

Sources of Variation of Fatty Acids in Milk of Indian Goat

V. Saroha¹, D. Kumar¹, A. Sharma², Jayakumar S², A.K. Tyagi³, Nagda RK⁴ and S.P. Dixit^{2*}

¹Deenbandhu Chhotu Ram University of Science and Technology, Sonapat, Haryana, India

²National Bureau of Animal Genetic Resources, Karnal, Haryana, India

³National Dairy Research Institute, Karnal, Haryana, India

⁴Rajasthan University of Veterinary and Animal Science, Vallabhnagar, Udaipur, Rajasthan, India

*Corresponding author: S.P. Dixit

Introduction

Milk is one of the essential parts in human diet rich in all nutritive components. Fatty acids (FA) in milk are becoming more and more important because of their link with certain diseases. Numerous studies, reviewed in Arnould and Soyeurt (2009), have reported that saturated fatty acids (SFA), and especially lauric (C12:0), myristic (C14:0), and palmitic acids (C16:0), have an unfavorable relation with some heart diseases, diabetes, and obesity (Haug et al., 2007). In contrast, unsaturated fatty acids (UFA) are reported to have a favorable effect on health, especially on cholesterol levels. However, some short- and medium-chain SFA, such as C6:0 to C10:0, which are well known for their role in the specific goat flavor (C8:0), seem to be of medical interest in humans (malabsorption syndromes, infant malnutrition, cardiovascular diseases, nonallergenic properties; Haenlein, 2004). Caproic acid (C6:0), caprylic acid (C8:0), and capric acid (C10:0) are more abundant in goats; they form 15 to 18% (compared with up to 10% in cow milk) of the total FA (Chilliard et al., 2006; Raynal-Ljutovac et al., 2008). Anti-carcinogenic and anti-atherogenic properties have also been attributed to conjugated linoleic acid (Lee et al., 2005; Bauman et al., 2006; Sooyeur and Genler, 2008). Moreover, fatty acids are reported to play an important role in the techno-functional properties of cheese making, including organoleptic properties and cheese yield. Milk fat content and composition can be modified by genetic and physiological factors as well as nutritional factors (Chilliard 2003). As result, research is currently being carried out on FA content of ruminant milk in different area of nutrition, physiology and genetics. The present study attempts on genetic and environment sources of variation of fatty acids in the milk of Sirohi breed of Indian goat to explore its modification to desired level.

Materials and methods

Data collection 700 milk samples of Sirohi breed of goat were collected during 2010-12 from All India Coordinated Research Project (AICRP) on goat from different villages of Udaipur, Ajmer and

Chitodgarh districts of Rajasthan. Samples were collected over 3 seasons viz. summer, winter and spring. Sodium Azide was added as preservatives so as to analyze the milk samples in the lab. 500 μ l of 10X solution was added in 100 ml of the milk collected. The samples were stored in refrigerator until further analysis.

Fatty acid analysis

The method chosen for Fatty acid methyl esters (FAME) preparation was of Fallon *et al.* (2007). FAME was prepared directly from milk without prior organic solvent extraction. The milk sample were placed into a 16 × 125 mm screw-cap pyrex culture tube to which 1.0 ml of the C13:0 internal standard (0.5 mg of C13:0/mL of MeOH), 0.7 mL of 10 N KOH in water, and 5.3 mL of MeOH were added. The tube was incubated at 55°C in water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min to properly permeate, dissolve, and hydrolyze the sample. After cooling below room temperature in a cold tap water bath, 0.58 ml of 24 N H₂ SO₄ in water was added. The tube was mixed by inversion and with precipitated K₂ SO₄ present was incubated again at 55°C for 1.5 h with hand-shaking for 5 s every 20 min. After FAME synthesis, the tube was cooled in a cold tap water bath. Three milliliters of hexane was added, and the tube was vortex-mixed for 5 min on a multitube vortex. The tube was centrifuged for 5 min in a tabletop centrifuge, and the hexane layer, containing the FAME, was placed into a GC vial. The vial was capped and placed at -20°C until GC analysis. The FAME was analyzed on gas chromatography equipped with an auto sampler injector. The FAME was separated in 60mm capillary column (60X 0.25mm 70 μ m) film thickness. Here FID is used as a functional unit. It works on polarizing voltage of 300V. The effluent from the column was mixed with hydrogen and air and get ionization. Then it will produce ions and electrons which can conduct electricity through the flame. A large electrical potential was applied at the burner tip and collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. Helium was used as carrier gas at a flow rate of 2mL/min. The injector and detector temperatures were 260°C and 270°C, respectively. The temperature program was as follows: the initial temperature was held at 60°C for 1 min after sample injection, then programmed to increase at 2°C/min to 240°C, and held there for 5 min. Sample (1 μ L) were injected by split injection (split ratio 10:1). Identification of FAME was performed from the retention times by using standards of 37 individual FAME (Supelco, Bellefonte, PA) was used to determine response factors. The peak areas in the chromatogram were calculated and normalized using response factors. The individual FA contents were expressed as weight percentages (g/100 g of FAME).

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response factors. The peak areas in the chromatogram were calculated and normalized using response factors. The individual FA content was expressed as weight percentages (g/100g of FAME). The conjugated linoleic acid (CLA) was estimated in the milk samples through spectrophotometry. Methyl ester CLA standard of 250mg/ml was diluted with hexane to a concentration of 25µg/ml. FAME was diluted 50 times and 230µl of each sample was loaded in the wells. In addition, wells with hexane only were used as blank. The optical density (OD) was taken at 233 wavelength. Value of CLA in µg/230µl was calculated from regression equation and then, the concentration of CLA in a given sample was converted to mg per gram of fat.

Grouping of fatty acids

All FAs were grouped according to their saturation level and chain length. Sum of saturated FA (SFA), sum of short chain saturated FA (C4 to C10, SCFA), sum of medium chain saturated FA (C12 to C15, MCFA) and sum of long chain saturated FA (C16 to C24, LCFA) were calculated. The remaining 2 group were the sum of monounsaturated FA (MUFA) and the sum of polyunsaturated FA (PUFA). 2 index, unsaturated index (UFA multiplied by 100/SFA) other index was related to rumenic and vaccenic FA (C18:2 cis9, trans11 multiplied by 100/ C18:1 trans11). The criteria for selecting these FAs depended upon their maximum contribution towards total percentage of FA, with the percentage more than 3.5 % (Arenas et al., 2007) and 3 additional FA of biological interest (CLA, vaccenic acid (C18:1) and linolenic acid (C18:3)) were also estimated.

Statistical Analysis

Statistical analysis was carried out by using mixed procedure of SAS (release 9.1) statistical package program. The mathematical model included fixed effects due to parity, year and season of sampling, region (cluster of villages), month of lactation, and random effects due to sire. Weight of doe was included as co-variate. The variation in 21 dependent variables (13 FA, 6 groups of FA and 2 indexes) due to factors included in the model was analyzed and Least square means were compared using Duncan multiple range test (DMRT).

Result and discussion

Descriptive statistics of fatty acids (FAs)

The Descriptive statistics for 36 FAs, 6 groups of FAs (SFA, MCFA, LCFA, MUFA and PUFA) and 2 indexes (UNSFA/SFA and C18:2/ C18:1) studied and shown in Table 1. Analysis of goat milk samples revealed the highest concentration of saturated fatty acids (SFA) out of total milk fatty acids (FA) with an average of 69.55% ranging from 43.26 to 88.05. Within saturated fatty acid, the major contribution was of palmitic (C16:0) 26.99% followed by myristic (C14:0) 11.77%, stearic (C18:0) 7.66% and capric (C10:0) 6.75%, respectively. The major contribution (45.56%) in total FAs was of

C12:0, C14:0 and C16:0. C18:0. Short chains FAs have a neutral or cholesterol-decreasing effect. Unsaturated fatty acids (UFA) are extremely important for human health. The highest monounsaturated fatty acid (MUFA) levels were those of oleic acid (C18:1 cis-9) – 19.08%. The average content of trans isomers of C18:1 varying between 0.240% and 19.94%. Polyunsaturated fatty acid (PUFA) contributes 3.96%. The total sum of CLA in studied goat milk was 4.87% and the biologically active isomer C18:2 cis-9, trans-11 contributes 2.9% and trans10 cis12 contributes 0.82%. For most of these traits, the estimates were within the range reported by Carta et al. (2008) in dairy sheep, Soyeurt and Gengler (2008) and Bilal et al. (2014) in dairy cattle, and Metka et al. (2006) and Maroteau et al. (2014) in goat.

Sources of variation of FAs

The effect of the different factors influencing the fatty acids has been presented in Table 2. The cluster of villages across different districts under study wherein Sirohi goats were reared had significant contribution towards the variability in CLA and its isomer cis 9 trans 11, capric, myristoleic, pentadecanoic, 10 pentadecanoic, heptadecanoic, cis 10 heptadecanoic, oleic, linoleic and arachidic and total short chain fatty acids (SCFA). The variation in feed and fodder resources, and management in different clusters might have been responsible for the variation observed in different FAs. This has indicated that grazing and housing periods for different flocks along with type of grazing pasture and feeding regimen during housing are important factors contributing in differences in these traits in different clusters. These results were in accordance with those of Stoop et al. (2008) and Bilal et al. (2014) in dairy cattle, Gonzalo et al. (1994) in Churra ewe flocks and those of Maroteau et al. (2014) in goat.

Month of lactation had significant effect on oleic and SCFA only. For rest of the traits under study, stage of lactation had little role in determining these traits. The parity of doe also influenced some of the fatty acids (palmitoleic, cis 10 heptadecanoic, arachidic, alpha-linolenic). The results revealed that physiological stage of doe as well as stage of lactation had little impact on most of the FAs. Similarly weight of doe also did not contribute much towards variation in the FAs under study except CLA isomer cis 9 trans 11. The little effect was also reflected by low value of coefficient of determination. Parity, stage of lactation and weight of doe are imperative sources of biological interest, but there is little information on their influence on fatty acids particularly in goat milk. Our results are in accordance with those obtained for age and stage of lactation by Kelsey et al. (2003) for FA in dairy cattle. On the contrary, for FA in dairy cattle, Cranix et al. (2008) did not find any significant effect of parity on FA, so further studies in other breeds of milking does would be needed to corroborate our results and to establish their physiological or metabolic meaning.

The year of sampling influenced CLA and its isomers, myristoleic, linoleic and arachidic FAs but not other traits under study. The year of sampling again emphasized the role of feed and fodder resources, and management in defining these traits. However, season of sampling influenced total of saturated FAs only particularly the total of medium chain saturated FAs (MCFA). Contrary to this, significant influence of season on 18 FA in ovine milk was reported by Fuente et al. (2009). However, they have included year effect in the model. The combined significant influence of herd-year-season (10-75% of total variance) was also reported by Bilal et al. (2014) with a higher proportion being explained for PUFA including CLA. For denovo synthesized FA (C4:0 to C14:0), the proportion of variance explained by cluster-year-season was less (10 to 20%), possibly because their production is less dependent on diet. The similar results were also revealed in the study of Bilal et al. (2014) in dairy cattle. Maroteau et al. (2014) in goat, estimated relatively higher estimates of heritability for these FA probably due to these reasons . They revealed highest values for 3 medium-chain SFA, C10:0 to C14:0 (h^2 from 0.26 to 0.38), and lower values for the UFA C18:1 and C18:2c9t11 (h^2 from 0.18 to 0.23). Similar trends were found in cattle for FA expressed on a milk basis (g/L) or on a fat basis (wt/wt%) by Bobe et al. (2008) and Stoop et al. (2008), with higher heritabilities for C10:0 (i.e., 0.71; Stoop et al., 2008) and C12:0 (i.e., 0.43; Bobe et al., 2008).

Sire of doe had major contribution in determining the variability in almost all the fatty acids and indices under investigation. This indicated the role of genetics in determining different fatty acids as most of the variability is being explained by sire component. The estimates of heritability of individual FA varied from 0.01 to 0.39 in dairy cattle (Bilal et al. 2014), from 0.18 to 0.38 in goat (Maroteau et al. 2014). Moreover, different results were found in the Churra sheep breed for UFA groups, in which estimates were not significantly different from zero. Saturated FA are the major constituent of milk fat, around 70 % in our study (Table 1), and individual SFA (i.e., C6:0 to C16:0) are synthesized de novo in the mammary gland and elongated by 2 main enzymes: fatty acid synthase and acetyl CoA carboxylase (Chilliard et al., 2001). In contrast, UFA (C18:1, C18:2c9t11), which are provided by the diet and directly taken from the blood after modification by biohydrogenation in the rumen might be less genetically determined and therefore less heritable compared with SFA. In the present study also, the estimates of heritability for group medium (0.16) and long chain saturated FA (0.02) were higher than those of unsaturated FA (0.01) but with very low precision due to very high standard errors probably due to small size data. Therefore the study for estimation of genetic parameters needs to be taken up in large sized well pedigreed flocks of Indian goats so as to formulate optimal breeding strategies for bringing about genetic improvement in desired fatty acids.

Fatty acid	variables	mean	minimum	max	SD
CLA	cla	4.873	0.393	16.724	2.878
cis9tran11	cis9tran11	2.943	0.0063	7.875	1.433
trans10 cis12	trans10 cis12	0.823	0.021	3.592	0.824
Butyric acid	c4:0	1.349	0.020	7.272	1.230
Caproic acid	c6:0	2.611	0.415	15.73	2.033
Caprylic acid	c8:0	3.660	0.463	9.722	1.612
Capric acid	c10:0	6.750	0.266	20.953	4.531
Undecanoic acid	c11:0	1.744	0.159	17.897	5.429
Lauric acid	c12:0	6.825	1.772	20.045	4.278
Short chain fatty acid	SCFA	13.461	2.239	33.631	6.818
Tridecanoic acid	c13:0	0.5886	0.136	14.386	3.276
Myristic acid	c14:0	11.770	0.315	24.881	3.899
Myristoleic acid	c14:1	1.353	0.113	15.465	2.600
Pentadecanoic acid	c15:0	1.667	0.0585	24.902	4.005
cis10-pentadecenoic acid	c15:1	0.494	0.121	11.133	1.278
Palmitic acid	c16:0	26.991	1.097	41.707	5.730
Palmitoleic acid	c16:1	2.731	0.206	19.129	2.379
Medium chain fatty acid	MCFA	20.056	7.470	45.270	6.096
Hepiadeconoic acid	c17:0	0.757	0.196	9.679	1.533
cis-10-heptadecenoic	c17:1	0.832	0.0567	18.287	2.233
Stearic acid	c18:0	7.665	0.3752	21.297	4.075
Elaidic acid	c18:1n9t	1.497	0.240	19.946	3.087
Oleic acid	c18:1n9c	19.088	0.783	31.947	6.222
Linolelaidic acid	c18:2n6t	0.735	0.141	6.778	1.526
Gamma linolenic acid	c18:3n6	1.682	0.0754	7.948	1.089
Linoleic acid	c18:2n6c	2.423	0.0257	15.063	3.015
Arachidic acid	c20:0	0.700	0.0957	13.892	2.532
cis-11-eicosenoic acid	c20:1	0.735	0.088	17.937	2.131
Linolenic acid	c18:3 n3	0.255	0.053	4.243	0.808
Heneicosanoic acid	c21:0	0.371	0.055	11.081	1.841
cis11,14 eicosadienoic acid	c20:2	0.215	0.102	2.887	0.878
Long chain fatty acid	LCFA	35.089	4.774	51.228	5.313
Saturated fatty acid	SFA TOTAL	69.595	43.263	88.053	5.446
Mono unsaturated fatty acid	MUFA	24.572	4.790	39.408	5.384
Polyunsaturated fatty acid	PUFA	3.966	0.592	18.309	3.593
Unsaturated fatty acid	USFA	28.502	10.449	45.741	5.236
Unsaturated index	USFA*100/SFA	41.595	12.575	84.303	10.196

Table1. Milk composition and percent contribution of each fatty acid in goat milk

Class	cla	Cis9trans 11	Trans10ci s12	C8:0	C10:0	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0
Cluster	***	*			*		*	**	**			**
1	3.74 a ±0.56	2.10 a ±0.29	0.76±0.1 4	3.10±0.3 3	6.67 ab ±0.95	12.13±0. 79	0.67 a ±0.44	0.96 b ±0.54	1.24 a ±0.23	25.96±1.17	3.21±0.57	0.51 a ±0.19
2	5.32 b ± 0.30	2.41 ab ±0.15	0.86±0.0 7	3.59±0.1 7	5.66 a ±0.50	11.70±0. 42	1.85 b ±0.23	1.40 ba ±0.28	0.36 b ±0.12	27.57±0.62	2.46±0.30	0.75 ab ±0.10
3	5.53 b ± 0.23	2.61 b ±0.12	0.92±0.0 6	3.85±0.1 3	6.81 b ±0.39	11.50±0. 33	1.36 a ±0.18	1.90 a ±0.22	0.54 b ±0.09	26.39±0.49	2.34±0.24	0.86 b ±0.07
4	4.52 a ± 0.24	2.20 a ±0.12	0.77±0.0 3	3.68±0.1 4	7.32 b ±0.40	12.19±0. 33	1.42 ab ±0.18	1.12 b ±0.22	0.43 b ±0.09	27.32±0.49	2.69±0.24	0.56 a ±0.08
month												
1	4.35±0.2 7	2.21 ±0.14	0.74 ±0.07	3.62±0.1 6	6.57±0.4 7	11.87±0. 39	1.30±0.21	1.50±0.2 6	0.51±0.1 1	26.47±0.57	2.82±0.28	0.66±0.09
2	4.96±0.2 6	2.38 ±0.13	0.87 ±0.06	3.52±0.1 5	6.10±0.4 3	12.06±0. 36	1.57±0.20	1.30±0.2 4	0.71±0.1 0	26.44±0.54	2.89±0.26	0.70±0.08
3	5.04±0.3 1	2.41 ±0.16	0.88 ±0.08	3.66±0.1 8	5.93±0.5 3	11.96±0. 44	1.06±0.24	1.36±0.3 0	0.66±0.1 3	26.72±0.65	2.89±0.32	0.72±0.10
4	5.12±0.3 3	2.49 ±0.17	0.94 ±0.08	3.27±0.1 9	6.85±0.5 6	12.33±0. 46	1.05±0.26	0.97±0.3 1	0.51±0.1 3	27.03±0.69	2.46±0.34	0.67±0.11
5	4.41±0.4 1	2.15±0.2 1	0.71 ±0.10	3.70±0.2 4	7.61±0.6 9	11.18±0. 58	1.65±0.32	1.60±0.3 9	0.82±0.1 7	27.38±0.86	2.33±0.42	0.61±0.13
year	**	**	*				*					
1	4.98 a ± 0.30	2.33 ab ±0.15	0.89 a ±0.07	3.68±0.1 7	7.16±0.5 0	11.78±0. 42	1.57 a ±0.23	1.27±0.2 8	0.50±0.1 2	26.47±0.62	2.74±0.30	0.78±0.10
2	4.16 b ±0.21	2.05 a ±0.11	0.71 b ±0.05	3.52±0.1 2	6.55±0.3 6	11.93±0. 30	0.99 b ±0.16	1.59±0.2 0	0.72±0.0 8	26.93±0.44	2.91±0.22	0.65±0.07
3	5.19 b± 0.45	2.60 b ±0.23	0.88 ab ±0.11	3.46±0.2 6	6.13±0.7 5	11.94±0. 62	1.40 ab ±0.35	1.18±0.4 2	0.71±0.1 8	27.02±0.93	2.38±0.45	0.58±0.15
parity											*	
1	4.78±0.3 0	2.24±0.1 5	0.88±0.0 8	3.23±0.1 8	6.37±0.5 2	11.25±0. 43	1.27±0.24	1.80±0.2 9	0.64±0.1 2	27.00±0.63	3.35 a ±0.31	0.80±0.10
2	5.05±0.3 2	2.27±0.1 6	0.78±0.0 8	3.52±0.1 8	6.30±0.5 3	12.24±0. 44	1.41±0.24	1.28±0.3 0	0.57±0.1 3	25.94±0.66	2.55 b ±0.32	0.69±0.10
3	4.80±0.3 0	2.35±0.1 5	0.87±0.0 8	3.74±0.1 8	6.70±0.5 2	11.81±0. 43	1.49±0.24	1.39±0.2 9	0.74±0.1 2	26.07±0.64	2.68 ab±0.31	0.60±0.10
4	4.67±0.3 4	2.43±0.1 7	0.88±0.0 9	3.48±0.2 0	6.81±0.5 8	12.12±0. 48	1.17±0.26	1.21±0.3 2	0.63±0.1 4	27.64±0.71	2.18 b ±0.35	0.65±0.11
5	4.57±0.3 3	2.35±0.1 7	0.73±0.0 8	3.81±0.1 9	6.90±0.5 5	11.98±0. 46	1.27±0.25	1.04±0.3 1	0.64±0.1 3	27.38±0.68	2.62 ab ±0.33	0.63±0.11
tok				**	**	**				**		
1	4.77±0.2 2	2.34±0.1 1	0.82±0.0 5	3.37 a± 0.13	6.57±0.3 8	11.41 a ±0.31	1.26±0.17	1.35±0.2 1	0.64±0.0 9	27.45 a ±0.46	2.70±0.23	0.67±0.075
2	4.78±0.2 8	2.32±0.1 4	0.83±0.0 7	3.74 b ±0.16	6.66±0.4 7	12.35 b ±0.39	1.39±0.21	1.34±0.2 6	0.64±0.1 1	26.16 b ±0.58	2.65±0.28	0.67±0.09
sos												
1	5.10±0.3 7	2.49±0.1 9	0.95±0.0 9	3.43±0.2 2	6.79±0.6 3	11.66±0. 52	1.36±0.29	0.90±0.3 6	0.66±0.1 5	26.96±0.78	3.02±0.38	0.63±0.12
2	4.53±0.2 7	2.19±0.1 4	0.73±0.0 7	3.63±0.1 6	6.35±0.4 6	11.67±0. 38	1.40±0.21	1.66±0.2 6	0.54±0.1 1	26.79±0.57	2.46±0.28	0.79±0.09
3	4.70±0.2 7	2.31±0.1 4	0.81±0.0 7	3.61±0.1 6	6.71±0.4 6	12.31±0. 38	1.22±0.21	1.48±0.2 6	0.73±0.1 1	26.67±0.57	2.54±0.28	0.60±0.09

Class	C15:1	C18:1	C18:1n9c	C18:2	C20:0	C18:2	SFA	SCFA	MCFA	LCFA	MUFA	PUFA
Cluster	*		*	**	*			**				
1	1.35 a ±0.31	2.39±0.49	16.03 a ±1.29	2.19 a ±0.42	0.50 a ±0.26	0.30±0.11	69.48±1.15	14.0 ab ±1.14	20.39±1.24	33.77±1.66	24.29±1.17	3.92±0.49
2	1.07 a ±0.16	1.39±0.26	19.14 b ±0.68	1.69 a ±0.22	0.99 b ±0.14	0.31±0.06	69.05±0.61	12.0 a ±0.60	19.74±0.66	36.61±0.88	24.92±0.62	3.39±0.26
3	0.90 ab ±0.13	1.28±0.20	19.67 b ±0.53	2.25 ac ±0.17	0.83 b ±0.11	0.29±0.04	69.89±0.48	13.2 b ±0.47	20.23±0.51	34.79±0.69	24.58±0.49	3.95±0.20
4	0.64 b ±0.13	1.30±0.20	18.99 b ±0.54	2.54 c ±0.18	0.52 a ±0.11	0.27±0.04	69.47±0.48	14.2 b ±0.48	19.02±0.52	35.43±0.70	24.24±0.49	4.03±0.20
month			*					*				
1	0.98±0.15	1.49±0.24	17.59 a ±0.63	2.28±0.20	0.65±0.13	0.24±0.05	69.88±0.56	13.86±0.56	20.11±0.61	34.81±0.81	23.69±0.57	3.89±0.24
2	0.84±0.14	1.70±0.22	18.17 a ±0.59	2.15±0.19	0.75±0.12	0.28±0.05	68.90±0.53	12.98±0.52	20.20±0.57	34.62±0.76	24.70±0.53	3.91±0.22
3	0.99±0.17	1.41±0.27	19.87 b ±0.71	2.00±0.23	0.75±0.14	0.34±0.06	69.16±0.64	12.89±0.63	19.57±0.69	35.42±0.92	25.38±0.65	3.58±0.27
4	1.08±0.18	1.76±0.29	18.87 ab ±0.76	2.22±0.25	0.76±0.15	0.24±0.06	69.04±0.68	12.87±0.67	19.28±0.73	35.99±0.98	24.63±0.69	3.82±0.29
5	1.06±0.23	1.61±0.36	17.78 a ±0.94	2.21±0.31	0.65±0.19	0.36±0.08	70.39±0.84	15.06±0.83	20.06±0.90	34.89±1.21	24.13±0.85	3.88±0.36
year				*	*							
1	0.90±0.16	1.74±0.26	18.48±0.68	2.46 a ±0.22	0.39 a ±0.14	0.34±0.06	68.54±0.61	14.24±0.60	19.28±0.66	33.85±0.88	24.54±0.62	4.27±0.26
2	0.91±0.12	1.64±0.18	19.06±0.49	2.41 a ±0.16	0.73 b ±0.10	0.26±0.04	69.55±0.43	13.40±0.43	20.01±0.47	35.32±0.63	24.96±0.44	3.94±0.18
3	1.16±0.25	1.41±0.39	17.83±1.02	1.65 b ±0.33	1.01 ab ±0.21	0.28±0.09	70.32±0.91	12.96±0.90	20.25±0.98	36.28±1.31	24.02±0.93	3.25±0.39
parity	*				*	*						
1	1.03 ab ±0.17	1.61±0.26	18.00±0.70	2.20±0.23	0.99 a ±0.14	0.33 ab ±0.06	68.90±0.62	13.09±0.61	20.39±0.67	34.80±0.90	24.70±0.63	3.82±0.26
2	0.70 a ±0.17	1.31±0.27	18.72±0.72	2.26±0.24	0.92 a ±0.15	0.32 ab ±0.06	68.92±0.65	12.79±0.64	19.79±0.70	35.30±0.93	24.65±0.66	4.13±0.27
3	0.80 ac ±0.17	1.44±0.26	18.26±0.70	2.07±0.23	0.57 b ±0.14	0.40 a ±0.06	69.33±0.62	13.84±0.62	20.00±0.67	34.31±0.90	24.23±0.64	3.86±0.26
4	1.32 b ±0.19	1.66±0.29	19.23±0.78	2.03±0.25	0.56 b ±0.16	0.19 b ±0.07	69.90±0.70	13.70±0.69	19.45±0.75	35.86±1.01	24.93±0.71	3.43±0.30
5	1.10 bc ±0.18	1.93±0.28	18.07±0.75	2.29±0.24	0.52 b ±0.15	0.22 b ±0.06	70.31±0.67	14.24±0.66	19.60±0.72	35.41±0.96	24.07±0.68	3.86±0.28
tok		*										
1	1.07±0.12	1.83 a ±0.19	18.35±0.51	2.18±0.16	0.71±0.10	0.29±0.04	69.54±0.45	13.31±0.45	19.55±0.49	35.57±0.66	24.74±0.46	3.78±0.19
2	0.92±0.15	1.36 b ±0.24	18.56±0.64	2.17±0.21	0.71±0.13	0.29±0.05	69.41±0.57	13.75±0.56	20.14±0.61	34.70±0.82	24.28±0.58	3.9±0.2
sos							*		*			
1	1.01±0.21	1.84±0.32	18.72±0.86	2.14±0.28	0.72±0.17	0.37±0.07	68.68 a ±0.77	13.74±0.76	18.61 a ±0.82	35.09±1.10	25.25±0.78	3.65±0.32
2	0.92±0.15	1.46±0.24	18.62±0.63	2.29±0.20	0.59±0.13	0.29±0.05	69.06 a ±0.56	13.31±0.55	19.92 ab ±0.60	34.92±0.81	24.28±0.57	4.01±0.24
3	1.05±0.15	1.48±0.24	18.03±0.63	2.08±0.20	0.82±0.13	0.21±0.05	70.68 b ±0.56	13.55±0.55	21.01 b ±0.60	35.44±0.81	24.00±0.57	3.60±0.24

*P<0.05 ; **P<0.001; ***P<0.001

Saturated fatty Acids (SFA) include short chain fatty acid (SSFA), Medium chain fatty acid (MFA) and Long chain fatty acid (LFA)

Unsaturated Fatty acid (UFA) = sum of MUFA and PUFA.

Mono unsaturated fatty acid (MUFA) include C12:1 , C14:1, C15:1, C16:1, C17:1 and the total of C18:1, C20:1 and C20:1.

Polyunsaturated fatty acid (PUFA) include C18:2 *cis*-9, *trans*-11, C20:2n-6, C18:3n-6, C20:2**Table2. Least square means of fatty acids by cluster, month of lactation, year, parity, type of kid (TOK), season of sampling (SOS)**

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