

Effect of Tanniferous Diet on Fatty Acids Profile of Camel Milk

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Abstract

Tanniferous tree leaves containing diet provided to camel could modify the fatty acid composition of its milk. For this experiment fifteen lactating camels (average BW 554 kg) in mid lactation stage were used to study the impact of tree leaves. Animals were blocked by weight and milk production into three groups, where these were offered roughage to concentrate ratio of 70:30. Roughage components included crop residues viz. groundnut (*Arachis hypogaea* L.) straw (GS), guar (*Cyamopsis tetragonoloba* L.) phalgati (GP) and khejri leaves (KL)/pala leaves (PL) in different ratio. Group T₀ fed with GS and GP ratio of 50:50, Group T₁ fed with GS, GP and KL ratio of 40:40:20 and Group T₂ were offered GS, GP and PL ratio of 40:40:20 for 6 months. Changes were observed in fatty acid composition with significant increase in C13:0 (Tridecanoic acid) in T₁ group as compared to T₀ and T₂ group. C16:0 (Methyl Palmitate) in T₀ and T₂ group did not differ significantly between themselves but were significantly higher ($P < 0.01$) than T₁ group. At reverse decrease of medium-chain fatty acids Cis-10-pentadecanoic acid (C15:1) was significantly ($P < 0.05$) lower in T₁ group as compared to T₀ and T₂ group but did not differ significantly between themselves. A significant increase was found in Linolenic acid (C18:3 cis 6,9,12,) in T₂ group than T₀ and T₁ and lowest alpha-Linolenic acid (C18:3 cis 9,12,15,) in T₂ group than T₀ and T₁. The predominant fatty acids (FA) in camel milk were myristic (C14:0), (cis-9 C16:1), Methyl Sterate (C18:0) alpha-Linolenic acid (C18:3) and oleic (cis-9 C18:1) acids was observed after 6 months of tanniferous tree leaves containing diet provision. As for other ruminants, it is possible to modulate the fatty acid composition of camel milk by the diet, but further trials for longer period and different tree leaves and other modulation in diet have to be implemented in camel.

Introduction

Camel milk is one of the main component of the human diet in arid region of Rajasthan. It has been regarded as a potential nutritional and therapeutic food source across various arid and semi-arid regions. The therapeutic effect of a regular camel milk (CM) consumption on different diseases is a strong commercial argument usually justifying the high price of the product on market compared to cow milk (Konuspayeva et al., 2021). The fatty acid (FA) composition of milk is one of the aspects linked to the discussion on the health effect of milk and milk products (Wahle and Heys, 2002). Fatty acids, the most important component of milk fat, constitute about 90% of its weight. Over 95% of the FAs are bound in triacylglycerols; remainder in mono- and diacylglycerols, phospholipids and cholesterol esters whereas free fatty acids are present in small proportions. Fatty acid composition of camel milk is characterized by a high proportion of monounsaturated fatty acids, is one of the parameters explaining its beneficial effect on the human consumers (Faye et al. 2008; Konuspayeva et al. 2008). Fatty acid composition, their position in the molecules of triacylglycerols, as well as the proportion of saturated and unsaturated FAs, affect nutritional, sensory and technological properties of FA extensively. The proportion of nutritionally desirable FAs to detrimental ones can be altered, utilizing factors that effectively change FA composition. These factors include feed, animal and environmental (Palmquist et al., 1993). To date, the greatest attention has been focused on feed factors. Their application has enabled

important essential changes in milk fatty acid composition. (Chilliard *et al.*, 2001, 2008; Kalač & Samková, 2010). Presence of a notable amount of α -linolenic acid in methanol and hexane extracts of the leaves of *Z. mauritiana* is fascinating towards exploring the potential uses of its leaves as a new dietary source of essential fatty acids (Ashraf, 2015).

Available information concerning modulation in camel milk by alteration in nutritional factors in camel diet is limited. Incorporation of tanniferous tree forages of hot-arid climatic regions at 20% level by replacing the basal crop-residues in the diet of lactating camel have positive influence on milk quality and its antioxidant properties. (Kumari *et al.*, 2023). This could potentially have far-reaching economic impact in the trade of camel milk and milk products and thus improve upon socio-economics of camel herders. There has not been much-documented research on the use of tree leaves as a feeding regime. Therefore, this research was conducted with the objective to evaluate change in dietary composition with tanniniferous tree leaves on the fatty acids composition of camel milk.

Materials and Methods

Fifteen multi-parous lactating camels in mid lactation were randomly divided into 3 groups of 5 camels each based on comparable milk yield, body weight, number of lactations completed and days in lactation. Feeding trial designed to compare effect of tanniferous tree leaves was initiated in September 2021 at ICAR-National Research Centre on Camel, Bikaner. Experimental animals were individually housed and provided with uniform management practices during 12 fortnights of study period. The animals were managed by the same workers and milked same parlour. Control group (T_0) was fed with a standard basal diet of roughage and supplemental concentrate in the ratio of 70:30 (ICAR, 2013), where in the roughage component included equal proportions (1:1) of Groundnut (*Arachis hypogaea* L.) straw (GS), Guar (*Cyamopsis tetragonoloba* L.) *phalgati* (GP) and designated as GG. Treatment group (T_1) received a similar ration with varied roughage combinations having tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios and referred as GGK whereas the other (T_2) group (GGP) received Pala leaves (PL) at GS40:GP40:PL20 ratios.

Data recording and sample collection

Milk samples were collected from two consecutive milkings on the last day of experimental period and mixed according to milk production. Samples collected were stored at -20°C until analysis for Fatty Acid composition. Milk fat separation was carried out by the rapid method for which refrigerated raw milk sample was tempered at 20°C for 20 min, and centrifuged and the upper top layer was removed for analysis.

Methyl ester

Methyl ester from fat was prepared according to O' Fallon *et al.*, (2007). After FAME synthesis, the tube was cooled in a cold tap water bath. After hexane addition 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 in Gas chromatography for analysis.

Gas chromatography

A fully automatic GC-MS system (TRACE 1300, Thermo-Fisher) equipped with ITQ 700 ion trap mass spectrometer having EI source emission equipped with a fused silica capillary column (100 m×0.25 mm×0.2 µm) of supelco (SPTM-260) was used for analysis. The carrier gas was helium and flow rate were 1 mL min⁻¹. The sample injection rate (split) was 1/10. The injector temperatures were 240°C and detector temperatures was 240°C. The column temperature was programmed to 70°C for 2 min, followed by a ramp of 10°C min⁻¹ up to 190°C, which was kept for 20 min. A second ramp of 5 ° C min⁻¹ was run up to 240°C for 10 min. The total analysis time was 50 min. The peak areas were determined using xcalibur Software. Injections of 1 µL were performed.

Statistical analysis

Statistical analysis was performed using SPSS version 24 (SPSS Inc., Chicago IL). The effects of tree leaves feeding on parameters were analysed by ANOVA with the Tukey post hoc test.

Results and discussion

The data used in this study are summarized in Table 1. There were minor effects of feeding tanniferous tree leaves on milk FA concentration. Concentration of saturated fatty acid C13:0 was significantly ($P < 0.05$) higher in T_1 group and at conversely concentration of C16:0 was significantly lower in T_1 group. C15:1 was lower when the diet containing KL was fed. Saturated fatty acids (SFA) contributes major part of total fatty acids in camel's milk like others species but it contains more unsaturated fatty acids as compared to other species as reported by Chamekh *et al.* (2020). The Concentration of some monounsaturated fatty acids (MUFA) like C15:1 was significantly lower in T_1 group. The MUFA and SFA contents were consistent with those reported by Konuspayeva *et al.* (2014). The concentration of C18:3 cis 6,9,12 (gamma linolenic acid) and was significantly highest and, C18:3 cis 9,12,15 (gamma linolenic acid) was lowest in T_1 group. The predominant FA in camel milk were myristic (C14:0), (cis-9 C16:1), Methyl Sterate (C18:0) alpha-Linolenic acid (C18:3) and oleic (cis-9 C18:1) acids. These results were consistent with previously reported findings in camel milk (Gorban and Izzeldin, 2001; Zou *et al.* 2013; Shamsi *et al.*, 2016,).

Chemical composition of *Z. mauritiana* leaves revealed methyl stearate – 15.59% and α-linolenic acid- 26.45% were the major components of methanol and hexane extracts, respectively. Other, important components identified in methanol extract were: palmitic acid 13.57%, squalene-12.09%, phytol-9.78%, methyl palmitate-7.81%, linoleic acid methyl ester – 5.98%) and Vitamin E-2.35%. On the other hand, a considerable quantity of palmitic acid (16.26%), squalene-12.83%, α-tocopherol-3.92%, γ-sitosterol-

2.72%, phytol- 2.52%, *transgeranylgeraniol*-2.34%, octacosane-2.04%, methyl palmitate-1.01% and myristic acid – 0.73% was detected in hexane extract. A considerable amount of α -tocopherol – 10.01%, stearic acid-5.82%, vitamin E-5.41%, *uneicosane*-4.79%, α - nonadecylene-3.77%, *bacchotricuneatin* C-3.48%, myristic acid-2.80% and lauric acid-1.66% was noted to be present in chloroform extract (Ashraf et al. 2015) reflecting fatty acid profile of the ingested browse material contribute to the diet, as it was evident in their composition in milk in the present study, especially Linolenic acid and α -Linolenic acid concentration in milk.

Thus, incorporation of alternate tanniferous tree forage seems to be a promising strategy for improving milk quality. This study was conducted to test the hypothesis that feeding tannins to dairy animals would favourably modify ruminal BH and thereby enhance the fatty acid profile of milk. There are only limited studies reporting effects of feeding tanniferous tree leaves (TTL) on milk FA profiles. The determination of fatty acid composition in different milk may provide potential nutritional supplements and development of alternative dietary fat sources for human health and optimum nutritional milk products for infant and toddlers. From this point of view, it appears that camel milk could be utilized as part of the diet or incorporated into functional foods for human nutrition benefits.

Table 1
Saturated fatty acids (SFA) in milk of Lactating camels on different diets (n = 5)

Fatty acids	T₀	T₁	T₂	P Value
C6:0MethylHexanoate	1.13 ± 0.017	1.05 ± 0.603	1.1 ± 0.04	NS
C8:0Methyl Octanoate	1.73 ± 0.482	0.77 ± 0.445	1.74 ± 0.473	NS
C10:0Methyl Caprate	1.28 ± 0.736	1.78 ± 1.025	0.01 ± 0.001	NS
C12:0Methyl Laurate	2.51 ± 1.446	1.02 ± 0.589	0.01 ± 0.0001	NS
C13:0 Tridecanoic acid	1.83 ± 1.057^a	11.11 ± 3.69^b	3.71 ± 0.069^a	*
C14:0Methyl Myristate	13.74 ± 0.257	7.96 ± 3.834	13.57 ± 0.202	NS
C15:0Pentadecanoic acid	0.37 ± 0.211	0.00 ± 0.00	0.41 ± 0.231	NS
C16:0 Methyl Palmitate	1.17 ± 0.124^b	0.00 ± 0.124^a	1.41 ± 0.124^b	**
C17:0MethylHeptadecanoate	0.00 ± 0.00	13.93 ± 8.042	0 ± 8.042	NS
C18:0Methyl Sterate	42.68 ± 1.556	20.49 ± 11.83	40.44 ± 0.02	NS
C15:1 Cis-10-pentadecanoic acid	1.21 ± 0.424^b	0.00 ± 0.00^a	1.32 ± 0.491^b	*
C16:1 Palmitoleic acid	0.21 ± 0.115	0.00 ± 0.00	0.00 ± 0.00	NS
C18:1 Oleic acid	3.13 ± 0.938	6.35 ± 3.666	5.91 ± 1.53	NS
C20:1Cis-11 Eicosenoic acid	1.75 ± 0.055	3.49 ± 0.932	1.7 ± 0.046	NS
C18:2 cis 9,12 Linoleic acid	0.29 ± 0.162	0.00 ± 0.00	0.00 ± 0.00	NS
C18:3 cis 6,9,12 Linolenic acid	1.77 ± 1.022^a	7.68 ± 0.375^a	17.97 ± 4.766^b	**
C18:3 cis 9,12,15alpha-Linolenic acid	24.54 ± 1.279^b	24.39 ± 1.204^b	10.63 ± 6.134^a	*
C20:4 cis 5,8,11,14 Arachidonic acid	0.78 ± 0.78	0.00 ± 0.00	0.00 ± 0.00	NS
T ₀ : Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)				

T₁: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios

T₂: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio.

Note

a, b, - Means superscripted with different letters within a row differ significantly from each other (P < 0.05)

Average fatty acids (%) of three different groups of lactating camels are summarised in Table 2. Total poly unsaturated fatty acids (PUFA) was also higher in T₁ group it may be due to inhibition of biohydrogenation (BH) of PUFA from feeds in rumen and by the use of tree leaves. The PUFA content was slightly higher than those observed by Haddad *et al.* (2010) and Konuspayeva *et al.* (2014).

Table 2
Average fatty acids (%) of three different groups of lactating camels

Fatty acids	T ₀	T ₁	T ₂
SFA%	66.44 ± 5.886	58.11 ± 30.184	62.4 ± 9.1941
MUFA%	27.38 ± 3.243	32.07 ± 1.579	28.6 ± 10.9
PUFA%	6.3 ± 1.532	9.84 ± 4.598	8.93 ± 2.067

From Fig. 1 it can be seen that linear scores of MUFA and PUFA was highest in T₁ group and lowest in T₀ group. SFA was highest in T₀ group.

Conclusion

Present study is suggestive that inclusion of taniniferous tree leaves at twenty percent affected fatty acid composition as there some alternation was observed in the concentration of Fatty Acids. Highest content of polyunsaturated fatty acids was fond in animals with 20% inclusion levels of tree leaves compared favourably with animals fed control diet. Therefore, it could be concluded that, tree leaves have the potential of meeting the nutritional needs of lactating camel in terms of milk fatty composition. Tree leaves can therefore be used as a suitable feed stuff for camel during lactation for improving the productivity of ruminant livestock. It could be recommended to adopt management practice system to improve production performance, milk quality, health and welfare in camel as dietary tannin have the potential to modulate Fatty Acid profile of milk, and this effect is dosage and source dependent.

Declarations

Conflict of Interest Statement

No conflict of Interest.

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Data Availability declaration

Not applicable

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Figures

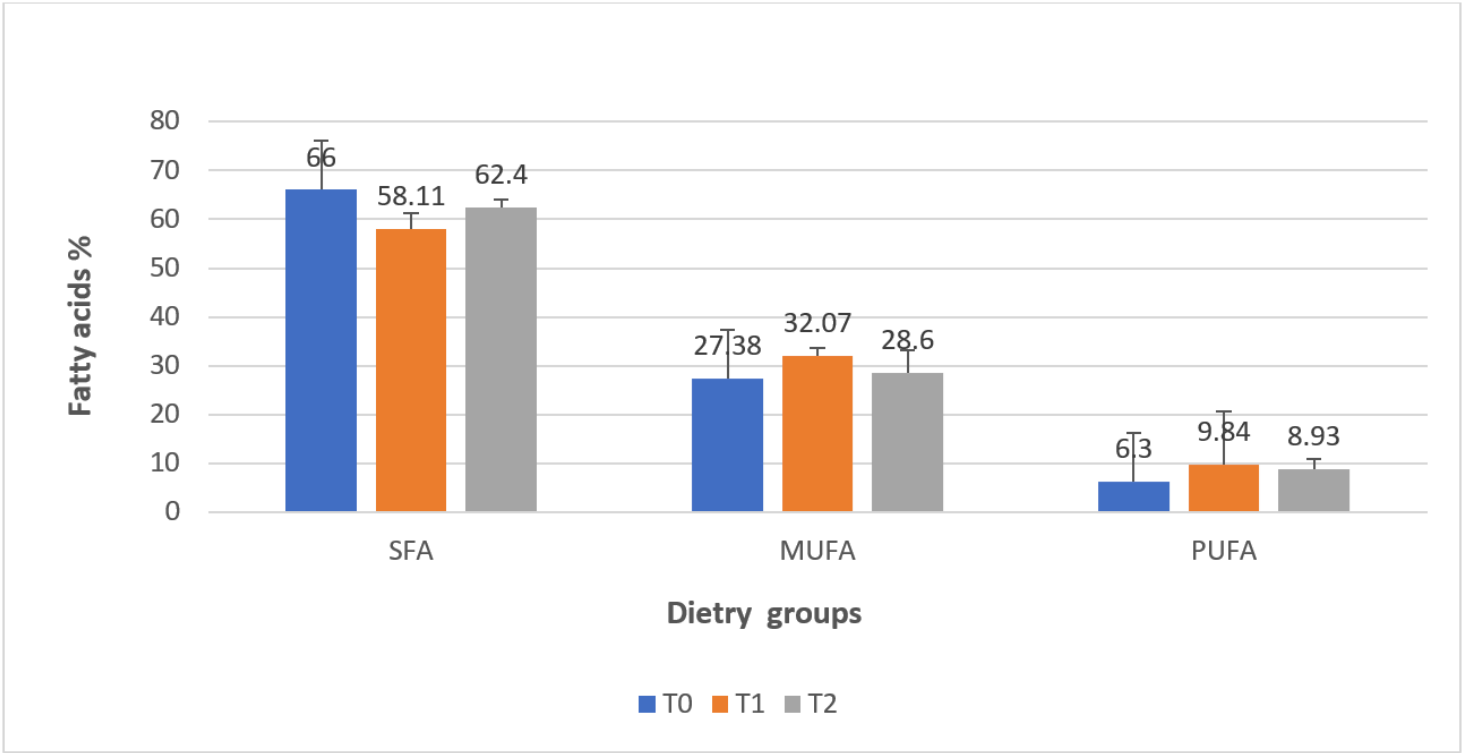


Figure 1

Fatty acids percentage in milk of lactating camels in different groups.