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Seasonal and lactational variations in fatty acid profile of milk in indigenous cattle

GEETESH MISHRA[1]✉, S C GOSWAMI[2], SANJITA SHARMA[2] , A K JHIRWAL[2] and RAVTARAM[1]

Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan 334 001 India

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ABSTRACT

The present study was conducted on quality and bioactive components in milk of Kankrej and Sahiwal indigenous cattle breeds at Livestock Research Station, Kodemdesar and College of Veterinary and Animal Science, Bikaner situated in hot-arid region of Rajasthan. Representative milk samples were collected and brought to the laboratory. The variance analysis observed significant effect of season on SFA of Kankrej and Sahiwal, and revealed that the total MUFA in Kankrej differ significantly. Significant effect of season on PUFA in Sahiwal and Kankrej cattle was also observed. Significant effect of season on Omega fatty acids in Sahiwal and Kankrej cattle except Omega-9 fatty acid in Sahiwal cattle was seen. The analysis of variance found highly significant effect of season on SCFA whereas non-significant effect on LCFA in Kankrej and Sahiwal cattle. Effect of season on MCFA was found significant in Sahiwal whereas non-significant effect was found in Kankrej cattle. The analysis of variance observed significant effect of various lactation stage on MUFA, Omega-9 and MCFA fatty acids for Kankrej and Sahiwal cattle and on SCFA in Sahiwal, and non-significant effect in Kankrej whereas significant effect on LCFA in Kankrej and non-significant effect in Sahiwal cattle.

Keywords: Kankrej, Milk fat, MUFA, PUFA, SFA, Sahiwal

Ruminant milk fat is most composite of every natural fat with great diversity of fatty acids. Near about 400 diverse FA (fatty acids) plasma lipids originating from the feed and *de novo* synthesis are in milk fat triacylglycerol (Jensen 2002) in the mammary gland but, only 15 FA above 1% (Parodi 2004). Short (SCFA) and medium chain (C4–14) FA accounts for about 60% of total FA (TFA) and are synthesized *de novo*; while, a half of C16:0 and all long chain FA originate from dietary lipolysis along with lipids of adipose tissue triacylglycerol (McGuire and McGuire 2000). In mammary gland, medium and long-chain fatty acids, specifically C18:0, can be converted into MUFA (Monounsaturated Fatty Acids). SFA, MUFA, along with PUFA (polyunsaturated fatty acids) constitute for 69%, 27%, and 4% of total milk fat, respectively (Jensen 2002). Apart from various factors viz. genetics, breed, feeding etc. season has marked effect on variation in the cattle milk FA (Lock and Garnsworthy 2003) that could be mainly attributed to the availability of feeds where proportion of green forages in diet enhance unsaturated FAs with simultaneous decrease in the SFA (DePeters *et al.* 2001, Shingfield *et al.* 2006). It is necessary to document seasonal variation in the FA composition of widely consumed mammals' milk by human beings that may be useful to modulate the quality for the health benefits. The key purpose of the current research has been to assess changes in fatty acids in various Sahiwal and Kankrej cattle milk with season and stage of lactation and ensure the quality of milk for human-being demand as well.

Present address: [1]Krishi Vigyan Kendra, Gudamalani Agriculture University, Jodhpur, Rajasthan. [2]Department of Livestock Production and Management, College of Veterinary Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan. ✉Corresponding author email: geeteshmishra09@gmail.com

MATERIALS AND METHODS

Animals and sampling: Milking animals (90) of two indigenous cattle breeds (45 each), viz. Sahiwal and Kankrej from University Livestock Research Stations at Bikaner were taken for the study. Milk samples were gathered from 45 animals from each cattle in morning and evening throughout the months of summer (from March to July), rainy (from August to November), and winter (from December to February) seasons, thus, accounting to 15 samples in every season. These 15 samples of individual breed were again divided into stage of lactation (Early, Mid and Late) in every season. All animals irrespective of species were hand milked. Morning and evening milk samples were pooled for each replicate in both the seasons. Milk samples were kept below 5°C until shifting to laboratory, as well as stored at –20°C for chemical investigation.

Feeding: A combination of dry fodder, concentrated feed, and *ad lib*. green feed was used for meeting the animals' nutritional needs. During the rainy season, Jowar was offered as green fodder; in winter season, oats were offered whereas, during summer, bajra was given to the

animals two times a day and wheat straw was used as dry fodder. The concentrate was provided at a rate of 1.25 kg/day to the animals with extra concentrate at a rate of 1 kg for 2.5 kg of milk produced over the daily production of 5 kg in order to fulfil their energy needs. The concentrate combination (Table 1) was provided in two portions, one at the time of morning milking and one at the time of evening milking. All feeds and feeding practices were same for all experimental animals.

Table 1. Composition of concentrate feed

Particular	Gold
Energy (KCal/kg) Min	2500
Moisture (%) Max	11
Crude protein (%) Min	20
Ether extract (%) Min	4.0
Crude fibre (%) Max	10
Sand silica (%) Max	3.0
Salt (%) Max	1
Calcium (%) Min	-
Phosphorus (%) Min	-
Vitamin A (1U/kg) Min	7000
Vitamin D3 (1U/kg) Min	1200
Vitamin E (1U/kg) Min	30

Procedure followed: The milk samples were thawed and gently mixed at 40°C in water bath and 15 ml was centrifuged at 4°C for 40 min at 10,000 g for separating the cream. Cream was separated, flushed with N_2 and stored at –20°C until analysis. FAME was prepared from milk fat samples by technique developed by O'Fallon et al. (2007) and analysed using GC-MS System.

Statistical analysis: Data were analyzed for variance based on following SPSS 20.0 using GLM.

RESULTS AND DISCUSSION

Effect of season on fatty acid profile: The Mean±SE value of total MUFAs, SFAs, PUFAs, ω-9, ω-6, ω-3, SCFA, MCFA along with LCFA in different season's, viz. summer, rainy and winter of Sahiwal and Kankrej are given in Tables 2 and 3. The analysis of variance had significant effect of season (P<0.01) on SFAs. Post-hoc DMRT demonstrated higher total SFAs in summer and lowest in winter in Sahiwal and Kankrej. The analysis of variance revealed significant (P<0.01) season effect on total MUFA for Kankrej whereas non-significant effect in Sahiwal. The post-hoc DMRT revealed that total MUFA was found highest in winter and lowest in rainy in Sahiwal and Kankrej. Significant season effect on total PUFA for Sahiwal and Kankrej was found. The post-hoc DMRT revealed that total PUFAs in Sahiwal and Kankrej were highest in winter whereas lowest in summer. The analysis of variance had (P<0.01) highly significant season effect on ω-6 along with ω-3 fatty acids in Sahiwal and Kankrej. Total ω-9 fatty acids differed significantly (P<0.05) with season in Kankrej whereas non-significant effect was observed in Sahiwal. The post-hoc DMRT revealed that total ω-3, 6 and 9 fatty acids of Sahiwal were found higher in winter than

Table 2. Effect of season on fatty acids in milk from Sahiwal cattle (Means±SE)

Fatty acid	Rainy	Winter	Summer	Level of significance
Saturated fatty acid	60.29^{a}±0.48	58.14^{b}±0.52	63.34^{c}±0.58	**
Monounsaturated fatty acid	28.28±0.34	29.32±0.32	27.96±0.30	NS
Polyunsaturated fatty acid	3.8^{b}±0.06	3.95^{b}±0.08	3.71^{a}±0.04	**
Omega-3 fatty acids	0.85^{a}±0.01	0.89^{b}±0.01	0.84^{c}±0.01	**
Omega-6 fatty acids	2.83^{a}±0.06	2.88^{b}±0.09	2.75^{a}±0.04	*
Omega-9 fatty acids	25.69±0.33	26.42±0.31	25.37±0.29	NS
Short chain fatty acid	11.08^{b}±0.21	10.02^{a}±0.17	12.35^{c}±0.31	**
Medium chain fatty acid	41.59^{a}±0.44	41.02^{a}±0.48	43.09^{b}±0.31	**
Long chain fatty acid	39.74±0.42	40.37±0.35	39.57±0.37	NS

[a, b, c] Differences significant at P<0.05; **, P<0.01; *, P<0.05.

Table 3. Effect of season on fatty acids in milk from Kankrej cattle (Means±SE)

Fatty acid	Rainy	Winter	Summer	Level of significance
Saturated fatty acid	61.85^{a}±0.62	60.81^{a}±0.54	63.29^{b}±0.58	**
Monounsaturated fatty acid	27.59^{a}±0.3	28.55^{b}±0.36	27.26^{a}±0.35	**
Polyunsaturated fatty acid	3.57^{c}±0.06	3.84^{b}±0.05	3.27^{a}±0.05	**
Omega-3 fatty acids	0.81^{b}±0.02	0.91^{b}±0.01	0.68^{a}±0.01	**
Omega-6 fatty acids	2.84^{b}±0.06	2.82^{b}±0.04	2.46^{a}±0.05	*
Omega-9 fatty acids	25.28^{ab}±0.28	25.74^{b}±0.34	24.61^{a}±0.34	*
Short chain fatty acid	11.66^{b}±0.2	11.5^{a}±0.22	12.41^{b}±0.2	**
Medium chain fatty acid	42.5±0.48	42.4±0.48	43.3±0.29	NS
Long chain fatty acid	38.84±0.27	39.25±0.37	38.09±0.38	NS

[a, b, c] Differences significant at P<0.05; **, P<0.01; *, P<0.05.

rainy and summer. The post hoc DMRT revealed that total ω-3, 6 and 9 fatty acids of Kankrej were found higher in winter than summer and rainy.

Highly significant season impact (P<0.01) on SCFA whereas non-significant effect on LCFA in Kankrej and Sahiwal were seen. Effect of season on MCFA was found significant in Sahiwal whereas non-significant in Kankrej cattle. The post-hoc DMRT revealed that total SCFA and MCFA content in Sahiwal and Kankrej was higher in summer than rainy and winter season. The LCFA content in Sahiwal and Kankrej was found higher in winter than summer and rainy season.

The outcomes were similar to the finding of Ahamad *et al.* (2013). Season affects the fatty acid composition due to fresh grass feeding in the spring and summer than in winter season (Chilliard *et al.* 2003). Talpur *et al.* (2009) and Jyotika (2016) reported higher MUFA in summer than winter season which could be attributed to feed resources and feeding pattern. This was contrary to our results, because sufficient green herbage was available for grazing in winter season. Moreover, in grass, the botanical composition differences may adjust the bacterial population in rumen and thereby lipid mobilization and affects the proportion of different FA (Collomb *et al.* 2008). Similar to our results, Saroj *et al.* (2017) reported 13–14% higher total SFA (Saturated Fatty Acids) content (fat g/100g) in summer as compared to winter in Sahiwal and Crossbred cattle. Total MUFA and PUFA concentration were 10–12% and 3–4% respectively and lower in summer than winter. MUFA and PUFA increased during winter season than summer in Sahiwal and crossbred cattle. Ferlich *et al.* (2012) noticed significant effect of season on PUFA and n6:n3 ratio in Czech Fleckvieh cows and Holstein cow. The season's impact may be described by certain management practices during the year. Farm involved in this study was located in arid region wherein cows are given access for pasturing from August to November. Temperature and humidity can have an impact on the milk fat profile of cows, either directly or by influencing the quality of their feed (Renna *et al.* 2010).

*Effect of stage of lactation on fatty acid profil*e: The Mean±SE values for total SFA, MUFAs, PUFAs, ω-9, ω-6, ω-3, SCFA, MCFA along with LCFA of lactation stages (early, late and mid) in Sahiwal and Kankrej are given in Tables 4 and 5. The analysis of variance revealed significant effects of lactation stage (P<0.01) on total SFA in Kankrej and Sahiwal. The post-hoc DMRT revealed that SFAs were higher in mid stage and lower in early stage of lactation in Sahiwal and Kankrej. The analysis of variance showed effect of various lactation stages (P<0.01) in Kankrej whereas non-significant effect was observed in Sahiwal. The post-hoc DMRT revealed that Sahiwal and Kankrej milk fat contained more MUFAs in early and late than in mid lactation stage. The analysis of variance observed non-significant effect of different lactation stages for Kankrej and Sahiwal. Also, Sahiwal and Kankrej milk fat contained more PUFAs in early and lower in late lactation stage. The analysis of variance showed significant effect of lactation stage on total ω-9, ω-3, and ω-6 fatty acids in Kankrej. Significant effect of lactation stage (P<0.01) on

Table 4. Effect of stage of lactation on fatty acids in milk from Sahiwal cattle (Means±SE)

Fatty acid	Early lactation	Mid lactation	Late lactation	Level of significance
Saturated fatty acid	58.28^{a}±0.54	62.15^{b}±0.59	61.34^{b}±0.61	**
Monounsaturated fatty acid	29.61^{c}±0.23	27.5^{a}±0.22	28.5^{b}±0.95	**
Polyunsaturated fatty acid	3.74^{a}±0.09	3.81^{a}±0.1	3.91^{a}±0.11	NS
Omega-3 fatty acids	0.85^{a}±0.03	0.86^{a}±0.03	0.87^{a}±0.03	NS
Omega-6 fatty acids	2.72^{a}±0.06	2.81^{a}±0.08	2.89^{a}±0.09	NS
Omega-9 fatty acids	26.79^{c}±0.23	24.85^{a}±0.20	25.86^{b}±0.23	**
Short chain fatty acid	10.62^{a}±0.29	11.55^{b}±0.32	11.28^{a}±0.35	*
Medium chain fatty acid	39.85^{a}±0.48	43.09^{b}±0.39	42.74^{b}±0.37	**
Long chain fatty acid	41.16^{c}±0.36	38.83^{a}±0.18	39.73^{b}±0.28	**

$^{a, b, c}$Differences significant at P<0.05; **, P<0.01; *, P<0.05.

Table 5. Effect of stage of lactation on fatty acids in milk from Kankrej cattle (Means±SE)

Fatty acid	Early lactation	Mid lactation	Late lactation	Level of significance
Saturated fatty acid	59.97^{a}±0.79	63.54^{b}±0.59	62.44^{b}±0.38	**
Monounsaturated fatty acid	28.83^{b}±0.28	27.09^{a}±0.48	27.48^{b}±0.26	**
Polyunsaturated fatty acid	3.47±0.08	3.54±0.09	3.7±0.10	NS
Omega-3 fatty acids	0.82±0.03	0.8±0.025	0.81±0.02	NS
Omega-6 fatty acids	2.53^{a}±0.06	2.64^{ab}±0.08	2.75^{b}±0.04	*
Omega-9 fatty acids	26.12^{b}±0.25	24.54^{a}±0.38	24.97^{a}±0.23	**
Short chain fatty acid	11.40±0.31	11.96±0.36	12.22±0.27	NS
Medium chain fatty acid	41.16^{a}±0.58	44.18^{b}±0.43	42.94^{b}±0.35	**
Long chain fatty acid	39.72^{a}±0.29	38.04^{a}±0.41	38.47^{b}±0.47	**

$^{a, b, c}$Differences significant at P<0.05; **, P<0.01; *, P<0.05.

total ω-9 fatty acid and non-significant effect on ω-6 along with ω-3 fatty acid in Sahiwal was observed. Post-hoc DMRT revealed that total ω-3 fatty acid of Sahiwal was higher in late and lower in early lactation stage; total ω-6 fatty acid in Sahiwal was found higher in late and lower in early lactation stage; total ω-9 fatty acid was found higher in early than late and mid stage of lactation in Sahiwal. On the contrary, the total ω-3 fatty acid in Kankrej was found significantly higher in mid than early as well as late lactation stage; total ω-6 fatty acid was found significantly higher in late while lowest in early lactation stage; total ω-9 fatty acid was found higher in early whereas lowest in mid lactation stages in Kankrej. The analysis of variance showed significant effect of lactation stage on SCFA, MCFA and LCFA in Kankrej and Sahiwal. The post-hoc DMRT revealed that total SCFA concentration in Kankrej was found significantly higher in late and lower in early lactation stage; MCFA was found higher in mid and lower in early lactation stage while LCFA was found higher in early and lower in mid lactation stage in Kankrej.

The total SCFA of Sahiwal was higher in late and lower in early lactation stage; MCFA was found higher in mid and lower in early lactation stage while LCFA was found higher in early and lower in mid lactation stage.

The difference in FA composition throughout different lactation phases in the present investigation was consistent with findings of Mele *et al.* (2009) and Soyeurt *et al.* (2008). From body fat stores, pre-formed LCFA is used for production of milk by cows during the first 100 days of lactation when energy balance is more likely to be negative, but *de novo* fatty acids contribute more to milk as lactation develops in the longer term (Palmquist *et al.* 1993). A study by Gottardo *et al.* (2017) found that SFA in milk grew until 120 days in milk and then declined; however, the same study found that the reverse pattern was observed for PUFA and MUFA. In contrast, the level of SCFA remained nearly constant during lactation, but the concentration of MCFA was lowest at 60 days in milk and grew steadily afterwards between 180 and 450 days. In terms of LCFA, their concentration in milk dropped until 150 days from the start of breast feeding and then slightly rose till the conclusion of lactation in the Alpine Grey, Simmental, Brown Swiss, and Holstein-Friesian cattle breeds.

It is possible to infer that seasonal grazing fluctuations have been proven as the key element influencing the milk fatty acid composition is seasonal variation that may be viewed favourably by consumers. Decline in saturated fatty acids and increase in poly-unsaturated fatty acids during winter might be due to increase in availability of green fodder. This study of variation in fatty acid with season and stage of lactation will be helpful in enhancing the health benefits of milk.

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