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Research Article

Fatty Acids Profile of *Himachali Pahari* Cattle and *Gaddi* Goat Milk

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Abstract

Fat is a most important constituents in milk and enhance the nutritional value of milk. The fatty acids profile of milk is associated with many factors such as animal origin, lactation stage, ruminal fermentation, feed-related factors, seasonal and regional effects. The fatty acids composition in *Himachali Pahari* cow, Jersey cross-bred cattle, *Gaddi* goat and local non-*Gaddi* goat milk was analyzed by gas chromatography-mass spectrometry (GC-MS). Maximum levels of saturated fatty acids (SFAs) were present in local non-*Gaddi* goat (78.80%) followed by *Himachali Pahari* cattle (72.58%), and Jersey cross-bred cattle (59.12%) milk. Extremely low levels of polyunsaturated fatty acids (PUFAs) were present in *Himachali Pahari* cattle, Jersey cross-bred, and local non-*Gaddi* goat milk. In *Himachali Pahari* cattle milk, saturated palmitic acid (22.57%), stearic acid (15.92%) and monounsaturated oleic acid (26.17%) were present. In Jersey cross-bred cattle, higher amount of saturated myristic acid (18.42%), stearic acid (29.04%) and monounsaturated oleic acid (31.26%) were detected. The fat present in goat milk was found to be a rich source of medium chain fatty acids. The occurrence of palmitic acid in higher amount might be responsible for intense flavour in local non-*Gaddi* and *Gaddi* goat milk but needs further research. Among all the breeds the saturated fatty acid (SFA's) content was higher whereas the polyunsaturated fatty acid (PUFA's) present in lower amount.

Keywords: *Himachali Pahari* Cattle; Jersey Cross-Bred Cattle; *Gaddi* Goat; Local Non-*Gaddi* Goat; Milk; Fatty Acids

Introduction

Dairy foods have been proven to be an excellent source of beneficial metabolites, such as CLA, n-3 and n-6 fatty acids (FA), antioxidants, phenols, flavonoids, and bioactive peptides [1-3]. The major components of milk are water, lactose, fat and protein, and are highly variable with respect to animal, management and environmental related factors [4]. Milk fat is the most complex of all the natural fats as it contains many different fatty acids. The fatty acids profile of milk is associated with many factors such as animal origin (breed and selection), stage of lactation, mastitis, ruminal fermentation, feed-related factors, seasonal and regional effects [5-8]. Almost 98% of the fats in bovine milk are made up of triacylglycerides [9] and the remaining approximate 2% is made up of diacylglycerides, cholesterol, phospholipids, and free fatty acids [10]. Feeding diets that are rich in readily fermentable carbohydrate and unsaturated fat leads to a reduced concentration of these fatty acids.

The fatty acids in milk fat are derived from four major sources, 1) directly from the ration being fed, 2) the microbial fermentation of feed-derived components in the rumen, 3) the mobilization of the animal's own body fat stores, or 4) from the *de novo* synthesis throughout the body, but mainly in the liver and the lactating mammary gland [11]. A cow's mammary gland synthesizes fatty acids that contain even numbers of carbon atoms. This so called *de novo* synthesis accounts for fatty acids with 4-14 carbons, such as myristic acid (14:0) as well as about half of the fatty acids with 16 carbons and are synthesized from acetic and β-hydroxybutyric acid [12]. Long-chain fatty acids >16 carbon atoms, such as palmitic (16:0) and stearic (18:0) acids, are generally derived from dietary sources and through the mobilization of body fat stores via lipolysis of adipose tissue triacylglycerides [13]. Triacylglycerol (TAG) plays an important role in improving digestion, absorption and metabolism when consumed by the infants [14].

Milk fat typically contains a high proportion of saturated fatty acids (SFA; 0.70-0.75; largely as a consequence of microbial biohydrogenation in the rumen) and monounsaturated fatty acids (MUFA; 0.20-0.25) and small amounts of polyunsaturated fatty acids (PUFA; 0.05) [15]. Generally, PUFA and MUFA are regarded as beneficial to human health and there is evidence of positive effects of trans-11 18:1 (the major trans fatty acid in milk fat on most diets) in animal models [16,17].

Goat milk is of particular economic interest in certain areas of the world. The production of this type of milk can be considered an alternative for consumers who have some type of sensitivity or allergy to dairy cow products [18]. One of the most important aspects of goat milk is its higher content of short chain fatty acids [19,20]. Medium-chain triglycerides in goat milk are also present in higher concentration as compared to cow milk, thereby reducing the synthesis of endogenous cholesterol [21]. The goat milk in view of its non-inflammatory, low-fat globules and abundance of nutrients viz., riboflavin, vitamin B-12, calcium, phosphorus, potassium, zinc and selenium is preferred for infants, nourishing and rejuvenating an overtaxed nervous system and boosting immunity [22]. Antioxidant, antimicrobial, and antihypertensive activities have been reported in herbal supplemented milk of *Himachali Pahari* cattle, Jersey cross-bred cattle, *Gaddi* goat and local non-*Gaddi* goat milk [23-27]. Herbal supplements, botanicals and nutraceuticals as alternative therapeutics are affluent resources of antioxidants and superior to synthetic antioxidants [28-30].

It is important to support local dairy producers and provide the important facts to the consumer on the benefits of milk consumption for their health. Thus, the objective of this study was to investigate fatty acids profile of *Himachali Pahari* cattle, Jersey cross-bred cattle, *Gaddi* goat and local non-*Gaddi* goat milk.

Material and Methods

Milk samples

Milk samples of *Himachali Pahari* cattle, Jersey cross-bred cattle, *Gaddi* goats and local non-*Gaddi* goats were obtained from Palampur (H.P) surrounding areas. Milk samples were collected in 250ml sterile container and were immediately transported in an ice box to the laboratory. The pH and titrable acidity [31] of milk samples were measured, and then milk containers were stored in a freezer at -20°C until required. Before the analysis, samples were allowed to thaw at room temperature for approximately 30 min.

Fatty acids analysis

Milk samples were brought to room temperature before analysis. Milk fatty acids were analyzed by a partially modified method originally described by [32]. Ten ml of milk sample was mixed with 66.5 ml of solvent mixture (methanol/chloroform/water, 35:17.5:14, *v/v*). The mixture was agitated for 30 min. followed by addition of 17.5 ml chloroform and 17.5 ml of 2% anhydrous sodium sulphate solution. The final mixture had 35:35:31.5 proportion of methanol: chloroform: water. This mixture was agitated for 1 min. and centrifuged at 3,000 × g for 15 min. The organic phase was separated using an injection needle. Chloroform (5 ml) was added to the remaining phase and again centrifuged as above. The organic phase was combined the previous one (a total of two extractions). One-gram anhydrous sodium sulphate was added to extract of total fats and left overnight. The extract was then filtered into pre-weighted test tubes and evaporated to dryness using a vacuum evaporator and the weight of fat extract was calculated from 10 ml milk sample.

Preparation of fatty acid methyl-ester

Fatty acids methyl esters were analyzed using a partially modified method, originally described by [33]. For preparing fatty acids methyl ester, 100 mg of milk fat was dissolved in 5 ml of methanolic sulfuric acid (4%, *v/v*) and heated to 50°C for 12 hrs. in nitrogen atmosphere. After this, the reaction mixture was dried by evaporation. Five ml of sodium chloride (4%, *v/v*) and 10 ml of hexane were added to the dried reaction mixture. Hexane (upper layer) was collected and to the remaining part again 10 ml hexane was incorporated. The above steps were repeated twice. Collected hexane was evaporated using rota-vapour. To the dried reaction mixture, added 5 ml of potassium carbonate (4%, *v/v*) and 10 ml hexane. Collected the hexane layer and repeated the extraction step twice as above. Ten mg dry sample was taken in vial and added 2 ml of DCM (Dichloromethane), then proceeded for further analysis in GCMS.

GC-MS analysis

The gas chromatography mass spectrometry (GCMS) analysis of the milk samples were performed using a Shimadzu QP 2010 equipped with AOC-5000 auto-injector using a ZB-5MS (J and W Scientific, Folsom, CA, USA) capillary column (30 m × 0.25 mm i.d., 0.25 µm thickness). The GC oven temperature was kept 70°C for 4 min. and then to 220°C at 4°C/min. Injector temperature, 240°C, Interface temperature, 250°C, acquisition mass range, 800-40 amu, ionization energy 70 eV. Helium was used as carrier gas. The resulting GCMS profiles were analyzed using NIST (National Institute of Standards and Technology) mass spectral library.

Statistical analysis

The statistical analysis was done by using SAS 9.2 statistical package. Results were presented as means and standard error of means. A *P-value* of 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

The major chemical components of milk include water, fats, proteins, carbohydrates, minerals, organic acids, enzymes and vitamins [34-36]. Milk is a complex fluid in which more than 100 separate chemical compounds have been found. Its major components are water, fat, lactose, casein, whey proteins, and minerals (or ash) in amounts varying with the milk of various species of animals [37].

Effect of lactation stages on milk pH and titratable acidity

Acidity and pH of milk samples were determined in fresh milk of *Himachali Pahari* cattle, Jersey cross-bred cattle, local non-*Gaddi* and *Gaddi* goats according to different stages of lactation as shown in table 1 and table 2.

Variation in milk pH tends to unstable the protein network which is influenced by hygienic and climatic conditions [38]. The significant differences in milk pH were only observed in *Himachali Pahari* cattle in which least pH (6.14 ± 0.028) was observed in early lactation (Table 1). However, the pH increased significantly (6.54 ± 0.018) in mid lactation, and again decline (6.35 ± 0.015) was observed in late lactation. Lactation effect was also observed on milk pH variations [39,40]. The pH [41] of milk was not significantly influenced by breed, highest in Red Sokoto (6.32 ± 0.08) followed by West African Dwarf (6.21 ± 0.10) and lowest in Sahel (6.21 ± 0.09) goats.

Lactation phase	*Himachali Pahari* cattle	Jersey cross-bred cattle	*Gaddi* goat	Local non-*Gaddi* goat
Early	6.14^{c} ± 0.028	6.21 ± 0.125	6.74 ± 0.072	6.81 ± 0.027
Mid	6.54^{a} ± 0.018	6.27 ± 0.012	6.73 ± 0.072	6.86 ± 0.022
Late	6.35^{b} ± 0.015	6.25 ± 0.029	6.71 ± 0.010	6.83 ± 0.019

Table 1: Effect of lactation stages on milk pH.
Values with different superscripts within column are statistically significant (a, b, c, d = $P < 0.05$).

Significant differences were observed in titrable acidity of *Himachali Pahari* cattle, and Jersey cross-bred cattle milk (Table 2). In *Himachali Pahari* cattle, significantly higher value was observed in early stage of lactation (0.31%). Significantly lower value of titrable acidity was recorded in mid and late lactations. In Jersey crossbred cattle, late lactation had significantly higher acidity followed by early- and mid- lactations (0.26%). No significant effect of lactation on titrable acidity was observed in *Gaddi* and local non-*Gaddi* goat milks. Titrable acidity has been used for many years to indicate whether milk has undergone bacterial degradation of lactose to lactic acid. The results obtained in the present investigation corroborate the findings of [42], who studied the relation between pH and titrable acidity in goat milk. Titrable acidity of Turkish shami goat milk ranged between 0.14-0.21 [43]. Another study reported slightly decreased acidity during lactation stage 2, then increase again in stage 3, with the progress of lactation (6.10 ± 0.55, 5.88 ± 0.66, 7.44 ± 0.70) [44].

Lactation phase	*Himachali Pahari* cattle	Jersey cross-bred cattle	*Gaddi* goat	Local *non-Gaddi* goat
Early	0.31^{a} ± 0.005	0.29^{ab} ± 0.010	0.22 ± 0.012	0.21 ± 0.016
Mid	0.25^{b} ± 0.017	0.26^{b} ± 0.004	0.18 ± 0.008	0.16 ±0.007
Late	0.27^{b} ± 0.025	0.31^{a} ± 0.009	0.20 ± 0.004	0.18 ± 0.007

Table 2: Effect of lactation stages on titrable acidity (%) in milk.

Values with different superscripts within column are statistically significant (a, b, c, d = $P < 0.05$)

Fatty acids in milk

The fatty acids composition in milk of *Himachali Pahari* cattle, Jersey cross-bred cattle, *Gaddi* goat and local non-*Gaddi* goat is presented in Table 3. The chromatogram of all the four breeds depicting different peaks of fatty acids at different Rt values are shown in figure 1.

In the present study, the main fatty acids content in *Himachali Pahari* cattle, Jersey cross-bred cattle, *Gaddi* goats and local non-*Gaddi* goat milk was analyzed by GC-MS. It was observed that high amount of saturated fatty acids is present in cattle and goat's milk whereas, polyunsaturated fatty acids present in lower amount. In *Himachali Pahari* cattle milk, saturated palmitic acid (22.57 per

Fatty acids	*Himachali Pahari* cattle	Jersey cross-bred cattle	Local non-*Gaddi* goat	*Gaddi* goat
Saturated fatty acids				
Caprylic acid ($C_{8:0}$)	1.21 ± 0.28	-	-	-
Capric acid ($C_{6:0}$)	15.74 ± 1.56	3.11 ± 0.06	15.08 ± 0.20	-
Lauric acid ($C_{12:0}$)	5.00 ± 3.52	4.35 ± 0.09	6.98 ± 0.69	-
Myristic acid ($C_{14:0}$)	9.60 ± 0.11	18.42 ± 0.20	15.07 ± 2.17	6.69 ± 0.51
Pentadecanoic acid ($C_{15:0}$)	2.54 ± 1.05	2.71 ± 0.00	1.24 ± 0.23	1.17 ± 0.01
Palmitic acid ($C_{16:0}$)	22.57 ± 0.42	-	25.05 ± 0.12	30.72 ± 1.46
Heptadecanoic acid	-	1.49 ± 0.01	-	
Stearic acid ($C_{18:0}$)	15.92 ± 0.20	29.04 ± 0.36	15.38 ± 3.05	18.79 ± 0.60
Heneicosanoic acid		-	-	
Monounsaturated fatty acids				
Palmitoleic acid ($C_{16:1}$)		-	-	
Oleic acid ($C_{18:1}$)	26.17 ± 0.62	31.26 ± 0.25	18.23 ± 2.46	27.44 ± 0.68
11-Octadecanoic acid ($C_{18:1}$)	-	7.50 ± 0.06	-	
10-Octadecanoic acid ($C_{18:1}$)	-	-		7.24 ± 1.00
Polyunsaturated fatty acids				
10,13-Octadecanoic acid ($C_{18:2}$)	-	1.84 ± 0.03	1.72 ± 0.35	-
Linoleic acid ($C_{18:2[9,12]}$)	1.15 ± 0.05	-	-	
SFA	72.58	59.12	78.80	57.37
MUFA	26.17	38.76	18.23	34.68
PUFA	1.15	1.84	1.72	0.00
Total	99.9	97.88	98.74	92.05

Table 3: Fatty acids profile of milk.

cent), stearic acid (15.92 per cent) and monounsaturated oleic acid (26.17 per cent) were present. The polyunsaturated fatty acid, α-linoleic acid was present in small amount only in milk of *Himachali Pahari* cattle. In Jersey cross-bred cattle, higher amount of saturated myristic acid (18.42 per cent), stearic acid (29.04 per cent) and monounsaturated oleic acid (31.26 per cent) were present.

The fat present in goat milk is a rich source of medium chain fatty acids. In local non-*Gaddi* goat, among saturated fatty acids capric acid ($C_{6:0}$), myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) was present in higher amount. Among monounsaturated fatty acids, only oleic acid was found to be present in local non-*Gaddi* goat's milk, which account for approximately 25 per cent by weight of total fatty acids. Our findings are similar to the studies carried out earlier [45]. In *Gaddi* goat milk, palmitic acid was present in high percentage in comparison to other milk types. Similar trend was also put forwarded by [46].

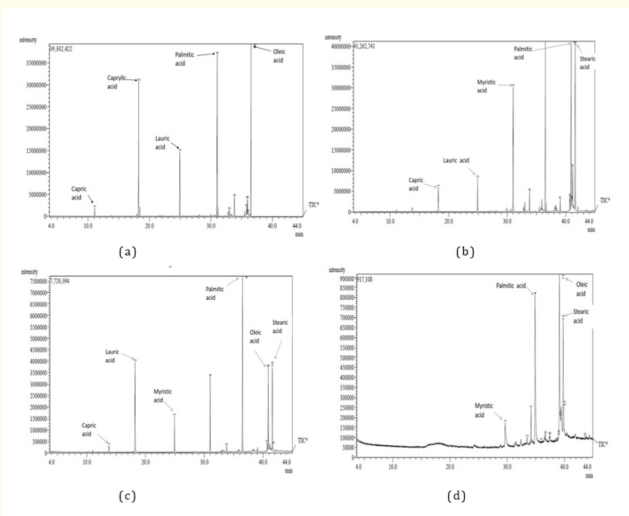

Figure 1: Fatty acids profile in milk of (a) Himachali Pahari cattle, (b) Jersey cross-bred cattle, (c) Local non-Gaddi goat, and (d) Gaddi goat.

Conclusion

From the present study, it may be concluded that there are noteworthy differences in the fatty acid profiles of *Himachali Pahari* cattle, Jersey cross-bred cattle, local non-*Gaddi* goat and *Gaddi* goats. Higher levels of saturated fatty acids (SFAs) were present in local non-*Gaddi* goat followed by *Himachali Pahari* cattle, and Jersey cross-bred cattle. Very low levels of polyunsaturated fatty acids (PUFAs) were present in *Himachali Pahari* cattle, Jersey cross-bred, and local non-*Gaddi* goat's milk. Moreover, PUFAs were not observed in *Gaddi* goat's milk.

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