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Comparative Analysis of Amino Acid and Fatty Acid Profiles in Mammalian (goat, sheep, cow and pig) Milk

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Abstract
Milk contains various bioactive components, including proteins and lipids, contributing to its nutritional value and potential health benefits. The study aimed to compare the amino acid and fatty acid profiles in the milk of goats, sheep, cows, and pigs. Raw milk samples from goats, cows, sheep, and pigs were collected from different local farmers, and their fatty acid and amino acid compositions were determined using standard methods. The fatty acid composition of milk from these mammals revealed variations in the levels of specific fatty acids, including heptadecanoic acid, palmitoleic acid, palmitic acid, stearic acid, myristic acid, pentadecanoic acid, behenic acid, oleic acid, caprylic acid, lauric acid, myristoleic acid, caproic acid, and butyric acid. The amino acid composition of milk showed significant differences ($p<0.05$) among the animals, with significant variations ($p<0.05$) in essential amino acids like leucine, phenylalanine, valine, and methionine. Knowing the relationship between milk composition and its physiological effects can inform dietary recommendations and product development strategies to promote human health and well-being.
Keywords: Milk, nutritional composition, fatty acids, amino acids, essential amino acids, and health

Introduction

Consumer curiosity about the potential health benefits of certain foods or physiologically active components has surged. Functional foods, in essence, are foods or dietary elements that offer substantial health advantages beyond basic nutrition (Kaur *et al*., 2014). Mammalian milk is a vital source of nutrition, providing essential nutrients crucial for growth and development. Knowledge of the composition of milk from different mammalian species is critical for assessing its nutritional value and potential health benefits. Apart from its vital active components like enzymes, antibodies, oligosaccharides, antimicrobial peptides, and hormones, milk primarily consists of an emulsion of oil in water (~88%), containing bioactive proteins, lipids, and carbohydrates (Landi *et al*., 2021). Milk proteins and their smaller parts (peptides) provide energy and essential building blocks, such as amino acids and amino groups necessary for nonessential amino acid synthesis (Goulding *et al*., 2019). Amino acids serve as the functional and structural components of proteins, categorized into two nutritional groups: non-essential, which the body can synthesize, and essential amino acids, which cannot be synthesized at a rate sufficient to meet metabolic needs (Alagawany *et al*., 2020; Eluu, Oko, Eluu, *et al*., 2024). The composition and abundance of amino acids in milk can vary significantly between different mammalian species, influencing the nutritional quality and biological activities of the milk (Guinee *et al*., 2007; Park *et al*., 2007). Similarly, fatty acids present in milk contribute to its flavor, texture, and nutritional properties. They are essential for energy metabolism and serve as precursors for synthesizing important molecules such as hormones and cell membranes. The biological functions can be categorized into regulating membrane structure and function, controlling intracellular signaling pathways, transcription factor activity, and gene expression, as well as overseeing the production of bioactive lipid mediators (Calder, 2015). The fatty acid composition of milk is influenced by factors such as diet, genetics, and lactation stage, resulting in unique profiles across different mammalian species (Bauman & Griinari, 2003; Palmquist *et al*., 1993). In this study, we compared the amino acid and fatty acid profiles in the milk of goats, sheep, cows, and pigs. We also discussed the many components of milk produced by mammals and their possible effects on human nutrition and health.

Materials and Methods

Sample collection

Raw milk samples from goats, cows, sheep, and pigs were collected from local farmers in Abakaliki, Ebonyi State, Nigeria, in sterilized bottles and preserved in a refrigerator before laboratory analysis.

Fatty Acid determination

Fat was extracted from the freeze-dried sample to analyze fatty acids using the method outlined by the Association of Official Analytical Chemists (1980), specifically designed for extracting fat from dried milk. The fatty acid composition was then determined using gas-liquid chromatography with an HP-5370A gas-liquid chromatograph equipped with a flame ionization detector. The milk fat was refluxed with a solution of 14% BF3-MeOH and benzene (15 ml and 5 ml, respectively) to prepare fatty acid methyl esters, following procedures outlined by the American Oil Chemists Society (1974). The fatty acid profile was quantified according to methods described by the American Oil Chemists Society (1977).

Amino acid determination

A total of 800 µL of 99% cold ethanol and 200 nmol of nor-Leucine (nor-Leu) as an internal reference standard were added to three sections of 200 µL of different types of raw milk to assess the composition of free amino acids. The mixture was centrifuged at 14,000 × g at 4 ◦C after homogenizing with a Teflon pestle. Exactly 3% sulfosalicylic acid (500 µL) was added to the supernatants after centrifugation to precipitate any leftover protein fraction. The supernatants were then freeze-dried. A post-column ninhydrin derivatization system was fitted to the Biochrom30 amino acid analyzer (Biochrom, Cambridge, UK) to analyze the supernatants.

Statistical analysis

The means ± standard deviation was used to express the data. Using the Statistical Package for the Social Sciences (SPSS) version 20, comparisons between groups were made using one-way analysis of variance (ANOVA). The least significant differences were identified at $p < 0.05$.

Results

Fatty acid composition of milk from different animal sources

Figure 1 shows the fatty acid compositions of milk from various animals, including goats, sheep, pigs, and cows. The result showed that heptadecanoic acid was most abundant in milk from sheep, followed by pig milk, with the lowest levels found in goat milk. Significantly higher levels of heptadecanoic acid were observed in sheep compared to goat and cow milk ($p<0.05$). Palmitoleic acid levels were similar across all animal species studied ($p>0.05$). Goat milk had significantly higher levels of palmitic acid than other animals, whereas the levels of palmitic acid in other animals did not differ significantly ($p>0.05$). Furthermore, levels of stearic acid, myristic acid, pentadecanoic acid, behenic acid, and oleic acid showed no significant difference among the different animal groups ($p>0.05$). However, the concentration of caprylic acid in cow milk was significantly higher than in pig milk ($p<0.05$). Meanwhile, there was no significant difference between goat, sheep, and pig milk ($p>0.05$). On the other hand, cow milk contained significantly higher levels of lauric acid than sheep and pig milk but similar to goat milk. The levels of myristoleic acid in goat, sheep, and pig milk were comparable. However, cow milk had significantly higher levels of myristoleic acid ($p<0.05$) than the other sources. Caproic acid levels were lower in cow milk, while they were similar ($p>0.05$) in milk from the other animals. Additionally, butyric acid was found in significantly higher amounts in pig milk than in milk from other animals.

Amino acid composition of milk from different animal sources

Figure 2 shows the amino acid compositions of milk from various animal sources. It was observed that leucine content was significantly higher ($p<0.05$) in sheep compared to other milk sources. Meanwhile, the values for leucine were comparable to those of milk from other animal sources. Lysine and tryptophan contents showed no significant differences across the studied animal milk ($p>0.05$). Isoleucine levels were significantly higher in sheep than in other sources, with cow and pig milk exhibiting similar levels, while goat milk varied significantly. Similarly, goat and sheep presented comparable concentrations of phenylalanine ($p>0.05$), while pig milk had significantly higher levels ($p<0.05$) than goat and sheep milk. Goats showed a significantly lower ($p<0.05$) valine and methionine concentration than sheep, pigs, and cows. Meanwhile, no significant difference ($p>0.05$) was observed in the valine and methionine contents of sheep, pig, and cow milk. Proline was more abundant in goat milk and least abundant in sheep milk, with pig and cow milk showing comparable amounts. Sheep milk had the highest amount of arginine and histidine, while the other sources' content of arginine and histidine did not differ significantly ($p>0.05$). Goat milk ranked last in its tyrosine content compared to sheep, pig, and cow milk. The value of cystine in goat milk was significantly higher than in the other animal milk. Alanine had significantly higher amounts in sheep milk when compared to other sources, followed by goat milk, which had significantly higher ($p<0.05$) than pig and cow milk. Among all the amino acids found in the different milk samples, glutamic acid concentration was the highest, with red Sokoto sheep and sheep exhibiting values of 16.2 g/100g and 15.6 g/100g, respectively. However, glutamic acid levels in sheep and goat milk were not significantly different ($p>0.05$), and comparable values were obtained in pig and cow milk. The level of glycine in goat, pig, and cow milk was similar but lower in sheep milk. Threonine, serine, and aspartic acid recorded significantly higher ($p<0.05$) concentrations in sheep, while cow milk had the lowest concentrations of the three amino acids.

Discussion

Fatty acids of varying lengths and degrees of saturation constitute the primary constituents of lipid molecules found in milk. Medium-chain fatty acids (C:16) are produced within mammary glands; meanwhile, long-chain saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids are transported to mammary glands through the bloodstream (Arsić *et al*., 2009). Consuming dairy fat constitutes the primary origin of heptadecanoic, which has been linked to enhanced insulin sensitivity (Hellgren & Nordby, 2017). The abundance of heptadecanoic acid, known for its antimicrobial properties and potential health benefits (Haug *et al*., 2007) was highest in sheep milk, followed by pig milk, and lowest in goat milk. This indicates the variability in fatty acid profiles among different mammals, which may have implications for the nutritional quality of milk consumed by humans. The significantly higher levels of heptadecanoic acid in sheep compared to goat and cow milk shows the importance of considering animal-specific differences in milk composition when evaluating nutritional adequacy and potential health benefits. Such differences may arise due to variations in animal diet, genetics, and metabolic processes (Palmquist, 2009).

The similarity in palmitoleic acid levels across all animal species suggests a consistent presence of this fatty acid regardless of the animal. Palmitoleic acid, an omega-7 monounsaturated fatty acid, carries notable significance in nutrition owing to its potential health advantages (Lee *et al*., 2015). Studies indicate its involvement in enhancing cardiovascular health, aiding metabolic processes, and mitigating inflammation. Additionally, dietary intake of palmitoleic acid has been associated with reduced risks of diabetes and inflammation. In pharmaceutical contexts, it is commonly utilized as an anti-thrombotic agent to prevent strokes (Hu *et al*., 2019; Lee *et al*., 2015).

Moreover, the lack of significant variation in stearic acid, myristic acid, pentadecanoic acid, behenic acid, and oleic acid levels among different animal groups implies a degree of uniformity in the composition of these fatty acids across mammalian milk. Stearic acid, commonly found in foods such as meat and dairy, is known to have a neutral or even advantageous effect on cardiovascular health, as it does not significantly elevate low-density lipoprotein (LDL) cholesterol levels (Mensink, 2005). Myristic acid, prevalent in dairy products and tropical oils, can potentially increase LDL cholesterol levels with excessive consumption; however, it plays essential roles in cell membrane function and lipid signaling (Saraswathi *et al*., 2022). Pentadecanoic acid, though present in small quantities in dairy fat and meats, exhibits potential anti-inflammatory and anti-diabetic properties, showing an inverse association with the risk of type 2 diabetes and cardiovascular disease (Santaren *et al*., 2014). Behenic acid, less commonly encountered in diets, has been investigated for its moisturizing properties in cosmetics and its stabilizing effects in pharmaceutical formulations (Cater & Denke, 2001). Oleic acid contributes to heart health by improving lipid profiles and demonstrating anti-inflammatory effects, potentially lowering the risk of chronic diseases such as cardiovascular disease and cancer (Bermudez *et al*., 2011; Pauwels, 2011; Pegoraro *et al*., 2024). However, the significant difference in caprylic acid concentration between cow and pig milk highlights potential species-specific variations in medium-chain fatty acid content, which may have implications for lipid metabolism and health outcomes (Houaga *et al*., 2018a, 2018b; Rooke *et al*., 2010).

Similarly, the higher levels of lauric acid in cow milk than in sheep and pig milk indicate the influence of animal-specific factors on milk fatty acid composition, with potential implications for nutritional quality and product development. Furthermore, the amino acid composition of milk from different animal sources provides valuable insights into their nutritional profiles and potential health benefits. The significantly higher ($p<0.05$) leucine content in sheep milk compared to other sources is noteworthy. Leucine is well known for being a dietary signal that regulates protein synthesis in skeletal muscle and other body tissues and is a building block for protein synthesis and muscle maintenance (Suryawan *et al*., 2011). Similarly, variations in isoleucine, phenylalanine, valine, and methionine levels were observed among the different animals. These variations may influence the nutritional adequacy and biological value of milk protein, affecting its suitability for specific dietary needs and metabolic requirements. Isoleucine, an essential amino acid crucial for protein synthesis and muscle metabolism, influences energy regulation and immune function. It contributes significantly to muscle growth and repair as a branched-chain amino acid (BCAA) alongside leucine and valine (Chou & Fasman, 1973; Dimou *et al*., 2022). Phenylalanine is a precursor for neurotransmitters and hormones like tyrosine, dopamine, epinephrine, and norepinephrine while also essential for melanin production, influencing hair and skin color. Valine, another vital branched-chain amino acid, aids in protein synthesis, muscle repair, and energy production, which is particularly beneficial for endurance athletes (DePietro & Fernstrom, 1999; Dimou *et al*., 2022; Fernstrom & Fernstrom, 2007). Methionine, an essential amino acid, is pivotal for protein synthesis and serves as the starting point for various essential molecules, including those involved in sulfur metabolism and methylation processes crucial for gene expression regulation and cellular functions (Geltink & Pearce, 2019). The higher proline content in goat milk compared to sheep milk was noted. Proline is a key amino acid in collagen formation and is essential for maintaining skin elasticity and joint integrity (Albaugh *et al*., 2017).

Furthermore, the significantly higher levels ($p<0.05$) of arginine and histidine in sheep milk highlight its potential role in cardiovascular health and immune function. Arginine is a precursor of nitric oxide, which regulates blood vessel function and blood pressure, while histidine is involved in immune responses and allergic reactions (Gambardella *et al*., 2020; Luiking *et al*., 2012). The variations in cystine, alanine, and glutamic acid levels among different animal milk show the complexity of milk protein composition and its implications for human health. Cystine is a sulfur-containing amino acid important for antioxidant defense and detoxification pathways, while alanine and glutamic acid play roles in energy metabolism and neurotransmitter synthesis (Bonifácio *et al*., 2021). Moreover, the differences in threonine, serine, and aspartic acid concentrations highlight mammalian-specific milk protein quality and functionality variations. Threonine is essential for protein synthesis and immune function, while serine and aspartic acid contribute to neurotransmitter production and cell signaling processes (Wu, 2010).

Conclusion

In conclusion, this study offers insights into the amino acid and fatty acid profiles that contribute to their health benefits. The findings provide the foundation for further research into optimizing dairy products for human consumption, considering consumers' diverse nutritional needs and preferences. Understanding the intricate relationship between milk composition and its physiological effects can inform dietary recommendations and product development strategies to promote human health and well-being.

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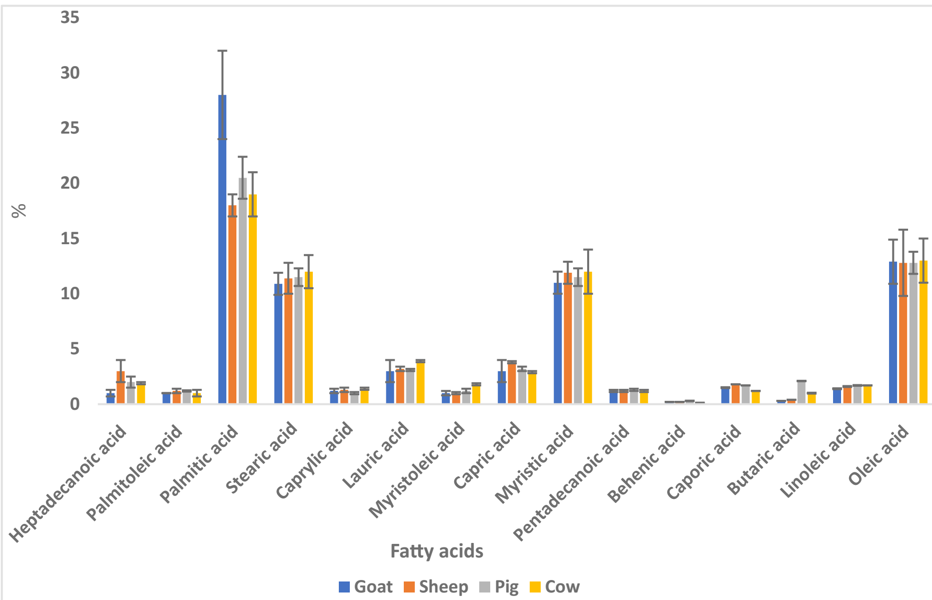

Figure 1: Fatty acid components in different animal milk

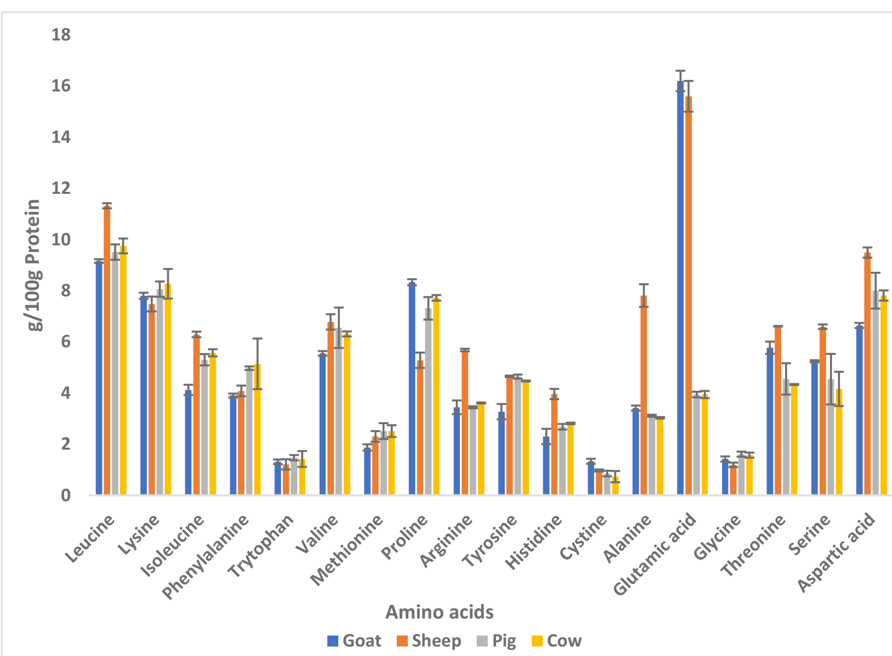

Figure 2: Amino acid contents of milk from different animal sources