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Differences in milk fat composition across selected mammals - A review

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SUMMARY: Milk fat composition varies significantly across species, influencing both the nutritional and sensory qualities of milk. The size of milk fat globules (MFGs) and the three-layer structure of the milk fat globule membrane (MFGM) differ among species, impacting digestion and nutrient absorption. Donkey and human milk, with smaller fat globules and outer glycoprotein layers, facilitate faster digestion. In contrast, the additional phospholipid layer found in ruminant milk hinders lipase activity, leading to reduced digestibility compared to donkey and human milk. Ruminant milk, with higher levels of saturated fatty acids (SFAs) (54.48-70.15 %) and conjugated linoleic acid (CLA) (0.72-1.69 %), contrasts with non-ruminant milk, which is richer in polyunsaturated fatty acids (PUFAs) (17.33-31.70 %) and has a lower Atherogenic Index (AI) and Thrombogenic Index (TI), suggesting potential cardiovascular health benefits. Various processing methods, including homogenization, pasteurization, boiling, cooling, and freezing, affect the size of the globules, the fatty acid profiles, and overall digestibility of the milk.

KEYWORDS: *Lipid digestion; MFGM; Milk fat; Non-ruminant; Phospholipids; Processing; Ruminant.*

RESUMEN: ***Diferencias en la composición de la grasa de la leche en mamíferos seleccionados: Revisión.*** La composición de la grasa de la leche varía significativamente entre especies, lo que influye tanto en las cualidades nutricionales como sensoriales de la leche. El tamaño de los glóbulos de grasa de la leche (GGL) y la estructura de tres capas de la membrana de los glóbulos de grasa de la leche (GGM) difieren entre especies, lo que afecta a la digestión y la absorción de nutrientes. La leche de burra y la humana, con glóbulos de grasa más pequeños y capas externas de glucoproteína, facilitan una digestión más rápida. Por el contrario, la capa adicional de fosfolípidos que se encuentra en la leche de rumiantes dificulta la actividad de la lipasa, lo que conduce a una digestibilidad reducida en comparación con la leche de burra y la humana. La leche de rumiantes, con niveles más altos de ácidos grasos saturados (AGS) (54,48-70,15 %) y ácido linoleico conjugado (ALC) (0,72-1,69 %), contrasta con la leche de los no-rumiantes, que es más rica en ácidos grasos poliinsaturados (AGPI) (17,33-31,70 %) y tiene índices aterogénico (IA) y trombogénico (IT) más bajos, lo que sugiere posibles beneficios para la salud cardiovascular. Varios métodos de procesamiento, incluida la homogeneización, pasteurización, ebullición, enfriamiento y congelación, afectan al tamaño de los glóbulos, los perfiles de ácidos grasos y la digestibilidad general de la leche.

PALABRAS CLAVE: *Digestión de lípidos; Fosfolípidos; Grasa de leche; MFGM; No rumiantes; Procesamiento; Rumiantes.*

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1. INTRODUCTION

Fat specifically refers to one subgroup of neutral lipids known as triacylglycerol. Milk contains 0.3 % to 9.0 % fat, depending on the species (Roy *et al*., 2020) and it plays an important role in the nutritional value, sensory perception and technological qualities of dairy products. Variations in milk fat composition among milch animal species significantly impact both milk characteristics and the quality of dairy products. Nowadays, there is a trend toward non-bovine milk due to consumer preferences and unsuitable natural climates for dairy cattle survival. Major milch species such as cows (*Bos taurus*), buffalos (*Bubalus bubalis*), camels (*Camelus*), goats (*Capra aegagrus hircus*) and sheep (*Ovis aries*) dominate the global dairy industry with unique contributions to milk fat profiles, while minor species such as donkey (*Equus* *asinus*) provide specific nutritional benefits and cultural significance in certain regions. Since 1961, the share of non-bovine milk in global production has increased from approximately 9 to 19 % by 2018. Over this period, buffalo milk production increased nearly threefold, camel milk almost doubled and goat milk saw a modest rise. However, comprehensive global data on milk production from other species like horse, donkey and deer are not currently available (Roy *et al*., 2020). Different animals can be categorized into ruminants and non-ruminants. The milk lipids of these two groups can be differentiated on a quantitative and qualitative basis (Singh and Dabas, 2023). In terms of quantitative differences, buffalo (7.1 % fat) and sheep (7.3 % fat) milk stand out for their higher fat content. In contrast, non-ruminant milk, like that of donkeys (0.76 % fat) and humans (3.8 % fat), contains comparatively lower fat levels (Nayak *et al*., 2020). The lower fat content in donkey milk results in fewer calories compared to other types of milk, making it a beneficial option for those on calorie-restricted diets. Chemically, donkey milk closely resembles human milk (Vincenzetti *et al*., 2021) while goat milk is more similar to cow milk (Park, 2017). Milk lipid composition determines qualitative differences (Singh and Dabas, 2023). Milk from ruminants and humans contains higher levels of triacylglycerols (95 to 98.2 %) (Singh and Dabas, 2023) compared to donkey milk, which contains 80-85 % triacylglycerols (Claeys *et al*., 2014). Additionally, the levels of free fatty acids (0.027-0.5 %) and phospholipids (0.26-0.6 %) are lower in ruminant and human milk (Singh and Dabas, 2023). Donkey milk, with its distinctive lipid profile, shows higher buffering properties due to its phospholipid content (5-10 %). Although it has not been explicitly stated, the high levels of free fatty acids (9.5 %) (Claeys *et al*., 2014) suggest significant lipolysis. If lipolysis were the cause of the FFAs, higher levels of mono- and di-glycerides would be expected, but these make up only 1.8 % of the total lipids (Uniacke-Lowe, 2011).

2. MILK FAT GLOBULES AND THEIR SIZE

Milk fat exists in tiny round droplets known as globules, with sizes ranging from 0.2 to 15 μm. Larger fat globules are typically associated with higher fat content. Different animals have varying globule sizes in their milk. For example, cow (2.5-5.7 μm), goat (3.5-5.7 μm), sheep (2.8-4.0 μm), camel (3.0-5.0 μm), human (4.2-5.1 μm), and donkey (2.0-3.0 μm) milk all have smaller globules compared to buffalo milk, which ranges from 4.1 to 8.7 μm (Roy *et al*., 2020; Thum *et al*., 2023). These size differences can affect how the fat is digested. Smaller fat globules are digested more rapidly compared to larger ones because they pose a larger surface area, facilitating increased adsorption of lipase (Chai *et al*., 2022). The fat globule size also influences the nutritional and functional qualities of milk.

The diversity in fat globule size can be influenced by factors like proteins, lipids, hormones, physiology, diet and genetics. Human and ruminant milk have a similar MFG structure, but differ in their protein profiles, concentrations, and the proportions of polar lipids. The distribution, profile, and size of polar lipids and proteins within the milk fat globule membrane vary across different species and breeds of animals. The release of the milk fat globules (MFG) from mammary epithelial cells (MEC) is driven by interactions among adipophilin (ADPF), butyrophilin (BTN) and xanthine oxidase/dehydrogenase (XDH). In this context, ADPF, BTN, and XDH form a complex at the surface of the fat globule, facilitating the attachment of the bilayer membrane to the lipid droplets (LDs). This interaction causes the membrane of the lipid droplet to deform, leading to the formation of LDs from secretory cells. Different species exhibit varying ratios of BTN/XDH, although this ratio remains consistent within breeds. In sheep and cow milk, the molar ratio of BTN/XDH typically ranges from 1:4 to 1:2. In contrast, BTN levels in human milk are approximately six times greater than those of XDH. In goat milk, the molar ratio of BTN to XDH is 1:1 (Zamora *et al*., 2009). Phosphatidylethanolamine (PE) is mainly located in the inner layer of MFGM and contributes to size variation in MFG. High PE can reduce the interfacial surface tension of lipids and produce fewer larger droplets. In human milk PE is lower compared to ruminant milk (Thum *et al*., 2023). In cow milk phosphatidylcholine (PC) levels were higher in small MFG (Logan *et al*., 2014). Prolactin and oxytocin hormones have a consistent influence across species. For humans, they are shown to have an effect throughout the lactation stages. When progesterone increases MFGs size can be decreased. Increased blood insulin levels lead to a reduction in milk fat globule (MFG) size across all milk species (Thum *et al*., 2023).

During the early phases of lactation, it has been shown that the negative energy balance in cows results in milk fat globules (MFGs) with less palmitic acid, more oleic acid, smaller globule size, and reduced fatty acid synthesis from scratch (Thum *et al*., 2023). In humans, the size of MFGs decreases from colostrum to transitional milk, and then increases in mature milk (Thum *et al*., 2023). Conversely, in bovine milk, the size of MFGs decreases from colostrum to mature milk. In addition, the rate of milk release may influence MFG size in bovine species (Thum *et al*., 2023). When cow, human and sheep were fed a diet of fresh pasture with added cereal concentrate versus a diet of corn silage with added soybean meal, there was a decrease in MFG size in their milk. However, in goat milk, pasture has a positive correlation with MFGs' size (Logan *et al*., 2014). Polyunsaturated fatty acids (PUFAs) have an inverse relationship with the MFGs size in all species of milk (Thum *et al*., 2023).

3. MILK FAT GLOBULE MEMBRANE

Milk fat exists in the form of triacylglycerol droplets enveloped by a complex membrane known as the milk fat globule membrane (MFGM) which originates from the mammary epithelial cell. The MFGM stabilizes the fat droplets and helps them integrate into the milk. In 1 ml of raw milk, there are roughly 10 billion fat globules. MFGM is a natural emulsifier which prevents the aggregation and merging of fat globules in milk while shielding them from enzymatic degradation (Singh and Gallier, 2017). The milk fat globule membrane (MFGM) makes up 2 to 6 % of the total mass of milk fat globules (MFGs), consisting of 60 % proteins and 40 % lipids. MFGM proteins represent 1 to 4 % of the total proteins in milk (Manoni *et al*., 2021). About 30 % of the membrane consists of lipids, which include 25 % phospholipids, 3 % cerebrosides and 2 % cholesterol (Mansson, 2008). Smaller fat globules have both a higher specific surface area and contain more MFGM than larger globules (Manoni *et al*., 2021). MFGM comprises three layers with an approximate thickness of 10-50 nm (Bernard, 2018). The inner triglyceride-rich core protects the core lipid and interacts with the membrane via hydrophobic tails of polar lipids which come from the endoplasmic reticulum. The outer proteinaceous core and glycocalyx layer come from secretory cells and are in contact with hydrophobic lipid groups. The macronutrient composition is varied, with an approximate ratio of proteins, lipids and carbohydrates of 4:3:1 (Venkat *et al*., 2024).

The structure of MFGM varies among milk species. In donkey and human milk, the milk fat globule membrane (MFGM) is composed of three layers: an inner protein layer, a middle phospholipid layer, and an outer glycoprotein layer. Glycoproteins, with their complex carbohydrate structure linked to branched oligosaccharides, may enhance digestion by binding to lipases. In ruminant milk, the outer layer of the MFGM includes an additional coating of phospholipids, which makes it more difficult for pancreatic lipase to access the triglyceride core. This phospholipid layer may act as a barrier, preventing pancreatic lipase from reaching the fat core (Santillo *et al*., 2018). As a result, the structural differences in the MFGM affect fat digestion.

Sun *et al.* (2019) characterized and compared the MFGM proteins of both Guanzhong goat and Holstein cow milk using proteomic techniques. A total of 776 MFGM proteins were detected and characterized (593 for goat milk and 349 for cow milk). Functional category analyses revealed that the MFGM proteins from both goat and cow milk were primarily composed of three abundant protein types: phosphoproteins, membrane-related proteins, and acetylation-related proteins. Goat milk exhibited significant differences in the number of proteins, Gene Ontology annotation categories, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in comparison to cow milk's MFGM proteins.

A qualitative and quantitative comparison of MFGM phosphoproteomics between colostrum and mature breast milk using a label-free quantitative technique was made by Yang e*t al*. (2020). Their results indicated that colostrum contained a higher number of phosphorylation sites. MFGM exhibits significant variations across different mammalian species and stages of lactation. The MFGM proteins in Murrah, Nili-Ravi, and Mediterranean buffaloes were identified using the tandem mass tag proteomic method. A total of 1250 MFGM proteins were detected, with 103 of them showing differential expression across the different breeds Li *et al.* (2021).

4. PHOSPHOLIPIDS

A phospholipid is composed of a glycerol molecule linked to two fatty acids at one end and a phosphoric acid with an organic alcohol group esterified at the other end. They possess a non-polar tail and a polar head, which contributes to their amphiphilic characteristics. This attribute influences their role, behavior and function. It is a crucial part of the lipid bilayer present in every cell membrane. Phospholipids (PLs), obtained directly from dietary intake or synthesized within the body, play a critical role in lipid digestion, transport and absorption in inflammatory processes. PLs make up 0.5-1.0 % of milk fat. Dietary PLs are found in many foods, including eggs, meat, soybeans, milk and dairy products and are generally ingested in modest quantities, typically ranging from 2-5 gm per day (Garcia *et al*., 2012).

Glycerophospholipids and sphingolipids are two main categories of PLs. The primary glycerophospholipids include phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and phosphatidylserine (PS). The other significant category of PLs is sphingolipids, with sphingomyelin (SM) being the most prevalent. Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin (SM) were the most abundant phospholipids in all species (Table 1) and made up 62 to 80 % of the total phospholipids. These were followed by phosphatidylserine (PS) and phosphatidylinositol (PI) at 12 to 15 %, along with minor classes such as lysophospholipids, plasmalogens and alkyl-acyl PC, which were below 5 % (García *et al*., 2012). PE and PC are zwitterions carry a neutral charge at pH 7, depending on the polar head group structure and the pH of the surrounding medium. At this pH level, PI and PS are both negatively charged (Garcia *et al*., 2012).

5. STEROLS

Sterols, which make up 0.3 to 0.5 % of the MFGM, are cyclic compounds with a 3-hydroxyl group in the β configuration (Dhankhar *et al*., 2020). This sterol is essential for the development of human newborns' brain and endocrine system, and acts as a precursor for vitamin D, steroid hormones and bile acids. The European Food Safety Authority (EFSA, 2012) reports that adding 1.5-2.4 grams of sterols and plant stanols to foods like margarine spreads, mayonnaise, salad dressings, and dairy products such as milk, yogurt, and cheese can lead to a clinically meaningful reduction in low-density lipoprotein (LDL) cholesterol, typically decreasing by 7 to 10.5 %. Cholesterol is the main sterol in milk fat, comprising approximately 95 % of the total, with minor quantities of other sterols which constitute 2 to 7 % (Martini *et al*., 2021) and vary among different species (Table 2). Specifically, cholesterol comprises 94 % of the total sterols in cow milk, 94 to 98 % in goat milk, 96 to 99 % in sheep milk and 88 to 95 % in human milk (Martini *et al*., 2021). The minor sterols found in milk are mainly intermediates formed during the synthesis of cholesterol. In ruminant milk, along with cholesterol, compounds such as dihydrolanosterol, 7-dehydrocholesterol, β-sitosterol, lanosterol, 3,5-cholestadiene-7-one, campesterol, and stigmasterol (plant sterols) are present. In contrast, non-ruminant milk, including that of humans and donkeys, contains cholesterol along with phytosterols (plant sterols) (Singh and Dabas, 2023). Lanosterol is currently being investigated for its possible preventive effects against colon cancer; whereas desmosterol is gaining attention for its ability to modify membrane fluidity, which may help to protect against Hepatitis C viral infection. Phytosterols are naturally occurring compounds found in the lipid-rich portions

Table 1. Composition of phospholipids of milk species

PLs	Cow	Buffalo	Goat	Sheep	Camel	Human	Donkey
Total phospholipid (mg/100 mL)	34.4-41.9	32.4-41.4	19.5-44.5	30.8-47.6	25.7-66.0	15.3-53.5	0.29-3.89
Phosphatidylcholine (PC)(%)	21.8-36.0	27.8-35.56	25.7-28.2	29.2-29.4	24.0	19-27.9	25
Phosphatidyl ethanolamine (PE)(%)	22.5-32.9	17.37-29.6	25.5-35.4	27.0-36.0	35.9	25.9-31.0	31
Phosphatidyl serine (PS)(%)	7.3-15.0	3.9-8.09	3.2-9.6	3.1-3.4	4.9	5.8-15.0	4
Phosphatidyl inositol (PI)(%)	4.2-11.2	4.2-12.55	1.4-5.6	3.4	5.9	4.2-5.0	4
Sphingomyelin (SM)(%)	22.5-28.4	13.48-32.1	27.9-35.9	11.56-28.3	28.3	12-31.1	36

(Cerbulis *et al*., 1982; Ma *et al*., 2017; Ali *et al*., 2018; Bourlieu *et al*., 2021; Singh and Dabas, 2023)

Table 2. Sterol composition of milk species

Parameters (mg/100mL milk)	Cow	Buffalo	Goat	Camel	Sheep	Human	Donkey
Cholesterol	13.1-31.4	4.0-18.0	10.7-18.1	31.3-37.1	14.0-29.0	14.0-20.0	0.9-2.9
Lanosterol	6.05	9.11	8.56	6.08	6.86	0.04	–
Desmosterol	0.62	2.77	1.36	0.92	0.41	1.50	–
Dihydrolanosterol	0.63	1.25	0.97	0.85	4.15	–	–
Lathosterol	3.15	7.50	3.85	3.63	1.81	0.04	–

(Goudjil *et al*., 2003; Gantner *et al*., 2015; Contarini *et al*., 2017; Hamdan *et al*., 2018; Dhankhar *et al*., 2020; Martini *et al*., 2021)

of all plants, and are thought to help reduce the risk of cardiovascular disease. Fatty acids also impact the cholesterol level in milk. Approximately 14 % of fatty acids in milk can raise cholesterol levels in the blood. Conversely, 45 % of these fatty acids help lower cholesterol, while 41 % are considered neutral (Pietrzak-Fiecko and Sadowska, 2020).

6. FAT SOLUBLE VITAMINS

Fat-soluble vitamins are nutrients that dissolve in organic solvents and are absorbed and transported similarly to fats. These vitamins, which include A, D, E and K, are essential in the diet to promote growth, reproduction and overall health. These lipophilic vitamins are found in either the aqueous phase or the lipid fraction of milk and associated with the MFGM and MFGM core. Vitamin-A, Vitamin D3, α-tocopherol and some riboflavin are primarily found in MFGM whereas, β-carotene and γ-tocopherol are mainly located in the MFGM core (Manoni *et al*., 2021). The lipid portion of milk acts as an important storage source for fat soluble vitamins. The number of fat-soluble vitamins in milk fat is linked to both the overall milk fat quantity and its composition. The distribution of fat-soluble vitamins in milk differs among mammalian species (Table 3) and is notably affected by the diet of the animal. Specifically, the levels of vitamins A, D, E and K in milk are directly affected by what the animal consumes. The main type of vitamin A found in milk is retinol esters. Vitamin A concentration is higher in smaller milk fat globules (MFGs) compared to larger ones, due to an inverse relationship between vitamin A content and MFG size (Manoni *et al*., 2021). This explains the higher vitamin A levels found in human and donkey milk. Vitamin D3's connection to MFGM is facilitated through its binding to β-lactoglobulin A. When heat treatments are applied, β-lactoglobulin A directly binds to the MFGM, forming a "bridge" that connects the MFGM with vitamin D3. This interaction highlights the indirect association between vitamin D3 and MFGM. Vitamin E, which includes four tocotrienols (α, β, γ, and δ) and four tocopherols (α, β, γ, and δ), is recognized for its antioxidant properties. Cow's milk contains primarily α- and γ-tocopherols. In contrast, human milk includes α-tocopherol, β-tocopherols, γ-tocopherols and δ-tocopherols, as well as γ-tocotrienol (Singh and Dabas, 2023). In milk, α-tocopherol levels increase by 17 mg for every 1 g of added milk fat. This compound primarily protects the MFGM phospholipids from lipid peroxidation (Manoni *et al*., 2021). Vitamin K, a lesser-known fat-soluble vitamin, is generally absent in defatted milk (Bernard *et al*., 2018).

7. FATTY ACIDS

Fatty acids, derived from the breakdown of glycerides, are long-chain carboxylic acids. Typically,

Table 3. Vitamin concentration of milk species

Vitamin (µg/100ml)	Cow	Buffalo	Sheep	Goat	Camel	Human	Donkey
Vit-A	41	30.7	44	40	26.7	60	58
Vit-D	0.08	–	0.18	0.06	0.3	0.06	2.32
Vit-E	113	26.6	110	0.04	1.78	237	5.2
Vit-K	1.1	–	–	0.3	0.04	0.2	nd

nd (no detected); (Haddadin *et al*., 2008; Ljutovac *et al*., 2008; El-Salam *et al*., 2011; Vincenzetti *et al*., 2021) these fatty acids are unbranched and have carbon atoms that are even in number. However, small quantities of branched odd-numbered fatty acids are also found in milk fat, which is synthesized by microbial flora in the rumen (Mollica *et al*., 2021). Long-chain fatty acids (LCFA) (C_{18-24}) are generally obtained through dietary sources; whereas short-chain fatty acid (SCFA) (C_{4-14}) and certain medium-chain fatty acids (MCFA) (C_{16}) are synthesized *de novo* (Felice *et al*., 2021). There are 2 types of FAs, essential and non-essential. Saturated fatty acids (SFAs) are categorized as non-essential nutrients because the human body produces them via lipogenesis. This process occurs primarily in the endoplasmic reticulum of liver cells, where carbohydrates are converted into fatty acids. However, humans lack the enzyme "Fatty Acid Desaturases" in the endoplasmic reticulum to introduce double bonds beyond the 9th carbon. As a result, linoleic acid and linolenic acid cannot be produced by the human body (Pelley, 2012) and must be obtained from external sources such as plants, where they are abundant and said to be essential FAs.

The composition of fatty acids in milk varies across species (Table 4), and significantly influences its nutritional value. Ruminant milk contains high levels of SFA due to the biohydrogenation of unsaturated fatty acids (USFA) in the rumen, which also results in higher levels of CLA. This composition is influenced by diet and dietary lipid supplements. In contrast, monounsaturated fatty acids (MUFA) are more prevalent in human milk, while non-ruminant milk is rich in polyunsaturated fatty acids (PUFA) because these animals lack the rumen necessary for PUFA biohydrogenation and can directly absorb PUFA from their diet (Felice *et al*., 2021).

From a human health perspective, an ideal fatty acid (FA) profile should consist of 30 % SFA, 60 % MUFA and 10 % PUFA. Donkey's milk, characterized by a high unsaturated-to-saturated fatty acid ratio, a significant content of PUFAs including ω-3, and a low ω-6/ω-3 ratio, is considered a beneficial dietary option for human nutrition. CLA isomers are generated in the rumen through the bacterial metabolism of dietary linoleic acid and alpha-linolenic acid (ALA) and they are also synthesized in the mammary gland by stearoyl-CoA desaturase acting on *trans*-11 C18:1 (*trans*-vaccenic acid) (Elsabaawy and Gad, 2021). The primary CLA isomers are *cis*-9,

Table 4. Fatty acid profiles of milk species

Composition (%)	Cow	Buffalo	Goat	Camel	Sheep	Donkey	Human
Butyric acid ($C_{4:0}$)	3.04	3.87	0.93	12.2	4.79	0.52	0.10
Caproic acid($C_{6:0}$)	2.17	2.31	2.51	0.01	2.80	0.89	0.11
Caprylic acid($C_{8:0}$)	1.41	1.34	3.15	0.26	2.31	5.57	0.19
Capric acid ($C_{10:0}$)	3.28	2.69	8.54	0.21	6.04	8.81	0.83
Lauric acid($C_{12:0}$)	3.76	3.25	3.15	0.91	3.75	7.41	1.91
Myristic acid($C_{14:0}$)	12.65	12.14	9.0	12.0	9.76	4.64	2.47
Palmitic acid($C_{16:0}$)	32.41	30.83	25.1	23.6	23.87	21.1	17.3
Palmitoleic acid($C_{16:1}$)	1.71	1.40	0.48	0.42	1.19	3.30	0.10
Stearic acid ($C_{18:0}$)	9.92	9.52	14.7	12.8	9.48	2.60	6.6
Oleic acid($C_{18:1}$)	20.11	26.86	19.9	15.5	25.11	26.8	32.7
Linoleic acid($C_{18:2}$)	1.43	0.26	3.07	3.19	3.65	9.03	28.0
Linolenic acid($C_{18:3}$)	0.68	0.75	0.84	2.12	0.71	7.16	1.23
Arachidic acid ($C_{20:0}$)	0.16	0.1	0.21	0.20	0.44	0.68	0.16
SFA	70.15	67.35	69.6	64.1	54.48	52.9	30.4
MUFA	25.84	29.68	23.8	25.7	27.06	29.77	37.1
PUFA	2.18	3.22	5.48	8.10	6.15	17.33	31.7
CLA	0.77	1.69	0.72	0.59	1.05	–	0.20
%$C_{16:0}$ at *sn*-2	38	37	36	–	29	54	74

(Claeys *et al*., 2014; Teng *et al*., 2017; Polidori *et al*., 2019; Gurler *et al*., 2023; Zapletal and Maj, 2023) *trans*-11 (rumenic acid) and *trans*-10, *cis*-12 (Mollica *et al*., 2021). Non-ruminants can also convert vaccenic acid into rumenic acid, but at a lower rate than ruminants. Rumenic acid (RA) constitutes the primary component of CLA, accounting for 90 % of the total CLA found in ruminant fat (Mollica *et al*., 2021).

The nutritional quality of milk fat was assessed using fatty acid profiles, based on the atherogenicity index (AI) and thrombogenicity index (TI) (Fernandes *et al*., 2010). This dietary fat significantly influences cardiovascular health, with specific impacts on atherogenicity and thrombogenicity (Pietrzak-Fiecko and Sadowska, 2020). Lower values of AI and TI are generally considered better for cardiovascular health. Non-ruminants (Donkey and human) milk contains the lowest AI and TI value (Table 5) and are better for cardiovascular health. These indices are calculated based on the fatty acid composition of a lipid sample, such as the fatty acids present in a particular type of oil or fat (Silva *et al*., 2019).

$$\text{AI(Atherogenicity Index)} = \frac{(\text{C12:0} + (4 \times \text{C16:0}) + \text{C16:0})}{(\text{MUFA} + \Sigma\omega6 + \Sigma\omega3)}$$

$$\text{TI(Thrombogenicity Index)} = \frac{(\text{C14:0} + \text{C16:0} + \text{C18:0})}{[(0.5 \times \Sigma\text{MUFA}) + (0.5 \times \Sigma\omega6) + (3 \times \Sigma\omega3) + \left(\frac{\Sigma\omega3}{\Sigma\omega6}\right)]}$$

8. LIPID DIGESTION

Lipid digestion starts in the stomach with gastric lipase, breaking down 10 to 30 % of lipids. In the small intestine, pancreatic lipase and bile salts continue the process. Bile salts help by removing surface materials from emulsified lipids. Most lipid hydrolysis happens in the small intestine, where triacylglycerols are broken down into *sn*-2 monoacylglycerols and free fatty acids. These then form mixed micelles, which carry them to the intestinal epithelium for absorption (Meena *et al*., 2014). Infants can absorb 2-monoglycerides containing palmitic acid at the *sn*-2 position more easily than free fatty acids (Santillo *et al*., 2018). Palmitic acid is predominantly found at *sn*-2 position in human milk; whereas it is primarily found at the *sn*-1 position in cow, goat and sheep milk (Claeys *et al*., 2014). The rate at which FFAs are absorbed from the position of *sn*-1 and *sn*-2 in fat molecules is determined by the length of their carbon chains. LCFAs are absorbed less quickly compared to shorter-chain fatty acids (Felice *et al*., 2021). MCFA are also easily digested and absorbed by the intestines. They are quickly used by the body for energy and various metabolic processes once they enter the bloodstream (Santillo *et al*., 2018).

9. EFFECT OF SELECTED PROCESSING ON MILK FAT

Various processing methods, including freezing, cold storage, homogenization, pasteurization and boiling, significantly impact the characteristics and qualities of raw milk fat. These processes affect the size and structure of fat crystals and the fatty acid profile, thereby affecting milk's texture and stability. Generally, lower particle sizes result in better stability of milk (Fan *et al*., 2022). Heating milk fat globules alone does not alter their size; although combining heating with homogenization, whether performed before or after heating, results in a reduction of globule size. Following homogenization, heating of milk at 65 °C and subsequently to 90 °C produced no notable difference in particle size (Fan *et al*., 2022). However, heating buffalo milk at 85 °C for 30 minutes after homogenization resulted in an increase in the size of fat globules. This occurs because different homogenization and heating methods alter the surface properties of the globules, causing them to clump together due to protein interactions (Mejares *et al*., 2022). Additionally, these processes can alter

Table 5. Atherogenicity and thrombogenicity index of milk species

Species	Cow	Buffalo	Goat	Sheep	Camel	Human	Donkey
AI	2.37	2.08	3.17	4.21	2.52	1.12	0.85
TI	1.63	9.89	2.06	2.30	3.43	0.84	0.59

(Konuspayeva *et al*., 2008; Fernandes *et al*., 2010; Pietrzak-Fiecko and Sadowska, 2020; Cavalcanti *et al*., 2021) the FAs content in milk, with variations across different milk types. Pasteurization and boiling generally increase levels of SCFA and MCFA; while LCFA typically decrease due to their breakdown (Khan *et al*., 2017). However, there are exceptions, such as butyric acid (C4), which decreases in buffalo milk after homogenization and subsequent heating (Fan *et al*., 2022). Similarly, in UHT-treated bovine milk, the levels of C8, C6, and C12 fatty acids are reduced. Some LCFAs (like C14 and C16) increase in both cow and buffalo milk subjected to heating at 65 °C for 30 min or boiling for 1 min (Khan *et al*., 2017). Homogenized milk was heated to allow lipoprotein lipase to hydrolyze the fat (Kilic-Akyilmaz *et al*., 2022). This enzyme preferentially hydrolyzes various FAs, such as C4, C6, C18:0 and C18:1, especially at the *sn*-1 and *sn*-3 positions. Consequently, the level of FAs in UHT-treated milk (135 °C for 15 seconds) was found to be 1.31 times higher than raw milk and 1.14 times higher than pasteurized milk (85 °C for 15 seconds) (Xu *et al*., 2020). Donkey milk has lower lipolysis than bovine milk, which was attributed to differing fat globule membranes and fatty acid profiles, including essential protective fatty acids (C6, C8, C10, C12 and C14) (Charfi *et al*., 2019). Heating at 68 °C and 75 °C led to a slight rise in FFAs in donkey milk compared to cow milk (Charfi *et al*., 2019). The heat treatment of milk samples (ghee) from cows, buffaloes, and goats not only releases fatty acids but also induces inter-esterification, leading to the formation of *trans* fatty acids (TFAs) when heated between 120 °C and 170 °C (Khan *et al*., 2018). This suggests that foods fried in ghee contain significant amounts of TFAs, ultimately increasing the risk of cardiovascular diseases, especially coronary heart disease. Initially, raw cow, buffalo, and goat milk contained 7.71, 7.12, and 6.82 % TFAs, respectively. However, when the milk fat samples were heated to temperatures between 125 and 175 °C, the total TFAs increased to 8.25, 7.82, and 7.61 % in cow, buffalo, and goat milk, respectively (Khan *et al*., 2018). When homogenized milk is heated, then cooled and stored at freezing temperatures, the size of its fat globules increases due to crystallization. At 4 °C, the milk fat globules appear smooth, round, and well-defined, with crystals likely forming in β' and β shapes inside. However, when the milk is stored at 20 °C, the globules undergo a transformation, becoming uneven and lumpy in texture. This occurs as more fat crystals form and protrude from the globules (Mou *et al*., 2021).

Homogenization, HTST pasteurization, boiling, cooling, and freezing all alter the composition of the milk fat globule membrane (MFGM) and impact overall digestibility. The digestion rates decrease in the following order: cooled, homogenized, HTST pasteurized, raw, frozen, and boiled milk, indicating that thermal processing reduces digestibility. However, homogenization improves lipolysis by producing smaller milk fat globules, which increase the surface area for enzyme action. Cooled milk is better digested than frozen milk, likely due to differences in fat crystal formation and lipid solidity in the digestive tract. This effect is influenced by factors such as the reduction in MFGs particle size, changes in MFGM phospholipid composition, and the formation of β' polymorph crystals in frozen milk (Mou *et al*., 2021).

10. CONCLUSIONS

Milk fat exists as globules ranging from 0.2 to 15 μm in size, with variations among species that impact digestion and nutritional qualities. Factors such as proteins, lipids, hormones, diet, and genetics influence the size of these globules, with adipophilin and butyrophilin playing key roles in their formation. Phospholipids, which are essential for lipid digestion and transport, contribute between 0.29 to 66.0 mg/100 ml across all species of milk. The major phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM), account for 19-36 % of this contribution. Sterols, mainly cholesterol, make up 0.3-0.5 % of the MFGM and are vital for brain and endocrine development. Fat soluble vitamins A, D, E and K, are stored in the lipid fraction and vary among species and according to diet. Fatty acids differ across species, affecting milk's nutritional value. Ruminant milk, which contains higher levels of saturated fatty acids (SFAs) (54.48-70.15 %) and conjugated linoleic acid (CLA) (0.72-1.69 %), contrasts with non-ruminant milk, which is richer in polyunsaturated fatty acids (PUFAs) (17.33-31.70 %) and has lower Atherogenic Index (AI) and Thrombogenic Index (TI), suggesting it may be more beneficial for cardiovascular health. Processing methods such as homogenization, pasteurization, boiling, cooling, and freezing affect milk fat characteristics, which in turn influence its stability and potential health benefits.

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AUTHORSHIP CONTRIBUTION STATEMENT

A.B.Vala: original draft, resources collections;

C. N. Dharaiya: review and editing;

B.M. Mehta: Conceptualization; writing – review and editing; supervision; resources.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest, and there has been no financial support for this work.

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