactoperoxidase (LP) along with other constituents are all contained in whey proteins [2]. Whey protein concentrations varies depending on its type (acidic or sweet), milk source (bovine, caprine, or ovine), phase of the year, the kind of feedstuff, lactation phase, and productivity.

2. Components and types of whey proteins

Acid whey, which has a pH of 5.1 or less, is made by acidifying milk directly, as is used in the production of cottage cheese. After rennet-coagulation, sweet whey with a pH of 5.6 or higher is produced, as it is used in most cheese-making processes around the world [3]. Whey protein (protein not precipitated by acid or rennet) accounts for about 0.6 to 0.7 percent of total milk content and accounts for about 20% of overall milk proteins. Whey proteins are high in nutrients that are beneficial to wellbeing of humans. α -lactalbumin, β -lactoglobulin, lactoferrin, lactoperoxidase, immunoglobulins, and glycomacropetide are among the proteins present in it [4]. Percentage of each component of whey protein is mentioned in **Table 1**.

The development of processing techniques, particularly those based on selectively permeable membranes, has allowed a range of whey protein constituents to emerge as common food additives in the last two decades. The membrane based processing techniques results in ultrafiltration (UF) to concentrate proteins and diafiltration (DF) to eliminate most lactose, minerals and low molecular weight components and hence producing versatile whey products with distinct qualitative and quantitative profiles of proteins, minerals, lipids, sugars and whey

| Sr. No | Component of whey | Amount | specie | Reference |
|--------|------------------------|--------------------|-------------------------------------|-----------|
| 1. | β -Lactoglobulin | 3.2 (mg/L milk) | Bovine, buffalo, caprine and equine | [5, 6] |
| 2. | Lysozyme | 0.0004 (mg/L milk) | Camel and bovine | [7, 8] |
| 3. | α -Lactalbumin | 1.2 (mg/L milk) | Camel, bovine and human | [9] |
| 4. | Lactoperoxidase | 0.03 (mg/L milk) | Camel and bovine | [10] |
| 5. | Glycomacropetide | 1.2 (mg/L milk) | Camel and bovine | [11] |
| 6. | Immunoglobulin M | 0.04 (mg/L milk) | Camel, bovine and human | [8, 12] |
| 7. | Immunoglobulin G | 0.7 (mg/L milk) | Camel, bovine and human | |
| 8. | Immunoglobulin A | 0.04 (mg/L milk) | Camel, bovine and human | |
| 9. | Lactoferrin | 0.06 (mg/L milk) | Camel, bovine and human | [9, 13] |

Table 1.
Approximate concentration of proteins in whey in different species.

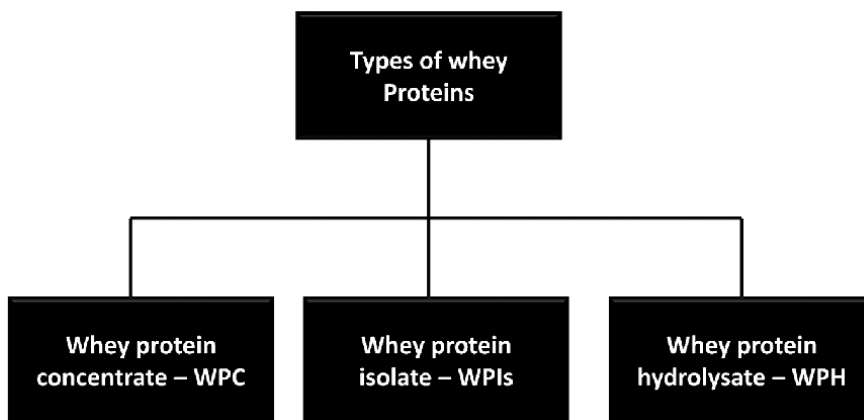


Figure 1.
Types of whey proteins; this classification is based on protein processing and refining [18, 19].

protein concentrate (WPC). There are WPC that produce 35, 50, 65 and 80% (w/w) protein, subject to their concentration. When the protein content reaches 90% (w/w), a whey protein isolate (WPI) is made, that is a high-grade and pure protein concentrate. When added to foods, each of these compounds function as carriers for the stimulation of a variety of biological characters. When whey is heated, α -LA denatures quickly, allowing the fraction to be divided by precipitation [14]. Whey protein hydrolysate (WPH) is the “predigested” type of whey protein since it has already passed through partial hydrolysis, which is needed for the body to consume protein. WPH is easier to absorb than the other two types of whey protein [15–17]. Flow sheet of all types of whey proteins is mentioned under **Figure 1**.

3. Physiological and biological roles of whey proteins

Whey protein is high in bioactive peptides, which can help with chronic disease control by nutrition. The conventional and evolving implications of whey proteins are elaborated in below para [20, 21].

Whey proteins and their constituents have been found to have a variety of health advantages. In a study, bovine whey proteins showed positive impacts on the lipid profile in serum and liver, including overall lipid and triglyceride alteration and reduction [22]. Enzymatic breakdown (natural digestion in the GI tract, enzyme mediated hydrolysis, milk fermentation) of whey proteins produces antihypertensive peptides (ACE-I) [23]. Whey proteins derivatives like short chain and peptides resistant to water have been shown in studies to reduce blood pressure and stress hypertension by increasing ACE inhibitory action [24, 25]. Whey proteins are a predecessor or foundation of glutathione (GSH) that performs oxidation against reactive oxygen species (ROS), responsible for tissue harm, especially in the central nervous system's neurons and glial cells [26]. Whey proteins are unusual in their capacity to increase glutathione (GSH) levels in different tissues that optimizes the functionality and structure of the immune system [27]. GSH defends cells from free radical destruction, contaminants, chemicals, infection, and UV exposure as part of antioxidant defense mechanism of body [28]. GSH levels are usually low in people who have cancer, HIV, persistent fatigue syndrome, or other immune-suppressing diseases [29]. GSH levels drop with age, and it's thought to play a role in diseases like Alzheimer's, cataracts, Parkinson's, and arteriosclerosis. Whey proteins provide a number of health benefits, including improving the acquired immune system. The most active fractions were whey protein extracts from β -lactoglobulin and α -lactalbumin, which had an additive impact on neutrophils, making them more sensitive to a subsequent stimulus [30]. Supplementing athletes with whey protein during rigorous endurance training can help them boost the immunity in between and afterward of workout and physical activity [29, 30]. In infants, an improved immune response may aid in the prevention of bacteriological and pathological syndromes, as well as illnesses including gastroenteritis. Thus, including whey proteins in one's diet can benefit people of all ages, not just those with a weakened immune system [31]. Whey protein has been shown to prohibit the progression of pathogenic bacteria, suggesting that it may be used to regulate the development and reinfection of *E. coli* (O157:H7), *Listeria monocytogenes*, and *Salmonella typhimurium* in ready to eat meat by using WPI as a defensive film covering ingredient [32–35]. Rises in the levels of alanine in plasma and aspartate aminotransferase activities, production of lactate dehydrogenase, and bilirubin concentration that refers to hepatitis indicators, as well as hyaluronic acid concentration which is fibrosis marker can be suppressed by a whey protein-rich diet [36, 37].

The active ingredient of whey protein found to have a vital role in growth and development of bones as well as a possible healing impact on osteoporosis by stimulating osteoblasts [38, 39]. The biochemical role of peptides in whey protein is as food additives is to improve calcium absorption (by preventing calcium phosphate formation) and avoid bone disorders [40]. Branched-chain amino acids (BCAAs) are well-known in whey proteins. While the need for BCAAs rises with endurance activity, whey protein may be used to supplement these BCAAs during the healing process to boost protein synthesis and muscle development [41]. Since both whey and skeletal muscle almost have identical amino acid profiling, it is highly effective to speedup muscle protein synthesis [42]. As a result, whey protein, by its necessary amino acids, aids in the production of skeletal muscle protein and the avoidance of sarcopenia (muscle wasting) after strenuous exercise [43, 44]. Current researches depicts that whey proteins may help athletes with resistance training boost their lean body mass and efficiency [45]. Increased brain tryptophan and serotonin activities boost cognitive function in stress-prone subjects because of efflux of whey protein in α -lactalbumin [46]. In milk, glycomacropeptides (GMPs) and whey protein concentrate may help probiotic bacteria like *bifidobacterium lactis* development. Gut wellbeing has been seen to be improved by a variety of probiotic bacteria [47]. Cancer prevention is essential. Whey protein in the diet can lower serum C-peptide levels and duodenal SREBP-1c mRNA profusion, as well as lessen the risk of duodenal tumors [48, 49]. Growth stimulator established in whey proteins showed positive impacts on various mammalian cells of the culture media. These growth stimulators work by suppressing protein degeneration and enhancing synthesis of protein and DNA inside the cells. As a result, whey protein can aid in tissue regeneration as well as the prevention of psoriasis and ulcers [50]. Incorporating whey protein concentrates and glycomacropeptides (GMPs) into the diet may increase satiety and reduce food consumption. GMP that activates cholecystokinin (CCK), which is a hormone to suppress hunger, is shown to have variety of important roles in gastrointestinal activity, including food intake control [51]. Various other proteins, in the patients suffering from type 2 diabetes, established to be overtaken by whey proteins for the improvement of postprandial lipemia, when taken as a complement to a fat-rich diet, likely due to the development of less chylomicrons or improved clearance of chylomicrons [25, 52] as shown in **Figure 2**.

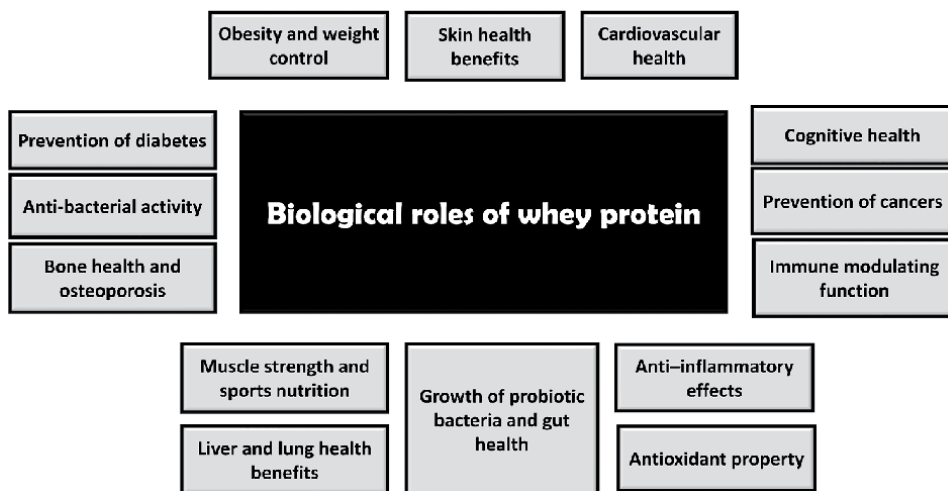


Figure 2. Biological roles of whey proteins summarized from different literature [53–60].

4. Inflammatory cytokines in inflammatory process

Cytokines are tiny secreted proteins (<40 kDa) that virtually every cell produces to control and affect immune response [20]. The discharge of pro-inflammatory cytokines causes immune cell initiation and development, along with the discharge of additional cytokines [61]. When the word “cytokine storm” first appeared, it was used to describe inflammation as a rapid immediate discharge of cytokines to activate an inflammatory mechanism [62]. Current work of scientists suggests that in every immune reaction, the simultaneous release of pro- and anti-inflammatory cytokines is needed [63]. Interleukins, chemokines, and growth factors are only some of the terms used to describe cytokines [64]. Super families of cytokines exist, which do not actually describe common genes but rather related structures [64]. Furthermore, the same cytokine may be generated by various cell populations. Cytokines have pleiotropic effects and their effects are dependent on the cell they are targeting [65]. Furthermore, various cytokines may have the same impact, making them redundant. However, they could have a synergistic influence. Finally, they can initiate signaling cascades, allowing even tiny amounts of protein to have catastrophic consequences [66].

5. Whey proteins leads to activation and production of pro-inflammatory cytokines

As cellular antioxidants struggle to keep up with ROS, the organism suffers from oxidative stress [67]. Inflammation causes damage to tissue by releasing free radicals from macrophages and neutrophils [68]. By transporting iron, whey proteins are essential for maintaining oxidative stress [69]. They produce the cytotoxic protection mechanism of neutrophils as well as free radicals [70, 71]. Whey proteins are involved in the preservation of inflammation through reducing lipid peroxidation and oxygen radicals via the production of antioxidant glutathione. Inflammatory cytokines are generated in large part by whey proteins. Whey proteins supplementation restored a high degree of IL-1 β and TNF- α . IL-1 β and TNF- α influenced the expression of chemokines and the adhesion of molecules required for the recruitment of inflammatory cells [72, 73]. According to a review, whey protein can increase neutrophil infiltration by inhibiting oxidative markers and reactive oxygen species. In whey protein treated mice, the number of bone marrow-derived dendritic cells, T-cells, and B-cells increased in response to CCL-21 and CXCL-12 [74]. Cytokines released by neutrophils increase the proliferative and remodeling processes of cells; via this, the earliest signals responsible for the activation of fibroblasts and keratinocytes are produced [75, 76]. Neutrophils are involved in the early stages of the anti-inflammatory cascade by removing bacteria and phagocytosing cellular waste [77]. Various experiments have shown that priming blood neutrophils with proteins can trigger oxidative explosion, chemotaxis, degranulation, and phagocytosis [78]. Whey protein promotes the development of inflammatory proteins such as IL-1 β , IL-8, IL-6 and TNF- α in macrophages [78, 79]. The initiation of NF- κ B, which contributes to the development of inflammatory cascades and the release of pro-inflammatory cytokines for instance IL-1 β , TNF- α , and IL-6 is one of whey protein's anti-inflammatory processes [80, 81]. Inflammatory cytokines such as IL-1, IL-10, and TNF- α were shown to be lower in the blood of mice feeding whey protein concentrate. Contrary to this, the amounts of cytokines such as IL-2, IL-4, IL-7, and IL-8, which are accountable for maintaining a healthy immune system, increased. The proliferative capacity of lymphocytes, monocytes, and macrophages in reaction to antigens was intensified [82].

6. Whey proteins role as anti-cancerous agents through production of inflammatory cytokines

Cancer is associated with a high rate of morbidity and mortality. According to a worldwide study, 27 main cancers were responsible for millions of deaths per year [81]. Dietetic modification has been proposed as an encouraging prophylactic strategy. Metabolites that modulate biomarkers of cancer began to emerge. Whey protein seems to be effective in this respect, and the assumptions have been supported by some data. In vitro, isolates of whey proteins had an impact on melanoma B16F10 cells that is cytotoxic in nature, as shown by the release of high caspase-3 [83]. According to a study performed on the rats suffering from gut cancer, fed whey protein hydrolysate, there were less tumor foci [84]. Before and after chemotherapy, a cervical cancer patient received whey protein (10 g three times quotidian) and a monthly testosterone infusion, which increased body lean mass, physical movement, and life quality [85]. Lactoferrin has been shown in vivo to stimulate cytokines in the guts and suppressing cancer growth in colon. Research established that consuming 3.0 g of bovine lactoferrin on a regular basis enhance the IFN levels that reduces the propagation of colorectal polyps [86]. While the available evidence is limited and the conclusion is far from definitive, but it might play a role of foundation for further research regarding anticancer experiments [21].

7. Whey protein as immunomodulators through maintenance of inflammatory cytokines

The complex network of lymphoid glands, effector cells, and molecules that make up the host immune defense is critical for survival [87]. Pathogenesis, metabolic, and degenerative disorders results as immunity lost. Dietary ingredients including probiotics, oligosaccharides, and β -glucans have been shown to have immune-boosting properties [88]. Whey proteins have been found to boost tolerance during childhood and thereby eliminate certain immune problems [89]. Whey proteins, upon digestion, found to activate variation among cells of immune system and recruitment of secondary lymphoid glands, according to a rodent report [89]. A comparative study among child's who fed whey incorporated formula were and the kids who consumed bovine milk, the chances of atopic dermatitis were 16 percent less in the former than later group [90]. Psoriasis patients' skin healed by taking 20 grams of whey protein isolate a day [21]. Although the psoriasis region and intensity score ranged from 4.9 to 28.8, it improved from 0.8 to 8.4 after 3 months of therapy. Higher glutathione levels and reduced systemic inflammation were linked to a reduction in autoimmune disorder symptoms [91].

8. Whey protein role in metabolic syndrome related to amelioration in pro-inflammatory cytokines

Hormones, cytokines and various other compounds involved to prohibit insulin sensitivity are produced by adipose tissue, an endocrine organ [66]. It is established that in the obese people, there is an increased discharge of adipocytokines through adipose tissue and macrophages [92]. Pal & Ellis [93] found that supplementing whey protein (54 g) for 12 weeks had little effect on pro-inflammatory markers (IL-6, C-reactive protein-CRP, and TNF- α) in overweight and obese participants. In rats with D-galactosamine-induced hepatitis and liver fibrosis, however, whey protein intake significantly decreased plasma amounts of pro-inflammatory cytokines

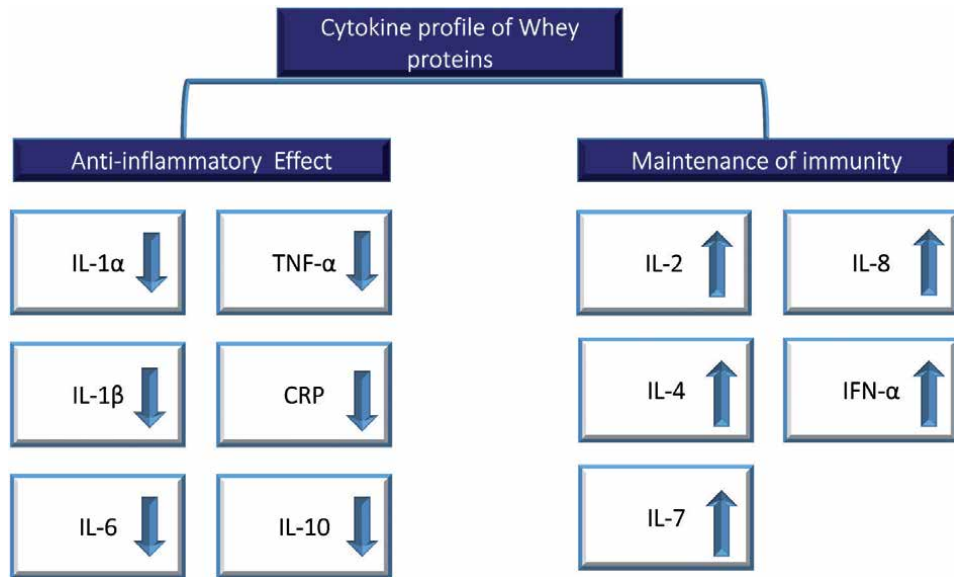


Figure 3. Cytokine profile related to whey proteins; by inhibiting IL-1 α , IL-1 β , IL-6, TNF- α , CRP and IL-10 whey protein show anti-inflammatory effect while release of IL-2, IL-4, IL-7, IL-8 and TNF- α showed its immunity related function [60, 96, 97].

(IL-1 β : 59 percent and IL-6: 29 percent) as paralleled to casein consumption [37]. Since consuming whey protein and its amino acids, a decrease in pro-inflammatory cytokines can be linked to a reduction in body weight gain [94]. In streptozotocin-induced diabetic rats, whey protein fed in 100 mg amount related to body weight in kg and results showed the decrease in number of oxidative stress markers, like; MDA, nitric oxide, and ROS concentrations, as well as pro-inflammatory cytokines (IL-1, TNF- α , IL-6, and IL-4) and raised glutathione amount [81]. A study in which rats were fed with a food of more carbohydrate content, no fat diet to cause fatty livers (nonalcoholic fatty liver model) plus orally directed whey protein (0.15 g/d/rat) for 28 days resulted in decreased MDA and elevated glutathione levels [95]. Hence it can be concluded that whey proteins plays a vital role to enhance endogenous antioxidant enzymes (glutathione peroxidase, catalase, and superoxide dismutase) and limits oxidative stress markers in obese, diabetic, or stroke patients, along with low expression of pro-inflammatory cytokines (IL-1, IL-6, and TNF- α) [94]. TBARs are commonly used to measure lipid peroxidation products in cell and MDA is used as an oxidative stress marker. Summary of all cytokines related to whey proteins is discussed in **Figure 3**.

9. Whey protein hydrolysate downregulate inflammation-related genes

Bioactive peptides with anti-inflammatory effects, as well as amino acids, are used in dairy protein hydrolysates [98]. After the consumption of milk or yogurt, postprandial mRNA levels of inflammatory markers were shown to be lower in an acute clinical trial [99]. These findings indicate that dairy nutrients, as shown in mice, may control the transcriptome [100]. Low-grade systemic inflammation has been related to endothelial dysfunction and the progression of atherosclerosis in obese and/or T2D people [101]. Reduced nitric oxide (NO) supply and hence endothelial nitric oxide synthase (eNOS) activity characterize endothelial dysfunction. The research showed that lower the availability of NO, enhances the invasion

of macrophages linked to adhering molecules like vascular cellular adhesion molecule (VCAM)-1, along with the discharge of proinflammatory cytokines like interleukins (ILs) and tumor necrosis factor (TNF- α) [102]. Antioxidant enzymes including superoxide dismutases (SOD) may even deactivate reactive oxygen species (ROS) that are connected to endothelial dysfunction [103]. Endothelial cells play an important role in inflammation. Dairy products' special protein and amino acid composition can influence cytokine gene expression and development. However, mechanistic experiments comparing various dairy protein compounds are rare [104]. Whey proteins and their chief amino acids, BCAAs, shown an increased anti-inflammatory capacity compared to other proteins.

Whey protein hydrolysate and BCAA have anti-inflammatory properties by lowering TNF and VCAM-1 expression and thereby attenuating TNF- α induced gene expression. Similarly, whey proteins were found to have an anti-inflammatory impact in Caco-2 intestinal cells by lowering IL-8 production [105]. Whey proteins' protective effects may be regulated by BCAAs. In HUVECs, BCAAs reduced the inflammatory reaction caused by TNF. In obese mice, induction of BCAAs, particularly leucine, reduced the appearance of genes involved in inflammation in the adipose tissue and liver, as well as macrophage infiltration [106].

In a rodent model of endothelial dysfunction, glutamine reduced the levels of circulating inflammatory markers (IL-6, IL-1, MIP-1, GM-CSF, MIP-2, IFN, and E-selectin) [107]. SOD2 expression is influenced by whey proteins and amino acids in the absence of TNF stimulation. WPH have been shown in to reduce inflammatory responses by constraining the nuclear factor κ B (NF- κ B) pathway [108]. Furthermore, glutamine, leucine, and proline have the ability to block the NF- κ B pathway [109]. NF- κ B is a transcription factor that controls the transcription of several inflammatory genes when it is triggered by proinflammatory signals like TNF- α , TNF, VCAM-1, and SOD2 are among the genes controlled by NF- κ B [110], and these were the genes most affected by whey protein combinations in this study. As a result, it is established that by a route of NF- κ B pathway, clear reduction in TNF-linked appearance of genes for inflammation through hydro-lysates and BCAAs of whey proteins could be achieved. Whey protein isolate, caseins, and WPC are entire proteins that can require other pathways. Autophagy, for example, has an effect on the functionality of mitochondria and oxidative stress, and that could be stimulated by proteins [111, 112].

10. IFN- γ , IL-8 and TGF- β expressions are downregulated by whey proteins

The most notable result of cow milk (CM0 whey therapy was the full abolition of interferon gamma (IFN- γ), as shown by extensive research. IFN- γ has a direct anti-proliferative effect on cancer cell lines. Studies have shown that mice lacking IFN- γ or IFN- γ receptor [113] as well as IFN- γ gene knockout (KO) mice grow cancer more quickly [114]. The late colitis stage and early neoplasms are reflected in the mouse examination and sample selection at 12th week. Dextral Sulfate Sodium (DSS) induced colitis was linked to an elevated level of IFN- γ , which was linked to decrease in weight and more death ratio [115]. Thus, the decreased IFN- γ amount results in the prohibition of immune cell recruitment to the inflammatory region and more tissue harm can clarify CM-mediated inhibition of inflammation. As a result, the neoplasm formation in CM-treated mice may have been influenced by lower mucosal inflammation. In a related vein, it has been shown that CM therapy reduced IFN- γ levels in chronic hepatitis B patients [116].

IL-8 has been shown to play a multifunctional role in cancer development, including encouraging tumor cell proliferation, improving cancer cell survival, and controlling adhesion and invasion [117]. Findings showed that cow milk (CM) significantly reduced IL-8 expression in colon tissue, suggesting that these results may reflect CM's anti-inflammatory mechanism. It was found in a report on immunomodulatory function of milk proteins that IL-8 was recruited by Caco2 gut cells lined with the both α -lactalbumin and β -lactoglobulin of a non-fat bovine milk (BM) whey [118]. This phenomenon occurred by the activation of Caco2 cells by IL-1 β . However, it is still needs to be confirmed that either the abstinence of β -LG in CM whey has any link to drop the IL-8 scale or graph in the CM treated population in model. Many studies have shown that all milk ingredients have a synergistic role in lowering IL-8 gene expression [118].

In addition, milk whey treatment reduced transforming growth factor (TGF- β) expression. TGF- β , a cytokine has double role in cancer growth and alteration in the TGF- β pathway within epithelial cells, promotes the enlargement and evolution of colonic tumors [118]. TGF- β signaling in tumor-infiltrating T-lymphocytes, on the other hand, led to the growth of dysplastic epithelial cells in experimental colorectal cancers (CRCs), according to contradictory findings [119]. Varying results on the role of TGF- β in CRC models could be described by the phase of cancer studied or the signaling pathway targeted by the cytokine in specific immune cells.

11. Conclusions

An interesting and verified phenomenon of bovine milk whey proteins is that they are known to act as anti-inflammatory and immune-stimulatory agents in connection with inflammatory and metabolic disorders.

According to molecular hypothesis, whey proteins decrease inflammatory cytokines (IL-1 α , IL-1 β , IL-10, and TNF- α) by molecularly inhibiting ACE and NF- κ B expression, promoting Fas signaling, and increasing caspase-3 expression, while elevating GLP-1, PYY, CCK, GIP, and leptin, as well as chelating and binding iron, manganese, and zinc.

In this chapter, we have described about whey proteins' essential significance in the molecular mechanisms associated with inflammatory health conditions. Whey protein also possesses many other impacts on different health issues on molecular level.

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Conflict of interest

Authors declare no conflict of interest.

Notes/thanks/other declarations

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Abbreviations

| | |
|----------------|--|
| ABTS | 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid |
| ACE | Angiotensin-converting enzyme |
| α -LA | alpha lactalbumin |
| AOM | Azoxymethane |
| B16F10 | Murine melanoma cell |
| BCAA | Branched chain aminoacids |
| Caco-2 | Cells human colon adenocarcinoma |
| CCK | Cholecystokinin |
| CM | Cow milk |
| CRC | colorectal cancer cell |
| CRP | C-reactive protein |
| Cxcl-12 | Motif Chemokine Ligand 12 |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| DSS | Dextran sulfate sodium |
| E.coli | <i>Escherichia coli</i> |
| E-selectin | CD62 antigen-like family member E |
| FRAP | Fluorescence recovery after photo bleaching |
| GI-tract | gastro intestinal tract |
| GLP-1 | Glucagon-Like Peptide 1 |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| GSH | Glutathione |
| HIV | human immunodeficiency virus |
| HSP70 | Heat Shock Protein |
| HUVECs | Human umbilical vein endothelial cells |
| IL | Interleukin |
| IFN | interferon |
| KDa | kilodalton |
| MDA | methylenedioxymethamphetamine |
| MIP-1 β | Macrophage inflammatory protein |
| NF- κ B | nuclear factor kappa-light-chain-enhancer of activated B cells |
| PYY | Peptide YY |
| SOD2 | Superoxide dismutase 2 |
| SREBP-1c | Sterol regulatory element-binding protein 1 |
| T2D | type 2 diabetes |
| TBARS | Thiobarbituric acid reactive substances |
| TGF- β | Transforming growth factor beta |
| TNF-a | Tumor Necrosis Factor alpha |
| UV | ultraviolet |
| w/w | weight per weight |
| WPCN | whey protein/casein mixture |
| (VCAM)-1 | Vascular cellular adhesion molecule |

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Section 2

Reproductive Biotechnology



Folliculogenesis, Fertility and Biotechnology in Dairy Cattle

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Abstract

The ovarian follicle population is formed by thousands of follicles, preantral and antral, where oocytes are included. During fetal life, the first follicles produced are preantral, and, as they undergo the development process, they reach the final stage of antral follicles, where a cavity/or antrum is developed. All this growth phase is called folliculogenesis, and this chapter will abord the most important aspects of this process. Moreover, not all follicles reach the preovulatory phase and can be fertilized, so we will discuss how reproductive biotechniques can positively influence the fertility of bovine females. We will also discuss the possibility of antral follicle count to influence reproductive performance and the correlation to biotechniques. Finally, we present alternatives on how to improve fertility and productive efficiency in dairy herds.

Keywords: dairy cattle, folliculogenesis, antral follicle count, fertility, embryo production

1. Introduction

Dairy farming plays an essential role in the global socioeconomic scenario, being one of the most traditional rural activities and fundamental for agribusiness. Milk is one of the most complete and most consumed food globally, in addition to providing a social function, generating thousands of direct and indirect jobs throughout the production chain. The development of new technologies should add more efficiency to the milk production chain, a constant challenge for several sectors involved in the segment.

The use of animal reproduction biotechnologies has contributed to the increase in animal productivity and has been one of the main responsible for the increase in milk production. The current scenario is the search for a model capable of providing high production efficiency concerning animal welfare without harming the environment and with the most advanced reproductive techniques for obtaining pregnancies and genetic improvement. In this way, ovarian physiology is a key aspect to contribute to the efficiency of dairy production.

The ovarian follicular population is characterized by the total amount of follicles present in the ovary. Each follicle contains an oocyte, so it is known that there are a great number of oocytes in the ovary. However, only a small portion of the ovarian follicles undergo ovulation. Therefore, the ovarian follicular reserve is an important

indicator of fertility in cattle, which may influence the applicability of reproductive biotechniques.

This chapter addresses the mechanism of folliculogenesis and the most recent research. It also brings discussions on how reproductive biotechniques can influence fertility in dairy cattle.

2. Oogenesis and folliculogenesis

The origin of the female reproductive system is still in embryonic life in the sublumbar region located caudally to the kidneys. The primordial germ cells, which will give rise to the germline formation, originate in the proximal epiblast and then move from the yolk sac to the gonadal ridges through the mesentery, around day 30 of germinal development [1]. At this moment, the Müller and Wolff ducts are still present, which will give rise to the female and male reproductive tract, respectively.

After the colonization of the ridges, around 35 days of gestation, the differentiation process begins by specifying the somatic cells of the ridge, where Sertoli cells will originate from the XY chromosome, and the granulosa cells will originate from the XX chromosome. There is an involution of Wolff's duct (or mesonephros) and development of Müller's duct (or paramesonephric) in the escarpments. In males, as they inherit the testicles determining factor (TDF) from the Y chromosome, Sertoli cells release the anti-müllerian hormone (AMH) and inhibit the development of Müller's ducts [2, 3]. After this process, they are formed as oogonia that through mitotic and meiotic divisions form a nest of oogonia in a tubular shape, and then a process of differentiation into oocytes begins [4].

Gonadal structures called germline cysts are elevated in the ovigerous cords and surrounded by a basement membrane shortly after colonization of the gonadal ridges by primordial germ cells. Meiotic divisions are initiated until the process is stopped in meiosis prophase I when primary oocytes are already formed. The primary oocytes are surrounded by a layer of undifferentiated pregranulosa cells [5]. The interruption of meiosis can last for years until a given follicle enters the growth process, resuming meiosis and continuing the follicular development through the primordial follicle until its final stage in the antral follicle [6].

It is known that folliculogenesis depends on interactions between the somatic cells of the follicle and the oocyte, so the communication between the granulosa and theca cells with the oocyte is essential for follicular development and growth to occur [7, 8]. The passage from the primordial follicle to the primary follicle is a transition phase and is characterized by the action of specific growth factors for each stage of folliculogenesis [9]. In bovine species, the so-called follicular growth waves correspond to a stimulus for the recruitment of preantral follicles.

Once the primordial follicle is recruited, whose granulosa cells are flat, it becomes the primary follicle and there is a transition between the flat cells to the cuboidal-shaped granulosa cells [9]. At this stage, the zona pellucida appears, which will remain around the oocyte throughout the follicle's development. Continuing to grow, the secondary follicle is constituted when the granulosa cells multiply and form two layers of cubic morphology, in addition to the emergence of the first theca cells [10].

The growth of these secondary follicles (when they reach approximately 4 mm in diameter) is regulated by the follicle-stimulating hormone (FSH), which has its receptors in the granulosa cells. When they reach a larger size (approximately 7–9 mm in diameter), they start to be controlled by luteinizing hormone (LH). At this stage, the follicle is already characterized as tertiary and has LH receptors in the theca cells that are already entirely organized, and the formation of the follicular antrum can be observed [11, 12]. The phases of follicular development are shown in **Figure 1**.

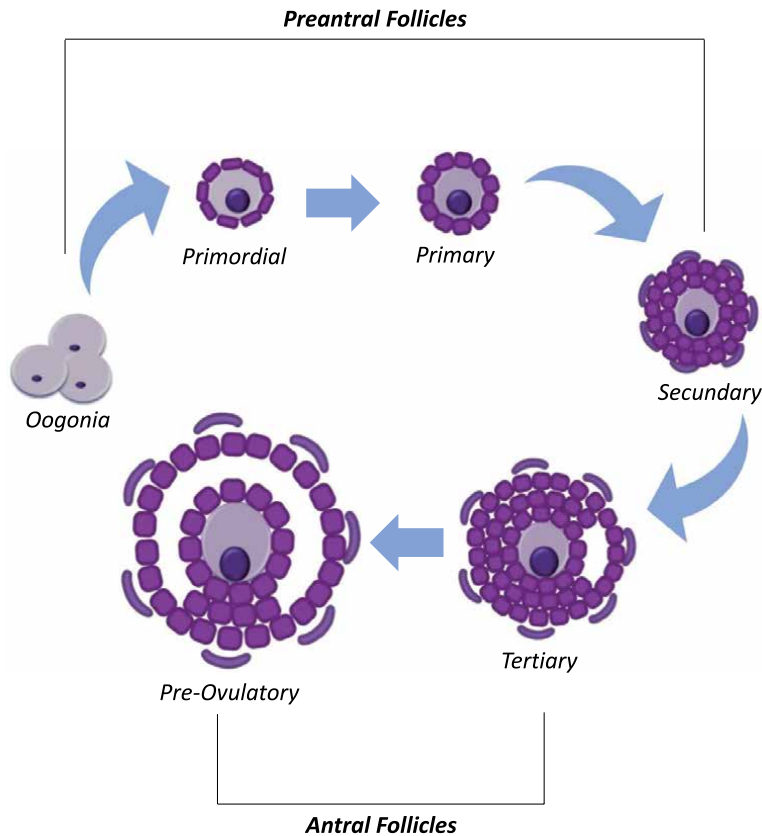


Figure 1.
Schematic sequence of complete follicular development.

3. Ovarian follicular population

The follicular population may vary between individuals, and some factors such as genetics, breed, age, species, and hormone levels can influence the number of follicles present in the ovaries [13]. For bovine females, it is estimated that the number of follicles at birth is about 235,000 [14].

An increase in the number of antral follicles present in the ovary and stimulated by gonadotrophin secretion is influenced by body development in heifers [15]. Endocrine activity at first seems to be controlled by suppressing negative feedback mechanisms until the heifer has a good body condition to initiate the estrous cycle and reproduction activities [16–18].

The first ovulation in heifers is marked by a gradual increase in LH secretion, which leads to the development of the antral follicles and the secretion of estrogen. At birth, antral follicles are not typically present in the ovaries, and the number of follicles increases when heifers reach 2 months of age. After that, the number of follicles declines at 5 months of age, and some changes continue to occur throughout the productive life of the female [19–21].

4. Antral follicle count and fertility in dairy cattle

Ovarian follicular population is highly variable among species, a concept that is well established [20–22] and has been already reported in cattle [23]. In recent

years, numerous studies have focused on the ovarian follicular population and its influence on reproductive activities, as well as on animal reproduction biotechniques [22, 24]. The antral follicle count (AFC) is a strategy to identify different profiles of cows, performing transrectal B-mode ultrasonography and counting all follicles larger than 3 mm [25]. The total number of follicles counted in the pair of ovaries is added up, and the cow is classified as low, medium, or high AFC. A feature in cattle is the high variability of AFC between animals, but it is known that there is high repeatability in the same individual [19, 26, 27]. The appearance of the ovary on ultrasound examination of cows with high and low AFC is presented in **Figure 2**.

Furthermore, according to [28], it has already been established that the concentrations of anti-müllerian hormone (AMH), which is released by the granulosa cells of growing ovarian follicles, are positively related to the entry into puberty of bovine females. In other words, the higher serum concentrations of this hormone are, the higher AFC will be [27]. AMH is a glycoprotein that belongs to the TGF- β growth factor family [29] and it is correlated with follicular growth [30]. AMH is in the granulosa cells and it is responsible for the growth of preantral and antral follicles [31] and follicular growth modulator through the control of ovarian follicular reserve depletion [32].

The intrafollicular AMH expression increases until the follicle reaches 5 mm in cows and then decreases as the follicle reaches the antral stage and increases in size [31]. The positive correlation of AMH with the ovarian follicular population has already been described in previous studies [33, 34]. Thus, the measurement of AMH can be a method of predicting AFC [35]. In *Bos taurus taurus*, *Bos taurus indicus*, and *taurus* \times *indicus* crosses, animals with a high plasma concentration of AMH present a greater number of antral follicles than those with a low concentration of this hormone [36].

Reproductive biotechniques, such as embryo transfer (ET) and IVEP, depends on the population of antral follicles present in the ovary of donor females to succeed. Among other factors that interfere with ET and IVEP, it is important to mention genetics, breed, and age [37, 38]. High AFC bovine females have been described to have a greater number of viable embryos produced *in vivo* per animal [39–41]. Similarly, in IVEP—ovum pick-up (OPU) procedures, high AFC animals resulted in a higher rate of blastocyst production than low AFC females [24, 42].

In contrast, a high conception rate was observed after the use of TAI in low AFC females *Bos taurus indicus* compared to high AFC animals [43, 44]. Additionally,

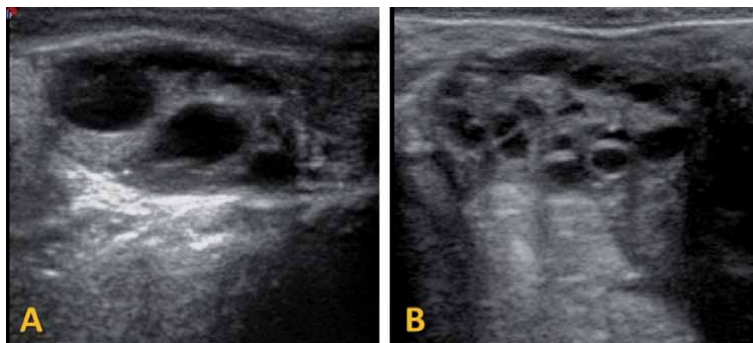


Figure 2. Aspect of the ovary on ultrasound examination of cows with different counts of antral follicles (AFC). On the right (A) cow with low AFC, and the left (B) cow with high AFC. Images were generated via the transrectal route with the equipment model S8v (SonoScape®) with a frequency of 8.6 MHz and a linear transducer of 5–10.0 MHz.

other authors [45] observed that high AFC *Bos taurus taurus* females had lower fertility and shorter reproductive life than females with low AFC, but contrasting data have been reported [46].

Donors with a high number of antral follicles have been selected, mainly for OPU-*in vitro* fertilization (IVF) procedures, due to the quantitative advantages for producing *in vitro* embryos. Because of the high number of calves generated from IVF, there is an increasing interest in studying the relationship between AFC and reproductive characteristics.

In summary, several studies have tried to verify the influence and the correlation of AFC in the reproductive performance of bovine females. So far, it is not possible to establish the role of AFC in fertility parameters due to the controversial results. Although it is quite predictable that AFC may be related to reproductive efficiency, a better understanding of the subject is necessary. Furthermore, considering basic research, it is necessary to elucidate some aspects of follicular physiology that remain unknown [47].

5. Reproductive biotechniques and fertility in dairy cattle

Increasing the productive efficiency of a herd is one of the great challenges for dairy cattle farming. In the past, genetic selection programs sought essential characteristics for increasing milk production, with effective gains in milk quantity and quality, but reproductive efficiency was disregarded. In recent years, several works have been presented to increase milk production and increase reproductive performance, a key association for efficient dairy farming.

Considering the importance of a sustainable, intensive, and economically viable production system, achieving the reproductive efficiency of the dairy herd is crucial for the effects on profitability by the number of offspring produced, genetic progress, and the shorter interval between lactations. This is a great challenge, as there is low heritability between production and reproduction traits. Therefore, the crucial importance of precise reproductive assistance is highlighted, providing maximum production efficiency in the smallest possible area and respecting the aspects of animal comfort.

5.1 *In vitro* embryo production (IVEP)

Despite the rapid development of the technique since its emergence in the late 1980s, until recently, IVEP was used only as a last resource when traditional techniques failed. However, the high genetic gains provided to the herds, obtaining a greater number of pregnancies concerning *in vivo* production, and lower costs due to high productivity have contributed to making IVEP the first choice in many dairy farms [48].

Holstein cows typically have lower oocyte production when subjected to IVEP. However, it is possible to obtain good results by performing a pre-selection of females with a high number of antral follicles using ultrasound. It is important to highlight that non-lactating females often have a higher number of follicles and oocytes.

Until a few years ago, some obstacles prevented the large-scale use of IVEP in dairy cattle. One of them was the large number of calves born from unwanted sex (male), which significantly increased the production cost. Another difficulty was the distance, often thousands of kilometers, between the laboratories and the properties where the recipient cows were located. The inefficiency of cryopreservation techniques for IVP embryos, especially when dealing with *Bos indicus* embryos,

limited their production and transfer connection. Thus, discarding untransferred embryos was a common practice.

These two major obstacles have now been overcome, making large-scale *in vitro* embryo production a reality. Some researchers [49] reported an IVEP program in which over 20,000 dairy embryos were produced with sexed semen (female). Embryos were transported through two or three days during the *in vitro* culture period using portable incubators. In just over a year, 8000 female calves were produced, with an average pregnancy rate of 39%.

In addition, some alternatives can be employed to improve the methods that assist in the recovery of better-quality oocytes and a higher competence in OPU to obtain more interesting results in embryo production. In this context, the follicular wave synchronization before OPU and consider the influence of the antral follicle population seem to be good alternatives [50].

5.2 IVEP and artificial insemination (AI)

Dairy European breeds, such as Holstein and Jersey, suffer great discomfort under high temperatures and high humidity conditions. Therefore, failures in cyclicity and the demonstration of estrus occur, making management difficult and compromising AI results. Furthermore, it is known that embryos are naturally more resistant to heat stress than gametes, which can suffer degeneration and further reduce pregnancy rates in the summer [51]. The transfer of embryos 7 days after fertilization avoids the harmful effects before this period, providing more advantageous rates than AI [52–54]. For dairy cattle, therefore, the use of transferred embryos seems to be the most viable option, especially in periods of excessive heat [55].

In AI, the number of descendants of genetically selected bulls is multiplied. IVEP, in turn, also generates descendants of females of high genetic merit, causing an even more significant impact on the improvement of a herd. Greater genetic gain is achieved in each generation with the transfer of embryos produced *in vitro* than with AI [48].

In some dairy farms, cows with better genetic potential are used as embryo donors and recipients. Thus, an efficient genetic selection from animals in the herd becomes possible. As for the economic aspect, with the number of pregnancies in IVEP, it is possible to produce embryos at affordable costs, making the embryo commercially attractive compared to semen [56]. Another advantage is the better use of high-value semen due to the possibility of fertilizing ten or more cows with a single dose.

5.3 Use of sexed semen in AI and timed artificial insemination (TAI)

The use of conventional semen, both in AI and *in vitro* fertilization, requires twice as many recipients compared to sexed semen [57]. By ensuring that almost all embryos are of the desired sex—female—the use of sexed semen significantly reduces the cost of production [58].

The most used technique for semen sexing is flow cytometry, which offers an accuracy of 85–95% [59]. However, during the sexing process, the sperm may be damaged, which might compromise their viability, reducing the fertilization potential and embryonic development [60]. The sexing process reduces sperm motility, compromising AI indices [61]. Related that the mean conception rate after AI between 2012 and 2016 was 56.9% with conventional semen and 47.3% using sexed semen. In IVEP, however, the method allows obtaining very satisfactory rates of blastocysts, with quality similar to those produced with conventional semen, since this technique requires fewer viable spermatozoa [62, 63]. Generally, the conception

rate obtained with sexed semen is 50–60% of the rates obtained with conventional semen in cows and 70–90% of conventional semen in heifers [64].

As there is a reduction in fertility using this semen, some strategies are currently suggested to improve conception rates in insemination programs that use sexed semen. First, it is recommended to use this semen in heifers and most in the first three services due to greater fertility. In AI programs with heat observation, the highest conception rates were achieved, with AI being performed between 16 and 24 h after the onset of heat [65]. Finally, in TAI programs, the best rates were achieved with semen deposition 60 h after removing the progesterone source [66].

5.4 Ovum pick up/IVEP vs. superovulation/embryo transfer (ET)

In the *in vivo* production of embryos, it is necessary to administer hormones so that superovulation (SOV) occurs and, subsequently, the transfer of the embryos. In Ovum Pick Up (OPU)/IVEP, however, obtaining oocytes and producing embryos do not require hormonal use. Furthermore, it is known that in *Bos indicus* animals, the number of embryos produced per aspiration session is higher than that of superovulation [67].

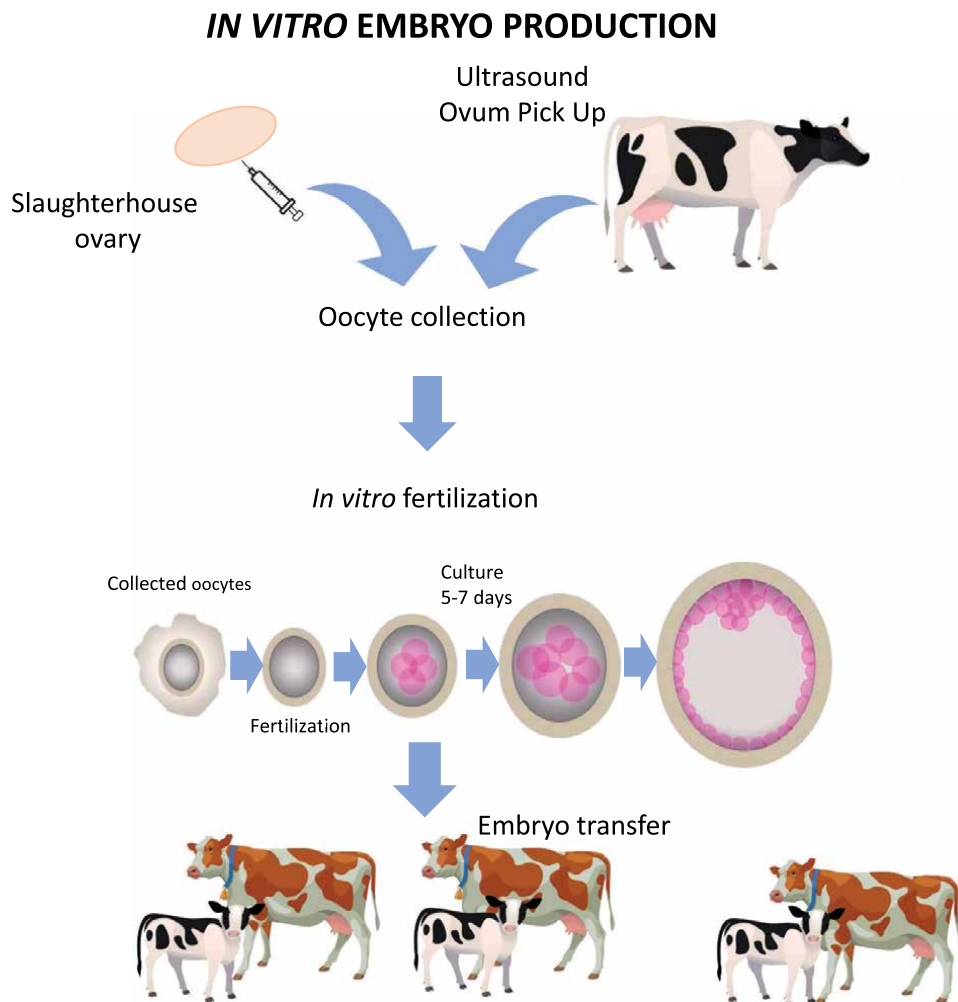


Figure 3. Schematic sequence of steps in the in vitro embryo production process (IVEP).

The *in vitro* technique also allows for less spaced collections of oocytes from donors. In general, the minimum interval is 15–30 days, and there is no limit to the number of aspirations performed on the same cow [68]. On the other hand, SOV requires intervals of 40–60 days and should only be performed three or four times before a period of several months apart [69].

The production of embryos by SOV also does not allow pregnant cows, while in IVEP, this is possible. Follicular aspiration can be performed as long as the ovaries can be manipulated without being subjected to excessive traction. The process flow of *in vitro* and *in vivo* embryo production is shown in Figures 3 and 4, respectively.

5.5 Cryopreservation of *in vitro* produced embryos

The cryopreservation of bovine embryos generated *in vivo* has protocols very well established and effective through a freezing process. However, despite the benefits obtained and the advantages of IVF already reported in previous topics, cryopreservation represents a challenge. The low cryotolerance of IVP embryos is a limiting factor for using the cryopreservation process associated with this process. IVP embryos are more susceptible to damage caused by cryopreservation when compared to those produced *in vivo*, as they present differences in morphological, metabolic, and chromosomal aspects of their structure [70].

The greater sensitivity of these embryos to low temperatures is mainly due to the greater accumulation of lipids in the cytoplasm [71]. Lipids, made up mostly of triacylglycerols, directly affect the survival of embryos during cooling, as they can undergo irreversible changes and severely compromise development. An alternative method to promote chemical delipidation of embryos and increase cryotolerance by decreasing lipid accumulation has been related [72]. Forskolin, for example, a compost derived from the Indian plant *Coleus forskohlii*, is able to promote intracellular lipolysis in swine [72] and bovine [73] embryos. When added to the medium at strategic periods of *in vitro* culture, this substance raises embryonic tolerance to levels that provide good pregnancy rates, even in *Bos indicus* embryos [73].

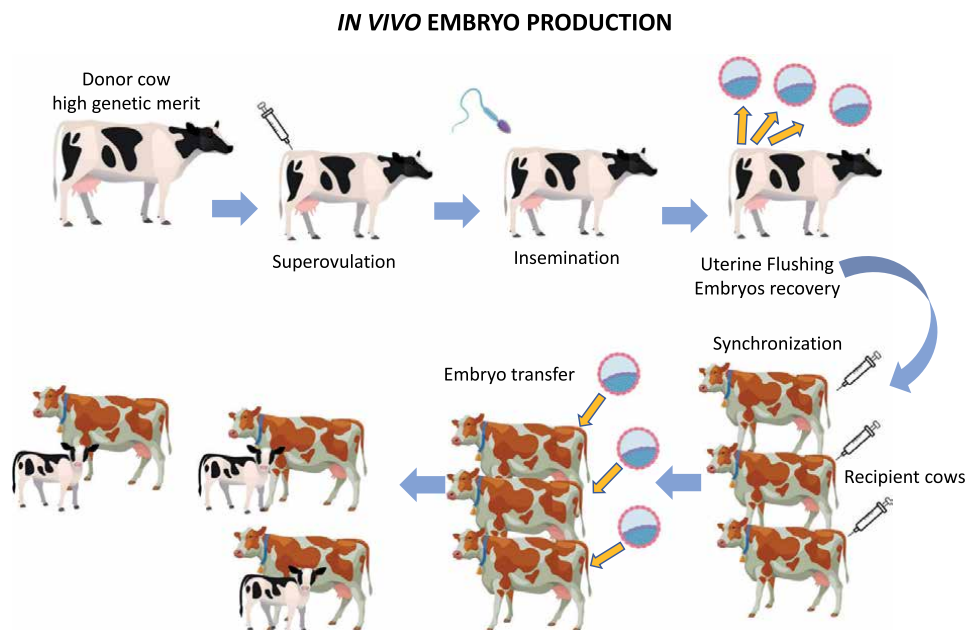


Figure 4. Schematic sequence of steps in the *in vivo* embryo production process (SOV/ET).

Among the cryopreservation methods, vitrification is the most used technique worldwide due to the speed of the process and its low cost [74]. On the other hand, direct transfer (DT), a technique used to simplify the *in vivo* post-thawing rehydration step of embryos, has its main advantage the low concentration of cryoprotectants reducing embryotoxicity [75]. Also, DT eliminates the evaluation process before the transfer, thus becoming a more practical way than vitrification [76].

6. Challenges of *in vitro* production embryos

The *in vitro* production technique comprises a greater number of steps than those necessary *in vivo*. Thus, skilled labor is necessary so that it is possible to obtain efficient results under controlled laboratory conditions. Due to the fixed costs of laboratory equipment, materials, and professionals, the number of embryos produced determines the commercial viability of the technique [77].

Due to the metabolic and morphological differences compared to those produced *in vivo*, the pregnancy rates are lower in the *in vitro* production of embryos. Furthermore, cryopreservation and rewarming processes are more critical for IVP embryos. Therefore, the use of cryopreserved *in vitro* embryos must be very judicious. Genetic growth must be considered together with the need for an adequate herd pregnancy rate to ensure milk production on the property. The most advantageous aspect of *in vitro* produced embryos refers to the wide success of using sexed semen in this biotechnique. In the current context, the efficiency of sexed semen in *in vivo* production is unsatisfactory.

Thus, if the proposal is to associate embryo transfer and sexed semen, the best strategy at the moment is the *in vitro* production technique. The use of cryopreserved or female sexed IVEP embryos has a precise indication for donor replacement and herd genetic improvement. AI with sexed semen and embryos produced *in vivo* is equally interesting biotechniques, and there may be an association between them all to ensure milk production, reproductive efficiency, and genetic improvement.

7. Challenges of dairy farming and the contribution of reproduction to increase productive efficiency

In order to minimize the effects of early embryonic loss, the Doppler ultrasound technique has been included in reproductive programs. This non-invasive and real-time biotechnology allows the characterization of blood perfusion of reproductive organs and tissues throughout the estrous cycle and pregnancy in cattle. One of its purposes is to accurately estimate the corpus luteum (CL) functionality for the selection of recipients and for the early diagnosis of pregnancy in TAI and TETF (Fixed Time Embryo Transfer) programs.

In addition to allowing for greater accuracy in the evaluation of the recipient, another feature of the Doppler is the diagnosis of pregnancy at 20–22 days, which is early compared to the conventional system performed at 30 days after insemination. Super-early resynchronization programs developed in heifers and cows are being introduced in dairy herds, as the reduction in the interval between two TAIs promotes gains in reproductive efficiency. Despite the correct evaluation being dependent on the experience and knowledge of the operator and the correct configuration of the equipment, the popularization of the technique is consolidated every day and presents good prospects for the future.

The current scenario of reproduction biotechnology demonstrates great potential for a sustainable increase in milk production, mainly due to the increase in reproductive and productive efficiency. Furthermore, the growth in the use of reproductive biotechniques is associated with the parallel development of a support network such as veterinarians, the pharmaceutical industry, disposable materials, equipment, and service providers. The generation of employment and the need to train human resources to meet the demand for activities are intended to provide social growth.

With the possibility of obtaining an accelerated genetic gain through the shortening of the generation interval, the use of prepubertal females, mainly in the production of embryos, has aroused great commercial interest and investment in research. The genetic potential of the female must first be evaluated in advance, that is, before total production. This is feasible thanks to progress in research with genetic markers for accurate prediction of the females that will be more efficient in milk production. It is also important to consider improving equipment for OPU (oocyte recovery by Ovum pick-up). There are currently fully adapted transducers for use in very young females. Despite the good number of aspirated follicles, a challenge in this category is the low blastocyst rate, promoting limited results in IVF.

Thus, to be viable for the use of these females, the next step is to develop protocols that improve the competence of the retrieved oocyte. Gonadotropin stimulus to increase the proportion (and size) of large follicles and synchronization of follicular waves before OPU to decrease immature oocytes have been investigated. A revolution in dairy farming that has become increasingly accessible is genomic selection which has significantly altered the global dairy industry. The reduction in the generation interval from 7 to 2.5 years and the reduction of costs with progeny tests were only the first benefits presented by the gene-editing biotechnology.

Silencing, altering or replacing genes that cause problems are effective strategies to increase the productive efficiency of the herd, selecting and breeding genetically superior animals. The generation gap is likely to narrow further as assessments gain wide acceptance, as genetic gains are cumulative across generations. Genetic progress is expected as continued genetic selection is implemented. Since 2009, more than one million animals have received genetic evaluations. Although these tests are carried out primarily on male animals, genotyping costs are currently economically viable. Currently, genomic selection programs are investing more in health traits (resistance to disease), reproduction, and selection for environmentally sustainable production, including reducing waste production and gas emissions.

This change of concept, which seeks longevity and animal welfare, is because, in recent years, there has been a decline in fertility and resistance in several populations, leading to a decrease in the profitability of the herds. The increase in slaughter rates, veterinary expenses, replacement costs, and reduced milk sales were just some of the consequences of the negative impact of years of selection focusing only on milk production and animal appearance. Furthermore, the adoption of a selection index, such as evaluating the quality and viability of embryos before the transfer, increases the efficiency of the process.

An example of this has been in North America, where the implementation of a genetic-based selection program for reproductive disorders is actively researched. A high and positive genetic correlation between retained placenta and metritis is being observed, implying selection of genes to improve one trait reflecting positively on the other. This demonstrates that the increased need for genomic traits for these traits contributes to the reproductive efficiency of dairy herds.

Other characteristics that have been valued in genomic tests are identifying biomarkers considered for genetic improvement, highly correlated with reproductive

performance, such as anti-müllerian hormone (AMH), and identifying relevant genes to reduce pregnancy losses. Identifying genetic markers related to the development and anticipation of the embryo and their selection to avoid embryonic losses can minimize economic damage. Another issue to be further elucidated shortly is whether genes relevant to embryonic development are positively associated with fertility traits. Estimates of the heritability of conventional reproductive traits are generally low. Even so, the progressive inclusion of genomic tests, as a routine in the field, has great potential for identifying superior animals. In the medium and long term, one perspective is that genetic improvement programs will bring consistent profitability for the dairy industry.

Genomic testing still faces challenges because a decisive outcome in the short term is unlikely. Genetic variation for economic characteristics is maintained by increased frequency of rare alleles, new mutations and changes in goals, and no selection management. Moreover, although genomic selection is being well applied at rates of genetic gain, we still know very little about the genetic structure that promotes this variation. The most relevant future challenge will probably be the incorporation of new characteristics in the selection index in breeding programs, overcoming a measurement difficulty or low heritability of them. Added to this, it is still uncertain whether traits produced over several generations emerged included in routine genomics, as gene frequencies change over time.

It is already known that the selection of some genes can directly or indirectly influence other aspects. The concern with creations called “ecologically correct” remains controversial. The inclusion of characteristics such as lower gas emissions can compromise herd productivity. It should be remembered that the increase in milk production per animal reduces the total production of residues in the atmosphere. In other words, it is something broader than simply a genetic alteration to favor an environmental issue narrowly.

Genomic testing positively changes productivity dynamics, but attention is needed to the consequences of these genetic manipulations. The pioneering application of genomic selection in cattle will lead to a series of unanticipated discoveries that could affect animals and society. An accidental finding was recently published in highly relevant research. It was discovered that two cloned bulls whose cell lineage had undergone gene editing, aiming at the characteristic of not having horns, were transgenic. The animals contained in their genome the genetic material of the bacterium used as a vector in gene editing. The Food and Drugs Administration (FDA) guarantees that intentional genomic alterations are safe for animals and anyone who consumes foods derived from them. However, there is still no universally accepted verification method for genomic editing.

Finally, with all the technological changes, the dairy herd has its premises, but the consumer market has also increased its requirements. Producers face the challenge that today there are claims for harmonic milk in ingestion (A2A2) [78], welfare for female producers, and respect for the environmental preservation area. People worldwide are looking for information about the products daily and are no longer limited to the final part of the milk production chain.

The increase in reproductive efficiency is a proposal fully adjusted to environmental sustainability. More productive herds require less area to generate more feed. Furthermore, the use of genetically improved animals according to the climatic conditions of each region prevents land competition with agriculture. As for differentiated milk production, the inclusion of bulls genotyped for the A2 allele of beta-casein accelerates aggregation of A2A2 animals in the herd.

Another critical aspect is the mandatory link between reproductive biotechnology and animal welfare. More productive animals only respond to greater reproductive efficiency if they have all vital requirements well met. Technological

innovations such as robotic milking, with the cow's autonomy about milking, signals a prospect of increased milk production with the same number of animals. A new change in concept which, adding welfare to the creation of dairy cattle will reflect positively on the profitability of producers.

8. Conclusion

All aspects of folliculogenesis remain a vast area to be studied, despite the notable progress made with previous research. It is not possible to determine the complete influence of AFC on female bovine fertility. The use of AFC as a tool to produce embryos *in vitro* and *in vivo* seems to be evident. However, further investigations need to be carried out for TAI and fertility. Despite the significant challenges of dairy farming, the development of reproductive biotechnologies, associated with the establishment of genomic analysis, has been used as a potential tool to increase dairy productivity, meet world demand, and meet the demands of the present consumer market.

Overcoming the main limitations of IVEP, together with the good results and its high applicability, has contributed to the use of biotechnology on a large scale. Thus, IVEP is no longer limited to elite animals or animals that do not respond to super-ovulation but actively contributes to the production, improvement, and profitability of dairy production.

The transfer of IVP embryos is a great strategy to reduce the cost of high genetic value semen, and it seems to be the most viable option in periods or regions of high temperatures. Thus, IVEP has benefited dairy farms of all sizes and animals of different breeds, whether *Bos taurus taurus* or *Bos taurus indicus*. However, factors such as nutrition and management must be considered before implementing this technique, as they directly influence reproductive efficiency.

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Section 3

Entrepreneurship Development

Livelihood Generation Avenues Using Earth-Worm Based Manure: A Study Referencing India

Analjyoti Basu and Jayanta Choudhury

Abstract

The present-day world focuses more on organic manures to get rid of the ill effects of chemical manures. In this context, Earth-Worm-based organic manure, vermicompost, and vermiwash are essential. The earth-worm-based manure replaces chemical manures and favors sustainable development through green practices. However, the present study is riding on the fact that vermi-products have good livelihood and entrepreneurship generating opportunities. The study captures the opportunities in four parts. The first part explored the potential entrepreneurial cum employment-generating opportunities through the works carried by different researchers, while the second captures the reports of different organizations and corporate bodies. The third part focuses on the opportunity analyzed through the author's field work's primary data. Finally, the fourth part tries to bring out all the possibilities of India's potential entrepreneurial cum employment generation possibilities by a modular approach.

Keywords: vermicompost, vermiwash, entrepreneurship opportunity, employment generation

1. Introduction

The present-day world is focusing on sustainable development. The key solution for it would be, using available local resources through participatory management. In this context, organic manures vermicompost and vermiwash are essential. They replace chemical manures and this is placing an important argument in favor of sustainable development through green practices.

Evidence from history indicates that way back in 1905, the movement for an organic method of agriculture practices was realized.¹ However, the yield from organic manure was less, and there was a need for more yields. This urge for more yield molded the Agri-system in the world to shift towards the chemical fertilizers. However, soon the ill effects of the chemical fertilizers emerged from all spheres. Chemical pesticides not only deplete the nutritional value of the food but also contaminate it. Research has consistently found pesticide residues in one-third of food consumed by a human. The results also indicated that the pesticides are also linked to more fatal diseases like—cancer, diseases linked to malfunctioning of both

¹ <http://www.icar.org.in/files/Base-Paper-Organic-Farming-%20Base-16-03-2015.pdf>.

the nervous system and reproductive system. Owing to the deadly side-effects of chemical pesticides, the world community is slowly and surely moving towards the option of a “Green production system”.² Different data throughout the world reflect the facts, and the tiltation is more visible after the 1990s. Until 2011, 162 countries practiced organic agriculture in 37 m Ha with Oceania, Europe and, Latin America are ranging from 12.1, 10.6 and 6.8 m Ha respectively (courtesy-ICAR). The data of business from organic farming is also not lagging. In the year 2011, the global sale of organic food and drink reached 62.9 billion US dollars.³

In terms of the Indian context, the story is quite different. At the time of independence, the country’s agricultural infrastructure was not organized and so was the food yield. So, India concentrated on more food yield to feed its large population and at the same time decrease the food import level to a minimum level. Research in the direction of more food yield brought “Green Revolution” in the 1960s, and there was an upsurge in the production of food in India. However, this development enhanced the use of chemical fertilizers in India, but the story of other parts of the world also prevailed in India [1]. It killed the beneficial soil organisms and destroyed their natural fertility, impaired the power of ‘biological resistance’ in crops making them more susceptible to pests and diseases. Chemically grown foods have adversely affected human health. In this position second green revolution came at the hands of organic farming, keeping in mind a new vermiwash model [2]. Its use showed significant positive effects on the betterment of humans and animals through the growth of good quality crops and fruits.

Based on the discussion above, the introduction of vermiculture, vermicompost and vermiwash are essential. Vermicompost is the excreta of earthworms, which are capable of improving soil health and nutrient status. Vermiculture is a process by which all types of biodegradable wastes such as farm wastes, kitchen wastes, market wastes, bio-wastes of agro-based industries, live-stock wastes, etc. are converted while passing through the worm-gut to nutrient-rich vermicompost. Vermi worms used here act as biological agents to consume those wastes and to deposit excreta in the process called vermicompost [1]. Vermiwash is the liquid extract of the earthworms [3].

The current discussion showed how organic farming is steadily arriving into the world and in the Indian agricultural arena siding the use of chemical fertilizers. However, the present study does not concentrate on the utility of the vermi-products (vermicompost and vermiwash) in providing good health of the soil, plants, animals, human beings and other living beings. It mainly focuses on the fact that vermi-products based (mainly-vermicompost and vermiwash) products have great employment-generating potentiality apart from their traditional green utility. The employment-generating potentiality is mainly necessary for India because a large population (even educated) is under the grip of unemployment [4].

The main objectives of the current study are the following:

1. To study different employment and entrepreneurship generation possibilities and the employment already generated through the use of vermi-products in different research works.
2. To study different employment and entrepreneurship generation possibilities and the employment already generated through the use of vermi-products in different corporate and organizational reports.

² <http://www.havahart.com/why-organic-harmful-effects-of-chemical-pesticides>.

³ <http://www.icar.org.in/files/Base-Paper-Organic-Farming-%20Base-16-03-2015.pdf>.

3. To explore the possible research works to be carried out on vermi-products by going through different research works of the researchers, and corporate and organizational reports.

The present study contributes to the existing literature in several ways. First, it investigates the employment generation and entrepreneurial idea generation paths by the use of vermiproducts. Although, several studies gave them ideas of employment and entrepreneurship through the use of vermin-products but the ideas are not presented collectively. The same thing could be spelled about the research avenues on Vermiculture. The present paper places a collection of researches that are carried out and that could be checked shortly. The study has therefore both academic and practical importance for the entrepreneurs, the existing organizations working on vermi-products, and also the policy makers. The research work is important for the existing organizations working on vermiculture because the work will guide them in capacity expansion, grow their business, and finally generate employment.

The study is divided into five sections. The present Section 1 is on introduction that is followed by Section 2 takes the present literature of vermiculture (i.e., vermicompost and vermiculture) into consideration. Section 2 is followed by Section 3 that discusses the research methodology. The Section 4 captures employment generation opportunities in four parts that is followed by the final Section 5 which is the conclusion part.

2. Vermicompost in literature

The utility of earth-worm-based manure vermicompost and vermiwash is felt from all spheres. Different union governments, scientists, environmentalists, and researchers have advocated for organic manure in place of chemical manures. This urge is being reflected in different literary and research works throughout the globe. Wani and Lee [5], Selden et al. [6], Yarger [7], Chakrabarty et al. [8], Adhikari [1], and Srinivasrao et al. [9] voiced for the utility of vermicompost. On the other hand, the usefulness of vermiwash is raised by Prabha and Varghese [10], Sundararasu and Jeyasankar [11], Khachi et al. [12], and Esakkiammal et al. [3].

Considering the international arena, the utility of vermicompost was well addressed by Wani and Lee [5], Selden et al. [6], and Yarger [7]. The more significant scenario of vermiculture is reflected in the research work carried out by Wani and Lee [5]. They felt that global productivity and environmental protection could be achieved by adopting ecological and sustainable farming practices. In this context, Selden et al. [6] said that vermicomposting is beneficial because of the benefits attached with it. According to them, it is faster than other traditional methods, and less space is required, and it has good positive environmental impacts. It reduces the number of green wastes that pollute landfills and sometimes different water bodies. The end product is rich in nutrients and boosts the productivity of the soil. The use of worms for organic matter production and application in small farms was examined by Yarger [7]. According to the author, vermiculture is having high utility from small firms' point of view. It recycles kitchen and livestock wastes into rich organic fertilizer and produces high-protein feed for poultry. Moreover, the worms and worm castings are sold, generating business options. Worms help to build the soil in the garden.

In line with the international researches, good numbers of research works are carried out by the Indian academic community. However, the research works of Chakrabarty et al. [8], Adhikari [1], and Srinivasrao et al. [9] are worth mentioning in this context. In terms of the Indian scenario, Adhikari [1] focused on the fact that

chemical fertilizers became an inseparable part of the Indian Agriculture system. Following the author, it could be said that the Green Revolution in 1960s has increased India's agricultural output. However, at the same time, it has enhanced the use of chemical fertilizers. On its part, the chemical fertilizers have adversely affected the health of Indians. In this scenario, the author felt that there should be more and more vermicompost type organic manure, which firmly places the author's favor for vermicompost and vermiculture that are promoters and protectors for crop plants. In this context, Srinivasrao et al. [9] in the Research Report for Central Research Institute for Dryland Agriculture (CRIDA)⁴ felt that as vermicomposting is being practiced for over two decades, such agricultural practices should come at the center stage of crop residue management and soil fertility enhancement. The research works also placed some bulletin documents useful for running different vermicompost production models and are useful for students, entrepreneurs, practitioners and field functionaries of the several development departments. Vermicompost and vermiproductions are not only for the growth of plants and good natural manure for the soil but have significant use in aquaculture was advocated by Chakrabarty [8]. Based on Chakrabarty and co-authors, there is an urgent need to enhance the culture of earthworms. The food is highly nutritious as they contain iron and amino acid.

Some worth-mentioning works on vermiwash as utility manure came in the works of Prabha and Varghese [10], Sundararasu and Jeyasankar [11], Khachi et al. [12], and Esakkiammal et al. [3]. The physicochemical characteristics, followed by the presence of micronutrients and macronutrients, proteins, and carbohydrates in vermiwash before and after inoculation of earthworm species were tested by Prabha and Varghese [10]. Further, they conducted a comparative study on the effect of vermiwash upon *Capsicum frutescens* growth parameters for root and shoot length, number of leaf counts after 30 days of the plantation. Their study revealed that the quality liquid manure vermiwash obtained from the degradation of jackfruit waste was an effective bio-fertilizer that would facilitate the increased uptake of the nutrients by the plants resulting in higher growth and yield and also improving soil health. In the same manner, the effect of vermiwash was experimented with by Sundararasu and Jeyasankar [11]. The results were not different from Prabha and Varghese. The results revealed that vermiwash spray enhanced the growth parameters and yield parameters in plant height, number of leaves, number of flowers and fruits per plant. Also, other results indicated that the flowering and the fruiting ratio were significantly increased in experimental plots. Few pieces of researches tested the combined effect of vermicompost and vermiwash. The work of Esakkiammal et al. [3] falls in that line. They tested the combined effect of vermicompost and vermiwash from the organic wastes by earthworms on lab bean. The combination showed maximum results on the growth and yield of lab beans. The study also indicated that vermicompost and vermiwash could be effectively used for sustainable plant production at low-input basis green farming. The comparative efficacy of different manures (where vermiwash was one of the manures) was carried by Khachi et al. [12]. They tested the comparative efficacy of bio-organic nutrients on cropping behavior and fruit quality of kiwifruit. In it, farm yard manure (FYM), vermicompost (VC), bio-fertilizers (BF), green manure (GM), and vermiwash (VW) was used. The research output reflected the fact that—using various sources of bio-organic materials under the organic farming regime, there will be sufficient improvement in fruit quality and plant nutrient contents.

⁴ Available from: http://www.aicrpda.in/aicrpda/attachments/555_CRIDA-Vermicompost-bk-ch%20srinivas.pdf

So, on totality, the result reflects those extracts from earthworms offer a valuable resource that could be effectively used for increasing the agricultural production, quality of agricultural output, and efficacy of the soil. Also, vermicompost and vermiwash can be economically and environmentally suitable for the soil environment.

However, there is a group of research works by different scholars that asserted towards linkage of vermiculture (i.e., vermicompost and vermiwash) in generating entrepreneurial opportunity and employment. The works indicated the linkages in three different ways. The researches of Tacon et al. [13], Stafford and Tacon [14–16], Nandeeshya et al. [17], Mahajan et al. [18], Cruz [19], Khwairakpam and Bhargava [20], Joshi and Aga [31], Kesavan and Swaminathan [21], Chakrabarty et al. [22], Charyulu and Biswas [23], Sinha et al. [24], Adhikary [1], Srinivasrao et al. [9], Tah [25], Bhat et al. [26], and Basu and Sahoo [27] gave ideas of direct, indirect and composite initiative (i.e., after adding other ways with vermiculture) in generating entrepreneurial opportunity and employment subsequently. The present study captures the avenues through the works of the scholars.

3. Research methodology

The paper presents the employment generation avenues by the use of vermi-products mainly through three ways. First, analysis of the existing literary works of different scholars was carried that gave different ideas of entrepreneurial opportunity, employment generation, and further research on vermin-products and related fields. Following the first part, the second part assessed the reports of the corporate and organizational reports for accumulating thoughts on the entrepreneurial opportunity, employment generation, and further research on vermin-products and related fields. The final part focused on the previous research and output of the research carried out by the author on the subject of vermiculture. The field study was carried out by the author and the output generated ideas for entrepreneurship and employment [28]. The sample selection and primary data collection process in the study are discussed below.

3.1 Sample

The purposive sampling technique [29] was used in the study [28]. The users of the vermi-products were targeted in the study. For those 45 respondents who were farmers, producers of vermin-products, and distributors were interviewed. They belonged to three districts, namely Cooch Behar, Jalpaiguri, and Darjeeling, the Northern part of West Bengal.

4. Employment generation avenues through use of vermi-products

The present section explores the potential entrepreneurial cum employment-generating opportunities through four sub-sections. The first sub-section places different researchers' works, which pulls up ideas on different entrepreneurship possibilities and subsequent employment generation. The second sub-section captures the reports of different organizations and corporate bodies and the possibilities of entrepreneurship and employment. In these two sub-sections, a part focuses on different research ideas that could help a good Food-For-Thought for the research fraternities. The third sub-section focuses on the opportunity analyzed through the primary data captured in the author's fieldwork previously. Finally, the

fourth part tries to bring out all the possibilities of India's potential entrepreneurial cum employment generation possibilities by a modular approach.

4.1 Employment, entrepreneurship and research opportunities based on the scholastic output

The Indian educational community carried out further research that gave rise to entrepreneurship ideas, followed by employment possibilities. The ideas are placed directly or indirectly in these researches. The researches of Tacon et al. [13], Stafford and Tacon [14–16], Nandeeshia et al. [17], Mahajan et al. [18], Cruz [19], Khwairakpam and Bhargava [20], Kesavan and Swaminathan [21], Chakrabarty et al. [22], Charyulu and Biswas [23], Sinha et al. [24], Adhikary [1], Srinivasrao et al. [9], Tah [25], Bhat et al. [26], and Basu and Sahoo [27] are mention-worthy in this direction. Direct employment generation ways through vermicompost or vermiwash came out in the works of Srinivasrao et al. [9] and Chattopadhyay [30], while the indirect possibilities came in the researches of Charyulu and Biswas [23], Adhikary [1], Tah [25], Srinivasrao et al. [9], Bhat et al. [26], and Basu and Sahoo [27]. The employment possibility created at the time of the production process of vermicompost or vermiwash is referred to as the direct employment possibility in the present study. On the other hand, the employment cum entrepreneurial possibility created indirectly is referred to as indirect employment possibilities.

The marketing, branding, after-sales service, etc., that does not come under the direct production process, comes under this category. However, in some cases, when vermicompost or/and vermiwash is combined with other forms of business, entrepreneurship cum employment possibility is generated. The combined efforts of business took a place in the works of Tacon et al. [13], Stafford and Tacon [14–16], Nandeeshia et al. [17], Mahajan et al. [18], Cruz [19], Khwairakpam and Bhargava [20], Joshi and Aga [31], Kesavan and Swaminathan [21], Chakrabarty et al. [8, 22, 32], Sinha et al. [24], Srinivasrao et al. [9], Tah [25], Bhat et al. [26], and Basu and Sahoo [27]. **Table 1** gives an overview of the possible entrepreneurship cum job opportunities.

The previous research works have opened doors for future research opportunities for the researchers' community. The works of Chandra [33], Behera et al. [34], Tharmaraj et al. [35], Sinha et al. [24], Nath and Singh [36], Adhikary [1], Chattopadhyay [30], Chatterjee and Thirumdasu [37], Chattopadhyay [38], Varghese and Prabha [10], and Esakkiammal et al. [3] are worth mentioning in this context. Based on the research needs, there is a good opportunity to research different state-funded research laboratories, higher education institutes, corporate laboratories, and any research bodies adjoined with Vermiculture-based research. These laboratories and organizations will also generate employment on their part. **Table 2** focuses on the research concepts based on different scholastic outputs.

4.2 Employment, entrepreneurship and research opportunities based on organizational/corporate reports

Different Annual Reports and Research Reports published by different corporate bodies and organizations are good resources in entrepreneurial cum employment generation opportunities. The reports focused and further advocated for the growth of organic farming in India. The good part of organic farming is captured by vermicompost and vermiwash [2]. So, a significant part of these reports focused on the ideas of entrepreneurial cum employment generation opportunities by using vermiculture. The present sub-section, through **Table 3**, places different entrepreneurial and employment generation avenues that came up in the mentioned reports.

| Entrepreneurship and employment idea | Description | Author | Opportunity type |
|--|--|--|-------------------------|
| Composite vermiculture, biomass and agriculture farm | Here biomass is generated as a byproduct of vermicomposting and is a good source of protein for fish | Tacon et al. [13], Stafford and Tacon [14–16], Nandeeshha et al. [17], Cruz [19], Khwairakpam and Bhargava [20], Joshi and Aga [31], Chakrabarty et al. [22] | Composite Initiative |
| Self-help Group for vermicomposting and nursery | Self-help groups for combined work for nursery raising, vegetable cultivation, and vermicomposting | Mahajan et al. [18] | Composite Initiative |
| Vermicompost in composite culture | Carrying out composite culture of medicinal, agricultural and plantation crops. | Kesavan and Swaminathan [21] | Composite Initiative |
| Composite vermicompost, poultry and fishery | Producing special feed using excess and old worms from vermipit, mixing it with poultry eggshell dust and plant rhizome to feed the fishes | Chakrabarty et al. [22] | Composite Initiative |
| Organic aquaculture with vermicompost and vermiproducs | Low-cost organic aquaculture in the vermiculture compound where vermicompost and vermiproducs (vermiwash, earthworm, cocoon, etc.) are produced. Here, adult earthworms, cocoons and vermiwash are utilized as live fish food, bait and fishmeal supplement and fish feed. Also, both solid and liquid animal excrement, silkworm pupae, earth worms' remains and human food grain products will form fish feeds | Chakrabarty et al. [22] | Composite Initiative |
| Juvenile fish rearing. | Applying vermiwash for juvenile fish rearing. | Chakrabarty et al. [8, 22, 32] | Composite Initiative |
| Attachment of insurance to vermicomposting | Insurance for the vermicomposting units for future production was lost | Charyulu and Biswas [23] | Indirect |
| Licensing and certification of vermicompost | Licensing and certification of vermicompost for marketing/exporting | Charyulu and Biswas [23] | Indirect |
| Vermicompost production unit combination with, pharmaceutical detergent, poultry and fishery | Earthworms 'biomass' are rich in protein and so, they have good use in industries like pharmaceutical, detergent, poultry, and fishery | Sinha et al. [24] | Composite Initiative |
| Vermimeal production | Vermimeal or earthworm meal production for livestock, birds and fish | Adhikary [1] | Indirect |
| Vermimeal plastic bags | Manufacturing special plastic bags for keeping vermimeal | Adhikary [1] | Indirect |
| Cold storage for vermimeal | Cold dry place for storage and maintenance of Vermimeal or earthworm meal | Adhikary [1] | Indirect |

| Entrepreneurship and employment idea | Description | Author | Opportunity type |
|--|--|--|-------------------------|
| Municipal waste for vermicomposting | Segregating municipal and/or other sources of wastes into categories and by vermicomposting manure | Chattopadhyay [30] | Direct |
| Construction Group for vermicompost unit | To construct various forms of vermicompost units, i.e., individual, larger-community based, biogas linked-community vermicomposting units | Srinivasrao, et al. [9] | Indirect |
| Office job | Book-keeping, record maintenance, computer operation in the vermicompost units | Srinivasrao et al. [9] | Indirect |
| Marketing Job | Marketing the products like vermicompost, Biogas plants output | Srinivasrao et al. [9] | Indirect |
| Exchange selling of vermicompost | Collecting semi/undecomposed material from the farmer community and getting decomposed vermicompost in return | Srinivasrao et al. [9] | Direct |
| Contract selling of vermicompost for farmers | Contract with the fruit and vegetable farmers and selling them from time to time | Srinivasrao et al. [9] | Indirect |
| Low or semi-skilled jobs | Accumulation of food waste, paper, cardboard, agriculture waste, manures, and biosolids | Srinivasrao et al. [9] | Direct |
| NGO job on popularizing effort | NGOs on popularizing vermicompost by organizing awareness campaigns in rural and urban areas | Srinivasrao et al. [9] | Indirect |
| Cottage Industry on Mass rearing | Cottage industry on mass rearing, maintenance of worm cultures, and tapping of organic wastes | Srinivasrao et al. [9] | Direct |
| Live-stock and calf rearing in vermicompost compound | For supply of the dung for the vermicompost units rearing the live-stock and their calf in the vermicompost compound | Srinivasrao et al. [9] | Composite Initiative |
| Organic waste recycling initiative | Organic waste recycling through vermicomposting in rural areas to create an asset at the farm level which can act as a source of nutrient self-sufficiency | Srinivasrao et al. [9] | Direct |
| Composite vermicomposting and mushroom production. | By applying modern scientific approach producing vermicompost and cultivating mushrooms at the same time | Tah [25] | Composite Initiative |
| Trainers for training programs | To train members of different Self-Help Groups (like women and youths), small and micro-industries for working on different works of vermicompost and vermiwash units like managing and collection of organic wastes, way of application | Srinivasrao et al. [9], Charyulu and Biswas [23], Tah [25] | Indirect |

| Entrepreneurship and employment idea | Description | Author | Opportunity type |
|--|---|---------------------|-----------------------------------|
| | of vermicompost for various crops, amount of application of vermicompost for various crops. Based on the agri-university schemes on vermicomposting, need for trainers for training and demonstration | | |
| Vermicompost in Greenhouse training | Training on using vermicompost in greenhouse potting medium consisting of sand, pine bark and peat | Bhat et al. [26] | Indirect and Composite Initiative |
| Contract selling of vermicompost for urban house-holds | Contract with urban-households having garden and selling manures to them | Basu and Sahoo [27] | Indirect |
| Training small-household on vermicompost production and vermi-bed construction | Training households in the urban and rural areas for vermicompost production and constructing small vermin-beds in their premises. Further, collecting the vermicomposts with the help of mobile applications from time to time | Basu and Sahoo [27] | Composite Initiative |
| Vermi-bed construction and Annual Maintenance Contract (AMC) | Constructing vermin-beds in the rural and urban households based on demand and further maintaining them through AMC | Basu and Sahoo [27] | Composite Initiative |
| Rural Tourism and Vermiculture | Keeping vermiculture units visit as an item of Rural Tourism. Further, to increase the enjoyment level the visitors could participate in the activities carried in the vermicompost/vermiwash production units | Basu and Sahoo [27] | Composite Initiative |
| Vermicompost units in Tea gardens | Tea leaves are important raw-materials for vermicomposting. So, the vermin-beds could be constructed in the tea gardens to reduce transportation and labor costs | Basu and Sahoo [27] | Composite Initiative |
| Vermicompost units with cow/buffalo sheds | Cow and buffalo dungs are important raw-materials for vermicomposting. So, the vermin-beds could be constructed along with cow/buffalo sheds | Basu and Sahoo [27] | Composite Initiative |
| Vermicompost units with food/ fruit processing units | Food and fruit wastes are important ingredients for vermicomposting. So, the vermi-beds could be constructed along with food/fruit processing units | Basu and Sahoo [27] | Composite Initiative |

Source: Author compilation based on earlier studies.

Table 1.
Entrepreneurship and employment ideas based on scholastic outputs.

| Author | Research description |
|--|--|
| Tharmaraj et al. [35] | Research on to check different types of vermicomposts based on physical properties like Porosity, water holding capacity and moisture content |
| Tharmaraj et al. [35] | Research on to Check different types of vermicomposts based on chemical properties like the presence of nitrogen, potassium, phosphorus and calcium |
| Behera et al. [34], Nath and Singh [36], Adhikary[1], Chattopadhyay [38], Chatterjee, Thirumdasu[37], Esakkiammal et al. [3] | Research on the combined effect of vermicompost with different manures in different proportions on different fruit, vegetables and the soil output. |
| Nath and Singh[36] | Research on the combined effect of vermicompost with different manures in different proportions on the safety of human and animal health |
| Sinha et al. [24] | Research on the vermicompost and the amount of PH to be increased in the soil |
| Sinha et al. [24] | Research on the vermicompost, the specific waste to make it and it's benefit on a specific soil |
| Sinha et al. [24] | Research on the vermicompost and its contribution in increasing specific microbes in the soil |
| Sinha et al. [24], Adhikary[1], Chattopadhyay [30] | Research on the vermicompost and the specific plant disease(s) to be suppressed |
| Adhikary[1] | Research on to check the amount to be administered in the field to Develop Biological Resistance in plants |
| Adhikary[1] | Researches on to check the amount to be mastered or mix with other substances or other manures in generating the ability to suppress plant disease |
| Adhikary[1] | Research on to stipulate the amount to be mastered or to mix with other substance or other manures to get rid of infection of insect and different pests |
| Chatterjee and Thirumdasu [37] | Research on creating vermicompost from different sources and their special match ability with a specific type of fruits and vegetables. |
| Varghese and Prabha [10], Chandra [33] | Research on Earthworms and their capability to decompose wastes and also specific wastes like a municipal waste. |

Source: Author compilation based on earlier studies

Table 2.
Research concepts based on scholastic outputs.

The reports mainly presented the actual happenings in terms of entrepreneurial and employment generation through the use of vermicompost and vermiwash. However, in some cases, they have given ideas of the entrepreneurial and employment generation possibilities. Following section Ci, the opportunities are segregated as direct, indirect, and composite initiatives.

4.3 Field survey and employment, entrepreneurship and research opportunities

The present sub-section is based on the author's previous fieldwork. This sub-section field survey was carried out by the author to assess employment, entrepreneurship, and research opportunities through vermicompost and vermiculture. The

| Entrepreneurship and employment ideas | Description | Organization/corporate report | Opportunity type |
|--|--|---|---------------------------------|
| Vermicompost Infrastructure building contract | Vermicompost infrastructure building contract through space for cattle, vermicompost bed, compost tank, vermiwash making, compost tea unit, etc. Further planting trees, digging irrigation well, starting water pumping infrastructure, digging percolation tanks for rainwater conservation, digging farm pond, 200 lit tanks (1 per acre) for liquid manure preparation. Also constructing containers for botanicals, NADEP tank, biodynamic compost beds, liquid manure tanks, cowpat pits, underground cattle-urine collection tank | National Horticulture Mission, Department of Agriculture and Cooperation Ministry of Agriculture, New Delhi ¹ | Indirect |
| Composite vermicompost, arecanut and cocoa- gardens and biomass generation | Arecanut and cocoa-gardens generate waste can be effectively utilized for production of vermicompost and generate biomass. Further arecanut-and cocoa-gardens waste can be utilized for the production of oyster mushroom and as livestock feed. | ICAR News, A science and Technology Newsletter [39] ² | Composite Initiative |
| Plastic or wooden model production | Vermicompost is often prepared in wooden or plastic model. | National Research Centre for Women in Agriculture, Indian Council of Agricultural Research, Bhubaneswar ³ | Indirect |
| Composite vermicompost production and ornamental plant cultivation | Vermicompost and vermiwash production and using the manures for the growth of ornamental plants | National Research Centre for Women in Agriculture, Indian Council of Agricultural Research, Bhubaneswar ³ | Composite Initiative |
| Transportation | Arranging transportation facility to transport vermicompost in different places | National Research Centre for Women in Agriculture, Indian Council of Agricultural Research, Bhubaneswar ³ | Indirect |
| Integrated manure management | Integrated manure management through producing Vermicompost, Neem cake, and Phosphate Rich Organic | Department of Agriculture, Cooperation & Farmers Welfare Ministry of Agriculture & Farmers Welfare Government of India ⁴ | Composite Initiative |
| Composite vermicompost production and exotic fish rearing | Producing vermicompost and also rearing the fast-growing exotic fishes | Kolkata Gazette, Department Agriculture, Government of West Bengal, India [40] | Composite Initiative |
| Composite hatcheries and vermicompost unit | Running the vermiculture hatchery and vermicompost unit in the same vermiculture unit | National Horticulture Mission, Annual Action Plan (2010–2011), Bihar, India [41] | Direct and Composite Initiative |
| Market Research | Market Research on Need assessment and market demand analysis | National Horticulture Mission, Annual Action Plan (2010–2011), Bihar, India [41] | Indirect |

| Entrepreneurship and employment ideas | Description | Organization/corporate report | Opportunity type |
|---|---|--|-------------------------|
| Group Formation for production and management | Formation of farmers group for production and management or by group of villagers. Further Equitable benefit sharing arrangements among the group members | National Horticulture Mission, Annual Action Plan (2010–2011) Bihar, India [41] | Direct |
| Procurement and rearing of suitable species | Procurement of suitable species of earth worms like <i>Eiscenia feotida</i> , <i>Periyonix excavates</i> , and <i>Udrelis eugine</i> and rearing them | National Horticulture Mission, Annual Action Plan (2010–2011), Bihar, India [41] | Indirect |
| Testing laboratories | Testing of the vermin-products in laboratories after production to stop adulteration of the products | National Horticulture Mission, Annual Action Plan (2010–2011), Bihar, India [41] | Indirect |
| Marketing Job | Packaging, branding Develop market linkages and marketing | National Horticulture Mission, Annual Action Plan (2010–2011), Bihar, India [41] | Indirect |

Source: Author compilation based on earlier studies.

¹Available from: https://midh.gov.in/technology/Organic_Management_NHM.pdf

²Available from: https://icar.org.in/files/ICARNews%20April-June%202016_for%20web.pdf

³Available from: <http://icarciwa.org.in/gks/Downloads/Technical%20Bulletins/Manual%20for%20Vermiculture%20Biotechnology.pdf>

⁴Available from: <http://agricoop.gov.in/sites/default/files/Krishi%20AR%202017-18-1%20for%20web.pdf>

Table 3.
Entrepreneurship and employment ideas based on different organizational reports.

sub-section is divided into two parts, i.e., Description and main outcomes, employment and research ideas.

a. Description and main outcomes

The author carried out the research in West Bengal, the eastern state of India (As previously discussed in Section C). Further, about 45 users (farmers, producers, distributors) of the product were interviewed from three districts, namely Cooch Behar, Jalpaiguri, and Darjeeling, the Northern part of West Bengal. The work was carried out to assess the users’ impression about the vermiproducs (vermicompost and vermiwash), study the present scenario of the market, and explore the possibilities of employment generation [28].

The outcome reveals several facets of the use of vermicompost and vermiwash, which in their part advocated for the use of organic manures. In words, they are as follows-

- Vermiwash is not harmful to the plants, instead is useful for the plants. It helps the vegetables fresh even after they are plucked from the main plant and the color of the vegetable remains unchanged. Also, it saves the plant from insects.
- The vermiwash and vermicompost help keep the land fertile for a long time though it may not produce is high yield compared to the inorganic manure or foliar spray.

- A greater amount of vermiwash is needed concerning the inorganic foliar spray for the field.
- Still, a small section of farmers has used the organic foliar in their land, so there are many opportunities for the businessmen and entrepreneurs to generate lots of business and good income from the business.
- Vermiwash and vermicompost are organic manures, so the crops grown by using them will not be harmful to people consuming different crops.
- Vermiproducts cost less than the inorganic foliar in the market and, if used regularly, in the long run, give a high yield from the field.
- The product has a high market opportunity and future. To maintain the productivity of their land, the farmers use cow dung as organic manure in a year. So, if cow dung could be replaced by vermiproducts, there will be an upsurge in the market for vermiproducts, for that high awareness, the drive is needed, and wherever the awareness drive is successful, the product has placed its feet.

b. Entrepreneurship, employment, and research opportunities

The entrepreneurship opportunities, followed by the employment, are generated from the needs of the people, gaps in a different process, and problems faced [42–45]. Based on the gaps and the study's problems, Basu and Panda [28], and the author in the present study carried the future entrepreneurial and research opportunities.

1. **Awareness drive for business growth**—Interview with the respondents revealed that farmers maintain the productivity of their land by using cow dung as organic manure (at least once in a year) in their fields. So, if the vermiproducts can replace the existing cow-dung manure as the organic one then the breakthrough could be easily achieved. For that high awareness, the drive is needed. If done, the sales of vermiproducts will soar up, and more business ventures on vermiproducts will come up.
2. **Small packages**—From the interview with the respondents, it came up that small package is not that available, and farmers are not interested in big packs all the time. It indicates that there is a demand for small packages, but supply is inadequate. It places the idea of small package supply, or production could be a good idea for an entrepreneurial venture.
3. **Production stages**—From collecting waste to selling the product to the market, different steps are followed for vermicompost production. They are collection of wastes (in form of cow dung, vegetable, flowers and fruit wastes and other organic wastes) from different locations, followed by sending the collected materials to different production centers, production of the final product, packaging and preservation of final products before sell, sending the final product to different distribution center and distributing the product to the final customer from different distribution center, and finally the after sales services (Refer **Figure 1**). Based on the stages, different works, followed by employment avenues, are as follows [28].
 - **Stage 1: Waste Collection** — The works attached in this stage are the collection of wastes (e.g., cow dung, vegetable, flowers and fruit wastes

and other organic wastes), (in some cases) semi-composting them to send them to the final production center.

- **Stage 2: Composting in production center** — The works joined in this stage are monitoring and taking necessary action in different levels of composting, Quality Control (QC) for different composting levels.
- **Stage 3: Packing final product** — The works joined in this stage are packing the final products in appropriate packaging packs, sample checking to eliminate adulteration, conservation, and inventory management by different packed products.
- **Stage 4: Transportation to the distribution center** — In this stage, the works are transportation to the distribution center, conservation measures for the unsold vermicomposts, and temporary inventory-related jobs.
- **Stage 5: After-sales service** — In this case, the works are to monitor and provide necessary suggestions to the customers, making the collection of customer grievances and escalating it to the necessary level, market research work to make necessary changes all the time, so it becomes more acceptable to the customers.

4. Research on Profile Card and Information Bank- After analyzing the interviews and going through different suggestions, the concept of “*Profile and Suggestion Card*” is being placed by Basu and Panda [28]. According to it, the product vermiwash contains different micronutrients like potassium, phosphate, sodium, calcium, copper, iron, magnesium and zinc in definite proportions (Refer-Appendix). These proportions are permanently fixed and are not changed from field to field. However, the field capacity and needs for nutrients may not be the same. For example, some of them may need more sodium than others. In that case, the fixed proportion in the vermiwash cannot provide the same. To get rid of the problem, soil testing, of each field is needed and on basis of it a Profile and Suggestion Card for each piece of farmland could (Refer **Figure 2**) be prepared. The criteria could be the specific land’s suitable vegetables, fruits and food grains; for a smooth yield of the vegetables, fruits and food grains the extra number of micronutrients needed; mapping suitable vegetables, fruits and food grains with the number of micronutrients to be used; the number of times specific crops could be grown; if micronutrient deficiency is thereafter the growth of any specific crop than the amount of micronutrient to be added back. So, experts, after testing the soil of different fields, can prepare the card. According to the authors, good research is needed for preparing the card. Further, they suggested using the card for customizing the vermiwash. After going through the above-described card and knowing the farmer’s choice for a specific crop, the vermiwash product could be customized by changing the proportion of the micronutrients used [28]. In this connection, the authors further suggested that the Government authority maintain “*Information Bank*” at their end. The Bank will keep all the mentioned information for their future use.

4.4 Employment, entrepreneurship and research opportunities through modular approach

The present sub-section accumulates the entrepreneurship cum employment generation ideas in a single platform through a Modular Approach [46, 47]. The

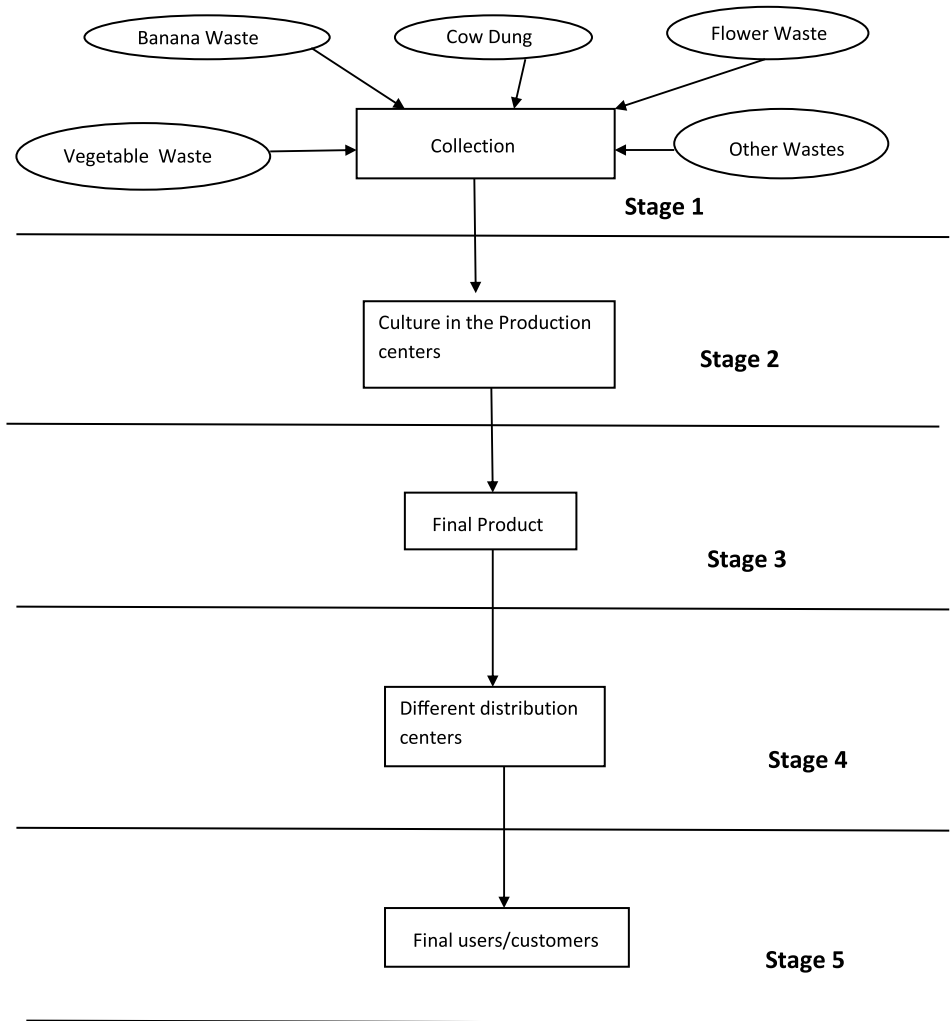


Figure 1. Stages involved in collection of wastes to the distribution of the wastes. (Source: Based on primary survey).

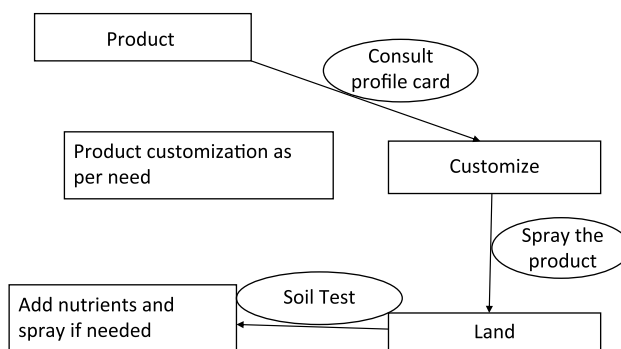


Figure 2. Use of profile and suggestion card. (Source: Basu and Panda [28]).

platform consists of direct ideas, indirect ideas, Composite Initiative, and the Research Avenues (for the research community) for employment generation (Refer **Figure 3**).

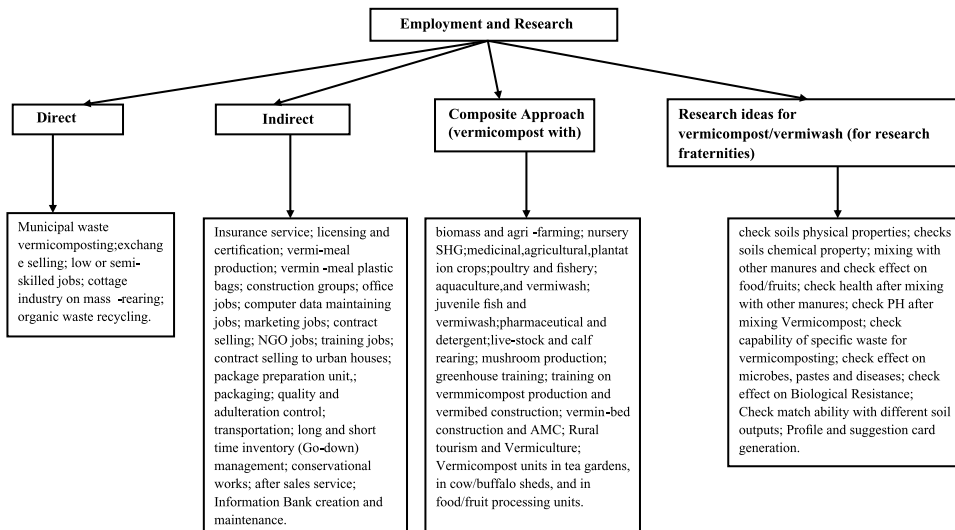


Figure 3. Employment and research ideas. (Source: Present study.)

5. Conclusion

The present research reveals that there are different ways to generate employment by producing verminproducts (i.e., vermicompost and vermiwash) as organic manures. Adding to the support site for the organic manure production the researchers and experts opined that environmental sustainability, growing health awareness, Governments aim in reducing heavy subsidies on food and fertilizers, the potentiality of high export earnings are the points of future opportunity for organic farming in the country. However, the present scenario of organic farming is quite gloomy. Based on the data of Agricultural and Processed Food Products Export Development Authority or APEDA, in 2007–2008, the total Gross Cultivated Area (GCA) under certified organic cultivation was only 0.31 percent (appx.), i.e., 0.45 million hectares. India ranked 33rd in total land under organic cultivation and 88th position for agriculture land under organic crops to the total farming area in 2007–2008. Following the researchers and experts, it could be said that lack of established markets having disjointed producers, processors and traders, poor quality management in production and processing, less incentives from the government on organic farming research, adulteration and poor quality of organic inputs, a large number of small farms with weak organizational build-up, high labor costs, high cost of organic food, lack of infrastructure facilities (like labs) and certification bodies and costly and complex organic certification process are the main problems of organic farming in India. However, at present, there is a strong need to cover-up the points of weaknesses with the points of strengths. The employment generation point places that lethal point in favor of organic farming (in terms of vermiculture) in India. If the data of APEDA Report 2007–2008 (as cited in Charyulu and Biswas [23]) is minutely checked, the opportunity is revealed. In terms of words, it could be said that less than one percent of the agricultural land in India is cultivated under organic cultivation. It means that 99% is still not cultivated under organic cultivation. In the present study, the employment generation possibilities through organic endeavors are considered.

based on this 1% cultivation but not 99%. So, it could be well imagined that if the rest 99% is brought under the organic endeavors in the future (at least partially or

once a year cultivation method) then there is a significant entrepreneurship cum employment generation possibility left. This could be achieved through a well-designed awareness drive for organic farming.

Appendix 1

| Items | Composition |
|-----------------------------|-----------------------|
| pH | 7.48×0.03 |
| Electro conductivity dS/m | 0.25×0.03 |
| Organic Carbon % | 0.008×0.001 |
| Total Kjeldal Nitrogen % | 0.01×0.005 |
| Available Phosphate % | 1.69×0.05 |
| Potassium (ppm) | 25×2 |
| Sodium (ppm) | 8×1 |
| Calcium (ppm) | 3×1 |
| Copper (ppm) | 0.01×0.001 |
| Ferrous (ppm) | 0.06×0.001 |
| Magnesium (ppm) | 158.44×23.42 |
| Manganese (ppm) | 0.58×0.040 |
| Zinc (ppm) | 0.02×0.001 |
| Total Heterotrophs (CFU/ml) | 1.79×10^3 |
| Nitrosomonas (CFU/ml) | 1.01×10^3 |
| Nitrobactor (CFU/ml) | 1.12×10^3 |
| Total Fungi (CFU/ml) | 1.46×10^3 |

Source: Field Study.

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Green Gold from Dairy Industry: A Self-Sustained Eco-Friendly Effluent Treatment Plant

Shaon Ray Chaudhuri

Abstract

The major bottleneck of dairy effluent treatment plant operation is the generation of 10 m³ of nutrient rich wastewater per m³ of milk processed resulting in an annual production of 7.93 tons of carbon dioxide equivalent (CO₂ e) gas during treatment in a 7–8 step process. It is an expensive, non-ecofriendly, laborious process which is often not adoptable by the small segment installations. A carefully selected tailor-made bacterial consortium in biofilm reactor within 4 h of incubation in a single step operation under ambient condition could transform the total volume of wastewater into ammonia rich liquid biofertilizer generating 0.79 tons/year CO₂ e gas. This biofertilizer replaces the use of fresh water and chemical fertilizer for agriculture, producing economic crops at par with chemical fertilizer. In certain cases, the production of crops is increased substantially over chemical fertilizer based growth. It reduced carbohydrate content of tuber crops. The generated liquid biofertilizer can overcome the shortage in fodder production without using chemical fertilizer and fresh water, hence solving one of the major concerns for sustaining the expansion of dairy industry, hence making dairy effluent treatment plant (ETP) operation an eco-friendly, self-sustainable operation.

Keywords: dairy wastewater, liquid biofertilized, CO₂ equivalent gas emission, fodder production, self-sustainable effluent treatment plant operation

1. Introduction

The dairy industry is a growing sector globally with ever increasing need for constant development to meet the needs of the population. It is one such food industry which serves the life from day 1 till life ends. The industry has its own issues that need to be addressed. Different countries have used different models to sustain its dairy industry. One of the common problem faced across the globe is the constant supply of adequate quantity of healthy fodder for the cattle [1]. The high price of the fodder often takes away any profit that would come out of the sale of the milk or milk product [2]. Quality of livestock and availability of land for fodder production are two major concerns that holds back the desired progress in the dairy sector in regions like India, Bangladesh, China, Mangolia, Philippines and Vietnam [3].

There are regions like Sri Lanka which have good livestock as well as land, yet have insufficient fodder due to lack of proper scientific management strategies [4].

The demand of milk in the domestic market is much more than the production, leading to import of dairy products in Sri Lanka. This harms the economy of the country due to spending of the limited foreign exchange. Adequate scientific input for agricultural produce increase can lead to addressing the need for nutritious food supply for its citizens, strengthening the agricultural activity without addition of excessive chemical fertilizer and strengthening the dairy sector through adequate fodder production. Dairy has been identified as a dynamic, integrated and integral part of rural economy of Sri Lanka by its government. The tropic environment results in experiencing different diseases, calling for adequate measures for improving the livestock as well as fodder producing pastures [5]. Adequate fodder plots are required for making the smallholder dairy operation profit yielding. Lack of ownership of adequate land creates hurdle in producing quality fodder by the farmers. Lack of quality feed round the year at reasonable prices prevents the farmers from realizing the genetic potential of high milk yielding animals. Adequate management of common grasslands can address this problem to a major extent.

The 10-Year Development Framework estimated 44% of agricultural land to be inadequately used, indicating towards a greater opportunity for further improvement of this fodder production practices. Unutilized state authority lands could also be put to use for fodder production. The Livestock Development Policy states that these lands will not be privatized but would be made available to private sector for production of superior quality fodder material. Providing access to the fodder cultivating land alone would not be sufficient for sustaining the economy [4]. In addition, access to modern technology would be needed.

Countries like India have developed to be the largest milk producer from a country with shortage of milk, due to operation flood (initiated in January 1970), with intense focus on development of dairy sector under the leadership of Verghese Kurien. One of the steps towards enhanced productivity of dairy industry was ensuring availability of cattle feed. National Dairy Development Board (NDDB) predicted the milk demand to be around 180 million tons in 2022. Increasing demand was expected to influence the milk prices globally. This called for a careful review of the requirements for the local dairy development.

The marginal dairy farmers with 2–3 animals form a major portion of dairy units in India. Their major investment is in the purchase of fodder. Hence a sustained fodder supply at affordable cost is a must to promote the dairy industry and the rural economy of a country. Such changes would have significant implications on the smallholder farmers who constitute about 75% of rural population, leading to alleviation of poverty. Moreover, it is these farmers who own major fraction of female cattle (60%) as well as land (33%). Feed is mainly the residue of crop, coarse grains, cereal remains/byproducts (brans, meal and cakes). Cooperatives have picked up the responsibility of production and supply of feed at subsidized rates to farmers. In spite of all these, the average milk production per animal per lactation (987 kg) is considerably lower than the global figure (2038 kg). This also depends to certain extent on the feeding practice, quality, availability and affordability in addition to the breed of the cattle. The land use for green fodder production varies between 15 and 20%. Insufficient fodder resources and absence of regulations to check the fodder quality is one of the major constraints in sustaining the milk production [2].

In Bangladesh too the cooperatives supply fodder to the farmers at subsidized rates from the agricultural byproduct growing in the locality. This ensures healthy livestock. The farmers in turn, use the cow dung for running the biogas digesters which provides the cooking gas and the source of illumination. The spent slurry from the digester is used in fish ponds to enhance fish production. The fish pond is dried and dredged ever 2–3 years and the mud scooped out of the pond bed is used as fertile soil on adjacent farm lands. Through this approach, the small scale dairy

practice becomes an eco-friendly integral part of the sustainable development of the rural community [6].

Rapid urbanization in China lead to expanding of the commercial activities outside the cities, resulting in rising prices of land, hence less available farmlands at affordable rates for dairy farming. This in turn lead to feed scarcity for the dairy. The model adopted in China to address this issue was to group the small scale dairy farmers in a designated region with all necessary infrastructure build up from the contributions of individuals, farmers and the local government. Each designated area with common facility for all (including milking and disease control), houses the cows which are managed by the households themselves (fodder production: from cultivation to processing) [7]. In order to solve the land problem in Mangolia, the state law has kept all grazing lands under its ownership, ensuring equal access to all [8].

In Pakistan the milk production faces a seasonal variation due to the variation in availability of green fodder. Though production of milk is highest between January to April, consumption is higher in summer months (May to August) due to dairy product consumption preferences. In order to solve this variation, there is a need for sustained production of green fodder round the year [9]. Philippines emphasizes on training of dairy farmers on different aspects including indigenous feed sources [10]. Thailand had its first dairy as early as 1962 which was handed over to the government on 1971. The government body has been operating since then with four objectives of which the first objective is farmer training in techniques of dairy farming, fodder production and disease management. The milk production expanded producing surplus milk. Cooperatives were formed to channel the produce to processing units. Some cooperatives have started feed mills to provide low price fodder. Superior quality of feed, concentrates and roughage has played an important role in improving milk yield of dairy farms in Thailand [11].

Provincial governments in Vietnam under its dairy development policies provides financial support for fodder production, among other things. Some provinces exempt agricultural land taxes while giving priority to making fodder production land available for the dairy farmers. Their decline in dairy industry was partly due to lack of fodder supply. Directed efforts are needed to designate priority fodder growing area in zones with ongoing dairy activities with special emphasis on the fodder type to be grown considering the seasonal variations, land type and irrigation facilities [12]. Unavailability of ownership land is the prime concern for lack of quality fodder production. Government intervention can improve the situation by leasing communal land and encouraging innovative methods for pasture development and fodder production [13]. One of the major component which could sustain expansion of dairy industry in different countries is a sustained production and supply of green fodder to the cattle at subsidized rates in order to cut down on the major investment of the small and middle segment farmers.

2. Rural dairy wastewater management

After petrochemical, dairy industry is a major sources of large volume of highly polluting wastewater generation. The volume of wastewater generated per unit of milk processed can go upto 10 times [14]. This is mainly from the cleaning activity at the processing plant with 2% of the total milk processed going into the wastewater. The quantity of milk to be processed is expected to rise to meet the demand of the rapidly expanding population [15]. Dairy industry wastewater is eco toxic unless adequately treated because of the biodegradable pollutants preset in it. This industry consumes a substantial volume of water and produces enormous

wastewater which seeps into the environment polluting fresh water reserves. The conventional dairy operation, to get the wastewater to discharge level, involves about 8 unit operations namely collection of wastewater in receiving tank, passing the wastewater through skimming tank to remove fats, subjecting the skimmed wastewater to anaerobic treatment, biological oxygen demand removal in aeration tanks, passing the water to settling tank, removing the settled sludge to sludge tank while passing a part of the sludge back to the aeration tank while the rest to the sludge drying bed. In addition, it might have separate arrangements for dewatering the sludge before sending to the sludge drying bed. This involves a large space and energy for transporting the wastewater between the units as well as in the aeration chamber. The treated water is mostly discharged into the environment while part of it might be used for non-potable application within the effluent treatment plant (ETP) premises.

Dairy wastewater is rich in plant growth nutrients like nitrogen, carbon and phosphorus. These pollutants are lost during conventional process of treatment due to biological action. A clear understanding of the pollutant to be treated [16], the microbial metabolic pathway that breaks down/converts the pollutant into plant acceptable form [17–19] and the kind of microbes known to harbor these pathways [17–20] can lead to development of processes which would convert the pollutant into a value added product [21]. The dairy wastewater with enhanced phosphate and nitrate along with a high carbon:nitrogen ratio [22] could be used directly for sustaining bacterial growth. This property causes nuisance when the wastewater is left unattended. However, the same raw material (entire volume of dairy wastewater) can be selectively converted into ammonia rich liquid using selected microbial consortium under ambient condition with no additional requirement of aeration [16, 20].

Ammonia is a preferred nitrogen source for plants than any other form. The microbes selected for the bioconversion should be able to degrade protein and lipid; remove phosphate while convert nitrate and nitrite into ammonia (ammonification) and not into nitrogen (denitrification) within the reactor. Therefore, the nutrient rich dairy wastewater gets converted to ammonia rich liquid by the enzymatic activities of microbes. This liquid demonstrates the properties of a liquid biofertilizer that can replace the use of fresh water and chemical fertilizer during agricultural [16, 20] operations. Lack of additional aeration and ambient operation ensures savings in energy expenditure.

Six well characterized bacteria (Firmicutes and Proteobacteria) from environmental origin were combined in definite proportion [16] and put as a biofilm in a single unit (well designed) biotreatment plant [21] that replaces the storage tank, skimming tank, anaerobic digester, aerobic digester, settling tank, sludge tank and sludge drying bed of a conventional dairy effluent treatment plant, hence reducing the space requirement for setting up an effluent treatment plant by about 80%.

The conventional treatment takes about 120 h [23] for treatment of the wastewater which is mostly discarded into the environment. Some part of it after further treatment is reused in plant operations. In the developed technology [16, 21], the organisms were placed as biofilm in the biotreatment plant. The biofilm formation ensures one-time bacterial inoculation with continuous operation for couple of years. Biofilm development with tailor-made consortium with synergistic interaction among the members ensures rapid stabilization of the biofilm (unlike industrial approach of using activated sludge for immobilization which take longer time for stabilization). This faster stabilization of the bacterial consortium is due to reduction in doubling time of the bacteria in the consortium [24]. In the case of the dairy wastewater treating consortium the doubling time in immobilized condition becomes 17 min and 10 s while the same under suspended condition was 78 min

and 56 s. This ensures rapid stabilization of the system with more biomass for active metabolism in biofilm based systems, hence improved performance.

In addition, bacteria in biofilm are more resistant to external perturbation, a property essential for treatment of wastewater whose nature might occasionally vary due to difference in the upstream operations. The current system takes only 4 h of incubation to convert the dairy wastewater into an ammonia rich liquid biofertilizer. This makes the current operation 30 times faster in dairy wastewater treatment than the conventional system. An added advantage of this system is no sludge formation unlike the conventional systems. Hence, if operated as per standard operating procedure, the system demonstrates stable performance over extended period of time.

Unlike the conventional dairy effluent treatment system which requires about 60 kW (kilo watt) of energy for treatment of 600 m³ of wastewater, the current system consumes only about 6 kW of energy for pumping the wastewater into the biotreatment plant. Since no aeration or temperature control is needed, hence the system utilizes only 10% of the energy of a conventional system resulting in a savings of approximately 90% on the carbon dioxide equivalent gas emission due to the effluent treatment plant operation [21].

The major drawback of this system is that it is only suitable for dairy installations in rural area which have large farm lands in their vicinity. The transportation of the large volume of liquid fertilizer would not be economically viable and hence direct use in adjoining farm lands is a must to ensure complete use of the by product (liquid biofertilizer) without damage to the environment. Through this approach, the major misuse (89%) of fresh water (for agriculture) can be stopped in agro-ecological zones of the globe. The fresh water can be set aside for portable applications while the plant growth essential nutrients along with the huge volume of the water (generated as wastewater) with suitable treatment could be used for such non-potable applications like agriculture. This would slow down the rapid depletion of fresh water reserves worldwide which is predicted to affect 40% of the world population by 2025 [25] unless checked immediately.

The treated water is suitable for irrigation purpose. In order to get the water to a nutrient free condition, it could be used for cultivation of yet another well-defined mixed consortium of bacteria and microalgae enriched from wastewater fed fish pond. This consortium with minimal aeration using bubbling and light could completely remove nitrate and phosphate with 93% removal of chemical oxygen demand and 87% removal of ammonia while growing in treated dairy wastewater for 48 h [26]. The consortium shows 67% enhancement in biomass with 42% enhancement of lipid and 55% enhancement of carbohydrate content when compared to biomass grown on wastewater fed aquaculture fish pond water (from where the biomass was originally enriched) as control [26].

3. Sustainable production of quality feed

About 80–89% of fresh water that is drawn every day is used for non-potable agricultural purposes in agro-economic regions [27]. This needs to be checked urgently to protect 40% of the world population from facing scarcity of fresh water by 2025 [25]. However, the agricultural practices cannot be avoided to sustain the lives of the ever increasing population of human. Similarly, fodder production is also a must, desirably green fodder for sustaining dairy industry directly, and the human population indirectly.

The agricultural practice consumes chemical fertilizer in addition to fresh water. However, only 12–30% of it is utilized while the rest pollutes the ground water

and the surface water bodies due to leaching [28]. Adequate scientific intervention leads to development of bacterial formulations which ensures entrapment of plant growth nutrients in the root zones by the microbes ensuring minimal leaching into the surrounding environment [29] and hence continuous access to nutrients during the growing season resulting in faster maturation of the crops [21, 28–30].

To address this requirement of sustaining agriculture without fresh water consumption and chemical fertilizer leaching, the liquid biofertilizer developed from bioconversion of dairy wastewater using well characterized bacterial biofilms was used for pot trial experiments followed by field trial experiments. A significant increase in production of mung bean (2.12 folds compared to chemical fertilizer) with enhanced chlorophyll content (1.4 folds compared to chemical fertilizer) of the leaves indicated health growth of the plants.

While the seeds serve as food for human consumption, its husk and the green plant serves as fodder. The maturation of plants was faster with liquid biofertilizer application showing shorter roots with fewer nodules than chemical fertilizer treated plants. The shorter roots with fewer nodules indicate easy access of nutrients in the root zone which neither needs to penetrate deep into the soil, nor establish association with rhizosphere bacteria for nitrogen fixation [16, 21].

In case of pot trial of sorghum sudan grass, there was 3.5-fold increase in biomass production compared to control (without fertilizer), hence ensuring enhanced supply of fodder per unit land without use of fresh water and chemical fertilizer. In case of field trial, sorghum sudan grass showed an increase of 2.53 folds within 2 months of growth.

In case of mung bean, the peak production was obtained within 18 days of podding and was significantly higher than chemical fertilizer grown plants. The production was increased by 2.09 folds if considered for the standard growing time of 65 days while was 1.56 folds higher when compared to chemical fertilizer grown production after 75 days. Faster maturation ensured higher fodder within shorter time with availability of land for the next crop.

In case of black gram a similar production (1.04-folds) was seen compared to chemical fertilizer during field trial. This biofertilizer enhanced the cob yield in maize (*Zea mays* var. Vijay) by 1.19-fold with associated biomass increase which serves as a fodder. The liquid biofertilizer caused 2.1–2.64-folds increase in biomass of lemongrass (*Cymbopogon citratus* var. Dhanitri and var. Krishna) with significant enhancement in oil content. Lemon grass addition to animal feed in definite proportion is reported to enhance nitrogen uptake, leading to healthier growth of animal [31–33]. When compared to chemical fertilizer, the liquid biofertilizer enhanced yield of ramie fiber (1.39-folds), sweet potato tubers (1.44-folds), cassava tuber (1.86-folds), yam tuber (2.55-folds) and elephant foot yam tuber (3.8-folds).

The yield was similar to chemical fertilizer in case of field pea seeds (1.16-folds), colocasia tubers (1.01-folds) and sugar cane (1.01-folds) indicating the biofertilizer to be as effective as chemical fertilizer. This biomass in case of colocasia showed significant increase (2.05-folds increase). Similarly, higher production was seen in case of sugar cane, cassava and sweet potato biomass, indication towards enhancement of fodder crops.

Through this approach, the fodder production around the rural dairies could be sustained round the year with no additional cost as a zero discharge technology considering the effluent treatment plant and the surrounding field [21]. The environment is protected as well as the fresh water reserves will be preserved. The access liquid biofertilizer could also be sold to the neighboring farm owners by the dairy effluent treatment plant owners at a very subsidized rate, which would be beneficial for both the seller and the buyer making the dairy effluent treatment plant operation self-sustainable.

4. Urban dairy wastewater treatment

The above approach can work only in case of rural dairies [21] with vast farm lands in its vicinity. However, microbial approach with tailor-made consortium can also work in case of urban dairies with space limitation. Appropriately designed consortium using well characterized microbes from activated sludge of dairy effluent treatment plant and other environmental origin can be combined in definite proportion to give a sludge free biofilm based dairy wastewater treatment system which within 20 h of incubation under ambient condition in a single unit operation can reduce the nitrate and phosphate substantially [20]. The treated water after further treatment with bacteria microalgae mixed consortium for 48 h was free of nutrients and suitable for discharge or reuse [26].

The biomass can be used as the raw material for biofuel production due to its high lipid and carbohydrate content. Another advantage of this technique is the growth of the consortium as attached biomass. That makes the harvesting of the biomass less energy intense (as centrifugation is not required) with no requirement for external supply of nutrients and fresh water for growing the microalgae. The dairy wastewater substitutes for the fresh water and the nutrient and hence makes algal growth for biofuel production an economically viable process. The requirement of 48 h compared to 5–7 days during conventional algae based wastewater treatment makes the process rapid and hence requiring less space for treatment. The total time required for the combined treatment of 68 h (bacterial plus the microalgae bacterial) is still less than the conventional system of 120 h [23]. In addition, each process is a single unit operation, hence saving in terms of land involvement.

The dairy wastewater could also be treated using pure bacterial isolates in biofilm reactors capable of removing ammonia, nitrate and nitrite within a much shorter time (depending on the initial pollutant concentration) [34] for reuse in aquaculture, again saving wastage of fresh water from being used for dilution of the wastewater before it could be used for aquaculture.

5. Conclusion

It can be concluded that microbial technology application for dairy wastewater treatment can lead to solving two of the major concerns of the dairy industry namely, (i) ensuring round the year green fodder for the cattle without wasting fresh water and using little chemical fertilizer, as well as (ii) making its effluent treatment plant operation ecofriendly and self-sustainable. The crux of the problem is in developing the right combination of microbes which would convert the pollutants into a plant usable form, resulting in little dead mass generation. This liquid biofertilizer, unlike the conventional organic fertilizers available, release nutrients at sustained, sufficient rate from the beginning of the cultivation resulting in higher yield. Hence health crop in high quantity can be produced from the byproduct (green gold) generated from the dairy effluent treatment process.

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Conflict of interest


There is no conflict of interest.

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Dairy Farming Innovations for Productivity Enhancement

Vidya Nimbalkar, Harish Kumar Verma and Jaswinder Singh

Abstract

Dairy farming innovations' implementation at every farmer's farm is the present day need; during the era of scarce natural resources coupled with population explosion, putting obvious pressure for more food production. Milk, produced from every single farm at micro level, is contributing to global economy at macro level. Dairy sector is facing the challenge of low animal productivity due to ineffective and poor farm management. This provides a big window for different innovations application to enhance animal productivity in developing nations where majority dairy farms are small scale and managed on traditional practices. Farm innovations are the novel practices/products/techniques suitable for particular area, physiological stage of animals and economically viable option to enhance the animals' per diem yield. Despite the prevalence of innovations, the scenario for its applicability is very dismal, majority of them are yet to reach masses at root level. Farmers' demographic, social and economic characteristics including adoption behavior, act as major impeding factors affecting impact of innovations. In this chapter, information on low cost and user friendly dairy farming innovations suitable for all kinds of farms, maintained under rural conditions existing in different tropical countries have been detailed for enhancing the animal productivity and henceforth farmers' socio-economic welfare.

Keywords: animal productivity, dairy farming, farmers' welfare, innovation need, low-cost innovations

1. Introduction

Farming is about feeding the world population that exceeds 6.9 billion people and is estimated to be more than 9 billion by 2050 [1]. In this scenario, striving to protect the natural resources (such as soil, water, and air) needed for current and future food production is not a new endeavor, although the present-day pressures on entire Earth's resources have generated widespread interest in agricultural productivity enhancement. Modern farming practices aids tremendously in boosting the food production across the world that too on diminishing cultivable land [2]. Increase in agricultural productivity achieved with altogether application of scientific knowledge and technological innovations [3].

The dairy world has also witnessed the rising trend of production and consumption and can be depicted as globally connected, composite and fast-changing sector of food production. Along with augmented supply of milk, rapid economic growth,

population expansion, increased urbanization etc. have also boosted up the demand for dairy products. With a growing middle class population having more disposable income, consumers seeking out healthy alternatives to fit in with a more active lifestyle, and a focus on natural ingredients, so milk and dairy products are growing in popularity. Presently the dairy world is serving over 7 billion consumers and providing livelihoods for approximately 1 billion people thrive on dairy farms [4]. Rearing of dairy animals always has a complimentary, supplementary and sustainable relationship with crops under mixed farming system prevalent in majority of the countries. However, as milk found the top most agricultural commodity in value terms and ranked third by production worldwide in 2013 [5], the valuable role of dairy sector in feeding the population of this planet can be understood without neglecting the need for sustainability at dairy farms as defined by U.S. Department of Agriculture (USDA) [2].

Being the chief source of income and food for a greater part of the rural poor [6], dairying is important for food security in many developing countries; also considered as one of the important sector for alleviating poverty, unemployment and reducing income inequalities. More balanced development of the rural economy is possible through the development of this sector [7]. The increasing importance of dairy to the world economy raises the importance of competitiveness among the countries. Globalization, trade liberalization and advancement in transportation and communication have given rise to an outstanding acceleration of market competition. This assures consumers to have a variety of goods and services to choose from, for a better standard of living with encouraging lower prices and lower fluctuations too.

Milk, produced from small as well as large scale farms at micro level, is contributing to every nation's economy, consequently global economy at macro level. There is a wide disparity of dairy farms in the world ranging from less than 3 cows per farm in some countries to over 1000 cows per farm in others, highlighting that milk production is performed distinctively in different countries. Discrepancies have also been noticed in terms of farm size, housing, milking and feeding systems. However, the world's average farmer keeps 3.2 milk animals with an average annual milk yield of approximately 2.2-ton ECM/animal/year [8]. During the era of global competition, achieving maximum productivity by using scarce natural resources is the biggest challenge among the dairy farmers, which can be addressed by implementing dairy farming innovations at every farmer's farm. Application of innovations at every stage of production since from cultivation of fodder till marketing of milk is the dire need of the present day.

Farm innovations are the novel practices/products/techniques suitable for particular area, physiological stage of animals and economically viable option to enhance the animals' per diem yield. Low cost and user friendly dairy farming innovations (technologies) suitable for all kinds of farms, maintained under rural conditions existing in different tropical countries are proved to be useful in enhancing animal productivity and henceforth farmers' socio-economic welfare. The term technology explains systematic application of scientific or other organized body of knowledge to practical purposes, which includes new ideas, inventions, innovations, techniques, methods and materials [9]. A decision made by an individual or group to use an innovation in a continuous manner termed as adoption. As, dairying has become a commercial enterprise and needs technology adoption for higher milk yield and lower per unit costs [10]; Innovations applicable for increasing net returns, reducing costs and optimizing production are discussed in this chapter; so that a common dairy farmer as well as consumer can contribute to a more resilient and more sustainable future for all of us.

2. Innovation needs

Though developing countries like India contribute above half in world milk pail, productivity per animal is poor compared to other countries. The huge production is purely number driven rather than productivity achievement. This leads to over exploitation of resources and more waste production particularly manure, which is really harmful to the planet looking towards environmental concerns. Low animal productivity might be a result of ineffective breeding, improper feed and fodder management, deficient veterinary care, poor farm management etc. Dairy farmer has to improve the amount of milk each animal produces, thereby reducing the amount of feed, water and space needed per liter of milk resulting in less manure production. This provides a big window for different innovation application to enhance productivity in such developing nations where majority dairy farms are small scale and managed on traditional practices. It is well recognized that sustainability—in its economic, social and ecological dimensions - in milk production vary across different dairy systems categorized on the basis of relevant socio-economic and farm characteristics of milk producing households. However, the sustainability studies concluded that market oriented farms with a high degree of technology adoption was the most economically, socially and ecologically sustainable farms.

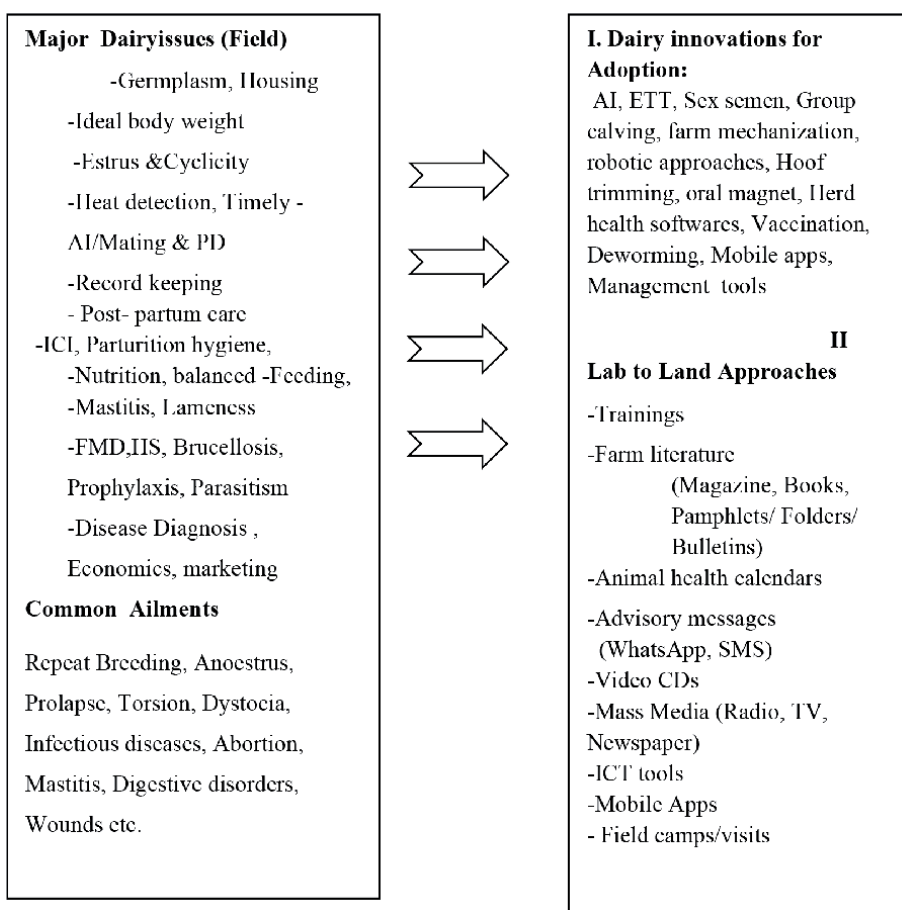


Figure 1.
 Dairy farming issues, innovations and lab to land approaches.

Technology adoption is associated with better milk yield and improved dairying is directly correlated with higher technology adoption, showing direct impact on income generation, poverty alleviation and animal protein availability [11] thus, to raise the milk production, improved animal husbandry techniques should be adopted in the small house hold dairy farms. To overcome the present challenges of this sector, technological innovations as well as ability to transfer these innovations from lab to field in dairy farming system is mandatory for achieving expected animal productivity, and lowering down the cost of production for greater economic returns to the farmers. The various dairy farming innovations addressed for sustainable dairy production are broadly discussed in this chapter. Various dairy farming issues, animal ailments along with dairy farming innovations and various lab to land approaches are presented jointly in **Figure 1**.

3. Breeding innovations

Breeding innovations generally known as cross breeding have resulted in profitable dairy farming with serious health and fertility concerns. Selection of good, diseases resistant and climate resilient breed coupled with adoption of scientific breeding innovations laid the strong foundation to the dairy farm to grow in future. Topography, soil type, feed and fodder availability must also be given due consideration while selecting the animals. Highly productive animal requires special care in terms of management, disease control and feeding strategies. Native breeds with quality germplasm would be more appropriate for local climatic conditions. Genetic up-gradation of non-descript animals by using local superior germplasm proves more beneficial in terms of sustainable production. However introducing exotic germplasm to a certain limit generally known as cross breeding have resulted in profitable dairy farming with serious concerns. Breeding innovations commonly introduced at field level are highlighted in this chapter.

3.1 Artificial insemination (AI) technique

Artificial Insemination (AI) is an Assisted Reproductive Technology (ART) used worldwide to deposit proven sire's stored semen directly into a cow's uterus. The technique is used as a rapid way to improve desired characteristics through intensive genetic selection. Advantages, such as facilitating the use of superior quality semen without the expense and risk of sire's ownership; reduction in the risk of introducing venereal diseases into the herd have achieved with this innovative technique. Being the quickest and most effective mean of breeding through AI, developing countries like India could witness position as the top most milk producing country of the world. Not only it exclude the need of keeping a bull for natural service but also helps in exploiting the excellent germplasm up to the fuller extent.

3.2 Progeny testing

Progeny testing is the practical and best technique, in which bulls are evaluated on the basis of their daughters' performance. When large numbers of animals are spread in many villages for a particular breed in its native tract, these villages can get AI services and progeny produced in this way is evaluated for their performance. Progeny testing is a practical and the best option for achieving genetic improvement in that breed.

3.3 Embryo transfer technology (ETT)

Embryo transfer technology (ETT) is one of the latest tools available for the faster improvement of livestock worldwide particularly for exploiting the genetic potential of high quality females and the males simultaneously. Prior to the development of this technology a limited number of off springs were achieved from a superior/high milk producing cow in her life time. Higher cost of technology with low conception rate might be the factors limiting its implementation.

3.4 Sexed semen

Sexed semen is processed semen of proven bull from where 'Y' chromosomes bearing sperm cells are removed through sorting process. Sexed semen predominant with 'X' chromosomes can ensure birth of female calf. Reduction in economic burden and production of more number of female calves as a future productive cattle are the main advantages popularizing this technology among dairy farmers. However, the higher cost of semen coupled with low conception rate are important factors to be considered before its use and that too in heifers or primiparous animals for better results.

3.5 Hormonal synchronization/protocols

Different hormone protocols are being adopted for getting group calving or desired calving in a year for efficient and controlled management. Such desired calving matches with market demand and season. It is planned administration of hormones with fixed time AI for specified calving.

In addition to this, the advanced reproductive techniques such as Multiple Ovulation and Embryo Transfer (MOET), ovum pick up technique and embryo manipulation (splitting, sexing and cloning etc.) offer possibilities for faster multiplication of superior germplasm from highly selected elite donors to achieve the target producing large number of superior bull calves/bulls and their adequate number of quality semen doses.

4. Feeding innovations (cost-effective feeding strategies)

Steady supply of quality feed and fodder assures productivity enhancement. Feeding constitutes about 60–70% of total cost of milk production in dairying. Feeding management plays a crucial role in exploiting real potential of dairy animals. Balanced feed (green and dry fodder along with concentrate ration) proves beneficial for sustainability as well as profitability of the farm. Fodder both green and dry needs to be grown inside the farm. High yielding fodder varieties like Bajra, Napier hybrids, Maize, Sorghum can be grown in fertile and well irrigated land, while Guinea/Rye grass can be grown in barren rain-fed land. In draught prone areas, planting of local fodder trees will sustain the animal production during scarcities. Some trees like *Prosopis cineraria*, *Leucaenale ucocephala* & *Moringa oleifera* are gaining popularity among fodder due to their high nutritional value. Further, slight improvement in animal nutritional status with additional supplementation can improve animal productivity with mere addition of cost. Different types of Animal Feed Innovations, easily applicable at every farm are discussed here in this chapter.

4.1 Baled silage

Silage, method of preserving surplus green fodder, predominantly adopted on large dairy farms as far as tropical countries are concerned. It is the product of controlled fermentation of green fodder retaining high moisture content. Many countries are propagating tube silage or bag silage, as one of the innovative technique of silage making, introduced for a marginal dairy farmer possessing one-two dairy animals and limited fodder acreage. Standard plastic tube/polythene bags of recyclable material are available in markets in India with a capacity of producing 500–1000 kg of silage. Baled silage is the latest upgraded innovation of fodder conservation. In this, forage is baled at higher moisture than forage to be stored as dry hay. The sealed airtight plastic bales remain sealed until they are required. The high moisture and lack of air promote fermentation within the sealed bale that preserves forage quality. Such baby corn silage bales of 50 kg are available for sale at a reasonable price on online portals like Indiamart.com.

4.2 Rumen inert protein (bypass protein)

Protein meals are subjected to suitable physical/chemical treatment, energy and nitrogen balance gets improved with only marginal increase in treatment cost. Chemical or heat treatments are the main methods used for protecting proteins. In this technique, part of the protein is not degraded in the rumen and it can be utilized more efficiently in the small intestine. This rumen inert protein commonly known as Bypass protein, that is a misnomer. This protein supplies more essential amino acids at the intestinal level, which can lead to increase in milk yield by 10–15% and growth rate by 20–25%.

4.3 Bypass fat

Dietary fat, that resists lipolysis and bio-hydrogenation in rumen by rumen microorganisms, but gets digested in lower digestive tract, is known as bypass fat or rumen protected fat or inert fat. Among all forms of bypass fat, calcium salts of long chain fatty acids (Ca-LCFA) has highest intestinal digestibility and act as an additional source of calcium. A simple cost effective indigenous technology has been developed for the preparation of bypass fat (Ca-LCFA) using vegetable fatty acids. Ration of the high producing animals should contain 4–6% fat, which should include fat from natural feed, oil seed and bypass fat in equal proportions. Bypass fat supplementation has proved beneficial without any adverse effect on the rumen fermentation, feed intake, digestibility of nutrients and different blood parameters of the dairy animals. Rise in milk is recorded by 5.5–24.0%. Improvement in post-partum recovery and reproductive performance of dairy animals are the added advantages of this innovation.

4.4 Total mixed ration

The term total mixed ration may be defined as, “The practice of weighing and blending all feedstuffs into a complete ration which provides adequate nourishment to meet the needs of dairy cows.” Each bite consumed contains the required level of nutrients (energy, protein, minerals and vitamins) needed by the cow. A 4% increase in feed utilization, greater accuracy in formulation and feeding, masking of the flavor of less palatable feeds (urea, limestone, fats, and some by-pass protein sources) and use of commodity ingredients can be expected while using TMR. While blending all the feeds together in a TMR, over mixing and under mixing of ingredients need to be avoided.

4.5 Buffers

Dietary changes like shift from hay to silage, feeding high level of grains/concentrate mixture cause increased acidity in rumen which may become detrimental for rumen microorganisms thereby affecting not only digestion but production and reproduction too. Buffers like Sodium bicarbonate, Magnesium oxide neutralize the acids produced by metabolism or fermentation. They are particularly required during hot weather when forage intake is lower and due to less chewing action natural buffer produced i.e. saliva is produced less.

4.6 Probiotics (prebiotics/synbiotics)

Probiotics are the live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal microflora. *Lactobacillus* spp. is the most prevalent probiotic bacteria, known as lactic acid producing bacteria (LAB). Control of diarrhea in calves, increased milk production and better composition, control of ruminal acidosis, control of growth of pathogens in rumen, reduced pathogen load are the advantages of the technology. The appropriate level of 20 g probiotic per day per animal is found effective. Prebiotic are the ingredients (like Fructo-Oligosaccharides (FOS), Mannan Oligosaccharides (MOS) etc) used to enhance the population of already present good bacteria and synbiotic pertains to combination of pre and probiotic.

5. Management innovations

Building a hygienic cow shed is another important aspect to be considered among the many factors that lead to the success or failure of dairy farms. Housing systems that require less labor, which provide a comfortable and healthy environment to animals, manage space including storage efficiently and take care of bio-security measures with easy modification and expansions are more profitable than heavy structures with huge capital investment. Sufficient sunlight, proper ventilation, clean, and dry flooring along with sufficient space for lying down and protection from adverse weather conditions are the basic necessities of animal housing. Further, an effective management program has to be developed, so that animals are prevented from falling ill and there is no need for antibiotics/medicines. The direction and orientation of shed plays an important role in keeping the animals healthy as well as reducing laborious work. Considering these factors, loose housing barn with open cattle shed are recommended here, as that can be easily adapted at small as well as marginal dairy farms.

5.1 Health tracking devices

Digital animal health tracking devices are getting attention now a days as they help farmers in tracking, monitoring and managing animal's health, nutrition, behavior, pregnancy, milking frequency, milk production anomaly and activity level in real-time. These smart animal wearing gadgets can be implanted in the cattle's ears, tail, legs, neck or any part of the body. For tracking the health and early diagnosis of medical condition in dairy animals, GPS-enabled digital chips have been implanted widely in India. A huge database will be generated if these devices are used efficiently. Accuracy in such data will guide in formulating strong and concrete policies for welfare of both human and animals.

5.2 Heat detection systems

Detection of heat is very important aspect of management for performing timely AI with successful animal conception. **Heat Detection System** is heat management software which monitors the cow's activity for the whole day, predicting heat on the basis of unrest and hyperactivity along with other features to check milk flow, conductivity for suspected mastitis. It has obvious advantage over visual heat detection which is based on observation, behavior and miss heats or false negatives leading to huge economic losses. Further, these gadgets help the farmers for fertility management to get the target of a 'calf at foot every year'.

5.3 Robotic milking machines

Innovation of robotic milking machines is useful in eliminating the pressure on physical labor and maintaining a hygienic milking process with remarkable improvement in milk production. These machines have cups with sensors that can be attached individually to cows' teats. The sensors play important role in detecting readiness of teats for milking and also identify impurities, color and quality of milk. Milk not fitted for human consumption, is diverted to a separate container. The machines automatically clean and sanitize the teats once the task is over. Few models of low-cost, non-electric milking machines are also developed considering locality and need of dairy farms. Innovation of mobile milk collection unit installed with Robotic milking machines and bulk coolers will introduce a way to produce clean and quality milk from small and marginal farms.

5.4 Waste disposal and management

Scientific disposal of excreta (Dung, Urine), other organic waste (aborted fetuses, dead calf/animals, placenta) demands utmost attention. Presently, there is not a clear cut policy for dung and carcass disposal. In majority of Asian countries, both these are disposed in open, which is a serious concern from zoonotic and infectious diseases point of view. Electric incinerator and community biogas plants can provide the tangible solution.

Technology of dung cleaning robot or manure robot is available for barn cleaning and scrapping the dung in slatted floors beneath the barn. Recently Manure eating robot has been launched for cow garden cleaning that cleans the barn/cow gardens.

5.5 Digital farm management

Completion of farm management includes accounting, finance, labor management and supply chain management. Dairy farm management softwares are the innovative tools available in markets for atomizing and digitalizing end-to-end production and operations activities. It provides a holistic view for entire farm activities, manage records, generate reports and detect inefficiencies; assuring profitable dairy farming.

6. Health care innovations

Reduction in milk production is the first sign of animal discomfort and illness; whereas getting back to this production is one of the major challenge and costly affair for small as well as marginal farmer. Also there is reduction in per lactation as well as life time production of that animal. Any kind of disease treatment compels

to use antibiotics. This part is of a global conversation about antibiotic resistance, which is a serious public concern shared by animal and human health experts. So, it's always better to prevent the occurrence of diseases rather to treat. This could be possible only through application of healthcare management innovations.

6.1 Vaccination

Livestock vaccination is considered an emerging innovation of socio-economic importance in the Indian dairy industry [12] and reported more profitable and sustainable than artificial insemination [13].

Majority of tropical countries like India are endemic to many diseases that cause severe economic losses due to drastic reduction in the production capacity. Some of the diseases are even highly fatal. Fortunately, vaccines are available for most of these diseases and can be easily controlled if timely vaccination is carried out in a mass scale, covering a large proportion of the susceptible population (at least 80%) [14]. Farmers must stick to the standard vaccination protocol recommended by the Government following all precautions and regularity in inoculations.

6.2 Teat dip

The teats of all the lactating dairy animals and dry cows (during first 10–14 days of dry period) are dipped regularly after every milking in a germicidal solution. The recommended teat dips are

1. Iodine (0.5%) solution 5 parts + Glycerine 1 part
2. Chlorhexidine (0.5%) solution 1 L + Glycerine 60 ml

The iodine teat dip is the best as it treats various types of teat lesions and injuries also. Post-milking teat dipping with 'Iodine-glycerine teat dip' for prevention of new mammary infections is also recommended by many research institutes. Studies have reported that the treatment applying the post-milking teat dip automatically via milking machines had the lowest number of new intra-mammary infections (IMI).

6.3 Mastitis diagnosis kit

Mastitis, one of the expensive diseases, affects economic returns of dairy farms heavily. Farmer has to suffer with huge financial burden due to sub-clinical mastitis (SCM) as it incur heavy losses related to culling, decreased production, decreased fecundity, and treatment costs. Diagnosis of mastitis at sub clinical stage and its management results in milk production rise with quality milk and safety to consumer health [15].

Innovation of mastitis diagnosis kit includes Sodium Lauryl Sulphate (SLS) Paddle with reagent and Bromothymol Blue (BTB) card. Such innovations can be used by the farmers at their own for early diagnosis and reducing the further incidence diseases for improving productivity [16].

6.4 Lameness management

Lameness is reported as the third most economically important disease in world after infertility and mastitis [17]. It is a major cause of involuntary culling after mastitis. About 90% of lameness in dairy cattle and buffaloes occurs due to foot lesions. Recommended guidelines for prevention of lameness include hoof

trimming of all the animals at every 6 months and footbath of size 3 m long, 1 m wide and 15 cm high. Formalin (39–40%) should be preferred for foot bathing as a 4% solution (120 L water +5 L of formalin) in the footbath. Concrete footbaths are best and cheaper. In case there are few animals (unorganized farms), formalin spray (40 ml per liter of water) can be used on 1st, 2nd, and 3rd day of every fortnight along with close monitoring of animal gait at the time of walking.

6.5 Oral magnet feeding

Hardware disease is a common term for *bovine traumatic reticulo-peritonitis*, which is usually caused by the ingestion of a sharp, metallic object. Due to industrialization and urbanization, it is commonly found in dairy cattle, than any other ruminants. It can be difficult to conclusively diagnose, but can be prevented by the oral administration of a magnet around the time that the animal reaches the age of 1 year. This innovative technology is beneficial to control Traumatic Reticulo-Peritonitis (TRP) that occurs due to the intake of any sharp foreign object such as nails, blades etc. along with feed by the animal.

7. Communication innovations

In this competitive world, farmers are not only looking for various information sources for carrying out their production and marketing tasks efficiently but also for ensuring delivery of safe and quality products to the consumers. Food safety for consumers is at greater risk because of the increasing globalization of food systems. Information and Communication Technology (ICT) has potential to mitigate the needs of both ends by introducing virtual platform for dairy product production and marketing. ICT based information delivery to dairy sector can significantly improve the quality of decision-making in dairy farming system. Mobile phones with internet facility have been one of those successful innovations which benefit a large number of people in the developing world. As worldwide acceptance for mobile phones has improved among all users, it can be used as a major tool for communication and dissemination of information for quality decision making. Different mobile apps, web portals such as epashupalan.com and expert systems are being used by dairy farmers. The mobile application for dairy farmers, named 'Pashu Poshan', is available on both web and android platform, can be accessed by registering on the INAPH portal (<http://inaph.nddb.coop>). Guru Angad Dev Veterinary and Animal Sciences University, Punjab, India has launched 'Precision Dairy Farming' mobile application dealing with important aspects of dairy farming including important milch breeds, breeding, feeding and housing management, record keeping, health management and economics.

7.1 Product traceability in dairy sector

Traceability is commonly defined as the ability to trace products back and forth throughout the supply chain, from farm or point of production to the end user. The growing complexity of food supply chains, the heterogeneity in food safety regulations across countries, and lack of uniform requirements from one commodity to another are some factors that explain why greater efficiency in food traceability systems has increased in recent years. Block chain technology to give real-time data about the products to customers has been introduced among dairy manufacturers, suppliers and other stakeholders. QR code provided on the packaging of the product can be scanned on personal mobile devices to get information on the origin

of the milk. Information about, how and where from the product has collected and packed, how old it is, what kind of transportation and cold milk chain facilities are used, is being provided on internet. However, scattered, diversified and unorganized dairy farming is the major barrier for deep penetration of this innovation at grass roots of the sector.

The application can be highly useful in organic milk production as demand for organic milk is increasing in the market. Organic milk is considered as the ultimate milk with almost nil risk of chemicals, drugs and also free from stress factors. However its production is quite cumbersome as it needs a lot of efforts, monitoring and adhering to the organic standards for a branded product. It fetches good value in the market and the product traceability is quite easy as its each and every production component is documented.

8. Marketing innovations

E-commerce market places have played revolutionary role in input availability and product sales in dairy sector. Modern equipment and advisory services have been made available at the doorstep to farmers and dairy manufacturers on their smart phones through online Business-to-Business (B2B) market places. Many Business-to-Customers (B2C) platforms have also emerged at a rapid pace. They have major role in picking fresh produce from farms and delivering them to the doorsteps of end users. These marketing innovations have reduced spacial barriers for both producers as well as consumers. Online portals like Indiamart.com, amazon.in, reliance fresh at relianceretail.com are the successful examples of innovative online marketing of various dairy products.

9. Factors impeding innovations

Despite the prevalence of innovations, the scenario for its applicability is very dismal, widening the gap between innovations developed and available; and innovations actually being adopted or used by the end users. Recommended innovations in dairy farming sector have not been adopted as widespread as it is anticipated and the correct level of adoption is far from desired. Though large scale innovative digitalization is happening in dairy sector considering the present need of time, it has yet to reach masses at root level. Factors like low socio-economic status, disrupted electric supply, and unavailability of reliable internet facilities in rural areas might be the cause of poor outreach of the innovations. Adoption is defined as a decision to make full use of an innovation as the best course of action available and the process starts with awareness of the new product and ends with routinized use of the new product by consumer [18]. So for increasing the adoption of innovation, it has to be diffused widely as, diffusion is the process by which an innovation is communicated through certain channels over time among the members of a social system.

Demographic, social and economic factors along with adoption behavior of farmers affect application of innovations in dairy sector. Farmer's education, knowledge, attitude, risk orientation, and innovation proneness controls adoption behavior [19, 20]. Constraints faced by the farmers such as lack of awareness, knowledge and skill of application can be considered as the major impeding factors in dairy technology adoption [21–23].

Attributes of innovation, known as characteristics of the innovation, also play influential role on farmers' technology adoption and usage decisions [24]. Five characteristics of innovations *viz.* relative advantage, compatibility, complexity,

divisibility (triability), and communicability (observability) have identified as factors affecting the rate of innovation adoption [25]. Relative complexity and profitability, risk and investment, technicality and reversibility of innovation can also significantly affect its diffusion and adoption. Majority farmers prefer adopting the only practice which needs no investment and technicality.

Low government policy support, insurance complexities and market fluctuations act as major barriers in adoption-decision making process; reducing the interest of young generation in dairy sector. Poor collective actions, low financial policy support and absence of fixed pricing assurance to milk are remained the major constraints and demotivating factors in moving this sector towards sustainability.

10. Policy barriers

Majority of policies and government schemes are suited for medium and large farmers as they are knowledgeable and can invest more in their farms. Small and marginal farmers are resource poor, less knowledgeable, low risk bearer and investor; possessing only 2–3 animals for family sustenance rather than income generating activity. However, their proportion as the dairy stake holders means a lot to the economy when it comes to scarce resources, as it is more than medium and large farmers. Hence the policies should focus more on such group of producers to change their attitude, knowledge and skill for introducing innovativeness among them and for motivating them towards sustainable dairy farming. The resistance against policy reforms in the northern Pakistan has been reported under the Dairy Science Park (DSP) as a conflict of interests among the weaker and power stakeholders across food value chain, and DSP has come up with the idea of the Triple Helix Model of Academia-Industry-Government Nexus of good governance [26].

11. Conclusions

This chapter entitled ‘Dairy farming Innovations for productivity enhancement’ has focused light on today’s dairy sector all over the world, describing the similarity and diversity in production and production performance. The innovation needs are justified for making the farming profitable for welfare of farmers and providing customer satisfaction by offering healthy, qualitative milk and milk products. Innovations from breeding, feeding, animal management, health care and preventive measures, waste disposal, product traceability and marketing features are discussed and explained with examples and success stories. Factors impeding innovations are discussed from point of innovation generation, diffusion till its adoption. Removal of these barriers and application of suitable extension approach with policy support will lead to more and more adoption for productivity enhancement and quality production. Recommendations have given for not only technology generation but also for its implementations.

12. Recommendations

In this innovative world, there is no single perfect technique or innovation which can cater to all the needs of farmers. Innovations must be considered with regards to their total cost for owner and end user. User friendly, economical, easily updated, accessible and locally available innovations, termed as ‘fit in situation innovation’, will be adopted at once and has more chances of popularization compared to the

one which has complex or more steps for executions. There is need for local and region specific technology generation and further its vast diffusion among similar socio-geographic regions. Innovation must reach the target people at right time; otherwise they are lying on shelves or in the books. The impacts of innovation application on the farmers' livelihoods should be adequately addressed and documented for different agro-ecologies of the world. There is a need for greater follow-up in tracking the adoption of technologies for sustainable farming systems and in the accountability of research efforts and policies for technology dissemination and adoption. Targeted efforts should be made in changing and building farmers' awareness, attitude and perceptions through training, demonstration, field visits, experience sharing etc. Already, huge dairy innovations have been made around the globe, but still they lack to be in rationale people for their usage. For that, strong extension is the need and call of the hour for getting more successful and sustainable farms, to break the ongoing trend of closure of dairy farms.

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Conflict of interest

“The authors hereby declare that there is no conflict of interest.”

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Section 4

Sustainable Development

Emergence of Regional Dairy Breeds

Livia Vidu and Stelian Baraitareanu

Abstract

This chapter covers the regional breeds in Europe of cattle, and buffalo that supply milk to the processing industry. Local breeds are evidence of the evolution of different species in different directions of breeding. The book chapter will give a brief description of the origin and cognition of different regional and local dairy breeds, the presentation of morphological and productive characters, as well as the perspective of rearing or conservation. The development of breeds in different directions of production was achieved under the impact of environmental factors, agricultural policies and market requirements. In this context, the factors will be analyzed in correlation with the spread of breeds in different regions, respectively countries.

Keywords: local breed, morphological aspects, origin of the breed, production, regional breed

1. Introduction

In this chapter we will try to present the regional breed groups (which are found in several neighbouring countries or in the same geographical area) important for milk production in south-eastern Europe. Dairy animals have provided food sources for humans since ancient times, as well as social means of subsistence. Humanity is currently going through a period of crisis caused by several factors, namely,

- climate change, declining water reserves and the fertile soil layer, which are causing food crises and even famines in some parts of the world;
- geopolitical changes, health crises and even wars generate delays or the impossibility of rhythmic food supply;
- the increase in the level of human well-being is followed by the increase in the consumption of food of animal origin and the use of cereals for the production of fuel.

In this context, milk is a staple food whose usefulness should not be forgotten, and dairy animals have their importance in food safety and security. The milk consumed by humans is produced by several species of animals (cow, buffalo, sheep, goat, camel, etc.), but it is recognized that cattle provides more than 90% of the world's milk.

Milk consumption has steadily increased from one century to another; currently a man consumes about 10,000 L of milk during lifetime.

This *white blood* has been used for its pleasant taste and therapeutic properties since ancient times. The nutritional value of milk is its ability to provide the necessary energy, plastic and bio-stimulating substances and to positively influence the health of the consumer. Today it is known that milk contains all the essential amino acids that the human body cannot synthesize, that it provides the necessary protein for children up to 6 years old and 50–60% of the necessary protein for young people up to the age of 20.

Statistics show that the world's population is currently growing by 80 million people a year, which should make us think and make us find other competitive sources of food. In this economic and social context, rethinking local or regional genetic resources is an important starting point in developing a food strategy.

The knowledge and quantification of the local genetic resources of the breeds with regional importance, perfectly adapted to the pedological and climatic conditions of the region, gives security and confidence for a sustainable future.

2. Regional breeds of dairy cattle

The main breeds of cattle were formed following a long process of evolution of the old local populations, under the influence of natural and artificial environmental factors. Going through the stages of agricultural development overlapped with the diversification of the productive skills of cattle with the specialization of production.

In the early eighteenth century, in Europe, began the process of gradual replacement of cattle breeds that had universal or multiple skills with specialized breeds for milk or meat. This trend appeared simultaneously with the industrial revolution, which generated large urban populations, involved in the production process, which also determined the change in the type of human diet, and consequently the need for larger quantities of milk and meat. At the same time, the draught force of animals began to be used less and less [1].

Later in the nineteenth century, the structure of the main modern breeds of today crystallized in Europe, such as the black-and-white spotted (Holstein, Friesian), white-spotted with red (Simmental), red (Danish red, Angler) and brown (Schwyz). As these breeds developed and penetrated most European countries, local breeds began to lose importance, their production became insufficient, they were used in crossbreeds to form new breeds, and over time some of them even disappeared. In 2007, around 20% of all breeds and animal populations worldwide were considered endangered, and in Europe around 130 breeds had already disappeared [2].

Regional or local breeds have emerged as a result of migrations that have taken place over the centuries from one country to another or from one region to another, along with the movements of the human population. Also, cattle were an important key of trade (currency exchange), they were used as ritual animals or transport. According to the FAO, there are 534 breeds of cattle in Europe, of which 464 are local or regional breeds [3].

In Western European countries, traditional products related to local breeds have been key points of the active breed conservation strategy. By promoting regional products, farmers have been encouraged to raise these breeds with low productivity.

Local breeds are defined as “breeds that occur only in one country”, while *Transboundary breeds* are “breeds that occur in more than one country”. Within this

group is identified—*Regional transboundary breeds*: transboundary breeds that occur only in one of the seven SoW-AnGR18 regions and *International transboundary breeds*: transboundary breeds that occur in more than one region [2, 3].

2.1 Red breed

The red breeds are a group of populations or breeds to which the local breeds have contributed, especially the grey steppe (Podolic cattle) and Angler and Danish Red breeds, which have appreciable qualities in the direction of milk production, but which lately are strongly competing with the spotted black and white breeds.

Geographical area. These populations or breeds are generally widespread in steppe areas or with drier climates in Bulgaria, Poland, Romania, the Republic of Moldova and Ukraine (**Table 1**). From a historical point of view, the formation period is in the second part of the nineteenth century, but for some, there are historical data even from the thirteenth century.

Morphological characterization. Body development is variable, wither height – 122–132 cm and body weight—350–450 kg, fine and rarely very robust, conforming to the morpho-productive type of dairy cattle. (**Figure 1**). The colour is uniform red, with shades from light red to cherry colour, often with light shades on the underside of the torso, the inside of the legs and in the earlobe, sometimes with white spots on the abdomen or udder. The apparent mucous membranes and hooves are grey-brown. The udder is well developed, globular in shape, glandular in consistency and relatively symmetrical [3, 4].

Productive characteristics. In general, the duration of lactation is short (250–300 days), with milk production between 2204 and 5033 kg of milk. The milk has a fat content of 3.8–4.2%. The age of the first calving is a variable, between 27 and 35 months. There are also breeds that, in addition to milk production, have also been exploited for meat, although the weight of calves at birth is 30–35 kg. Slaughter yield between 40 and 50%, depending on age and condition; very well fattened animals achieve a slaughter yield of 51–52%.

The future of breeds. In general, these breeds have a vulnerable or critical status, due to very small numbers, with a risk of extinction. Some red cattle populations have also disappeared because they are susceptible to bovine leukosis, as was the case of Red cattle of Dobrogea. Some countries have ongoing programmes to protect local race, benefiting conservation strategies in vivo and in vitro.

| No | Country | Cattle breed name | Milk yield per lactation (kg milk /lactation) |
|----|---------------------|---|---|
| 1 | Bulgaria | Balgarsko cherveno govedo (Bulgarian red cattle) | 4000 |
| 2 | Czech Republic | Ceska Cervinka (Czech red) | 2204 |
| 3 | Poland | Polska czerwona (Polish red) | 4000 |
| 4 | Republic of Moldova | Roşia de Stepă moldovenească (Moldavian red steppe) | 3600 |
| 5 | Romania | Roşia Dobrogeană (red cattle of Dobrogea) | 3800 |
| 6 | Ukraine | Ukrainian red dairy (Ukrainska chervona molochna) | 5033 |
| | | Red steppe (Chervona Stepova) | 3944 |

Table 1.
 The red breeds of south-eastern Europe [2].



Figure 1.
Red steppe breed from Ukraine [2].

2.2 Pinzgau breeds

The Pinzgau breed was formed as a result of a successive process of crossbreeding and purebred breeding, which began around 800 BC, with the Celts bringing robust cattle to the Hohe Tauern area of present-day Salzburg [4, 5]. Cattle of Celtic origin were crossed over time with local red cattle, with Bern type imported from Switzerland, and after 1740 the population grew in purebred. Over time, some varieties or sub-breeds have been created, such as Pongau, which resembles the Pinzgau breed, but which is distinguished by the darker body colour and a more massive body development; Mölltal, widespread in Carinthia, whose body development is lower.

Geographical area. The animals of this breed were formed in the pedoclimatic conditions specific to the Austrian Alps, the lands of Salzburg, Tyrol and especially Pinzgau, hence the name of the breed. Studies have led to the idea that the breed comes from the old local populations, belonging to *Bos taurus brachycephalus* in the mountains and *Bos taurus primigenius* var. *Hani* in the plain area. Since 1820, the Pinzgau breed has been spread in all the provinces of the Austro-Hungarian Empire in the regions that now belong to Romania, Slovenia, the Czech Republic and Slovakia. At that time, it was the most widespread regional breed, adapted to the local natural environment (mountains, pastures, forests) [4–6].

Morphological characterization. Pinzgau animals have a harmonious conformation, good body development, wither height: 133–150 cm and body weight: 550–630 kg in cows and 1000 kg in bulls, differ depending on the area, altitude and technology used. The head is large, the neck short, the trunk short and deep. The legs are short and strong with hard hooves horn, which allows easy movement in mountainous areas. Pinzgau cattle were raised with horns, which is a distinct beauty, but they were also used for traction and then had yokes and harnesses. The colour is red, with a white stripe on the back and belly, tail and udder always white, head and legs always red and white rings around the legs (**Figure 2**).

Productive characteristics. The Pinzgau breed falls into the intermediate morphological type, has a good ability to capitalize on fodder from alpine pastures; it is very suitable for grazing. The first calving occurs at 31–32 months, being considered a late breed, with a production life between 5 and 7 years. A very important feature of this breed refers to the high content of k-casein in milk, which provides a better



Figure 2.
 Pinzgauer breed from Austria [2].

efficiency of transformation of milk into cheese (**Table 2**). Calves at birth weigh between 31 and 36 kg. Pinzgau cows are good mothers and have a good milk yield, which ensures a high weight gain in calves. In most Pinzgau breeding countries, it is used for milk, but also for meat [4–6].

The future of breeds. In general, the number of Pinzgau cattle has decreased greatly, being a breed with a special state, in some countries, the status is even critical. In the future, purebred breeding is expected to happen on the current distribution areas, especially in the mountainous area of the Alps and the Carpathians, at altitudes of 400–1600 m.

2.3 Grey steppe breeds or Podolic cattle

The cattle from the grey steppe or Podolic cattle breeds group represent some of the oldest regional cattle, which comes from *Bos taurus primigenius* and was

| No. | Country | Cattle breed name | Milk yield per lactation (kg milk / lactation) | Milk fat (%) | Milk protein (%) |
|-----|---|----------------------------|--|--------------|------------------|
| 1 | Austria (Salzburg, Pinzgau; Upper Austria; Styria; Tyrol) | Pinzgauer | 5300 | 3.92 | 3.25 |
| 2 | Germany | Pinzgauer Doppelnutzung | 5500 | 4 | 3.5 |
| 3 | Italy (Province of Bolzano) | Pinzgauer | 4000 | 4 | — |
| 4 | Slovakia (North Slovakia regions) | Slovenský Pinzgauský | 3900 | 3.9 | 3.3 |
| 5 | Slovenia | Cikasto govredo | 2500 | 4 | 3.6 |
| 6 | Romania (Transylvania, Apuseni mountains) | Pinzgau de Transilvania | 4173 | 3.88 | 3.25 |

Table 2.
 The productive characteristics of the Pinzgau breed [2, 4, 5].

formed in the pedoclimatic conditions specific to the steppe area. Human intervention in the formation of these breeds is insignificant over the centuries undergoing little change in terms of morphological appearance and production skills.

Geographical area. Podolian or grey steppe cattle belong to the group of primigenius cattle, about the origin of which there are several theories. The first hypothesis of the origin of these breeds refers to the migratory peoples who brought the animals from the steppes of Central Asia over 1000 years ago, and then were subjected to a process of acclimatization. Another theory claims that this group of breeds appeared with the great migration in the fourth century, and other researchers consider the grey cavities appeared after domestication in the Carpathian Basin. Etymologically the term 'Podolic' comes from the Podolia region of Ukraine. During the 14th and 15th centuries, Podolic cattle were widespread in the Carpathian Basin and the Pannonian Plain, extending as far as Vojvodina. 100 years ago, the group of Podolian cattle was found in Ukraine, Romania and Hungary; later it spread to the Balkan countries (Bulgaria, Serbia) and to the south (Croatia, Greece, Italy) [2, 7].

Morphological characterization. Podolic breeds are characterized by a body development varying from weights of 480–850 kg and height at withers of 137–150 cm, animals are tall and leggy, have well-developed front part of body (lionic aspect inherited from wild ancestors) and thick skin (adaptation to cold winters). The udder is mainly rounded with glandular tissue in a small proportion. The conformation of the breed is robust, the body shape is rectangular and the limbs are strong and long with wide and strong joints. The colour is grey or silver-grey, with darker shades to bulls on the sides of the trunk and on the top. The nasal mirror, horns, tail tuft and hooves are dark or black. Calves at birth are reddish in colour (**Figure 3**) [8].

Productive characteristics. Podolic cattle has a morpho-productive type specific to breeds with universal production characteristics, of which the traction aptitude is unmatched by other modern breeds. It withstands long and difficult roads, withstands good weather conditions and has modest demands on maintenance conditions. It is a late breed in which the reproductive maturity is reached at 30–32 months, with the productive life of up to 11–13 years and even more (15–16 years) and fertility is very high (99–99%). Typically, milk production varies between 980 and 2950 kg and a fat content of 3.6–6%, but in very good conditions of maintenance it can achieve more, and the lively temperament (**Table 3**). Animals of Podolic breed are hardy animals with low nutritional requirements and good viability. Calves at birth are small in size; calves have a body weight of 20–38 kg at



Figure 3.
Grey steppe breed from Romania.

| No | Country | Cattle breed name | Milk yield per lactation (kg milk/lactation) |
|----|---|---------------------------------------|--|
| 1 | Bulgaria (Regions of Plovdiv, Haskovo, Burgas, Smoljan, and Stara Zagora) | Iskarsko govedo | 1900 (4.3% fat) |
| 2 | Italy (Basilicata, Calabria, Apugli, Campania) | Razza Podolica | 1200 (4.29% fat) |
| 3 | Serbia (Vojvodina) | Podolsko govece Sivo-stepsko govece | 980 (4.24% fat) |
| 4 | Hungary (Bocfölte, Western Hungary) | Hungarian grey | 2100 (4.2% fat) |
| 5 | Romania | Sură de stepă (Moldova, Danube Delta) | 2222 (4.4% fat) |
| 6 | Ukrain (Donetsk, Kyiv, Sumy, Kherson and Dnipropetrovsk regions) | Ukrainian grey | 2950 (4.5% fat) |

Table 3.
The productive characteristics of the grey steppe breed (Podolic cattle) [2].

birth, and in appropriate growth conditions, achieve an average daily growth rate of 0.7–0.8 kg and a body weight of 130–150 kg at the age of 6 months [1, 7, 8].

The future of breeds. Grey steppe breeds are distinguished by robustness, low requirements for feed rations and housing conditions; they are not sensitive to tuberculosis or leukosis, so they can be true genetic reserves for the future. Most breeds that are part of the Podolic cattle or grey steppe group have a very small number of animals, so it is necessary to implement in vivo conservation programmes or cryo-programmes. It is very suitable for growing on organic farms and for the recovery of milk in traditional products or with controlled origin, given that milk has special organoleptic qualities.

3. Regional dairy buffalo breeds

Of the total world milk production, buffalo milk is the second most important source, after cow's milk. Most developed countries face an overproduction of cow's milk, which is why some of them impose quantitative restrictions and qualitative requirements. Under these conditions, an important alternative source of milk is that obtained from buffaloes. World milk production has doubled in recent decades, and it is noteworthy that 12% of it is provided by buffaloes. India and Pakistan produce about 60 and 30% of total buffalo milk production respectively. Buffalo milk contributes to about 55 and 75% of total local production in India and Pakistan, respectively. In the NWF Province (NWFP) of Pakistan and elsewhere in the Indo-Pakistan sub-continent, buffalo farming is practiced on non-scientific lines. The major causes associated with the under-developed buffalo farms in Pakistan have been identified as:

- i. calf losses, irregular breeding, imbalanced feeding
- ii. ungainly loans and
- iii. a hostile marketing system.

These three causes at commercial buffalo herds lead to annual losses to the tune of US\$ 17.38 [9].

Buffalo milk products are obtained by specific processes in countries in the Caucasus, Asia, where the consumption of sour milk, butter and yogurt is very popular.

In Italy, the buffalo milk industry is highly developed due to the production of the well-known mozzarella cheese. Buffalo milk and dairy products have become very popular in many European countries, and buffalo milk production is now prevalent in countries that previously had no history of this production such as Germany, Ireland, the United Kingdom and even the United States.

Buffaloes adapt easily to environmental conditions, have low requirements for feeding and housing conditions, do not require special care for pathogens, and are resistant to disease. They are rustic animals and suitable for organic farming.

Geographical area. European buffalo breeds belong to the Mediterranean type and are widespread in countries such as Italy, Romania, Bulgaria, Turkey where specific (local) breeds are raised—Mediterranean breed (Italian, Romanian, Bulgarian, etc.). Mediterranean buffaloes come from *Bubalus bubalus*; in *Italy*, they were introduced by the Crusaders at the beginning of the thirteenth century. Improving production capacity was achieved in the twentieth century in the direction of milk production [10].

The Bulgarian Murrah breed comes from the local Mediterranean buffalo, which was crossed with the Murrah breed, imported from India. The formation of the breed by crossing practiced between the local buffaloes and the Murrah breed began in 1962. Thus, in 1962, imports of the Indian Murrah breed were made, which continued until 1975 [10–12].

The Romanian buffalo breed was approved in 1987 [1, 13], and had an increase during the period until 1990 (228,000 heads), after which the number decreased (10 times). Regarding the appearance of buffaloes on the territory of today's Romania, the following are the existing hypotheses (**Figure 4**):

- by the South, from Turkey, Greece and Bulgaria, being brought by the Turks, through the Balkan Peninsula from where they passed into Dobrogea and the Danube Plain;
- by the West, from Hungary, being brought by the Huns and spread by the Avars in the area of Transylvania.



Figure 4.
Romanian buffalo breed from Romania.

| Morpho-productive characters | Average values | | |
|---|------------------|-------------------|-----------------|
| | Romanian buffalo | Bulgarian buffalo | Italian buffalo |
| Body weight (kg) | 490 | 530 | 650 |
| Height at withers (cm) | 132 | 138 | 140 |
| Age at first calving (months) | 43 | 37 | 32 |
| Lactation period (days) | 273 | 278 | 270 |
| Average amount of milk per lactation (kg) | 1960 | 1800 | 2175 |
| %Fat | 7.85 | 8 | 8.15 |
| %Protein | 4.39 | 4.55 | 4.65 |

Table 4.
The productive characteristics of the Buffalo breeds [2, 13].

Breeds characterization. The buffalo breed in Italy has superior performance to the breeds in Romania and Bulgaria, which proves the effort made by Italian farmers in modernizing breeding technologies and improving the breed. (**Table 4**).

Compared to the average milk production of 1800–2200 kg and a fat content of 7–9% (**Table 4**) in Bulgaria, Italy and Romania, there are many herds with average productions of over 2000 kg, there are animals with productions of over 3000–4000 kg of milk, with an average fat content of up to 11%, aspects, which indicates the special genetic potential and the possibilities for future improvement of these populations [14–17].

4. Conclusion

Regional dairy breeds and regional transboundary breeds represent an invaluable genetic heritage, as they are the reservoir of genes for different types of production, perfectly adapted to climatic conditions, resistant to disease, even if productive performance is lower compared to breeds of universal importance. It is appreciated that milk obtained from animals of these breeds is suitable for obtaining specific or traditional products, so agricultural policies must encourage farmers to grow these breeds, thus achieving active conservation.

Conflict of interest

The authors declare no conflict of interest.

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The Interactions of Some Minerals Elements in Health and Reproductive Performance of Dairy Cows

Imad M. T. Fadlalla

Abstract

The dairy business is recognized as an important economic and nutritional resource. The food and agriculture organization (FAO) estimates that there are about 245 million dairy cows in the world. Milk production offers enormous health, sociological, and economic benefits around the world. In emerging economies, livestock serves a range of purposes, including providing a source of household income, a financial asset for women, food security, risk management, and a direct connection to human health. With a total value of \$628.27 billion USD in 2018, the dairy industry provides a major economic impact. The dairy business supports over a million employment, either directly or indirectly. In terms of public health, the dairy business plays a role. While dairy products are a beneficial part of a balanced diet, zoonotic and food-borne diseases originating in dairy animals can be dangerous to humans. Milk production performance in emerging countries is poor. Non-infectious infertility in dairy cattle is most commonly caused by energy, protein, and mineral deficiencies. According to the current review, minerals, play an essential role in animal production and reproduction issues. This chapter discusses the linkages of several mineral elements in health and reproductive performance that affects the dairy industry.

Keywords: minerals, health, reproductive performance, dairy cows

1. Introduction

Ruminants, like all other animals, must receive all the essential dietary nutrients, including water, protein, carbohydrates, fats, minerals, and vitamins in optimal amounts to maintain health, growth, and to reproduce at their potential levels. Climatic factors may modify the amount of certain nutrients that animals require and affect the responses to adequate diets. The responses to supplements will depend upon whether other essential nutrients are adequately supplied and whether animal management practices will allow responses. For example, feeding mineral supplements to cattle will not increase growth if protein energy is lacking [1, 2].

Mineral deficiencies and imbalances for herbivores are reported for almost all tropical regions of the world [3, 4]. Many reports from Africa, dating back to the

early part of this century, have revealed the beneficial effects of minerals supplementation on the overall performance, performance calving percent, and reduce mortality.

Grazing livestock does not usually receive mineral supplementation except for common salt and must depend almost exclusively upon forage for their mineral requirements, only rarely; however, can tropical forages completely satisfy all mineral requirements. Deficient levels of certain elements were noted for many forages in the world. Mineral deficiencies in soil and forages have long been held responsible for low production and reproduction problems among grazing tropical cattle. Plants withdraw essential elements from the soil solution in quantities to satisfy their own requirement as well as satisfy many of the requirements of grazing livestock. Besides essential plant elements, plants also withdraw Selenium (Se), Cobalt (Co), and Iodine (I) which are essential for the grazing ruminants. The soil-plant relationship is direct in that the plant must obtain all the mineral nutrients from the specific soil with which it has contact.

At least 16 minerals, elements are nutritionally essential for ruminants. There are 7 macro-elements, therefore, Calcium (Ca), Phosphorus (P), Sodium (Na), Chloride (Cl), Magnesium (Mg), Potassium (K) and Sulfur (S), and 9 trace or micro-elements, Iron (Fe), Iodine (I), Zinc (Zn), Copper (Cu), Cobalt (Co), Molybdenum (Mo), Manganese (Mn), Selenium (Se) and Fluorine (F). Others possibly required, based on limited evidence, are aluminum (Al) arsenic (As), Boron (B), bromine (Br), Cadmium (Cd), Lithium (Li), Nickel (Ni), Lead (Pb), tin (Sn), Vanadium (V), Silicon (Si) and Chromium (Cr).

Cattle play an important role in the livestock economy. The low performance of milk production and reproductive problems are common causes of profit loss to the farmers. During an animal's lifetime, a longer dry period and fewer calving's and lactations result in significant economic losses. Reduced calf crops hamper selection efficiency in long-term dairy herd improvement, whereas infertile animals result in a direct loss of milk production [5].

Minerals, in addition to energy and protein, play an important role in animal production and reproduction. Mineral deficiencies can create reproductive problems in livestock since minerals are essential for their health and reproduction. Infertility and reproductive abnormalities in our livestock herd have become a major issue. Both macro and trace minerals are essential for the synthesis of structural components of the body as well as the appropriate functioning of enzymes, hormones, vitamins, and cells. Mineral insufficiency and toxicity are localized problems. Minerals can have a positive or negative impact on an animal's physiological well-being, depending on the situation.

Approximately 5% of the body weight of an animal consists of minerals. Investigations have shown that ruminant livestock may deteriorate and fail to achieve a responsible level of productivity because of deficiencies and excesses of minerals in soils and plants. Furthermore, such deficiencies or excesses may give rise to problems of reproduction and to clinical signs indicating mineral deficiencies, such as wasting diseases, loss of hair, depigmented hair, skin disorders, non-infectious abortion, diarrhea, anemia, loss of appetite, bone abnormalities, tetany, low fertility, and pica [6, 7]. Soil fertility is defined as the ability of soil to supply proportionate and sufficient nutrients and water to plants [8, 9].

Minerals in the ration of dairy animals are important for regulating biological activities, growth, production, and reproduction. However, animals are ingesting a little quantity of minerals through feeds and fodder in the tropical climate. Also, soils from all over the country are getting depleted for one or more mineral elements in soil, plants, and animals [10]. In dairy buffaloes, the average intake of zinc was higher by 72.5% than National Research council (NRC) requirements for dairy cattle. The

average intake of copper was higher by 40% than National Research council (NRC) requirements. Increasing Cu intake decreased postpartum uterine involution and ovulation intervals ($r = -0.31, -0.32; P < 0.01$). Intake of Zn and Cu have favorable effects on various reproductive parameters and blood levels of macro-minerals [11].

Micronutrients, particularly mineral elements are inevitable for the normal metabolic and physiological processes of animal systems. The importance of minerals in regulating biological systems, growth, production, and reproduction is well documented [12, 13]. Hence, dairy cows depend on forages for their mineral requirements [14]. Garg et al. [15] and [16] both found high rates of forage and blood serum samples with Cu and Zn levels below the threshold levels. Cu and P deficiencies were found in fodder samples collected from the pasture by Miles and McDowell [17]. Cu, Zn, P, and S levels in soil, vegetation, and dairy cows have been decreased across the country [10]. The quantity of minerals, thus, present in forages may not be sufficient for optimum growth, milk yield, and reproduction when those were fed to dairy cows [14].

Macro minerals and trace minerals are equally important as they take part in the formation of the structural component of the body and the proper functioning of enzymes, hormones, vitamins, and cells [18].

Minerals are structural components of the body and play an important role in enzymes, hormones, body fluids, and tissues, as well as cell replication and differentiation regulators. Minerals are necessary for all animal physiological activities, including reproduction [19].

2. Minerals physiology

It must be realized that there are two categories of trace elements action: physiological and pharmacological. A trace element acts as an essential nutrient by preventing or reversing a deficiency, no effect is apparent when supplementation is given to an organism that is not deficient; this action is described as pharmacological. In contrast, the pharmacological action of an element is independent of the nutritional status [20].

2.1 Calcium (Ca)

Major Functions are bone and teeth formation; nerve function; muscle contraction; blood coagulation, cell permeability, essential for milk production. Absorption takes place in the duodenum by active and passive absorption (diffusion). Vitamin D required and the ratio of Ca:P is important. Excretion by feces as a major path, the urinary loss is minimal. Vitamin D involved in absorption & bone deposition; excess P and Mg decrease absorption; Ca:P ratio should not be below 1:1 and over 7:1 (1:1 to 2:1 for monogastric) [21].

2.2 Phosphorus (P)

The major functions are bone and teeth formation, phosphorylation, high energy phosphate bonds, Po; important in acid-base balance, a component of RNA, DNA, and several enzyme systems [2, 22]. Approximately 20% of the phosphorus content of the body is outside the skeleton, and less important function, occurring in nucleo-protein, ATP and other energy-rich compounds, energy and intermediate of carbohydrates metabolism [23, 24]. Absorption takes place in the duodenum, vitamin D is required, and a ratio of Ca: P (1:1) is important. Excretion through feces as a major pathway, and urine from a high concentration diet. Excess calcium and magnesium decrease P absorption [25].

2.3 Cobalt (Co)

Rumen micro organisms use cobalt for the synthesis of vitamin B₁₂ and the growth of rumen bacteria, components of adenosylcobalamin and methylcobalamin [22, 26–28].

Absorption as part of vitamin B₁₂, absorbed in the lower portion of the small intestine. Excretion mainly through feces, urine, and around 1–12% could be via milk [29].

2.4 Copper (Cu)

It is a co-factor in several oxidation-reduction enzyme systems such as hemoglobin synthesis, bone formation, maintenance of myeline of nerves, and hair pigmentation [30–34]. Absorption, principally in the small intestine, in ruminant only 1–3% of copper absorbed [35–37]. Excretion, via feces is the major route [38–40]. The dietary content of components known to reduce the availability of Cu, e.g. S, Mo, Zn, Cadmium, Fe or soil have been reported. The inorganic sulfate potentiates the adverse effect of Mo on the utilization of Cu by sheep [41–43].

Molybdenum does not interfere with Cu transport to the liver, but interferes with the synthesis of the plasma enzyme ceruloplasmin, the resultant apoceruloplasmin-like substances, with fewer Cu atoms per molecule and less enzyme activity, are catabolized more rapidly than holo-ceruloplasmin [38]. Pope [44] indicated that levels of forage Mo as low as 2, 0 ppm can be antagonistic to Cu metabolism. The inclusion of 10% of 3 diverse soils in the diet of sheep reduced the coefficient of true absorption for Cu by at least 50% [45]. Dick [46] in experiments with sheep receiving 30 mg Cu/day found that diets containing about 90 gm calcium carbonate/kg halved the hepatic retention of Cu. Campbell et al. [47] showed that liver Cu reserves in calves at pasture were depleted by a daily dose of Fe. Mills and Dalgarno [48] found that cadmium at 7 mg/kg added to the diet of the pregnant ewes reduced the liver-copper stores of her offspring. Consumption of rations containing 40, 220, or 420 mg Zn/kg reduced the fraction of dietary Cu retained by the liver of growing lambs [49].

2.5 Manganese (Mn)

It is essential for normal bone formation, activator and constituent of the enzyme systems, involved in oxidative phosphorylation, amino acids metabolism, and fatty acid synthesis [50–52]. In vitro experiments have shown that Mn in plasma became bound to two proteins, to α_2 -macro-globulin, in its divalent Mn²⁺ state and to transferrin in its trivalent Mn³⁺ state [53].

Most of the pathological changes that developed appear to be related to defective synthesis of mucopolysaccharides and glycoproteins and probably reflects the importance of the role of Mn in several glycosyl-transferase enzymes involved in their synthesis [54].

Absorption, is throughout the small intestine [55, 56] (Watson et al., 1973). Excretion, via feces with very small amounts in urine [57, 58]. Excess calcium and phosphorus decrease Mn absorption [2, 59].

2.6 Zinc (Zn)

It is a component or co-factor of several enzymes, including peptidase and carbonic anhydrase, needed for bone and for normal protein synthesis and metabolism (Prask and Plocke, 1971; Mills et al., 1967).

In dairy buffaloes, the average intake of zinc was higher by 72.5% than NRC requirements for dairy cattle. The average intake of copper was higher by 40% than National Research council (NRC) requirements. Increasing Cu intake decreased postpartum uterine involution and ovulation intervals ($r = -0.31, -0.32; P < 0.01$). Intake of Zn and Cu have favorable effects on various reproductive parameters and blood levels of macro-minerals [11].

A sensitive response to zinc intake is exhibited by the activity of the alkaline phosphatase of serum and bones, and by the activity of the pancreatic carboxypeptidase A, and perhaps by the biopotency of insulin [60, 61] (Kirchgissner et al., 1976b; Roth et al., 1974).

Absorption takes place in the rumen and small intestine [62–64]. Excretion is via feces, and small amounts in urine [65–68]. Miller et al. [69] strongly suggested that endogenous fecal losses of zinc are influenced by the nature of the diet and greatly reduced when zinc intake is low. Estimates of urinary Zn passes ranged from 0.004–0.019 mg/kg liver weight/day for cattle [70].

2.7 Magnesium (Mg)

Essential for normal skeletal development, as a constituent of bone; enzyme activator, primarily in the glycolytic system, helps to decrease tissue irritability. Absorption Throughout digestive tract, major site reticulorumen. Excretion via urine, feces & milk, urine major pathway. Sources are Magnesium oxide, Magnesium sulfate, Magnesium chloride, Magnesium carbonate. Interrelationships and toxicities are excess upsets Ca and P metabolism; toxicity not likely [21].

2.8 Iron (Fe)

Body Fe content is approximately 3–4 g, which almost corresponds to a concentration of 40–50 mg of Fe per kilogram of body weight [71]. The majority of Fe in the body is contained within hemoglobin [72]. Fe is an essential component of myoglobin [73]. Fe is also necessary for growth, development, normal cellular functioning, and synthesis of some hormones and connective tissue [72, 74, 75]. Absorption throughout the gastrointestinal tract, major sites are duodenum and jejunum. Excretion by feces, urine, sweat, and hair, hemorrhage can be a major loss. Sources are ferrous sulfate, ferrous carbonate, leafy plants, meats, legume seeds, and cereal grains. Cu is required for proper Fe metabolism. Too much Fe may be deleterious interfering with P, Cu, and Se [21].

3. Mineral sources

3.1 Forages

Only, rarely, can tropical forages satisfy all mineral requirements [76]. Feedstuffs of plant origin are generally poor in minerals. The commonly used fodder, cereals, cereal by-products, and vegetables derived concentrates do not contain sufficient minerals to meet the optimum requirements of farm animals [77, 78]. Mineral element concentrations in forages are influenced by a variety of factors, including soil, plant species, maturation stage, yield, pasture management, and climate. The impact of soil chemistry and features on the emergence of mineral issues in grazing ruminants has been studied [51, 58, 79]. Plants withdraw essential elements from the soil solution in quantities to satisfy their own requirement. Besides essential plant elements, plants also withdraw selenium (Se), Cobalt (Co), and Iodine (I), which are essential for the grazing requirements.

It is generally accepted that the herbs and legumes are richer in several mineral elements than grasses. As the plant matures mineral contents decline due to the natural dilution process and translocation of nutrients to the root systems.

In most circumstances the concentrations of phosphorus P, K, Mg, Na, Cl, Cu, Co, Fe, Se, Zn and Mo, decline as plants mature. Forage Ca concentrations is less affected by advancing maturity [2, 80].

Climate as well as forage management and yield influence plant mineral composition. In Africa, uncontrolled heavy grazing pressure causes many palatable genera to disappear and to be replaced by high lignified species. Grazing pressure also changes the leaf/stem ratio radically, therefore having a direct bearing on the mineral content of the plant. Increasing crop yield removes minerals from the soil at faster rates. Over liming can accumulate Se or Mo toxicity in livestock by increasing plant concentration of these elements [58].

Bhanderi et al. [81] discovered that the phosphorus level in concentrate ingredients was high (0.32–0.67%) but low in dry roughages (0.06–0.20%). Copper was abundant in greens (12.31 ppm). The zinc content of wheat straw was found to be low (19.71 ppm), but it was high in manganese (47.88 ppm) and iron (630.24 ppm). Cobalt was detected in abundance in lucerne and chikori green (>0.35 ppm).

Copper (Cu) content was found below the critical level (<8 ppm) in all types of straws and concentrate ingredients, except cottonseed cake. Zinc (Zn) content was below a critical level (<30 ppm) in all the straws except paddy straw. Greens and cakes were found to be a better source of Zn as compared to crushed grains. The Mn levels in the district ranged from 36.47–478.12 ppm in straws, 62.64–132.99 ppm in green fodders, 13.18–75.74 ppm in concentrate ingredients. These findings are in agreement with the observations of Youssef et al. [82], Yadav et al. [83], and Mandal et al. [84]. Cobalt content in feed and fodder resources was found in the range of 0.18 ppm to 0.71 ppm. Concentrates and roughages contained 0.25–0.67 and 0.06–0.20% P, respectively.

Ruminant production in different regions of the world are depends on forages to satisfy all their nutritional requirements. Forage and soil mineral imbalances are common, and forages are frequently low in essential trace minerals [14, 85].

Mineral shortages, such as those of the major elements Ca, P, Mg, Na, S, and the trace elements Co, Cu, I, Mn, Se, and Zn, can impact the output of grazing animals at pasture in most parts of the world [86–92].

The nutrition of grazing animals is a complicated interaction of soil, plant, and animal. Forage Cu^{2+} concentrations were found to be sufficiently high to meet the demand of animals (8 mg/kg) during both seasons (winter and summer) [34]. Forage Co^{2+} levels were deficient for ruminants during both seasons because these were lower than the critical level [93]. Except for P and Cu, Co^{2+} insufficiency is the most common mineral deficiency in grazing animals [94]. Plant absorption of Co^{2+} is influenced by soil Co^{2+} and Mn^{2+} concentrations. Mn^{2+} deficiency in the soil reduces Co^{2+} uptake in forages. High levels of Mn^{2+} were observed in the soil in this study, which could have resulted in reduced Co^{2+} absorption by plants and, as a result, low levels in plant tissues. McKenzie [95, 96] claims that soils with high levels of manganese oxide bind free soil Co^{2+} to their surfaces, resulting in reduced Co^{2+} availability to plants. Low Co^{2+} concentration of soil was also a possible explanation of the high level of Mn^{2+} in forage as these elements antagonize the soil [95, 96]. Several factors including soil, plant species, pasture management, and climate, may affect the likelihood of Zn^{2+} deficiency in ruminants. Cox [97] reported the low level of Zn^{2+} in soil and plants. Plant maturity has also been reported to affect Zn^{2+} concentration of forage [31, 98–100].

The mineral composition of plants also termed the ionome [101], is an integrated outcome of interactions between endogenous plant processes and the

environment [102]. Leaf mineral composition, the leaf ionome, reflects the complex interaction between a plant and its environment including local soil composition, an influential factor that can limit species distribution and plant productivity. Land plants require several inorganic mineral nutrients that they must take up from the soil solution into roots and partition appropriately within the plant [103–105].

Kitchell (1963) reported an average Co concentration of 0.11 ppm in dry matter in mixed pasture herbage, and mean Co levels of 0.10, 0.20, and 0.26 in the hay by Zacherl et al. [106], Schiller et al. [107], and Kocialkowski et al. [108]. Grassland grasses have a lower Co concentration than legumes, while legumes have a greater Co level [109–111]. As a result, if legumes are present in the forage, animals are less prone to acquire Co deficiency. Cereal grains, especially maize, are poor suppliers of Co, with concentrations typically ranging from 0.01 to 0.06 ppm. Wheat bran contains 0.12 to 0.16 ppm, while leguminous seed and oilseed meals contain 0.2 to 0.3 ppm Co [58].

A pasture's various plant varieties provide grazing animals with a reliable source of mineral nutrients. Ruminant production is hampered by an insufficient supply of mineral nutrients. A proper analysis of the mineral levels in the animal body, forages, and soil is required to determine whether animals have an acceptable requirement for mineral nutrients. Although grazing livestock obtains its mineral requirements through forages, they are unable to fully meet the demands of animals in terms of mineral elements [3].

Co levels in forage varied from 0.42 to 0.60 mg/kg. These levels, however, were higher than the threshold level of 0.01 mg/kg [34]. The amounts of forage Co discovered in our study were greater than those previously reported by Khan et al., [112] and Espinoza et al. [113]. Co deficiency has been observed to be the most limiting factor for animals grazing in various areas.

Mn concentrations in forage ranged from 153.43 to 215.20 mg/kg. According to McDowell, all mean Mn readings were over the threshold limit of 20 mg/kg [4]. These values for ruminants were higher than those found by previous studies [114] in various parts of the world. These values, however, are within the range reported by Pastrana et al. [115].

3.2 Soil

In some cases, a soil assessment might reveal the presence of important minerals deficiency in animals. Soil concentrations of Co, Mo, and I reflect the plants' concentrations of these elements to a certain degree. However, several factors affect forage mineral uptake from the soil, including, the yield of the plant, stage of maturity, species and strain deficiencies, climatic and seasonal conditions, chemical forms of minerals, and factors of the soil, including pH and degree of aeration and water logging. Soil analysis though useful for pasture fertilization has been eliminated in some investigations because of its direct relationship to the mineral content of herbage growing on the soil (Gitter et al., 1975). For instance, a plant growing on cobalt-deficient soil may not necessarily be deficient in Co, nor would a soil rich in Co necessarily yield plants with high levels of Co (Lattuer, 1983). However, in the Netherlands, soil analysis is preferred to that of forage analysis to establish a cobalt deficiency [116].

Hartmans [117] reported that the availability of Cu in the soil does not show any positive relationship with the Cu status of the animal. Data from Brazil, Bolivia, Guatemala, Malawi, and Florida (USA) have indicated that mineral correlation among soil, plant, and animal tissue concentration was highly variable among locations, and are often low and nonexistent [4].

The soil was deficient in P. The overall mean concentration of P (9.42 ± 0.59 ppm) in soil, whereas, in plants the overall mean of P (2913 ± 470 ppm). The dairy cattle

blood serum was found to be deficient in P. Overall incidence (%) of deficiency in cattle blood serum was found to be 74.50% in P. The incidence of deficiency in cattle blood serum was found to be above 70%. In cattle blood serum, P (2.90 ± 0.380 mg/dL). A significant positive correlation was found between plants and cattle for P [118].

Productive and reproductive performance of dairy cattle is mainly dependent on their nutritional status. Here, the role of soil and the nutritional quality of plants plays a very important role. Animals are malnourished particularly with regards to lack of micro and macro mineral of soil, and feed. The availability of minerals in soil depends upon their effective concentration in soil which is influenced by pH, moisture, organic matter, leaching, and the presence of other elements [119, 120]. The concentration of minerals in plants is affected by the soil-plant interaction, pH, species, stage of maturity of plant, etc. [2]. There appears to be a definitive role of minerals deficient soil to cause deficient levels in feeds (McDowell and Conrad, 1990).

The concentrations of some trace minerals varied greatly among seasons and sampling periods. Seasonal effects were found in all soil microminerals except zinc, while forage iron, zinc, and selenium were affected by seasonal changes. All soil mineral levels except cobalt and selenium were sufficiently high to meet the requirements of plants for normal growth during both seasons (winter and summer). During the summer, forage Zn levels were marginally insufficient, but all other forage micro-minerals were within the necessary range for ruminants in both seasons. Although fodder microminerals were within the range required by ruminants, they were not high enough to prevent ruminant predisposition to nutrient deficiency-related illnesses.

Soil materials high in clay were highest in all analyzed elements Speirs et al., (1989). The overall range for cobalt in soils on a worldwide basis is 0.1–70 ppm [98] and the average amount is 8 ppm [121].

Mean copper contents for uncontaminated soils worldwide range from 13 to 24 ppm, but the overall range for world soils is higher (1–140 ppm) depending on the nature of the soil parent materials [98]. Mn levels in world soils range from 7 to 9200 ppm, with an estimated grand mean of 437 ppm [98]. Manganese is found in soils and minerals mostly as Mn^{2+} , Mn^{3+} , and Mn^{4+} [98, 122] but only Mn^{2+} is absorbed by plants [121]. The overall zinc content of soils ranges from 10 to 300 ppm [121].

Co deficiency in ruminants is relatively widespread, resulting in loss of appetite, progressive emaciation retarded sexual development, muscular atrophy, or anemia. Sometimes the only symptom may be very slow growth or none. It has been shown that vitamin B₁₂ is synthesized in the rumen by micro-organisms, hence the ruminant's special requirements for dietary Co. The average content of Co in the lithosphere has been estimated to be about 40 ppm and the total Co content of soils is usually in the range of 1–40 ppm (Almond [123], Hawkes [124], Swaine [125]). Many pasture soils in different parts of the world are known to have too low a Co content. However, many instances of Co deficiency have been reported from soils of 2–5 ppm or less of total Co (Bear [126], Stewart [127], Sullivan [128], Young). The level of deficiency lies about 0.25 ppm in mineral soils [129].

The average occurrence of Cu in the earth's crust has been estimated to be 70 ppm and its total content in soils ranges usually from about 2 to 100 ppm have been reported (Muff [130], Layering [131], Mitchell [132], Vogt). The content of Cu in most normal plants is usually within the range of 5 to 25 ppm but varies with plant parts and species, state of maturity, soils, etc. Bear [126] gives average Cu plant contents varying from 12 to 20 ppm.

The typical Mn content in the lithosphere is 1000 parts per million, whereas total Mn concentrations in soils range from less than 100 to several thousand parts per million. The total Mn concentration of the soil, on the other hand, cannot be used to predict the availability of this element to plants due to many factors that influence uptake. Among the factors affecting Mn availability, soil pH and the oxidation-reduction conditions may be the most important since the forms in which Mn occurs in soils are strongly related to these factors.

The lithosphere's Zn content has been estimated to be around 80 ppm, while total Zn content in soils has been recorded to range from 10 to 300 ppm, with occasional lower and higher levels. However, because of various factors impacting Zn availability to plants, the total level of Zn in soils has been found to be an inconsistent indicator of soil Zn status. Zn shortage can occur in a variety of soil textures, but it is most common in sandy soils. Viets et al. [133] found that Zn is more readily available in acidic soils than in alkaline soils. Zn content in plants typically ranges between 20 and 100 ppm dry weight, however it varies by plant species and plant sections. Plant maturity, the nature of the soil in which it grew, meteorological circumstances, and, of course, Zn fertilizer all have an impact on the Zn content of plants.

The effects of sample times on soil Co, Fe, and Mn were not significant. In this study, all mean soil Fe and Mn values were higher than the requirements of forage crops, whereas the opposite was true for soil Co. The influence of sample times on forage Fe and Co was found to be non-significant, however, forage Mn showed a constant increase in forage Fe and a drop in Co and Mn with sampling time. From this pattern of nutrient transfer from soil to forage, a toxic range of these nutrients is possible for the animals at any time during the year.

The soil was deficient in P. The overall mean concentration of P (9.42 ± 0.59 ppm) in soil, whereas, in plants the overall mean of P (2913 ± 470 ppm). The dairy cattle blood serum was found to be deficient in P. Overall incidence (%) of deficiency in cattle blood serum was found to be 74.50% in P. The incidence of deficiency in cattle blood serum was found to be above 70%. In cattle blood serum, P (2.90 ± 0.380 mg/dL). A significant positive correlation was found between plants and cattle for P [118].

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Analysis of variance shows the non-significant effect of sampling periods on soil Co concentration. Mean soil Co concentrations varied from 0.665 to 0.789 mg/kg during all sampling intervals. All mean soil Co values were higher than the critical level of 0.1 mg/kg as described by McDowell et al. [17].

Soil Mn concentration values ranged from 54.75 to 69.38 mg/kg. These levels, however, were higher than the essential limit of 5 mg/kg proposed by Rhue and Kidder [134]. Similar soil Mn concentrations were observed by Prabowo et al. [135]. The current study's results, however, were lower than those reported by Espinoza et al. [113].

4. Mineral elements requirements

No organism can attain its full potential of growth and performance unless its requirements for all essential nutrients are met. Variation in the composition of the body and its secretion and in the absorption of an element from the gut presents problems in terms both of the amount of basic information needed to give reliable mean values and of allowing for the variation in dietary requirements of different animals in specific productive status [22, 136].

The following minerals are known to be required by one or more animal species for a normal life, processes, Co, I, Fe, Cu, Zn, Mn, Se, Cr, F, Mo, and Si [80, 137]. Other possibly required based on limited evidence are aluminum (Al), arsenic (As), boron (B), bromine (Br), cadmium (Cd), lithium (Li), nickel (Ni), lead (Pb), tin (Sn) and vanadium (V). These elements are referred to as newer trace elements [2, 138–140]. Inadvertent high soil ingestion is favored when soil has a weak structure and poor drainage, high stocking rate, high earthworm population, and during months when pasture growth is poor [2, 141]. Rosa [142] reported that the inclusion of 10% Costa Rican soil in the diet of sheep decreased apparent and true phosphorus absorption. Mertz [143] illustrated that soil is a very important source of trace elements, as well as iron (Fe). Fonseca and Lang (1976) reviewed that Mn accumulation by plants was dependent upon soil pH.

Langlands et al. [144] studied the effects of stocking rate and soil ingestion on Cu and Se status of grazing animals, it was concluded that both Fe and Cu concentrations in the tissues of grazing sheep may be decreased when stocking rate is increased. Suttle et al. [45] conclude that the Co antagonists Mo and Zn are biologically available in the soil and their ingestion from soil contamination of herbage may be a factor in the etiology of hypocuperiosis.

In temperate areas, frequent glacial coverings removed the older soils, and in the early stages of rock washing to the soil, there was continued slow release of soluble ions [145, 146]. Young and alkaline geological formations are generally more abundant in most trace elements than the older, more acid, coarse sandy formation [117] Dutoit et al. [147] in South Africa performed soil and herbage analysis, and also used cattle blood as an indication.

All elements present a lower concentration than 50 mg/kg body weight, on average, maybe called trace elements. For trace elements to be essential, too low a

concentration in food must result in deficiency symptoms that can be prevented or cured by dietary addition of this element [148, 149]. All elements known to participate as activators or components of enzymes in biochemical reactions and to be thus deeply engaged in metabolic functions are considered essential [150].

Many factors affect mineral requirements including nature and level of production, age, level and chemical form of elements in the feed ingredients, interrelationship with other nutrients, supplemented mineral intake, breed, and animal adaptation [151, 152].

Adequate intake of forages by grazing ruminants is essential to meet mineral requirements. Factors that greatly reduce forage intakes such as low protein (> 7.0%) and energy content and increased degree of lignification, likewise reduce the total mineral consumed [2].

The dietary requirement of P for lactating cows with milk yield 20 kg/day was 44–51 g/day. The dietary requirement of P for lactating ewes with milk yield 3 kg/day and live weight 40 kg was 3.7 g/day, and for sheep at 13 weeks of pregnancy and live weight 40 kg was 1.4 g/day [22]. For lactating cows of 500 kg live weight and 20 kg milk/day, the net requirement for Cu was 5.5 mg Cu/day, and the dietary requirement was 138 mg Cu/day, and the relative requirement was 8–11 (19.2–15.2) mg Cu/kg diet dry matter. For lactating ewe of 75 kg live weight and 3 kg milk yield, the net requirement was 1.24 mg Cu/day, and the dietary requirement was 1.24 mg Cu/day, and the dietary requirement was 20.7 mg Cu/day.

The Co dietary requirement of sheep and cattle fed pasture diet was 0.08–0.11 mg/kg DM. 0.08 mg regarded as marginal, 0.11 mg as adequate. The requirement for Zn will be fully met by rations providing approximately 30 mg Zn/kg DM [22].

The requirements of Mn for growth may be rations providing 10 mg/kg DM, about 20–25 mg/kg DM is needed to permit optimum skeletal development. Experimental results so far available suggested that this should be adequate to meet requirements for reproduction [22]. **Table 1** showed some mineral requirements and critical level for ruminants [2].

National Research council (NRC) [153] has recommended for dairy cattle the level range from 0.3 to 0.4%. Increasing the concentration of dietary phosphorus above the requirement (more than 0.38–0.40%) does not improve reproductive performance [154].

Depending on the stage of lifecycle and dry matter consumption, the required Zn dietary value for dairy cattle is normally between 18 and 73 ppm. Cu, Cd, Ca, and Fe interact with Zn metabolism and limit its absorption [18]. Dairy cows require 40 parts per million of zinc in their diet [153]. The maintenance requirement for absorbed Mn was defined at 0.002 mg/kg of body weight, whereas the growth requirement was set at 0.7 mg/kg of growth, pregnancy at 0.3 mg/d, and lactation at 0.03 mg/kg of milk [153]. Cattle may require up to 50 mg of Mn/Kg of DM during pregnancy because it aids in skeletal cartilage growth and fetal bone formation [155]. A cobalt deficiency ultimately resulted in vitamin B12 deficiency. Manganese, Zinc, iodine, and monensin may reduce cobalt deficiency. The dietary requirement for a lactating cow is 0.11 ppm of the ratio of dry matter intake (Balamurugan et al. [18]).

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| Elements | Animal requirements | | | Tissue | Critical levels |
|----------|---------------------|-------------|-------|--------|-----------------|
| | Dairy cows | beef cattle | sheep | | |
| P | 0.34 | 0.17–0.59 | 0.16 | Plasma | 4.5 mg/100 ml |
| Cu (ppm) | 10 | 3–10 | 0.38 | Serum | 0.65 µg/ml |
| Mn (ppm) | 40 | 20–50 | 7–11 | Liver | 6 ppm |
| Zn (ppm) | 40 | 20–40 | 20–40 | Serum | 0.6–0.8 µg/ml |
| Co (ppm) | 0.10 | 0.05–0.10 | 30–33 | Liver | 0.05–0.07 ppm |

Source: McDowell et al. [17]; McDowell et al. (1976).

Table 1.
Mineral requirements and critical level for ruminants.

at 0.03 mg/kg of milk [153]. Cattle may require up to 50 mg of Mn/Kg of DM during pregnancy because it aids in skeletal cartilage growth and fetal development.

5. Roles of minerals in health

Clinical signs of mineral deficiencies in animals gave a good indication of the mineral status. Alopecia, parakeratosis, hair discoloration, weakness, and fertility were the major clinical signs at the different locations. In this study, Zn deficiency symptoms reported by Pallauf and Kirchgessner [156]; Kirchgessner et al. [60, 61], and Schwarz and Kirchgessner [157], were low growth, weight loss, alopecia, and severs parakeratosis. Idris et al. [1] observed that clinical signs of Cu deficiency were anemia, stunted growth, hair discoloration, infertility, and diarrhea. Impaired growth and poor reproductive performance were the most significant features of Mn deficiency in ruminants [22, 54, 56].

All the diseases recorded in the farm health records were reported. Type of veterinary health care, cow calendar, vaccination program, and clinical signs of mineral deficiencies for each survey were also reported. Repeat breeder's percent, average calving interval, average conception rate, percent and age of delayed puberty heifers, and average milk yield for each survey were reported. As stated by Underwood (1983), changes in an animal's appearance or level of production can often be early identification of diet inadequacy. Severe or acute deficiencies of minerals are often characterized by specific clinical signs, but disorders are often mild or marginal, expressed only as vague unthriftiness, or suboptimal growth, fertility, or productivity. These changes are often non-specific and indistinguishable from those resulting from inadequate energy, protein, or vitamins, or from parasitism, or toxic plants. Therefore, it often becomes necessary to resort to chemical analysis in order to adequately determine mineral insufficiencies (Underwood, 1983).

In fact, approximately 5% of the bodyweight of an animal consists of minerals. Investigations have shown that ruminant livestock may deteriorate and fail to achieve a responsible level of productivity because of deficiencies and excesses of minerals in soils and plants. Furthermore, such deficiencies or excesses may not only result in low production but may give rise to problems of reproduction and to clinical signs indicating minerals deficiencies usually encountered worldwide, such as wasting diseases, loss of hair, depigmented hair, skin disorders, non-infectious abortion, diarrhea, anemia, loss of appetite, bone abnormalities, tetany, low fertility and pica [6, 7].

A dietary deficiency of either Ca or P is sufficiently prolonged, however, results in skeletal abnormalities, subnormal growth, depraved appetite, rickets, and

osteomalacia [23]. Uncomplicated Ca deficiency symptoms included hemorrhages and delayed coagulation of the blood (Martin, 1937; [13]). P deficiency in dairy cows resulted in a reduction in milk yield, loss of condition, depraved appetite, and temporary sterility [23, 158].

Zinc deficiency symptoms reported by Pallauf and Kirchgessner [156], Kirchgessner et al. [60, 61], and [157] were anorexia, low growth, weight loss, alopecia, severe parakeratosis, and epidermoid lesions [159, 160]. Apart from parakeratosis, the most characteristic consequence of Zn deficiency is the abnormal ossification of the skeleton, whereas poor ossification has been found in rats and growing pigs [161].

Copper deficiency causes, anemia, stunted growth, bone deformation, change in hair color, infertility, diarrhea, enzootic ataxia or swayback in sheep, or fatting disease in cattle [1]. Mo-deficiency in cattle is characterized by intense diarrhea, and a change in coat color.

Mg deficiency is uncommon [162, 163]. The early and moderate symptoms of Mg deficiency include loss of appetite, nausea, vomiting, fatigue, tingling or numbness, rapid heartbeat, delirium, hallucinations, retention of sodium, low circulating levels of parathyroid hormone, and weakness [164–166].

The effect of cobalt deficiency in cattle and sheep are those of vitamin B₁₂ deficiency and range from a mild deficiency with an ill-defined and transient thriffliness with no clear clinical signs to moderate or severe deficiency with appetite failure, emaciation, and listlessness accompanied by characteristic pallor of the skin and mucous membranes caused by progressively increasing anemia [28] (ARC, 1989).

Impaired growth, development of skeletal abnormalities, poor reproductive performance, and ataxia of the newborn are the most significant features of manganese deficiency in ruminants [54, 56] (ARC 1989). Low Mn levels in the body (Mn deficiency) have been linked to hypercholesterolemia, impaired glucose tolerance, dermatitis, hair color changes, skeletal abnormalities, infertility, deafness, and impaired synthesis of vitamin K-dependent clotting factors [167–169].

Iron (Fe) is necessary for growth, development, normal cellular functioning, and synthesis of some hormones and connective tissue [72, 75]. In the case that the body supply of available Fe is too low, this led to a condition known as Fe deficiency. Fe deficiency causes an inadequate amount of hemoglobin to meet body's oxygen transport needs. When the deficiency becomes severe, the condition is diagnosed as Fe-deficiency anemia [170, 171]. The most common symptoms of Fe-deficiency anemia are tiredness and weakness due to the inadequate oxygen supply to the body's cells and paleness due to the decreased levels of oxygenated hemoglobin. The other symptoms include fatigue, dizziness, hair loss, twitches, irritability, impaired immune function, pagophagia, and restless legs syndrome [170, 172, 173].

6. Roles of minerals in reproductive performance

Minerals are required in reproductive processes because of their role in maintenance, metabolism, and growth [174]. Requirements for minerals are influenced by several factors that include age, stage of pregnancy, and stage of lactation [175]. Apart from energy and protein, mineral deficiencies such as calcium, phosphorus, iron, zinc, and copper have been reported to be a risk factor for placental retention [18], repeat breeding in dairy cows [176] (Kumar, 2014), abortion [177], and weak calf syndrome [177, 178]. Minerals are divided into two categories based on their requirements: macro minerals, which require more than 100 ppm in the diet and include calcium, phosphorus, magnesium, potassium, sulfur, sodium, and chloride; and micro minerals, which require less than 100 ppm in the diet and include

calcium, phosphorus, magnesium, potassium, sulfur, sodium, and chloride. Trace or micro minerals, such as cobalt, copper, iodine, iron, manganese, selenium, and zinc, fall into this category and are required in amounts of less than 100 ppm in the diet [18].

Mineral deficiency has also been strongly associated with decreased reproductive performance in dairy cows. Inactive ovaries (anaestrous) delayed sexual maturity and low conception rates have been reported when phosphorus intakes are low. Other minerals such as copper, manganese, and cobalt deficiencies have been associated with impaired ovarian function, silent anestrus and abortions.

Dekruif [179] illustrated that for optimal reproductive performance, cows must conceive within 80–85 days of calving. Ward et al. [180] illustrated that the calving interval is the best index for monitoring herd reproductive status. The calving interval is 477–523 days. Chantaraprateep and Humbert [181] reported that a repeat breeder cow is a cow that inseminated more than three times and is still not pregnant.

For cattle, the female age at puberty are 6–10 months, the usual age at first service is 14–22 month, the length of the oestrus cycle is 21 days, the oestrus cycle type is polyestrous, the duration of oestrus is 18 hours, the gestation length is 280 days, and the first postpartum oestrus used for breeding is first after 42 days [182].

Many factors influence the amount of time between parturition and first oestrus in tropical cattle, including endocrine events, management, nutrition, heat and humidity, genetic-environmental interactions, illnesses, and internal and external parasites. Nutritional factors that result in reduced hemoglobin level (trace minerals deficiencies, and parasites infestation) also cause prolonged postpartum anoestrus and infertility [183].

Calving interval is taken as the best index for monitoring herd reproductive status [180]. According to Chantaraprateep and Humbert [181], a repeat breeder cow is a cow that is inseminated more than three times and still not pregnant. The normal gestation length is 280 days [182], and a cow with good reproductive performance would conceive within 80–85 days after calving [179]. For Zebu cattle, age at puberty, first oestrus, and first calving were found to be 858, 930, and 1185 days respectively [184, 185]. McDowell (1968) stated that the calving to oestrus was 56 days. McDowell (1972) found that the incidence of anoestrus was only 13% at 80 days postpartum intervals can be attained even in a hot climate.

Progesterone concentrations in the plasma of recently calved cows and delayed puberty heifers were low. According to Sijiu and Beixeng [186], before the first oestrus, plasma progesterone concentrations were 3.9 ± 0.3 nmol/L, and reached 16.6 ± 3.2 nmol/L on day 15 of the cycle. Eduvie et al. [187] stated that a progesterone concentration of 1.59 nmol/L was taken as indicative of attainment of puberty. According to Hansel and Alila [183], the length of the period from parturition to first oestrus varies greatly in cattle in the tropics and is influenced by many factors including, endocrine events, management, nutrition, heat and humidity, genetic-environment interacting, diseases, and external parasites, similarly interval from calving to first oestrus and subsequent pregnancy rare are influenced by prepartum and postpartum nutrition [188–190], sucking status [191, 192].

The major causes of non-infectious infertility in dairy animals are due to energy, protein, and mineral deficiencies mainly of calcium, phosphorus, trace minerals (copper, cobalt, zinc, iodine, and manganese), and other salts [193]. The mean blood zinc levels were higher ($p < 0.05$) in anestrus than in subestrus and/or repeat breeding cows and buffaloes. Further, the plasma phosphorus, copper, and cobalt concentrations were found to be non-significantly higher in repeat breeders than in anestrus or subestrus cows and buffaloes were deficient in some animals indicating its role in causing infertility in dairy animals.

The plasma levels of phosphorus were found to be non-significantly higher in repeat breeders than in the anestrus or subestrus cattle. Among trace minerals, plasma zinc levels were lower, while copper and cobalt levels were higher in repeat breeder cattle as compared to anestrus or subestrus ones [194]. Kumar et al. [195] and Butani et al. [196].

The mean of studied essential trace minerals in retained placenta (RP) revealed a decline in Zn^{2+} , Cu^{2+} , and total iron (TF) by 68.9%, 65.7%, and 19.4% respectively. Additionally, all studied minerals exhibited a significant reduction in both non-retained placenta (NRP), and RP groups compared to heifers (HEF) group. Also, it was reported that buffaloes with RP are significantly deficient in Zn^{2+} which has an important role in preserving the uterus following parturition as it helps in the healing process and immune system during the convalescent stage [197]. TF in the case of RP was lower than NRP which attributed to Cu^{2+} reductions as required for the biosynthesis of hemoglobin.

The mineral profile of anestrus cattle results showed that the mean Copper (ppm) for anestrus island cattle was 0.04 ± 0.005 and for coastal cattle was 0.02 ± 0.004 . The mean Manganese (ppm) values recorded were 0.031 ± 0.007 in the case of island cattle and 0.024 ± 0.003 for coastal cattle. The mean Zinc (ppm) value in the case of the island and coastal cattle was 0.33 ± 0.11 and 0.33 ± 0.07 respectively. Statistical analysis of the data revealed that there was no significant difference in the values of Copper, Manganese, and Zinc between the cattle of both coastal and island ecosystems.

The mineral profiles of repeat breeder cattle depicted the mean Copper (ppm) for repeat breeder island cattle was 0.05 ± 0.006 and for coastal cattle was 0.03 ± 0.005 . The mean Manganese (ppm) values recorded were 0.023 ± 0.005 in the case of island cattle and 0.021 ± 0.004 for coastal cattle. The mean Zinc (ppm) value in the case of the island and coastal cattle was 0.33 ± 0.12 and 0.32 ± 0.09 respectively.

The level of P in cattle of island ecosystem was found to be 3.54 mg/dl, whereas, in coastal ecosystem, the values were 3.60 mg/dl. The present value of P corroborates the finding of Ramakrishna [198].

The serum concentration of zinc (0.32 to 0.33 ppm) was against a normal range of 0.8–1.2 ppm which speaks of a mild deficiency of zinc in the animals. Comparable values have been reported by Sahoo et al. [199]. The serum levels of copper were well within the physiological range (0.7–1.5 ppm) indicating a normal copper level. The observation found the support of Sahoo et al. [199]. The serum manganese during the present study was slightly below the normal range of 0.4–0.8 ppm. A comparable range of manganese levels had been reported by Sahoo et al. [199]. However, lower values had also been reported by Modi et al. [200].

One of the primary constraints limiting livestock production is inadequate nutritional resources on a year-round basis [201]. Many reports reviewed the beneficial effects of mineral supplementation on reproductive performance among animals [143]. Mineral deficiencies in soils and forages have been responsible for low production and reproduction problems among grazing tropical cattle [2]. Molybdenum-induced interference in luteinizing hormone (LH) that delays puberty in heifers is caused by a disruption in ovarian steroid secretion [202].

Zinc importance in spermatogenesis coupled with its synergic role in uptake by spermatozoa of vitamin A, particularly as vitamin A plays an essential role in the attainment of puberty and the maintenance of both libido and integrity of testicular germinate epithelium (Hurley and Doanc, 1989). Master and Moir [203] showed that ewes given a Zn diet (4 mg/kg) produced lambs that were 17% lighter at birth than those receiving an adequate Zn diet (50 mg/kg).

Macpherson et al. [29] showed that the increase in calving rate to the first insemination in dairy heifers is by correcting a selenium deficiency before mating.

Mohammed et al. [204] reviewed that cows with blood selenium concentration > 169 ng/ml had twice the risk of developing cystic ovaries than cows with levels <108 ng/ml. Tasker et al. [205] reported that Se supplementation improves the conception rate. Deficiencies of specific minerals such as Ca, P, Cu, Fe, and I may cause post-partum anoestrus [206] (Surendra Singh and Vadnere, 1987).

The animals showed general weakness, parakeratosis, achromotrichia, and infertility [207]. Suttle [208] reported that the economic importance of Cu deficiencies has been emphasized by the discovery of unsuspected cases of loss, increased susceptibility to cattle. Ingraham et al. [209] illustrated that Cu and Mg supplementation improves the conception rate. Lavin et al. [210] showed that plasma Cu values decreased with advancing gestation, and increased after calving, and were lowest in cows returning repeatedly to service. Dhoble and Gupta [211] discussed the role of Ca and P low values in postpartum anoestrus. Fisher and Macpherson [212] reported that cobalt treatment had a significant effect on ewe serum vitamin B12 and methyl-malonic acid concentration. Cobalt deficient ewes produced fewer lambs and had more stillbirth and newborn mortalities than cobalt sufficient controls.

7. Conclusion

Mineral diet can have a significant impact on animal reproduction, as evidenced by the current review. This study would highlight the role of soil minerals in farm animal reproductive function, including infertility, met energy-protein requirements, and mineral deficits. Mineral deficiencies may be a major cause of infertility in dairy cows in different parts of the country. Exotic and crossbreeds showed increased severity of infertility than local breeds. This reflected the increased mineral requirements of these breeds. Compared to milkers, dry cows had severe trace element deficits.

The bulk of the animals with low fertility was found to be weak in P, Cu, Zn, Mn, and Co. Analysis of soils and plants could provide some insight into mineral levels in animal blood. With the use of clinical symptoms and serum analysis, an accurate picture of the animals' mineral status could be established. Satisfying the animal's energy-protein requirements is insufficient to express the animal's maximum reproductive potential. Mineral supplementation in animal diets, particularly those of plant origin, should be addressed.

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Predicting Trends, Seasonal Effects, and Future Yields in Cow's Milk through Time Series Analysis

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Abstract

A dairy is a place that is used for handling milk and milk products. Dairy products are basically based on milk. Milk is used to prepare dairy products, such as butter, cheese, and milk powder. There is always a great demand for milk and milk products among people. This study attempted to investigate the trends in the actual yield of cow's milk production at Andassa dairy farm. We used secondary data for the study of the daily milk production of cows at Andassa dairy farm. The specific objectives of the study were—to identify whether the milk production is time-dependent or not; to predict in which season the milk production is high or low; to examine the daily trend analysis of milk production; to fit the appropriate model; and to forecast the milk production for the future. The study was conducted based on quantitative variables. So, the dependent variable is the average daily milk, and the independent variable is the time measure at which milk production is measured each day. The study used both descriptive and inferential statistics to analyze the data that were collected from the dairy farms in the sector. This study covered a total of 179 days of milk production. The results reveal that the milk yield of cows is declining, and that milk output is time-dependent, according to the time series plot, and that the model is ARIMA.

Keywords: milk production, time series analysis, forecasting

1. Introduction

A dairy is a place that is used for handling milk and milk products. Dairy products are basically based on milk. Milk is used to prepare dairy products, such as butter, cheese, and milk powder. There is always a great demand for milk and milk products among people. Most of the time, milk is used as a complete food for infants. It is used in all homes, hotels, and restaurants as well as in milk products. Most countries are expanding their production systems to increase the supply of milk and fulfill the needs of the people [1].

Unless it is produced and handled under sanitary circumstances, milk is an essential route for the spread of harmful germs to humans. As a result, sanitary milk production must be prioritized in order to give more high-quality milk to the general population. Consumers need clean, healthy, and nutritious food, which has been produced and processed in a safe, sanitary manner, and is free of pathogens [2]. As a result, premium milk production is required to meet consumer demand. Milk that is

free of pathogenic bacteria and hazardous poisonous compounds, free of silt and extraneous elements, of good flavor, of normal composition, adequate in maintaining quality, and low in a bacterial count is considered to be of high quality; on the other hand, the superiority of milk has persisted deprived [3]. A number of technological innovations now permit automated daily monitoring of cow performance [4]. The use of automated milk yield recording systems for early detection of diseases requires a statistical model to forecast expected performance and actual performance. Previous research on modeling milk production in cows has focused on fitting linear or nonlinear deterministic models to daily, weekly, or monthly milk measurements. Milk production is an integral part of the Andassa agricultural farming system. Even though the area has potential for milk and dairy products, little is known about the existing dairy production system, constraints, and opportunities associated with dairying in this area. It is essential that researchers and dairy development agents understand the existing situations in order to design relevant development strategies for the specific regions [5]. Therefore, the main objective of the study was to predict trends, seasonal effects, and future yields in cow's milk through time series analysis.

2. Data and methods

This study was conducted at Andassa dairy farm, which is located in the Amhara region in northwestern Ethiopia. At Andassa dairy farm, milk production was recorded in liters per cow on a daily basis. This study employed only secondary data obtained from this dairy sector.

2.1 Statistical method

A time series is a set of ordered observations of quantitative variables taken at successive points in time. In other words, it is a set of observations recorded over time, which is usually at equal intervals. Stationary is a critical assumption in time series models. Stationary implies homogeneity in the sense that the series behaves in a similar way regardless of time, which means that its statistical properties do not change over time. Trend analysis is the characteristics of a time series that extends consistently throughout the entire period of time under consideration. In this scenario, trend analysis was used to anticipate the future amount of milk products based on the historical trend of milk production. In this case, we will look at a linear trend and estimate it using the least square estimation method, double moving average, and double exponential smoothing [6].

2.2 Autocorrelation function and partial autocorrelation function

The two moments of any random variable, namely its mean and variance, are well known, and since we established in the introduction that a time series is a realization of a stochastic process, this holds true for any time series. In Box–Jenkins model, the partial autocorrelation plot or partial correlogram is also often employed for model identification [7].

3. ARMA model

The ARMA model is a mixed model in which the series is partly autoregressive and partly moving average. As a result, we get a very generic time series model, as shown below.

$$Y_t = \Phi_1 Y_{t-1} + \Phi_2 Y_{t-2} + \dots + \Phi_p Y_{t-p} + e_t + \Theta_1 e_{t-1} + \Theta_2 e_{t-2} + \dots + \Theta_q e_{t-q}$$

We say that $\{Y_t\}$ is a mixed autoregressive moving average process of order p and q , respectively. We abbreviate the name to ARMA (p, q).

4. ARIMA model

ARIMA models are the most general class of models for forecasting a time series that can be stationary via transformations, such as differencing and lagging. Determine the order(s) of difference required to stabilize the series before determining the best ARIMA model for it.

4.1 Box: Jenkins modeling method

The Box–Jenkins model is one of the classes of models to choose from the systematic approach to identify the correct model form. There are statistical tests for the validity of the model. Using this test, it is possible to identify the best appropriate Box–Jenkins model for fitting the data on the milk production at Andassa dairy farm.

5. Result and discussion

From the average daily milk production table, the minimum and maximum records of milk production at Andassa dairy farm are 715.0 and 2295.0, respectively, and the mean for the average daily milk production is 1416.9 (**Table 1**).

Test of randomness by using the different sign test.

Test of hypothesis.

H0: Data daily recorded in the average milk production is random.

H1: $\neg H_0$.

Test statistics.

$$Z_{cal} = \frac{w - E(w)}{\sqrt{Var(w)}}$$

where $E(w) = \frac{1}{2}(N - 1)$

$$var(w) = \frac{1}{12}(N + 1)$$

From this, we have the number of points increase $W = 81$

$$E(w) = \frac{1}{2}(N - 1) = \frac{1}{2}(179 - 1) = 89$$

$$var(w) = \frac{1}{12}(N + 1) = \frac{1}{12}(179 + 1) = 15$$

| Variable | N | N* | Mean | SE Mean | St DEV | Minimum | Q1 | Median | Q3 | Maximum |
|----------|-----|----|--------|---------|--------|---------|--------|--------|--------|---------|
| Average | 179 | 0 | 1416.9 | 28.4 | 380.0 | 715.0 | 1180.0 | 1370.0 | 1780.0 | 2295.0 |

Table 1.
Average daily milk production.

$$\sqrt{\text{Var}(w)} = \sqrt{15} = 3.87$$

Therefore: $Z_{cal} = \frac{81-89}{3.87} = -2.0672$.

Note: Level of significance used in this case is $\alpha = 0.05$.

Test rule:

We reject the null hypothesis since $|Z_{cal}| = 2.0672 > Z_{\frac{\alpha}{2}} = 1.96$. Thus, at five percent (5%) level of significance, the data on the average daily milk production is not random. This means that there is a systematic pattern. Meaning, the dissemination of the data is not balanced and it must be transformed into random form by using different methods.

6. Stationary time series

This can be judged by looking at its time plot. The time plot appears similar at different points along the time axis, and the time series plot is as shown in **Figure 1**. The average daily milk production of a cow (Y) is decreasing over time (t) and is not stationary; we must change the time series plot into a stationary form using the differencing method.

6.1 Stationary through differencing

After differencing the data in lag two, the data becomes a stationary series, as illustrated in **Figure 2**. This plot shows that the series varies around the mean. This indicates that it does not deviate or drift from the mean, and the time plots appear to be similar in various places. As a result, the time series is stationary, suggesting that the fluctuation in the average daily milk production of cows is not far apart from one another.

6.2 Stationary trend and difference

The trend analysis plot reveals that it is not stationary, implying that it will require differencing to become stationary. The trend analysis becomes stationary after differencing the data by lag two, as illustrated in **Figure 3**. The amount of average daily milk output is declining, as shown by the fitted trend in **Figure 4**. For

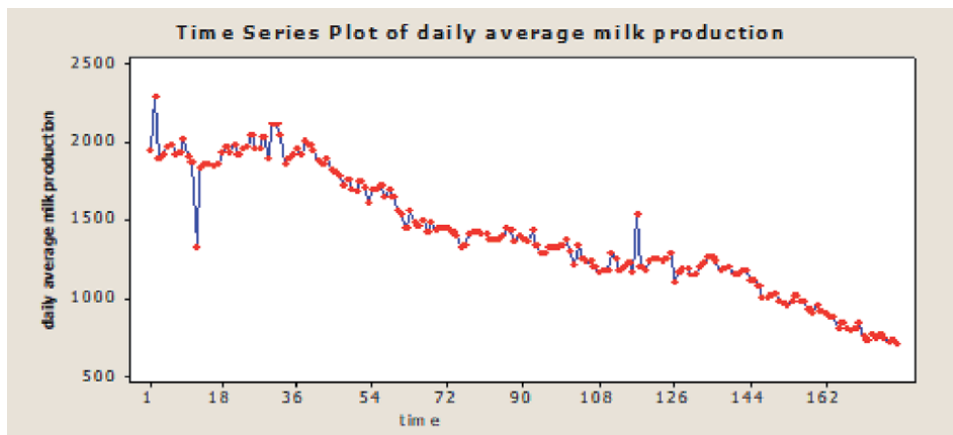


Figure 1.
Average volume of milk production (litter).

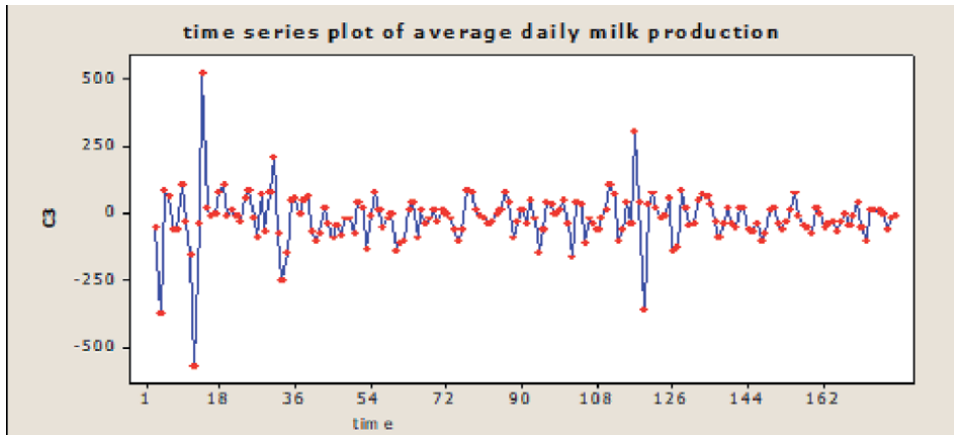


Figure 2.
 Stationary time series plot.

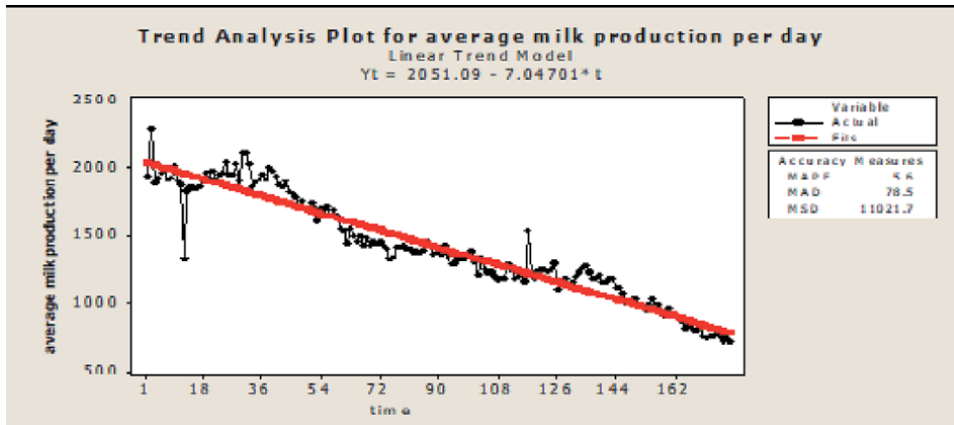


Figure 3.
 Trend analysis.

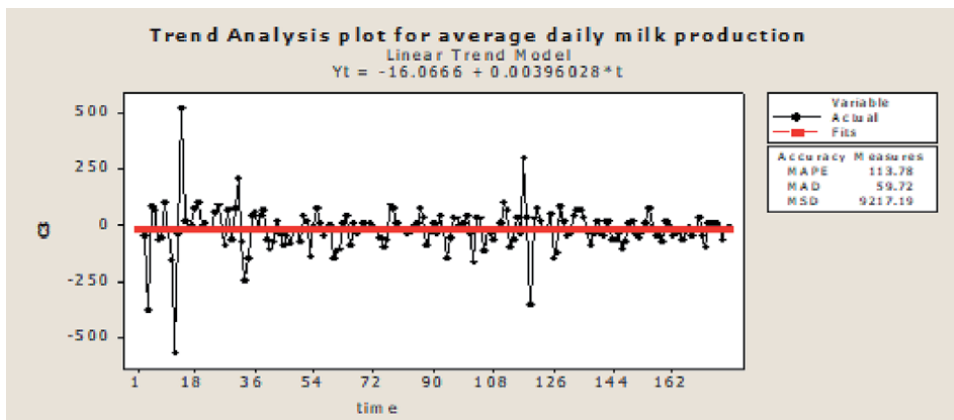


Figure 4.
 Stationary trend analysis.

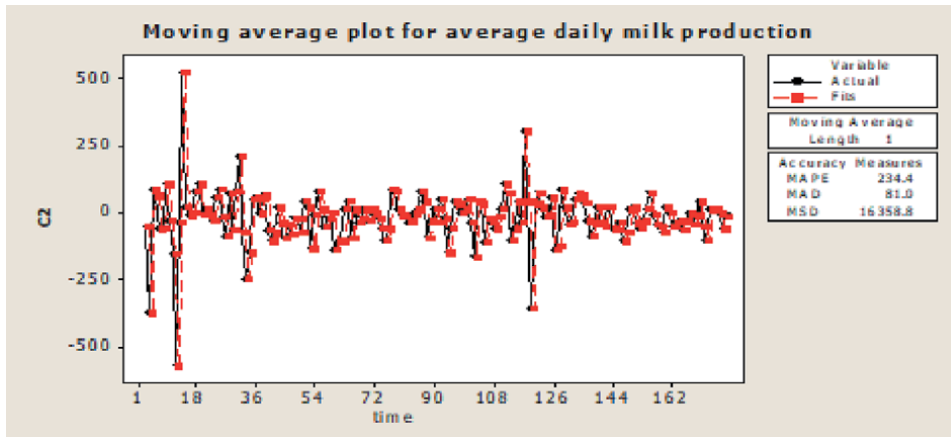


Figure 5.
Moving average plot.

179 days of data, the slope of the trend is -16.066 , which represents the rate at which the amount of average milk per day is decreasing. This also implies that the average daily milk consumption decreases over time.

6.3 Moving average

Figure 5 depicts the moving average plot after the data has been transformed into stationary form by differentiating the observations.

| Accuracy | Measures |
|----------|----------|
| MAPE | 234.4 |
| MAD | 81.0 |
| MSD | 16358.8 |

For all those three measures, the smaller value is a better fit for the model, that is, $MAD = 81.0$ is a better fit for the model.

7. Daily milk production autocorrelation function

There is a lag in the numbers. As a result, we must test the AR model to ensure that it is enough. That is, the autocorrelation graph in **Figure 6** shows that the average milk output is one point outside the lower bound, which is AR [1].

7.1 Partial autocorrelation function: for average daily milk production

In Box–Jenkins models, the partial autocorrelation plot or partial correlogram is also often employed for model identification. In the same way, there is one lag number. As a result, we must test the MA model to ensure that it is adequate. That is, under the partial autocorrelations graph, the average daily milk output shows that there is a point outside the bottom boundaries, which is MA [1]. In other words, the distributions of average daily milk output are neither balanced nor equal, as observed in **Figure 7**.

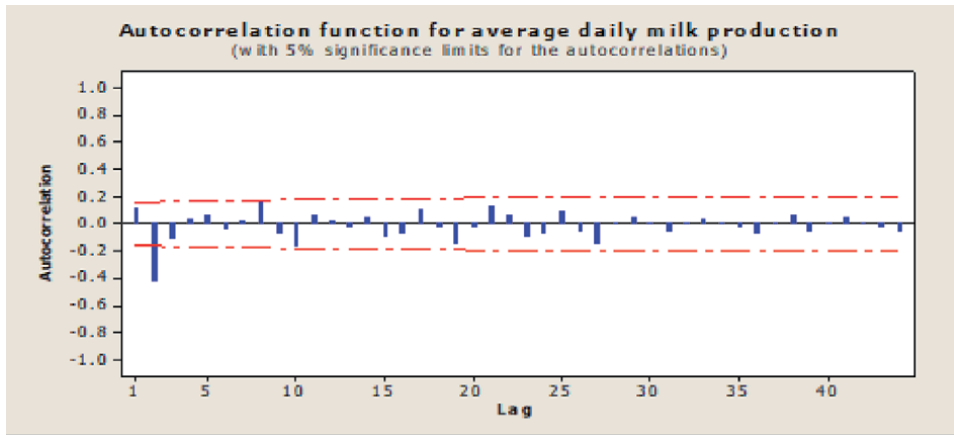


Figure 6.
 Autocorrelation plot.

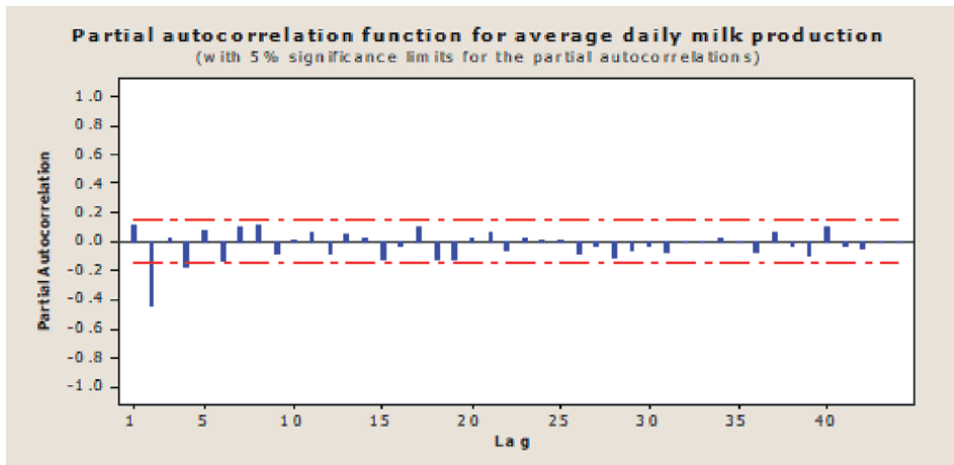


Figure 7.
 Partial autocorrelation plot.

7.2 ARMA process

By combining the autoregressive and moving average processes, we obtain a very general time series model, ARMA (1,1).

7.3 ARIMA process

ARIMA (p, d, q) stands for autoregressive integrated moving average process, where d denotes the number of times the data is differenced before it is an ARMA (p, q). As a result, the ARIMA model is ARIMA (p, d, q) = ARIMA (1,2,1).

7.4 ARIMA model: for average daily milk production

Final estimates of parameters.

| Category | B | Std. Error | T | P-value |
|----------|---------|------------|-------|---------|
| AR [1] | -0.2066 | 0.0742 | -2.79 | 0.006 |

| Category | B | Std. Error | T | P-value |
|----------|--------|------------|---------|---------|
| MA (1) | 0.9977 | 0.0004 | 2352.08 | 0.000 |
| B_0 | 0.2512 | 0.1447 | 1.74 | 0.084 |

| Improved box-pierce χ^2 statistic | | | | |
|--|-------|-------|-------|-------|
| Gap | 12 | 24 | 36 | 48 |
| χ^2 | 67.1 | 92.0 | 102.9 | 109.0 |
| DF | 9 | 21 | 33 | 45 |
| P-value | 0.000 | 0.000 | 0.000 | 0.000 |

As we have seen from the MINITAB output, the ARIMA model (1, 2, 1) equation is described as follows.

$$Y_t = -0.2066Y_{t-1} + 0.9977e_{t-1} + e_t$$

7.4.1 Testing of parameters

The final estimates are those that reduce the sum of squared errors to the point where no other estimates yield lower sums of squared errors. As shown in the MINITAB output, a model should include significant parameters. The p-value of ARIMA (1, 2, 1) is less than the significance level (=0.05). This means the parameters are significantly different from zero and have the smallest sum-squared error possible. Then it has parameters that are statistically significant. As a result, the model is adequate.

7.4.2 Forecasting

The process of obtaining the forecast point and the final model in its original form is as follows.

$$Y_t = -0.2066Y_{t-1} + 0.9977 e_{t-1} + e_t$$

| 95% Bounds | | | |
|------------|----------|-------|---------|
| Period | Forecast | Lower | Upper |
| 180 | 26.663 | 0 | 273.768 |
| 181 | 54.100 | 0 | 369.879 |
| 182 | 82.662 | 0 | 460.574 |
| 183 | 111.242 | 0 | 541.606 |

We can use the 95% confidence interval (CI) defined above to assess the accuracy of the anticipated number. We can state that the forecasted value is accurate since the entire forecast values are found between the lower and upper intervals.

8. Conclusions

The average amount of milk produced at Andassa dairy farm is dropping. The data for 179 days reveal a high degree of variability in daily milk production

compared to other days, implying that the amount of milk produced varies greatly from day to day. Because the slope of the trends over the 179 days is -16.066 , the amount of milk is falling. The daily milk production graph in the autocorrelations and partial autocorrelations graphs reveals that the top and lower boundaries do not encompass the entire observation. For ARIMA (1, 2, 1), a parameter with a p-value less than the level of significance (0.05) is a parameter. This indicates that the parameter is significantly distinct from zero and has the smallest value.

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Sequencing the ‘Dairy Mind’ Using Mind Genomics to Create an “MRI of Consumer Decisions”

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Abstract

We present the research methodology that generates an integrated database of the mind of a dairy consumer, regarding nine different dairy products. The set of studies deals with a variety of end products, presenting alternative messages about each product. Respondents rate combinations of messages, that is, vignettes, which are created using an advanced form of conjoint analysis. OLS (ordinary least-squares) regression is used to deconstruct the ratings at the level of the individual respondents, producing a coefficient value for each message that was tested. Cluster analyses revealed three distinct mind-sets around dairy products: a strong focus on flavor, a strong focus on health, and a strong focus on price. This chapter demonstrates how the science of Mind Genomics is further applied through a typing tool, known as PVI (personal viewpoint identifier). The PVI is able to identify the mind-set of any individual that provides a binary response to six short questions. The chapter concludes with a vision for the future of the Mind Genomics research methodology in the fields of science and business.

Keywords: consumer, behavior

1. Introduction

When one thinks of large-scale ‘consumer research’ in the world of products, such as dairy products, one is limited by that which exists, that which works, and of course that which one can afford. It should come as no surprise that the armory of knowledge about consumers and dairy come at once from observations of trends in the market, and at the same time large-scale segmentation studies, wherein the respondent is asked many questions about habits, practices, beliefs, and so forth; for instance, for dairy products, such data ground up into segmentation studies [1–3]. The result of both large-scale tracking of consumer behaviors, whether purchase or expressed attitudes, and deep studies of attitudes and behaviors produce for us a bewildering array of numbers, statistics, points to be talked about in presentations, and indeed a panoply of what might be called interesting consumer information.

Whereas there is an ongoing focus on the bigger world in which dairy ‘plays’, there is a parallel world of scientists focusing on the product itself. These are typically so-called sensory scientists, who study the properties of foods, using

laboratory methods to describe the food and the person-food interaction. In the words of R&D director, Dr. Al Bowles 'across the world there are literally file cabinets of data from research on the properties of dairy products' [personal communication to HRM, 1996].

Despite the increasingly impressive array of information about the world of dairy, whether from the trade and social research (e.g., brand tracking), and from the laboratory (preferences for specific sensory aspects of dairy products), there is an information desert when it comes to the mind of the dairy customer, not so much regarding behavior and to general attitudes, but rather to the specifics of a variety of different dairy products. By this we mean that we know a great deal of what's happening in the market, and in the product, but precious little about what is going on deeply in the mind, for dairy products and actually for most products, and even situations which constitute the warp and woof of everyday life.

2. The worldview of Mind Genomics, an 'MRI of the Mind'

The emerging science of Mind Genomics was created to understand the way people make decisions. Rather than asking people to provide 'logical' and presumably realistic answers to questions about the world of their everyday, Mind Genomics goes in a different direction. Instead of one question at a time, Mind Genomics presents people, the participants in an experiment, to respond to combinations of messages, for example, about dairy, in a short experiment. The pattern of responses to the combinations of messages (so-called vignettes) ends up revealing the aspects of the experience, which really make a difference to the respondent [4, 5].

This paper brings together half a decade of work, beginning in the early years of the twenty first century. The studies focused on discovering the mind of the dairy consumer, or more correctly, the mind of the consumer as it is turned by instruction to dairy products. The original studies were part of larger efforts to map the mind of the consumer, regarding foods, snacks, beverages, and healthful items as a particular focus. We obtained an abstract from simple results of those studies, in a so-called 4×4 design (four aspects of the product and four alternatives to each aspect.).

The Mind Genomics 'project,' as it has evolved since its introduction in 1993 [6], has concentrated on identifying how ordinary consumers 'weigh' the different aspects of a product or service, to come up with a simple overall judgment. Thus, Mind Genomics may be considered a science, which has emerged from multi-attribute measurement [7-9].

The importance of dealing with compound stimuli cannot be overestimated. High school science textbooks often discuss a strategy of science, which begins by identifying what is to be studied, and then move on to how the scientist isolates the factor(s) to be studied, reducing all extraneous variability until all which remains is the object, process, or whatever other name is given to the center of focus. The effort is to reduce the 'noise' so that the 'signal' can emerge. When the topic involves thinking behavior, wherein one cannot possibly attain that idea 'quiet' situation, the alternative strategy is to test many hundreds or even thousands of situations containing the 'signal' or factor to be measured, and then hoping the random variability contributed by uncontrolled factors, which is the human element, can be averaged out. Thus, the opposing strategies are to suppress the noise or average it out through replication. There is however a third strategy, one patterned after the MRI, magnetic resonance imaging, in the world of health. The strategy is to take many 'pictures' of the same situation or 'thing' from different angles, and recombine them afterwards to produce a deeply detailed, focused picture. It is this third strategy that Mind Genomics adopts.

The objective of a Mind Genomics study is to discover how the respondent 'weights' the different inputs. The higher weights, as discussed below, mean that the message or element in the test stimuli more strongly 'drives' the response toward a defined 'high' point, such as likely to purchase, likely to crave, likely to like the product, and so on. The lower and negative weights mean that the message of element in the test stimuli is irrelevant or may even drive the rating to the low end of the scale, such as would not purchase, not likely to crave, do not like the product, and so on. Mind Genomics studies typically focus on the positive coefficients only, values higher than 0. Values of 0 or lower mean that either the respondent feels that the message drives the response to the lower anchor (viz., the negatives, such as 'do not like the product,') or the respondent often feels that the element is simply irrelevant.

Beyond the creation of a database for each element showing how that element 'drives' the response, the Mind Genomics project focuses on the discovery of underlying mind-sets, that is, groups of individuals in the population who think about the product in the same way. Although we are 'taught' that one can divide people by WHO THEY ARE, such divisions are scarcely useful when it comes to understanding the preferences of people toward products, whether these preferences pertain to product features, product 'benefits', product 'packaging,' and so forth. Until the development of Mind Genomics, there appears to be no efficient, standard way to uncover the latent mind-sets.

3. Background to the studies presented in this chapter

During the first decade of the 21st century, from 2001 to 2005, author HRM was involved in the development of Mind Genomics as an integrated database of the mind [5, 10]. During those 4 years, Moskowitz and colleagues created the It! studies, each It! study comprising 20–30 parallel studies of a food or beverage. The studies themselves were designed according to a common experimental design, typically comprising four basic questions (silos), and nine answers (elements) to each basic question. Each respondent evaluated a total of 60 vignettes, each vignette comprising 2–4 elements, at most one element from each silo. Furthermore, each respondent evaluated every element the same number of times. One of the important innovations of the Mind Genomics approach is that each respondent evaluated the same number of vignettes, 60, but the 60 vignettes for each respondent differed from the 60 vignettes for every other respondent. Indeed, the Mind Genomics algorithm ensured that most of the vignettes in a study were seen at most two or three times across the several hundred respondents, and the several thousand vignettes.

The design, permuted orthogonal design [11], was the most important feature of the Mind Genomics approach, instantiated in these different sets of 60 vignettes each, one set for each respondent. The second innovation providing the statistical power, and the potential for deep understanding was that the 60 vignettes were arrayed according to an experimental design, at the level of the individual respondent. This is called the within-subjects design. Each respondent could be investigated alone, without the need of other respondents.

The mathematical structure of the vignettes, comprising individual, permuted experimental designs, means that the 36 independent variables, so-called 'elements', were likely to be statistically independent of each other, whether the data were considered in their original form for one respondent (4 questions, 9 answers per question), or the data combined the results from any combination of respondents.

The respondents evaluated each rating on an anchored 1–9-point scale, a so-called category, or Likert Scale. The scale allows the respondent to act as a measuring instrument. However, for the subsequent analysis, the 9-point scale was divided into two parts. Ratings of 1–6 were converted to 0 to denote little or no level of the attribute being rated (e.g., desirability or crave-ability of the product denoted by the scale). Ratings of 7–9 were converted to 10 to denote a great deal of the attribute being rated. The conversion of ratings from the more granular 1–9 scale to the less granular 0/100 scale was done following the basic worldview of consumer researchers, wherein managers prefer all-or-none or yes-no answers. The binary transformed ratings, generating a 0/100 output, makes it easy for the manager to understand and use the data. Some of the granularity is lost, however. A good practice is to work with at least 5–10 respondents with different patterns of response AFTER the binary transform has been done.

The mathematics of the design allowed the researcher to use OLS (ordinary least-squares) regression to the presence/absence of the element (Eq. (1)):

$$\text{Rating} = k_0 + k_1(A_1) + k_2(A_2) \dots k_{36}(D_9) \quad (1)$$

The foregoing model allows the researcher to learn, quite quickly, which of the elements (A1–D9) are key to driving the rating.

Moving beyond the general model, the Mind Genomics software created individual level models, one model or equation for each respondent. This analysis is possible because the underlying experimental design was ‘complete’ for each respondent. That is, one needed only the ratings from each respondent to create an equation for that respondent. The individual models were then clustered [12], so that similar patterns of the 36 coefficients were put into the same cluster or group. Clustering itself is a form of exploratory data analysis. The objective of clustering is simply to identify, in the manner of a heuristic, generally groups showing distinct, and interpretable patterns. The composition of the clusters is a function of the data itself, and the form of clustering.

Each data set was subjected to the same clustering approach whereby the first two clusters were generated, and then three clusters. We chose the fewest number of clusters, subject to the requirement that the clusters could be interpreted, that is, in such a way that it told a coherent, seemingly reasonable story. Generally, the ‘three-cluster-solution’ best fulfilled the joint goals of parsimony (fewer clusters are better) and interpretability (the clusters told a story, which made sense at a logical level).

4. Understanding the results

We begin the analysis with a summary table showing how the Mind Genomics process identified the relevant mind-sets for 10 dairy products. **Table 1** shows the different, emerging mind-sets for each product. The remainder of this chapter will discuss how these mind-sets were discovered and used for understanding how people think of dairy products, and how the minds of 41 students were ‘sequenced’ to identify the pattern of mind-sets for dairy for each student.

We now go in depth to show how these mind-sets were developed, and the rich data underlying the table. **Table 2** shows the three clusters emerging from the clustering for healthful yogurt. The clustering was done on all 36 elements, in the original study, to generate either two or three different clusters (mind-sets). Author SD then selected 16 elements from the data to present. Keep in mind that each data table, **Table 2**, is a reduced version of a larger 4 × 9 table. The 16 elements were

| | MS1 | MS2 | MS3 |
|-----------------------------|---|--------------------------------------|---------------------------------------|
| Cheese: healthful | Function-oriented: health & wellness | Traditionalists | Flavor seekers/quality-oriented |
| Ice cream: novelties | Quality seekers | Flavor seekers | Feature-oriented |
| Milk | Flavor seekers | Function-oriented | Traditionalists |
| Milk: healthful | Flavor seekers | Function-oriented: health & wellness | None |
| Shake | Flavor seekers | Traditionalists | Value seekers |
| Shake: healthful nutritious | Flavor seekers/function-oriented: health & wellness | Function-oriented: health & wellness | Flavor seekers/focused on burning fat |
| Smoothie | Flavor seekers | Function-oriented | Experience-oriented |
| Yogurt | Flavor seekers | Value seekers | Traditionalists |
| Yogurt: beverage | Flavor seekers | Value seekers | Traditionalists |
| Yogurt: healthful | Flavor seekers | Value seekers | Traditionalists |

Table 1.
 The different mind-sets (MS) for 10 dairy products.

| Healthful yogurt | | MS1 | MS2 | MS3 |
|---|---|-----|-----|-----|
| Base size | | 80 | 60 | 89 |
| Additive constant | | 30 | 55 | 39 |
| What are the sensory features of the product? | | | | |
| E1 | The incredibly rich, indulgent flavors like Boston cream pie, mocha fudge, and strawberry a la mode | 21 | | 19 |
| E2 | The delicious, classic fruit flavors like raspberry, strawberry banana, and blueberry | 13 | 9 | 22 |
| E3 | Smooth and creamy, premium blended custard style yogurt | 13 | | 14 |
| E4 | Thick with lots of real fruit at the bottom | 3 | | 21 |
| What vitamins and nutrients does this product contain? | | | | |
| E5 | Provides essential vitamins your body needs, including A, B12, C, and E | 23 | | 7 |
| E6 | Provides essential minerals your body needs, including potassium, magnesium, and zinc | 18 | 7 | |
| E7 | Contains the essential nutrient choline ... shown to improve memory and learning | 18 | 1 | |
| E8 | Contains essential omega-3 fatty acids, which may reduce your risk of heart disease | 15 | 13 | |
| What are the benefits of using it? | | | | |
| E9 | With ingredients that restore and maintain a healthy balance in your digestive system | 12 | 7 | 1 |
| E10 | May reduce your risk of high blood pressure and stroke | 11 | 2 | |
| E11 | Fills that empty spot in you ... just when you want it | | | 4 |
| E12 | Builds and maintains strong bones | 2 | 6 | 3 |

| Healthful yogurt | | MS1 | MS2 | MS3 |
|--|---|-----|-----|-----|
| What are the functional features of this product? | | | | |
| E13 | As part of a low fat, low cholesterol diet, may reduce the risk of some forms of cancer | 15 | 6 | |
| E14 | All natural ... no artificial flavors, colors, or sweeteners | 13 | 1 | 10 |
| E15 | Low fat with only 2 g per serving | 10 | | 4 |
| E16 | Made with the freshest milk | 3 | | 10 |

Strong performing elements are highlighted. Elements with coefficients or negative values are shown as blank cells.

Table 2. Summary data for the healthful yogurt product. The table shows the original 4 × 9 design, truncated to a 4 × 4.

then associated with four questions, so that each ‘question’ was associated with four different, but related elements. This analysis was done AFTER the research was completed. The four questions and the four answers to each question, created on a post-hoc basis, do not affect the results at all, but simply represent an easy way to deal with the data for subsequent analyses.

Table 2 presents the data in three columns, one column for each mind-set. The base size shows the number of respondents in the cluster. Note that the clustering program attempts to separate the respondents into either two or three groups based upon the pattern of the 36 coefficients. The base sizes do not have to be equal. Furthermore, the clustering program is ‘agnostic’ in terms of the ‘meaning’ of the elements and the reason for their membership in a cluster. The only consideration is the satisfaction of the mathematical criterion.

The additive constant is the estimated value of a vignette without any elements. Since all vignettes comprised elements, the additive constant is a purely estimated parameter. The OLS regression relating the presence/absence of the 36 elements to the binary response returns with one number for each element. This element is the coefficient. The coefficients can be both positive and negative. For positive coefficients, the interpretation is that putting the element into a vignette sways an additional percent of the respondents to assign the rating of 7–9. Furthermore, the coefficient, whether positive or negative (see below) can be added to the constant to estimate the percent of times that the vignette would be assigned a value of 7–9 on a 9-point scale, when the vignette comprises the specific element(s).

For example, for Mind-Set 1, the additive constant is 30. A vignette comprising E1, E5, E9, would be expected to get ratings of 7–9 (30 + 21 + 23 + 12) or about 86% of the time. The same vignette would be far lower in Mind-Set 2 because two of the coefficients are either 0 or negative (not shown), and only one coefficient (E9) is positive.

Looking at mind-set 1, we see that the additive constant for healthful yogurt is 30. In the absence of elements, we expect 30% of the responses to be ratings of 7–9, and the other 70% of response to be 1–6. Again, keep in mind that the additive constant is a purely theoretical parameter, computed by the OLS regression. Mind-set 2 is a bit more positive. The additive constant is 55, meaning that in the absence of elements, we expect to see 55% of the responses from the mindset to be between 7 and 9. Finally, Mind-set 3, with 89 respondents, shows an additive constant of 39, in the middle. This implies that in the absence of elements, we see 39% of the responses from this mindset to lie between 7 and 9.

Our first conclusions are that there are three interpretable mind-sets. The basic interest in healthful yogurt spans a range from low (mind-set 1) to reasonably high (mind-set 2). What we do not know is the nature of the mind-sets. The remainder

| Healthful yogurt | | MS1 | MS2 | MS3 |
|---------------------------------------|---|-----|-----|-----|
| Base size | | 80 | 60 | 89 |
| Additive constant | | 30 | 55 | 39 |
| Mind-set 1 | | | | |
| E5 | Provides essential vitamins your body needs, including A, B12, C, and E | 23 | | 7 |
| E1 | The incredibly rich, indulgent flavors like Boston cream pie, mocha fudge, and strawberry a la mode | 21 | | 19 |
| E6 | Provides essential minerals your body needs, including potassium, magnesium, and zinc | 18 | 7 | |
| E7 | Contains the essential nutrient choline ... shown to improve memory and learning | 18 | 1 | |
| E8 | Contains essential omega-3 fatty acids, which may reduce your risk of heart disease | 15 | 13 | |
| E13 | As part of a low fat, low cholesterol diet, may reduce the risk of some forms of cancer | 15 | 6 | |
| E2 | The delicious, classic fruit flavors like raspberry, strawberry banana, and blueberry | 13 | 9 | 22 |
| E3 | Smooth and creamy, premium blended custard style yogurt | 13 | | 14 |
| E14 | All natural ... no artificial flavors, colors, or sweeteners | 13 | 1 | 10 |
| E9 | With ingredients that restore and maintain a healthy balance in your digestive system | 12 | 7 | 1 |
| E10 | May reduce your risk of high blood pressure and stroke | 11 | 2 | |
| E15 | Low fat with only 2 grams per serving | 10 | | 4 |
| Mind-set 2 | | | | |
| E8 | Contains essential omega-3 fatty acids, which may reduce your risk of heart disease | 15 | 13 | |
| E2 | The delicious, classic fruit flavors like raspberry, strawberry banana, and blueberry | 13 | 9 | 22 |
| Mind-Set 3 | | | | |
| E2 | The delicious, classic fruit flavors like raspberry, strawberry banana, and blueberry | 13 | 9 | 22 |
| E4 | Thick with lots of real fruit on the bottom | 3 | | 21 |
| E1 | The incredibly rich, indulgent flavors like Boston cream pie, mocha fudge, and strawberry a la mode | 21 | | 19 |
| E3 | Smooth and creamy, premium blended custard style yogurt | 13 | | 14 |
| E3 | Smooth and creamy, premium blended custard style yogurt | 13 | | 14 |
| E14 | All natural ... no artificial flavors, colors, or sweeteners | 13 | 1 | 10 |
| E16 | Made with the freshest milk | 3 | | 10 |
| E14 | All natural ... no artificial flavors, colors or sweeteners | 13 | 1 | 10 |
| Not strong in for any mind-set | | | | |
| E11 | Fills that empty spot in you ... just when you want it | | | 4 |
| E12 | Builds and maintains strong bones | 2 | 6 | 3 |

Strong performing elements are highlighted. Elements with coefficients or negative values are shown as blank cells.

Table 3. Summary data for the healthful yogurt product. The elements have been sorted from highest to lowest rating.

of the table shows coefficients from 16 of the 36 elements, or 44% of the original data, the specific elements in the table being chosen because it is 'actionable', viz., describing the nature of the product. Some elements score very strongly across all mind-sets. An example is 'The delicious, classic fruit flavors like raspberry, strawberry banana, and blueberry.' Some elements score very strongly, but perhaps only with one mind-set. They may or may not even score positively among other mind-sets. A good example is 'Contains the essential nutrient choline ... shown to improve memory and learning', performing well in mind-set 1, virtually irrelevant in mind-set 2, and perhaps totally irrelevant, and even damaging in mind-set 3.

Table 2 can be made more informative by sorting the table by mind-set; this can be done based upon the strong performing elements (coefficient ≥ 8). The sorted table shows the strong performing elements for each mind-set, with elements performing strongly in two mind-sets appearing twice or thrice, once for each mind-set in which the element performs strongly. The duplicates are not important. **Table 3** shows the sorted data.

When we look at the 10 tables of data, we see 10 different sets of mind-sets, generally three mind-sets for a study, but sometimes two mind-sets. We look at the mind-sets for the 4×4 matrices, and in our analysis develop a name for each mind-set, based upon the elements that perform most strongly. It is important once again to reiterate the fact that the clustering program does not name the mind-set. Rather, the researcher does. All that the clustering does is to create the different groups based upon statistical criteria.

5. Finding these mind-sets in the population using the PVI

Researchers are accustomed to working with mind-sets. The notion that people radically differ from each other in how they react to simple stimuli is an old one, embodied in aphorisms and folk wisdom. What is novel, however, is the rather unpleasant realization that there is generally no simple set of rules, which one can use to put a new person into a mind-set. There is the ever-present wish that people who are 'alike' in who they ARE (e.g., age, education, gender, residence, shopping behaviors, and so forth) will share similar mind-sets. Thus, the standard method of cross-tabulating individuals to search for clues to the potential membership in one or several mind-sets is to use the easy-to-collect information about the person. As we will see below, in a study of 41 students, similar in age, education, and so on, this is not the case. Birds of a feather may flock together, but they think disparately.

Many marketers and scientists have 'complained' that the mind-sets provide valuable information, but they need the mind-sets to be generalized. For reason of cost and simply the marginal knowledge imparted by each new respondent, most Mind Genomics studies comprise at most 300 respondents. A great lesson can be learned about mind-sets with as few as 40–50 respondents. The base size of 50–100 suffices to reveal the nature of the mind-set, and often to define it, but does not let the researcher or businessperson make full use of the mind-sets for other purposes. A method is needed to assign new people to mind-sets that have already been discovered.

One original method was to work with large samples of 300+ respondents, discover their mind-sets, and then, during the research, purchase a great deal of additional information about these same 300 respondents. A data analysis would be then hired to create ad hoc models attempting to relate mind-set members to some combination of purchased information. Occasionally the predictive methods worked, but most often the collection of the ancillary data was expensive; the number of variables to collect was unknown as subject to many vagaries occurring when the data were collected and required significant analytical effort.

During the past 5 years, author HRM and colleagues, especially author Gere, have collaborated to create an easier system based upon a Monte-Carlo method. The original summary for data, showing the coefficients for the three mindsets, for example, is perturbed to create 'noisy' data. A decision tree is created to determine the assignment of a new respondent to one of the three mind-sets, based upon the perturbed data. At the end, a synthesized decision tree is created, comprising six of the 16 elements. The respondent uses a 2-point scale rate for each of these six elements. The pattern of the ratings assigns a respondent to one mind-set or of the two or three mind-sets emerging from the study.

Figure 1 shows an example of the first part of the PVI. The left-most rectangle shows the introductory information about the respondent. The respondent identity (name) is never collected, but there is an option to collect the respondent phone number and email address. This option must be accepted by the respondent who participates in the study, viz. so-called opt-in. Should the respondent refuse to provide the information when requested, PVI is instructed to close, going no further, and thus respecting the respondent's desire for privacy.

Each of the 10 studies generates six questions, based upon the elements in the study, but with the option to edit the elements, as well as edit the two-point rating scale. Not shown is the option to ask four simple questions for each product PVI, each question having up to four answers, one of which must be selected.

Each of the 10 studies is set up separately, and then added into the PVI tool. Thus, for this project with 10 different dairy products, the PVI comprised the information rectangle (left), and 10 columns, one column corresponding to each product in the set of studies.

| | | |
|---|---|--|
| <p>The purpose of this study is to assess consumer mindsets around various dairy products.</p> <p>The information I am giving you is about my attitudes towards a specific topic(s). As part of the exercise, I am giving you information about myself. This information will be used to provide better choices in products and services, based on your answers.</p> <p><input type="checkbox"/> I Agree to Participate <input type="checkbox"/> I Do Not Agree to Participate</p> <p>Follow Up for Research and Marketing Purposes *Required <input type="radio"/> Allow <input type="radio"/> Not Allow</p> <p>Day Of Week Taken *Required DAY</p> <p>Approximate Time Taken *Required SELECT RANGE</p> <p>Email address *Required Enter Email</p> <p>Year of Birth *Required YEAR :</p> <p>Country *Required United States</p> <p>Postal Code *Required Enter Postal Code</p> <p>Gender *Required GENDER</p> <p>Ethnicity *Required NOT SPECIFIED</p> | <p>SMOOTHIES</p> <p>No Specialty Questions for This Study</p> <p>RELAXES YOU AFTER A BUSY DAY <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>SMOOTHIES ... A DELICIOUS SLUSHY WITH FRUIT FLAVORS <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>YOU CAN IMAGINE THE TASTE EVEN BEFORE YOU DRINK IT <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>SMOOTHIES BLENDED WITH FRUIT, ICE, AND JUST A LITTLE FROTH <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>100% NATURAL AND ORGANIC <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>LOWFAT SMOOTHIES ... HIGH IN VITAMINS, MINERALS, AND REFRESHINGLY GOOD <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> | <p>HEALTHFUL NUTRITIONAL SHAKE</p> <p>No Specialty Questions for This Study</p> <p>WITH SOY ISOFLAVONES... SHOWN TO MODERATE SYMPTOMS OF MENOPAUSE AND DECREASE BONE LOSS <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>MAKES YOU FEEL FULL SO YOU EAT LESS <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>A GOOD SOURCE OF FIBER, IMPORTANT IN REDUCING YOUR RISK OF CHRONIC DISEASES LIKE HEART DISEASE AND DIABETES <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>INDULGE YOURSELF IN DELICIOUS FLAVORS LIKE DEEP, RICH CHOCOLATE AND MOCHA FUDGE <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>LOSE WEIGHT FAST <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> <p>CONTAINS SOY PROTEIN... CLINICALLY PROVEN TO REDUCE THE RISK OF HEART DISEASE <input type="radio"/> DISLIKE <input type="radio"/> LIKE</p> |
|---|---|--|

Figure 1. Example of the PVI, showing the introductory panel, and two panels for products, a smoothie and a healthful, nutritional shake.

The researcher setting up the study can instruct the PVI to randomize the order of the studies when desired, to randomize the order of the questions within the study, when desired, and even to randomize the full set of 60 questions. The latter, full randomization, makes the task difficult for the respondent to ‘game.’

The time to complete the introductory panel is approximately 45 s. The evaluation for each panel takes approximately 15 s. Thus, for the introductory panel and for the 10 product panels, the total time is approximately 195 s or 3.5 min. The time suffices to ‘sequence’ the mind of the respondent on the 10 dairy products, that is, to discover what is important. The PVI typically takes about 3–4 min for 10 different products (as well as the information page.)

The researcher can set up the PVI to drive three additional steps, each of which is optional. **Figure 2** immediately provides the feedback to the respondent regarding the assignment of the respondent to the proper mind-set for each product.

| | |
|--|--|
| <p>Study Name YOGURT MS1: FLAVOUR SEEKERS Mindset 1 Feedback FLAVOUR SEEKERS Mindset 1 Video Mindset 1 Link Leave this page Mindset 2 Name MS2: VALUE SEEKERS Mindset 2 Feedback VALUE SEEKERS Mindset 2 Video Mindset 2 Link Mindset 3 Name MS3: TRADITIONALISTS Mindset 3 Feedback TRADITIONALISTS Mindset 3 Video Mindset 3 Link</p> | <p>Study Name ICE CREAM MS1: QUALITY SEEKERS Mindset 1 Feedback QUALITY SEEKERS Mindset 1 Video Mindset 1 Link Leave this page Mindset 2 Name MS2: FLAVOUR SEEKERS Mindset 2 Feedback FLAVOUR SEEKERS Mindset 2 Video Mindset 2 Link Mindset 3 Name MS3: FEATURE-ORIENTED Mindset 3 Feedback FEATURE-ORIENTED Mindset 3 Video Mindset 3 Link</p> |
| <p>Study Name YOGURT BEVERAGE MS1: FLAVOUR SEEKERS Mindset 1 Feedback FLAVOUR SEEKERS Mindset 1 Video Mindset 1 Link Mindset 2 Name MS2: VALUE SEEKERS Mindset 2 Feedback VALUE SEEKERS Mindset 2 Video Mindset 2 Link Mindset 3 Name MS3: TRADITIONALISTS Mindset 3 Feedback TRADITIONALISTS Mindset 3 Video Mindset 3 Link Leave this page</p> | <p>Study Name MILK MS1: FLAVOUR SEEKERS Mindset 1 Feedback FLAVOUR SEEKERS Mindset 1 Video Mindset 1 Link Mindset 2 Name MS2: FUNCTION-ORIENTED Mindset 2 Feedback FUNCTION-ORIENTED Mindset 2 Video Mindset 2 Link Leave this page Mindset 3 Name MS3: TRADITIONALISTS Mindset 3 Feedback TRADITIONALISTS Mindset 3 Video Mindset 3 Link</p> |

Figure 2.
PVI output with partial feedback for one respondent (four of 10 products).

1. Provide information, that is, feedback, about the different mind-sets, those to which the respondent is assigned, and those to which the respondent is not assigned.
2. Path to a landing page to which the respondent can be automatically directed after being assigned to the proper mind-set. Only one landing page can be selected, however. The researcher must select the specific product which determines the landing page.
3. Path to a video to which the respondent can be automatically directed after being assigned to the proper mind-set.

6. Creating an integrated database from the set of PVI

We conclude the empirical section of this chapter with the creation of an integrated database, comprising the information about each respondent who participates. The database comprises the information about the respondent herself or himself, such as age, gender, country, and other material collected at the start of the PVI. **Figure 3** shows part of the database.

Each row of the database comprises the information about the respondent, the name of the individual study on which the respondent is being 'typed,' the mindset name, as well as other information not shown. The other information comprises the mind-sets, the feedback, the six questions and their answers, and the (up to) four questions and answers that could be asked for each product at the start of the PVI for that product.

The first objective of the database is to advance science. **Tables 4** and **5** show the results from one small study conducted with 41 students at Ryerson University, who participated in a larger study, from which these data were abstracted. Had the study been limited to 10 products, each respondent would have seen the products in random order, the questions within the products in random order, and the entire sequence might have lasted less than 4–5 min. The actual study comprised the 'typing' by all 41 students on the full set of 67 products.

It is clear from **Tables 4** and **5** that groups and individuals show a preponderance of the group of mind-sets encompassed by the term 'flavor seeker'. Yet there are other mind-sets, and a few respondents who fall into these other mind-sets. **Table 5** shows the mind-set memberships for 10 of the 41 respondents. Most of the respondents fall into the group called 'flavor seeker'. In general, for these dairy products, about 60% of the time a respondent will fall into one of the groups that can be defined as 'flavor seekers.' The rest of the time, the respondent will fall into different groups, whether these be traditionalists, value seekers, health seekers, and

| StudyName | RespID | Age | Gender | Ethnicity | CountryCode | MindsetName |
|-----------------------------|--------|-----|--------|-----------|-------------|---|
| HEALTHFUL CHEESE | 5179 | 23 | F | White | USA | MS1: FUNCTION-ORIENTED: HEALTH & WELLNESS |
| HEALTHFUL MILK | 5179 | 23 | F | White | USA | MS1: FLAVOUR SEEKERS |
| HEALTHFUL NUTRITIONAL SHAKE | 5179 | 23 | F | White | USA | MS3: FLAVOUR SEEKERS/ FOCUSED ON BURNING FAT |
| HEALTHFUL YOGURT | 5179 | 23 | F | White | USA | MS1: FLAVOUR SEEKERS/FUNCTION-ORIENTED: HEALTH & WELLNESS |
| ICE CREAM | 5179 | 23 | F | White | USA | MS2: FLAVOUR SEEKERS |
| MILK | 5179 | 23 | F | White | USA | MS1: FLAVOUR SEEKERS |
| SHAKE | 5179 | 23 | F | White | USA | MS2: TRADITIONALISTS |
| SMOOTHIE | 5179 | 23 | F | White | USA | MS3: EXPERIENCE-ORIENTED |
| YOGURT | 5179 | 23 | F | White | USA | MS3: TRADITIONALISTS |
| YOGURT BEVERAGE | 5179 | 23 | F | White | USA | MS2: VALUE SEEKERS |

Figure 3.
 Part of the database created for the study, for one respondent.

| | Total | Female | Male | A18–20 | A21–24 | A25+ | Asian | Black | Latino | Other | White |
|----------------------------|-------|--------|------|--------|--------|------|-------|-------|--------|-------|-------|
| Total | 410 | 340 | 60 | 160 | 180 | 70 | 160 | 50 | 20 | 60 | 110 |
| Total % in 'flavor seeker' | 60% | 60% | 60% | 61% | 59% | 60% | 66% | 60% | 55% | 57% | 53% |
| Flavor seekers | 185 | 152 | 29 | 74 | 79 | 32 | 80 | 20 | 8 | 27 | 44 |
| Flavor seekers/function | 48 | 42 | 6 | 19 | 20 | 9 | 23 | 8 | 2 | 5 | 9 |
| Flavor seekers/focused | 10 | 10 | 0 | 3 | 6 | 1 | 2 | 2 | 0 | 2 | 4 |
| Flavor seekers/quality | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| Function-oriented: health | 54 | 41 | 11 | 21 | 23 | 10 | 22 | 3 | 3 | 6 | 17 |
| Traditionalists | 35 | 29 | 4 | 16 | 12 | 7 | 8 | 4 | 2 | 10 | 11 |
| Quality seekers | 31 | 26 | 4 | 11 | 15 | 5 | 13 | 4 | 1 | 6 | 7 |
| Value seekers | 23 | 20 | 3 | 7 | 13 | 3 | 6 | 5 | 2 | 2 | 8 |
| Function-oriented | 17 | 15 | 2 | 5 | 9 | 3 | 4 | 3 | 1 | 2 | 7 |
| Experience-oriented | 4 | 3 | 0 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 2 |
| Feature-oriented | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

Table 4. *Distribution of groups into different mind-sets. Groups with one respondent are not shown.*

| Panelist # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Total in 'flavor seeker' | 70% | 80% | 60% | 60% | 50% | 80% | 80% | 50% | 60% | 60% |
| Flavor seekers | 5 | 6 | 4 | 2 | 3 | 7 | 6 | 3 | 5 | 4 |
| Flavor seekers/function | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 2 |
| Flavor seekers/focused | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Flavor seekers/quality | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Function-oriented: health | 1 | 1 | 0 | 1 | 0 | 2 | 1 | 0 | 2 | 1 |
| Traditionalists | 0 | 0 | 2 | 4 | 2 | 0 | 0 | 1 | 1 | 0 |
| Quality seekers | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Value seekers | 2 | 0 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 1 |
| Function-oriented | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Experience-oriented | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Feature-oriented | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Table 5. *How 10 different panelists fall distribute into the mind-sets.*

so on. From this small sample, the hierarchy of memberships in the different mind-sets is not clear. That is, when the respondent does not fall into the 'flavor seeker' group, it is not clear the next likely group to which the respondent might file.

7. Discussion

The 'emerging' science of Mind Genomics traditionally has focused on how people think about one product. The notion of creating a set of Mind Genomics studies appears to be first attempted with the It! studies beginning in 2001. In those studies, the effort was made to identify fundamental mind-sets of respondents across 20–30 different foods and beverages [13].

These early studies opened the way to thinking both about a 'wiki' of the mind for a set of different foods, and the potential of typing a person on these different foods. The early thinking, however, was simply to discover a limited set of overarching categories. Thus, in the first study, the efforts revealed three groups of mind-sets for foods, based on one's desire for the food. The set of 30 foods was encompassed in the so-called Crave It! Study [14]. The three mind-sets were called Elaborates (focusing on the description of the food), Imaginers (focusing on the description of the ambience, and other ancillary factors), and Classics (focusing only on the food itself). These three mind-sets appeared in consecutive studies, albeit in different proportions.

Around 2008, when marketers began to think about using Mind Genomics to sell foods, the notion of typing the same person on a set of related foods began to emerge. The standard question was the same: Across different foods, is there a single mind-set segment which best describes a single individual? And thus, was born the idea for this paper, namely, create a typing tool, the PVI, personal view-point identifier, which could 'sequence' a person's mind, assigning the respondent to different and appropriate mind-sets for each of a set of identifiable products.

As noted in the introduction to this chapter, the evolution of Mind Genomics studies quickly revealed just how easy it was to dig deeply into the granularity of a person's mind on a specific topic. The simplicity, rapidity, and sheer efficiency of a Mind Genomics study soon make it less rewarding to investigate one product with excruciating thoroughness. One might consider that response to Mind Genomics to be more of an indication of personality than a description of the scientific project, but the reality is that it appeared possible to create powerful, granular data at an 'industrial level.' It was easy to investigate 10, 20, 30, or more products or situations (e.g., insurance, anxiety, health issues) as it did to investigate one product or situation. One needed simply to create more studies, launch them in parallel with as many respondents as one wanted, and as many of the types of respondents as were thought to be need. The only constraint was money.

The question arose, however, about interconnecting these results, not at the general level, but at the level of the individual. If one could mind-type a person on 10, 20, or even 100 or more products or situations, was there any way to integrate the data? It was not feasible to run a person on 100 studies, each lasting 3–4 min, simply because of fatigue, boredom, and resistance. Working with 100 products, each study requiring 3–4 min, means that to do the original study at an industrial scale, we would require 300–500 min, or 5+ h. One could, however, create the simply typing tool, the PVI, with each part of the PVI lasting 15–30 s. The typing tool could be run in one long, relaxed, stretched session, lasting about 30–55 min.

The data for this study comes from the typing of 41 students from Ryerson University, done by author SD as part of her senior capstone project. This chapter demonstrates the relative simplicity and power emerging from the research ability to corral data from different studies, reshape the results, and use the resulting data to create a new data set, and in turn to create a new PVI. The PVI, whether for all the products or simply for the 10 dairy products, allows us to type new people in a reasonably short session, to identify relations between who the person is and how the person thinks.

Looking backward at the effort as it applies to the knowledge of thinking, it seems possible now to erect large-scale databases of the mind literally from the 'bottom-up', in short spans of time, with efficiencies never-before realized. One can imagine the power of science, whether food science, medicine, social science, legal science, and so on, when it is possible for practitioners to create these large-scale structures, with the PVI attached, literally one can type millions of people, to understand the covariation of the mind with behavior, with health, and so on, almost ad infinitum.

8. Conclusion

To get a sense of an investor looking at the value of mind-typing a person on a set of different products, consider this scenario, doable now, and most likely the case in the not-too-distant future. Imagine a store with 'beacons', receivers and senders of information. Imagine these beacons linked with computer screens, with the computer screens placed near different parts of the dairy case(s). A shopper who has gone through the PVI exercise, and had her or his mind 'typed,' whether for dairy alone or for many foods, would have a card in her or his bag or wallet. The information in the card would identify the mind-set of the person for the different types of items in the dairy case, or even for the different types of items in the entire store.

One might then imagine the beacon 'reading the card', to discover the mind-sets of the individual with that card. The person herself or himself would not be the relevant information, and thus would remain private. All that would be required would be 'knowing' the mind-set of the person for the particular product. Privacy would be an issue and certainly the massive computations to generate a cogent recommendation for this individual would not be necessary. All the relevant information is stored on the card, that is, the relevant information about what to say to the shopper for the product to be sold. An offer about the product might be made, or the salient messages about the product would appear on the respondent's smart phone, or on electronic signage above the product. The scenario just painted means true individualization of the shopping experience, with the right words, cogent messages, and even electronic, storable coupons.

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Edited by Muhammad Subhan Qureshi

This book discusses advances in the dairy industry in various parts of the world. It is divided into four sections on “Dairy Technology”, “Reproductive Biotechnology”, “Entrepreneurship Development”, and “Sustainable Development”. Chapters address such topics as regional dairy breeds, farming innovations, mineral nutrition, folliculogenesis, fertility in dairy cows, milk hygiene, and much more.

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