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RESEARCH ON DIFFERENT TYPES OF PROTEIN IN COW'S AND SHEEP'S MILK ACCORDING TO DIFFERENT INFLUENCING FACTORS (SEASON, FEED RATION, BREED, PHYSIOLOGICAL CONDITION)

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Abstract

This paper focuses on the dynamic of milk protein in cows and sheep and explores the factors that influence milk protein content. Milk protein plays a crucial role in determining the quantity and quality of milk products, and therefore, various studies have been carried out to investigate the factors that influence milk protein content. To achieve this, we plan to monitor several farms raising different breeds of cows and sheep over a period of time to determine the changes of protein content. The breeds of cows that will be monitored include Holstein, Brown, and Romanian Spotted breeds. While the breeds of sheep that will be monitored have not yet been determined. Previous research has shown that optimizing the nutrition and management of cows and sheep can increase milk protein content. However, further research is necessary to better understand the complex dynamic of milk protein in both species and to develop best practices for improving milk production and quality.

Key words: *cow, milk production, protein, Romania, sheep.*

INTRODUCTION

Milk is commonly defined as a whitish liquid produced by the mammary glands of female mammals after parturition. Physiologically, it is a complex biological fluid that contains a precise balance of essential nutrients such protein, fat, lactose and minerals. The main function of milk is to provide a source of nutrition to support the growth and development of new-borns in mammals (Martin et al., 1999). Milk has taken on a sacred meaning in some ancient cultures, such as Egypt, Iran and India, due to its close relationship with the cow. Before the scientific revolution and industrialization in the 19th century, the production of dairy products such as fermented milk, butter, and cheese already played an important role in human life (Konte, 1999). Nowadays, the dairy industry aims to meet the demands of consumers who are seeking innovative products with consistent quality. Therefore, it must harness the full potential of this seemingly simple but complex raw material. To succeed in the production and sale of dairy products derived from sheep and cows, it is crucial to have access to accurate information on the composition and physicochemical characteristics of the milk from these animals. This data is essential for the effective development of cattle and sheep supply chains and for the commercialization of their products. Sheep milk is used exclusively for cheese production, in order to ensure consistent quality and yield of cheese, particularly the levels of fat and protein, as these parameters have a significant impact on cheese yield (Pellegrini et al., 1997). Therefore, given the scarcity of available data on the variation of protein content in sheep's milk according to various factors, we conducted a bibliographic search to establish links between these variables and those of cow's milk. This research serves as only a preliminary introduction to our study, which will be conducted in a second phase.

MATERIALS AND METHODS

During the initial phase of preparing this review, the topic to be addressed was selected based on the work of several authors. After analysing the existing literature, it was decided that a review article was necessary to gather the scattered information in the current scientific literature on the variation of protein in cow and sheep milk according to several extrinsic and intrinsic factors. The following scientific databases were used to search for relevant articles using appropriate keywords: Web of Science, Science Direct, Scopus, and PubMed. Criteria were established for the inclusion of each publication in this review, prioritizing the most recent and relevant articles for the topic being addressed. The final version of this article includes references dating from 1987 to 2020, with particular attention given to publications from the last 15 years, which represent over 50% of the selected sources.

RESULTS AND DISCUSSIONS

1. Factors influencing milk production

As reported by several authors, the composition of milk varies according to various factors.

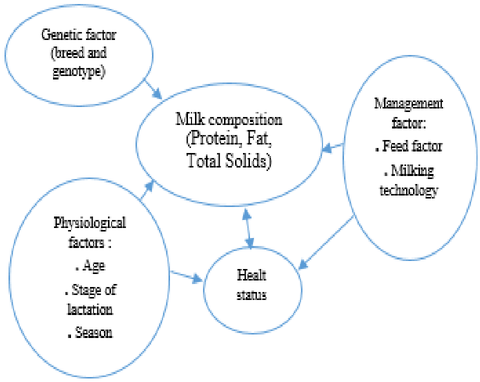

Figure 1. Factors that influence milk quality

Genetic factor

Several studies (Bencini & Pulina, 1997; Bencini, 2001) have established that there was a significant influence of breed on the chemical composition of sheep and cow milk. Considerable variations in milk production have also been observed between and within the same breed. According to Giambra (2011), selection can be used to improve milk production. In fact, selection for milk production has resulted in the creation of dairy breeds and sheep that produce more milk than meat or wool breeds.
For instance, the Awassi dairy breed is capable of producing up to 1000 liters of milk during a lactation period, while the Dorset Poll, a meat-producing breed, only 100 to 150 liters of milk per lactation. There is an inverse correlation between milk production and the concentration of fat and protein in milk. As animals produce more milk, the fat and protein content tends to decrease. This trend is observed not only between more or less productive dairy breeds, but also within a herd, among animals producing more milk, as well as in the same animal producing at different levels throughout its lactation (Casoli et al., 1989) This same author examined the milk composition of 12 breeds of sheep and reported a significant variation in the concentration of fat, ranging from 4.6% in Kurdish Iraqi ewes to 12.6% in Dorset ewe treated in America. The concentration of proteins was less variable, ranging from 4.8% to 7.2% in Armenian Corriedale sheep.

Nutritional factor

The quality and the quantity of consumed food, as well as the amount of ingested water, are key factors that have a significant impact on milk production. According to observations reported by (Saley & Steinmetz, 1994), healthy cattle and sheep with adequate nutrition produce more milk.
Additionally, higher quality nutrition can lead to slight increase in protein and casein content in milk.

Lactation stage and rank

According to research conducted by Casoli et al. (1989), the level of total solids, protein, lactose, and fat in milk tend to gradually decrease during the first few months and towards the end of the lactation period. Data gathered from literature suggest that the peak of milk production generally occurs the second or third lactation period.

Health status

The animal's health status, particularly in the case of udder infection, has a significant influence on milk composition. Most parasitic disorders such as trypanosomiasis, gastrointestinal parasitism, and external parasitism have an impact on milk production.

Milk composition

Sheep's milk differs from cow's milk in its higher content of total solids and essential nutrients. Although the lactose content of sheep's milk is comparable to that of cow's milk, the levels off fat and protein are significantly higher. Therefore, the proportion of lactose to total solids is lower in sheep's milk compared to cow's milk, at 22 to 27% versus 33 to 40%, respectively. The physical properties of sheep's milk, such as its density, viscosity, refractive index, titratable acidity, and freezing point, are higher than those of average cow's milk, as reported by Haenlein and Wendorff (2006). The lipids present in sheep and goat milk also exhibit superior physical characteristics compared to those of cow's milk, although the differences vary depending on the ratios, as emphasized by Anifantakis (1986) and Park (2006). However, some reports indicate that the quality of the different milks does not show significant differences.

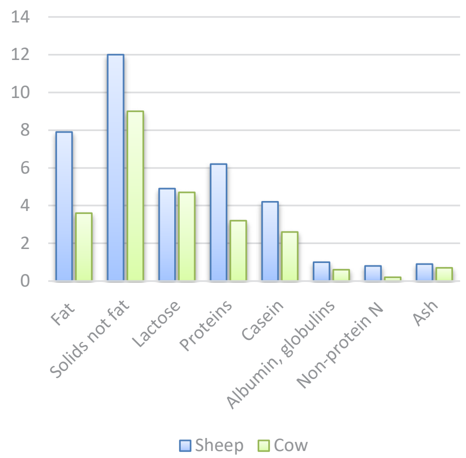

Figure 2. Average composition of basic nutrients in sheep and cow milk (%)

Lipids

Lipids are considered the most essential constituents of milk because they influence the economic nutritional, physical, and sensory properties of dairy products (Anifantakis, 1986; Park, 2006). The energy value and nutritional properties of milk are mainly attributed to the fat content, which also plays an important role in milk processing. The synthesis of milk fat occurs in the mammary epithelial cells, where lipids gradually accumulate and form inclusions that move to the upper part of the cell. The size of fat globules in milk varies from less 0.1 μm to around 18 μm, according to El-Zeini (2006). Mehaia (1995) reported a mean size of fat globules in decreasing order as follows: cow, sheep, and then goat. However, this observation is not entirely consistent with another research (Anifantakis, 1986). In sheep's milk, fat is mostly composed of triacylglycerols, representing about 98% (compared to 95% in cow's milk according to Jensen, 2002), which contain a large number of esterified fatty acids (Ramos & Juarez, 2011).

In addition to triacylglycerol's, sheep's milk contains other simple lipids such as diacylglycerols, monoacylglycerols, and cholesterol esters, as well as complex (Park, 2006; Haenlein & Wendorff, 2006).

The structure of triacylglycerols plays an important role in the rheological properties of milk fat and its behaviour during melting and crystallization.

Furthermore, the composition of triacylglycerols is interesting as it allows determining the origin of milk (Park et al., 2007). The chromatographic profile of sheep milk triacylglycerols present similarities with that observed in cow milk (Precht, 1992).

Moreover, sheep milk presents unique characteristics of fatty acid composition, with higher levels of linoleic acid, similar to those observed in goat milk, as well as a higher concentration of linolenic acid and polyunsaturated fatty acids overall.

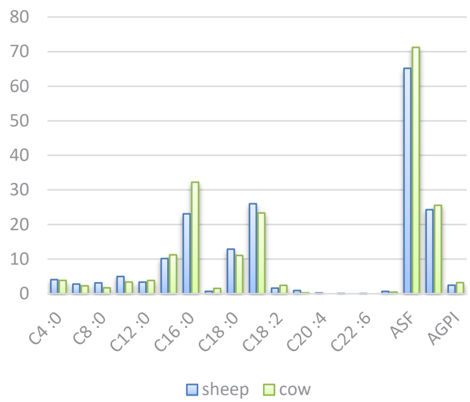

Figure 3. Fatty acids profile of cow and sheep milk (% total fatty acids)

Proteins

According to a study conducted by Park and colleagues in 2007, the most important proteins found in sheep's milk are similar to those found in cow's and goat's milk. Therefore, it is important to obtain simpler information about the composition for physiological and technological aspects. This same study shows that, in average, sheep's milk contains 5.8% protein, which is higher than that of goat's milk (4.6%) and cow's milk (3.3%). Nearly 95% of the total nitrogen in sheep's milk is protein-based, while the remaining 5% comes from other sources (Park et al., 2007). In contrast, goat's milk has a higher level of non-protein nitrogen and less casein than sheep's and cow's milk, which may result in lower cheese production resulting in a less structured texture (Guo, 2003). Sheep's milk, on the other hand, is particularly suitable for coagulation (Park et al., 2007). Several factors can influence protein content, such as lactation stage, season, age, and animal nutrition (Park et al., 2007). Furthermore, the specificity of small ruminants like sheep relies on the organization and mineralization of casein micelles, which are highly mineralized in sheep's milk (Pellegrini et al., 1994). There is also a small amount of a soluble protein with antibacterial properties called Lactoferrin. According to Kinsella and Whitehead (1989), there is a strong correlation between the major whey proteins, namely α-lactalbumin and β-lactoglobulin, and the nutritional value and functional properties of milk. Based on research conducted by Caboni and colleagues in 2019, sheep's milk is rich in lactoferrin and proteins with antibacterial and anti-inflammatory effects. This lactoferrin has also antioxidant, and anticancer effects.

According to research conducted by Zheng and colleagues in 2020, lactoferrin has the ability to attenuate oxidative stress in the hippocampus. Sheep's milk protein contains an important endogenous amino acid called proline, which plays a crucial role in the synthesis of arginine and polyamines, as well as in the activation of mTOR cellular signalling. This signalling initiates the protein synthesis process, including collagen. Proline and hydroxyproline are present in higher quantities in sheep's milk proteins.

According to some studies, the concentration of lactoferrin in cow's milk is inversely proportional to the somatic cell count (SCC) (Hagiwara et al., 2003; Rainard et al., 1982). Studies have also shown that lactoferrin concentration increases during natural or experimental mastitis (Chen et al., 2004; Rainard et al., 1982; Schmitz et al., 2004). In these cases, high levels of lactoferrin appeared to be due to increased synthesis by mammary epithelial cells and/or release by neutrophils present in inflamed tissues, suggesting that lactoferrin could be considered an acute phase protein in cow's milk.

Several factors influence lactoferrin and SCC concentrations. While SCC is regulated by cellular components transported by the bloodstream, lactoferrin is synthesized directly by the mammary gland (Molenaar et al., 1996). Increased somatic cells in milk are triggered by compounds from bacterial cells walls or bacterial metabolites, as well as endogenous components such as cytokines or complement components (Kehrli & Shuster, 1994). Increased cytokine levels during certain physiological processes could also influence lactoferrin synthesis (Baumrucker, 2005).

A study conducted on goats by Hiss et al. (2008) showed a correlation between high lactoferrin concentration and high SCC levels. These researchers suggested that increased lactoferrin levels could play a protective role for the mammary gland, as high somatic cell levels are often associated with the presence of subclinical infectious processes. Lehmann et al. (2013) found that when comparing IgG concentrations during lactation in two groups was not statistically significant, unlike lactoferrin. Thus, when the mammary gland is affected by processes that lead to an increase in SCC, IgG levels are not as strongly affected as lactoferrin levels. This observation could be explained in the mammary gland while IgG primarily comes from blood. Increases in IgG and SCC levels in milk have been widely documented in cases of natural and induced mastitis in cows (Caffin & Poutrel, 1988; Lehmann et al., 2013). It has been reported that levels of IgG, lactoferrin, and bovine serum albumin increase in response to mammary pathologies in cows (Levieux et al., 2002). However, in study of goats with subclinical mastitis, the SCC rate was high, but the IgG rate in milk was even lower than in healthy goats (Ferrer et al., 1997). Such contradictory differences could be related to many factors, such as species specificity, breed, source of infection, and intensity of mammary lesions, among others. Several studies, mainly conducted on cow's milk, have demonstrated a correlation between an increase in SCC and a decrease in milk production. High levels of somatic cell count negatively affect milk protein content, which can lead to a decrease in cheese quality (Barbano et al., 1991). The implication of SCC as an indicator of mastitis or hygienic quality may be different in sheep milk compared to other ruminants, given that in some countries such as France and the United States, values of up to 1 million cells/ ml can be considered normal (Raynal-Ljutovac et al., 2005), even in healthy sheep and goat milk, especially towards the end of lactation. Therefore, Riggio et al. (2010) reported that the SCC level may be high even when the sheep are not infected, suggesting that a healthy animal could be wrongly diagnosed as infected if the SCC level is exclusively taken into account.

a. Casein

Caseins are phosphoproteins that are synthesized in the mammary gland in response to lactogenic hormones and other stimuli.

They are secreted in the form of colloidal aggregates called micelle, which are responsible for most of the unique physical properties of milk (Melanie et al., 1999). Caseins are abundant in milk and play an important role in the dairy industry, making them of interest to biochemists who study them potentially as the most widely studied food proteins (Swaisgood, 2003). Casein represents the main class of proteins present in sheep milk, constituting between 76% and 83% of total proteins (Treacher & Caja, 2002). Among these proteins, the same types as in cow's milk are found, namely αs1-Cn, αs2- Cn, β- Cn, and κ-casein (Table 1).

Table 1. Comparaison of casein profiles for cow's and sheep's milk (%) (Balthazar et al., 2017)

Casein	αS1 casein	αS2 casein	β casein	κ casein
Cow	37	7	42	9
Sheep	6.7	22.8	61.6	8.9

Each of these fractions has a variable proportion in milk and exhibits polymorphism in most animal species (Vera et al., 2009).

The heterogeneity of caseins is determined by various factors, such as the presence of genetic variants, discrete levels of phosphorylation, variation in the extent of glycosylation of the κ-casein fraction, and the coexistence of protein with different chain lengths (Park et al., 2007).

Casein κ is one of the most extensively studied caseins, likely due to its crucial role in micelle stability and dairy processing. Unlike the others caseins (α and β), it contains carbohydrate residues within its structure (Fox & Mulvihill, 1992). In the presence of calcium phosphate, caseins form stable micelles in a colloidal phase that is balanced with the soluble phase of milk. This balance can be adjusted by altering factors such as temperature, pH, and the addition of salts. When lactic acid bacteria convert lactose to lactic acid, the pH of the milk decreases, leading to decalcification of the casein micelles. Alternatively, casein micelles can be destabilized using an enzyme, such as chymosine. Caseins and whey proteins are the main sources of protein in milk, rich in essential and non-essential amino acids, with a high biological value and good digestibility, absorption, and utilization.

b. Whey proteins

Whey proteins from sheep's milk represent between 17 and 22% of all the present proteins (Ramos and Juarez, 2011). The two main proteins found in whey are β-lactoglobulin (β-Lg) and α-lactalbumin (α-La), while immunoglobulins, serum albumin, and protease-peptones are present in lower concentrations. The latter are produced during the breakdown of β-casein by plasmin. The raw milk of various mammalian species constitutes an important source of protein for human nutrition. Table 2 indicates the total amino acid content (free plus protein-bound) per 100 g of milk from cows and sheep raised in Italy.

Although no qualitative differences were observed between the two types of raw milk, quantitative differences were observed. Glutamic acid and glutamine are the most abundant amino acids (approximately 21.6% and 19.6 %, respectively) in both types of milk. The amino acids leucine, lysine, and aspartic acid were also present in sufficient quantities (approximately 7 to 8%) in both types of milk, while proline was more abundant in raw sheep milk (9.3%) than in cow milk (8.9%). The other amino acids did not account for more than 5% of the total protein content, including tyrosine and serine. Furthermore, raw sheep milk had higher content of valine, threonine, and phenylalanine than raw cow milk. The amounts of methionine and cysteine did not exceed 4% of total protein for both types of milk, confirming a low content of sulphur-containing amino acids. In summary, the results indicate that both types of raw milk contain approximately 40% essential amino acids relative to total protein, thus confirming their protein quality. The results also suggest that the amino acid content varies depending on the mammalian species from which the milk is sourced.

Table 2. Amino acid composition of sheep and cow milk proteins, adapted from (Molik et al., 2012)

Amino acid	Cow	Sheep
Ile	4.01	4.22
Leu	8.81	7.91
Lys	7.79	7.47
Phé	4.67	4.76
Thr	4.87	5.06
Trp	-	-
Val	4.79	5.01
Ala	3.31	3.64
Arg	3.33	3.40
Cys	0.87	1.06
Gln	21.67	19.69
Gly	1.80	1.74
Pro	6.58	9.31
Ser	5.96	5.69
Tyr	5.00	5.04

Amino acids are the fundamental building blocks of all proteins, playing a crucial role in the structure and function of cells. However, amino acids also have other important biological functions. In particular, some amino acids are precursors to molecules such as antioxidants, which are essential for protecting cells against damage caused by free radicals. Glutathione, which is made up of the amino acids glutamic acid, cysteine, and glycine, is a naturally tripeptide that is found in most animals, plants, and microorganisms, and it plays various roles in cells, according to Meister and Tate 1976. These roles include its use as coenzyme, interaction with peroxides and free radicals, contribution to the formation of mercapturic acid, and its ability to protect membrane lipids and proteins. However, the presence of rapid turnover of the GSH tripeptide in some tissues suggests that is quickly broken down into its constituent amino acids and regenerated within the cell, as proposed by Meister in 1973. Researchers such as Meister in 1973, Griffith et al. (1979) have proposed that GSH also plays a role in amino acid transport, working in collaboration with the enzyme 7-glutamyl transpeptidase located on the outer surface of plasma membranes. 7-glutamyl transpeptidase is the only biological pathway currently known for breaking the γ-glutamyl bond of GSH. Additionally, it is possible to translocate glutathione out of cells (as reported by Bannai & Tsukeda in 1979, and Griffith et al. in 1979), and it can be utilized (metabolized) by tissues with high glutathione peroxidase activity, such as the kidney. The levels of glutathione were measured in the whole blood and plasma of lactating Holstein cows. Samples were taken from both the internal iliac artery and mammary vein to measure the arteriovenous differences across the vascular bed supplying the mammary gland. The results showed that plasma glutathione levels were very low and there was no significant arteriovenous difference in the levels of this tripeptide across the mammary gland. However, there was a significant disparity between the levels of glutathione in whole blood and plasma, with blood concentration being approximately 200 times high. Additionally, there was a significant arteriovenous difference in glutathione levels across the mammary gland. Studies conducted on living subjects have shown that insufficient levels of cysteine in plasma may limit milk protein synthesis. Using arteriovenous concentration differences and estimated blood flow, it has been calculated that the amount of glutathione absorbed by the mammary gland from whole blood is more than sufficient to explain the amount of cysteine secreted in milk. The mammary tissue of cows has a high activity of GTP cyclohydrolase, according to a study by Baumrucker and Pocius in 1978. This activity allows the tissue to use GSH to synthesis milk proteins. In vitro, studies have shown that the mammary gland has a low capacity for the absorption of cysteine and glutamate compared to other amino acids, according to research by Clark and al, 1978 and Derrig and al in 1974. Cysteine absorption from plasma is often in deficiency, with venous concentration higher than arterial concentration. The use of blood glutathione by the mammary gland as a source of amino acids could be the cause of these results.

Non-protein nitrogen

About 5% to 6.8% of the total nitrogen present in sheep's milk is considered non-protein nitrogen, consisting mainly of urea (45%), free amino acids (16%), creatine (2.4%), creatinine (1.7%), ammonia nitrogen (1%), uric acid (2.1%), and others compounds whose nature remains unknown (Park, 2006). Compared to cow's milk, sheep's milk contains higher amounts of urea and uric acid.

The remaining 20% of milk proteins include whey proteins such as β-lactoglobulin and α-lactalbumin as well as other proteins such as immunoglobulins, serum proteins, fat globule membrane proteins, transferrin, lactoferrin, β2-microglobulin, several enzymes, peptides, and proteolytic products. Protein levels and amino acid profiles vary considerably by species, as well as growth rate, and are influenced by genetic, physiological, nutritional, and environmental factors.

Water

Water is the predominant nutrient for all animals, and it is abundant in milk, representing approximately 88% of its composition. The amount of water in milk is regulated by the synthesis of lactose by secretory cells in the mammary gland.

Carbohydrates

Lactose is the main carbohydrate present in milk. It is formed when molecules of D-galactose combine in a β-galactoside 1,4 linkage. Its concentration in milk varies slightly, ranging from 4.5 to 5.2 g/100 g, and unlike fat content, its concentration cannot be easily modified by diet or by breed of animal. Lactic acid bacteria use lactose as a substrate during fermentation, which leads to the production of lactic acid, resulting in the characteristic texture and flavour of fermented products such as yogurts and cheeses.

Lactose plays a key role in the production of fermented milk as the amount of lactic acid produced by lactic acid bacteria depends on several factors, including bacterial strain, operating parameters, and the available amount of bacterial lactose. Additionally, the buffering capacity of milk also plays an important role in this process, as described by Fillion in 2006.

CONCLUSIONS

In conclusion, milk production for human consumption is subject to strict standards to ensure the health and safety of consumers. The quality and quantity of milk production can be affected by various factors, including race, species, health, nutrition, and age of the animal. Cow milk remains the most commonly produced and processed milk worldwide, but the milk from other mammals such as goats, sheep, and buffalo are of significant economic importance in certain regions, particularly those in the Mediterranean basin. Overall, continued research is necessary to understand the complex factors that affect milk production and quality in different species and to develop best practices for ensuring the availability of high-quality milk for human consumption. The knowledge of variability of sheep casein is still incomplete, which has been confirmed by the discovery of several new variants of casein during screening of milk samples from different breeds by isoelectric focussing (IEF). Therefore, this technique could be widely exploited for typing lactating ewes based on milk protein polymorphisms in a first step, followed by molecular methods. This allows for more complete picture of milk protein genes in sheep and consideration of milk protein variants/haplotypes in scientific breeding programs aimed at preserving biodiversity and/or improving dairy breeds for specific milk protein production.

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