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Seasonal variations in the fatty acid profile of cow, buffalo, and goat milk

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Abstract
The research on seasonal fluctuations in the fatty acid profile of milk among different indigenous livestock species. Milk from three different species was collected from the cattle yard of the institute for the research work. The fatty acid profile was evaluated over two seasons: three months in the summer (May, July, and August) and the winter (November, December, and January). The data were statistically evaluated using two-way, completely randomized ANOVA and were represented in tabular and graphical form. The significance level was set below 5% (p<0.05). The Fatty Acid Profile of milk revealed that palmitic acid was abundant in all species in both seasons. C16:0 was found in significant concentrations in cow milk during the summer and buffalo milk during the winter. The C18:1 level was higher in all species during the winter than in the summer. All medium-chain fatty acids (C6, C8, C10, C12, and C14) were abundant in sheep milk during the winter season. During the summer, C14 levels were high in all species.

Keywords: Fatty acid, milk, season, summer, winter

1. Introduction
One of the essential foods for an infant's growth is milk. Different species have different milk compositions solely because of the different requirements of the young ones of particular species. Differences in nutrient needs among newborns of different species include variations in growth rate after birth, baby's maturity and body composition at the time of birth, and specific environmental conditions of the natural setting [16].

Milk is classified as a complete diet since it contains all of the necessary elements a newborn need [4]. It is regarded as nature's most comprehensive biological fluid essential for the growth and feeding of children and adults [13]. Milk is defined as a typical secretion of all mammals' mammary glands that is essential for the sustenance of their young ones [6]. Milk is the most crucial food for a child throughout his or her first years of life [8]. Because of the range of nutrients in milk, it is regarded as one of the healthiest foods for infants and adults [1]. Milk contains various nutrients and bioactive substances, including high-quality fat, a unique fatty acid composition, and best-quality protein such as amino acids, whey, and casein. These nutrients and bioactive chemicals have a significant impact on human health. In addition to these nutrients, milk provides a healthy balance of vitamins and minerals needed for daily metabolic activities. These contain vitamins A, D, and B complex, calcium, phosphorus, and magnesium. Because of the distinctive makeup of milk, it is crucial to consume it according to dietary recommendations. Consuming insufficient milk and other dairy products while an adult increases the risk of developing primary health conditions, including rickets and osteoporosis [17].

In general, milk contains 85% water; the rest consists of milk solids. Out of the 15% of milk solids, about 4% are fat, while about 9% are Solid Not Fat (SNF). SNF includes 4.8-5% lactose, 3.4% protein and less than 1% minerals [2]. Milk fat is the most complex natural fat because of the wide range of constituent fatty acids. 69% of the fatty acids in milk fat are saturated, 27% are monounsaturated, and 4% are polyunsaturated. One of the main fatty acids found in mammalian milk, linolenic acid, has many health advantages, such as preventing cancer, heart disease, and high blood pressure [19].

Fat contributes to the distinct flavour and nutritional benefits of the milk. Milk fat is a rich source of fat-soluble vitamins like A, D, E, and K and essential fatty acids like linoleic acid, linolenic, and arachidonic acid [15]. The composition and the yield of milk fat are highly variable among individual animals and within different breeds of the same animal species. Therefore, it significantly impacts the manufacturing and processing of dairy products.

The average fat concentration in cow milk is 4.5% fat and in goat milk is approximately 3.2-4.2%. Various factors influencing the quality and quantity of milk fat include the breastfeeding stage, season, bread, and seasonal fluctuations [18]. Several studies have indicated that short-chain fatty acids in milk are more abundant in the summer than in the winter [9].

Milk is vital in enhancing health because of its excellent nutritious value and food security, especially in impoverished countries. Poverty and malnutrition, the two leading causes of death in developing nations, can be addressed by improving livestock breeds, dairy technology, and milk quality [16]. Dairy cattle make a significant contribution to dairy production. According to OECD-FAO Agricultural Outlook [11], 2020-2029, worldwide cattle milk contributes to 81% of the total milk production, as it is the most reared livestock species in the world. Globally, several breeds of different livestock species have been developed based on their needs. Cow, buffalo, and goats are the primary milk producers in the world [3].

The dairy sector is the primary sector among the allied sector of agriculture, and it accounts for 5% of the country's GDP and directly supports over eight crore farmers. India is the top milk producer in the world, accounting for 23% of all milk produced worldwide. According to a report prepared by NDDB, the nation's milk production reached 209.96 million tonnes in 2020-21 and has grown at a compound annual growth rate of approximately 6.2% compared to 2014-15, when the annual milk production was 146.31 million tonnes. According to the data, buffaloes produce roughly 51% of milk production, followed by crossbred cows at 24%, whereas indigenous cows account for 20% of total milk production in the country. Goat milk accounts for 3-4% of total milk production across the country, and exotic cows contribute 3% of total milk production [7].

The content of milk varies among different animal species [16]. This variation is because of the different variable components that affect the quality and quantity of the milk. Some of these variables include seasonal and environmental variations, the type of feed used, the animal's age and health, and the stage of lactation. These elements also impact the quality of dairy products like butter and other cheeses [10].

A very scarce amount of data is available on the seasonal fluctuation of the nutritional profiles of milk of different livestock species. In order to gather data to analyse variations in the nutritional profiles of milk among the aforementioned species in winter and summer, the nutritional profiles of three milk-producing livestock species (cows, buffaloes, and goats) were examined.

2. Materials and Methods

2.1 Research design

Three livestock species were selected to quantify the fatty acid from the milk, i.e., Cattle, Buffalo, and Goat, as they form the major pie of milk production in India. A total of 30 female lactating animals were selected, ten from each species. In cattle (Sahiwal), buffalo (Murrah) and goat (Gohilwadi) indigenous breed selected. The milk samples were collected in the evening from all the selected animals to maintain uniformity. This study was conducted in college of dairy science, Amreli, for six months.

2.2 Sample collection

Milk samples were collected in labelled sterilized bottles with specific codes from three species, including cow, buffalo, and goat, from Amreli district's local dairy farmers. For sample collection, a direct method of milking was done. From each animal, 100 ml of milk was collected. The samples were collected in sterilized labelled bottles and were kept in the refrigerator at 4 °C for further analysis.

2.3 Fatty acid profile

The estimation of fatty acid was done in two phases. The first phase, Extraction of milk fat was performed using the Röse-Gottlieb method was performed in the dairy technology division, college of dairy science, Amreli. After Fatty acids profile of samples in methylated form were analyzed in capillary column using gas chromatograph (Thermo Fisher Scientific-Trace 1110) equipped with flame ionization detector in NDRI, Karnal.

2.4 Preparation of fatty acid methyl esters

Methylation of above samples for making esters was done by using the sealed tube method Approximately 0.2 g of the melted ghee sample was introduced into a freeze drying tube by means of pasture pipette directly to the bottom without sticking ghee inside the inner wall of tubes followed by addition of approximately 0.3 ml of 0.2 N sodium methoxide and 0.1 ml of benzene by making about double the quantity of solvents (sodium methoxide and benzene) to that of fat. The tube was sealed and placed in an oven maintained at 75 °C and shaking was done at regular intervals of 10 to 15 minutes. The methylation was completed in an hour, as evident from the change of two phase system into one phase system. At the time of analysis, ester tubes were broken and sample was injected into the GC column without any further treatment.

Specification of Gas chromatography

Glass column	**TG-5 MS (30 m, I.D:0.25 mm)**
Sample volume	3µl
Injector temperature	230 °C
Carrier gas flow rate	30 ml/min.
Detector temperature	240 °C.
Carrier gas	Nitrogen
Nitrogen pressure	3 psi
Air pressure	1 psi
Hydrogen pressure	2.5 psi

2.4 Statistical analysis

Data were analyzed statistically using Microsoft Excel. Two-way ANOVA was used, and differences were considered significant at $p < 0.05$. Data is represented in both tabular and graphical forms.

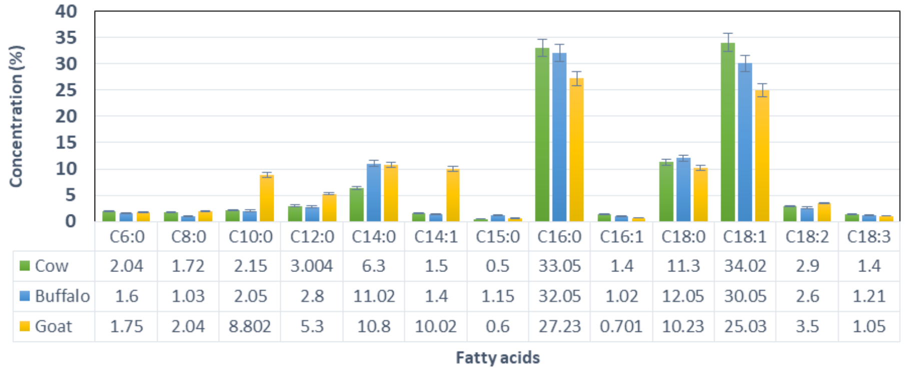
3. Result

The concentration of medium-chain and short-chain fatty acids in milk was estimated using gas chromatography. The results obtained were used for the comparative study. From the obtained data, it was evitable that there was a difference in the fatty acid concentration among the milk of different species. Table 1. Shows the mean of each fatty acid in different species in both seasons, i.e., summer and winter. The concentration of different short-chain fatty acids was in a minimal amount. Out of all the long-chain fatty acids estimated, the concentration of C16, C18:0 and C18:1 were more than other medium-chain fatty acids. The difference was observed in goat milk, where the concentration of C10 was approximately four times higher than that of cattle and buffalo

milk.
Similarly, the concentration of C14: was found to be ten times more in goat milk than in the milk of cattle and buffalo. Mean values of different fatty acids concerning seasonal variation are graphically represented below in Fig. 1. Conclusively, the concentration of C14, C16, and C18:0, and C18:1 was higher in cattle and buffalo milk than the other fatty acids. In the case of goat milk, the concentration of C10, C14:0, C14:1, C16:0, C18:0, and C18:1 was higher than the other estimated fatty acids.

Table 1: Mean of fatty acid profile of milk in different species

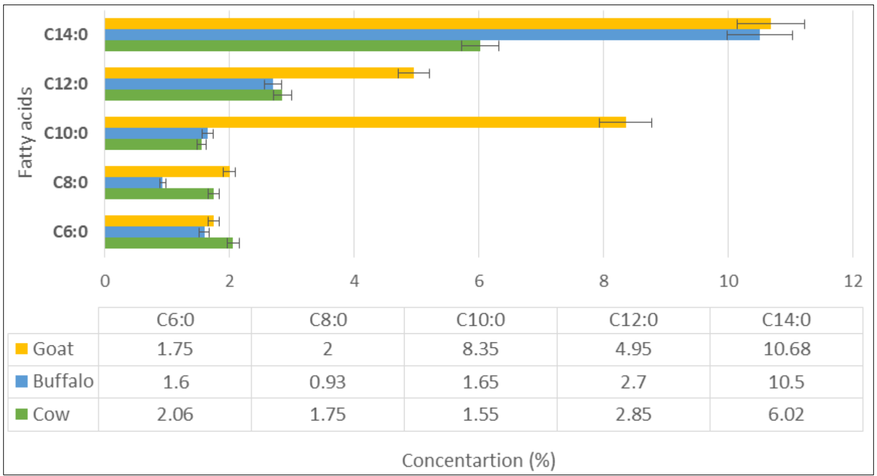
F.A (%)	Cow	Buffalo	Goat
C6:0	2.04 ±0.05	1.6±0.011	1.75±0.04
C8:0	1.72±0.01	1.03±0.05	2.04±0.013
C10:0	2.15±0.04	2.05±0.04	8.82±0.05
C12:0	3.004±0.04	2.8±0.01	5.3±0.02
C14:0	6.3±0.01	11.02±0.03	10.8±0.05
C14:1	1.5±0.014	1.4±0.058	10.02±0.09
C15:0	0.5±0.05	1.15±0.07	0.6±0.05
C16:0	33.05±0.03	32.05±0.014	27.23±0.089
C16:1	1.4±0.01	1.02±0.05	0.701±0.03
C18:0	11.3±0.08	12.05±0.011	10.23±0.055
C18:1	34.02±0.012	30.05±0.08	25.03±0.098
C18:2	2.9±0.05	2.6±0.01	3.5±0.01
C18:3	1.4±0.02	1.21±0.05	1.05±0.041



	C6:0	C8:0	C10:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Cow	2.04	1.72	2.15	3.004	6.3	1.5	0.5	33.05	1.4	11.3	34.02	2.9	1.4
Buffalo	1.6	1.03	2.05	2.8	11.02	1.4	1.15	32.05	1.02	12.05	30.05	2.6	1.21
Goat	1.75	2.04	8.802	5.3	10.8	10.02	0.6	27.23	0.701	10.23	25.03	3.5	1.05

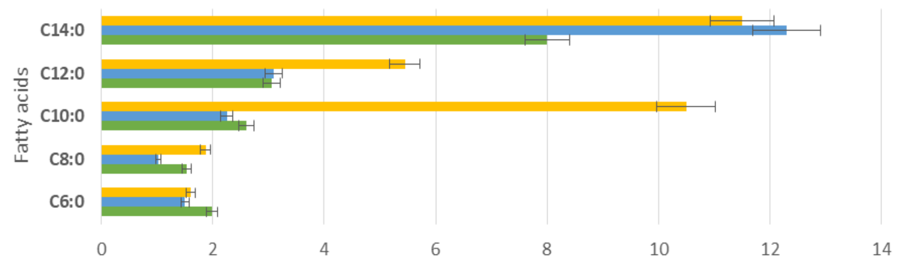
Fig 1: Mean of Fatty acid profile of milk in different species

In Figure 2, the concentration of the medium chain fatty acids among different species during the winter season is represented graphically. Medium-chain fatty acids include C6, C8, C10, C12, and C14 containing fatty acids. After comparing the data, it was evident that the concentration of C6 was found to be highest in cow milk, C8 was highest in goat milk followed by cow milk with a slight difference, C10 was highest in goat milk, C12 was again high in goat milk followed by cow and buffalo milk with slight variation and C14 was high in goat milk than in buffalo milk and in cow milk. Overall, all medium-chain fatty acids were high in goat milk during the winter season.
Similarly, the concentration of the medium chain fatty in the milk of different species was compared and is shown in Figure 3. Among all medium-chain fatty acids, the concentration of C14 is high in all species during the summer season, and C14 is high in buffalo milk, followed by goat milk. The concentration of both C12 and C10 were higher in goat milk. C8 is high in goat, followed by cow and buffalo milk, and C6 is high in cow milk.



	C6:0	C8:0	C10:0	C12:0	C14:0
Goat	1.75	2	8.35	4.95	10.68
Buffalo	1.6	0.93	1.65	2.7	10.5
Cow	2.06	1.75	1.55	2.85	6.02

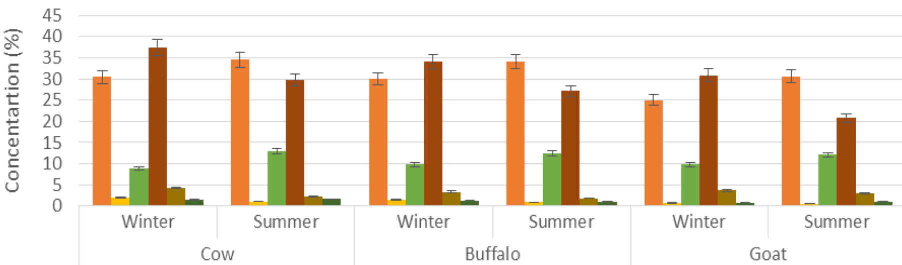
Fig 2: Mean values of medium chain fatty acids during winter



	C6:0	C8:0	C10:0	C12:0	C14:0
Goat	1.6	1.87	10.5	5.45	11.5
Buffalo	1.5	1.02	2.25	3.1	12.3
Cow	1.98	1.53	2.6	3.05	8

Concentartion (%)

Fig 3: Mean values of medium chain fatty acids during summer



	Cow Winter	Cow Summer	Buffalo Winter	Buffalo Summer	Goat Winter	Goat Summer
C16:0	30.5	34.5	30	34.03	25.05	30.6
C16:1	1.95	1.05	1.5	0.9	0.7	0.55
C18:0	8.9	13.05	9.8	12.5	9.85	12.07
C18:1	37.45	29.8	34.05	27.1	30.85	20.75
C18:2	4.25	2.3	3.35	1.85	3.7	3.01
C18:3	1.45	1.61	1.3	1.05	0.81	1.05

Fatty acids

Fig 4: Unsaturated fatty acids during winter and summer

Figure 4. Provides a clear comparison of seasonal variations in long-chain fatty acids among three species. The concentration of C16 is high during summer in cow milk, whereas in winter, it is higher in buffalo milk. C18 is high in cow and buffalo milk during the summer, and in winter, it is high in buffalo and goat milk.

4. Discussion

Of all natural fats available, milk fat is the most complex, with a great variety of fatty acids. Out of total milk fat, 69% is contributed by saturated fatty acids, 27% by monounsaturated fatty acids, and 4% by polyunsaturated fatty acids. Linolenic acid is one the major fatty acid in mammalian milk that imposes a great variety of health benefits, including prevention against cardiovascular diseases, cancers, increased blood pressure, etc. [14].

Milk and its products are the most nutritious in the human diet. The exclusive nutritional quality of milk and milk products is due to their fat portion. Milk fat comprises more than 400 fatty acids. Several factors are responsible for variations in fat concentration, including animal breed, diet, animal health, season, and lactation stage. Heart diseases, cancer, obesity, and diabetes account for more than 80% of deaths worldwide. One of the essential factors in controlling the prevention of all these health-related issues is the fat, type of fat, and amount of it to be consumed. Milk fat consists of approximately 400-500 fatty acids. Mono Unsaturated Fatty Acids are beneficial as they increase the circulatory concentration of HDL cholesterol and decrease the circulatory concentration of LDL cholesterol in the blood. Oleic acid (C18:1) is one of the MUFA found in higher concentrations in the milk of all mammals. Other MUFAs found in small amount includes 14:1 and 16:1. Saturated fatty acid (SFA) is the most durable component and primary fat component of the human diet [12, 13]. However, these FA's are responsible for significant health hazards, including heart diseases, obesity, etc. One of the most important SFA is C16:0. SFAs are responsible for increased LDL blood concentration [9].

According to a study conducted by Saroj., *et al.* (2017) [19], variations in the fatty acid composition of different species occur due to different seasons. Results of the present study for C6:0, C8:0, C10:0, C12:0, and C14:0 for all species are similar to Saroj *et al.* (2017) [19]. Total SFA's in the present study ranged between 55-65g/100g during summer and <60g/100g during winter in all species. These results are also in accordance with Saroj *et al.* (2017) [19]. Medium and long chain saturated fatty acids, i.e., myristic, palmatic, and stearic, and long chain unsaturated fatty acids, i.e., oleic acid. These fatty acids make up more than 65% of the TFA. The present study's results are similar to those reported by Rodríguez-Alcalá. *et al.* (2009) [16] and Naz., *et al.* (2009) [20]. C16:0 and C18:1 were the highest among all fatty acids in all species, with the highest C16 recorded in summer than winter and high C18:1 in winter than summer. These results are favored by Saroj *et al.* (2017) [19]. 3-5% of TFA comprises PUFA in milk in almost all species.

5. Conclusion
The present study revealed that the concentration of MUFA is higher in winter in all species, and these results are similar to Haenlein (2004) [5]. Diet and seasons positively affect the fatty acid composition of milk in all species due to grazing during the summer and spring seasons.

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