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Milk fatty acid profile of Malabari goats

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Abstract
This research was conducted to analyse the normal fatty acid profile of Malabari goat milk. Six Malabari does on the sixth day of lactation were selected to conduct the trial. Milk samples were collected fortnightly on 14th, 28th, 42nd and 56th day. Fat separation was carried out according to ISO-IDF (2001) using n-pentane and diethyl ether after adding ammonium hydroxide solution to the milk. Lipid extraction was carried out, fatty acid methyl esters were prepared and gas chromatography analysis of the samples were done. The fatty acid profile of normal goat milk from Malabari goats were elucidated. The fatty acid detected in the milk samples of were categorised to short, medium and long chain fatty acids. The fatty acids like C14:0 (Methyl Myristate) and C15:0 (Methyl pentadecanoate) showed a significant difference (p<0.01) between the fortnights with the exception of a few. Similarly, C18:1(c9) (Methyl oleate) and C18:3 (Methyl linoleate) showed a significant difference (p<0.05) between most of the fortnights. The medium chain fatty acids (MCFA) and long chain fatty acids (LCFA) exhibited a significant difference (p<0.05) between most of the fortnights.

Keywords: Malabari, milk fatty acid profile, short chain, medium chain and long chain fatty acids

1. Introduction
Livestock production is the most widely adopted agriculture practice by marginal and subsistence farmers, particularly in the developing parts of the world. Among the different livestock, goat is particularly important in the economy of the rural people in developing countries. Goats are mainly reared by small and marginal farmers and landless laboures.
Goat is generally described as a 'poor man's cow'. They form an integral part of the livelihood of the poor in rural India because of their adaptation to extreme climatic conditions and low maintenance cost. Goat milk composition differs from other mammalian milk in terms of alkalinity, better digestibility, buffering capacity and its nutraceutical properties. Hence, milk of different breeds of goat is endorsed for children, aged and unhealthy people. The important components that affect the milk yield and composition of the goats are breed, nutrition, stage of lactation, environment and health condition of animals (Park *et al*., 2007) [14].
Due to acceptability, attractive odour and taste of the goat milk, it can substitute cow milk. It has less allergenicity and higher digestibility (Jandal, 1996; Park *et al*., 2007) [9, 14]. Goat milk contains both polyunsaturated fatty acid (PUFA) and conjugated linoleic acid (CLA) which are proved to have potential health benefits. Health benefits of omega-3 PUFA includes decreased risk of cardiovascular diseases, increased development of brain and visual ability in small children and modulation of inflammatory disorders. Health benefits of CLA includes anti-carcinogenic and anti-atherogenic properties (Kinsella, 1986; Whigham *et al*., 2000) [10, 16].
Hence, this research was taken up to elucidate the normal fatty acid profile of milk of Malabari goat breed of Kerala, India, which would be helpful in improving the quality of goat milk and the products derived from goat milk.

2. Materials and Methods
2.1. Animal selection
The study was conducted in six Malabari goats in the first week of lactation, which were randomly selected from the Goat Farm, Instructional Livestock Farm Complex (ILFC), College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India. The animals were fed as per the recommended feeding regimen of the goat farm.

2.2. Analysis of fatty acid profile of goat milk
Milk was directly collected into the 50 mL Eppendorf tubes without any contamination. Milk

samples were collected fortnightly at 14th, 28th, 42nd and 56th day. The samples were stored in deep freezer at -20 °C until further processing.

2.2.1. Fat separation and derivatization methods

Fat separation was carried out according to ISO-IDF (2001) using n-pentane and diethyl ether after adding ammonium hydroxide solution to the milk. Milk fat separation was also carried out using the rapid method proposed by Feng *et al.* (2004) [6]. 30 mL of raw milk was centrifuged at 17,800 × g for 30 min at 4 °C (Eppendorf Centrifuge 5430 R). The fat layer was transferred to a micro-tube and left at room temperature for approximately 30 min before being micro-centrifuged at 19,300 ×g for 20 min at 4 °C. After the second centrifugation, the top layer was removed for analysis (Luna *et al*., 2005) [12].

2.2.2. Lipid extraction technique

To one gram of fat, 18 ml of hexane: isopropanol (HIP-3:2) was added, the mixture was homogenized with a Polytron (Kinematica, USA) for 30 seconds, and the suspension was filtered through a sintered glass Buchner funnel (medium porosity) fitted with a ball joint for use with pressure (10). The homogenizer, funnel, and residue were washed three times with 2-mL portions of HIP, by re-suspending the residue each time and letting the solvent soak for two minutes before applying air pressure. If desired, non-lipids in the extract could be removed by mixing the pooled filtrates for at least one min with 12 mL of aqueous sodium sulphate (prepared from one gram of the anhydrous salt and 15 mL of water). The two layers that formed were each about 18 mL in volume. The lipids were in the upper, hexane-rich layer. No precipitate was visible at the interface followed as per (Hara and Radin, 1978) [8].

2.2.3. Fatty acid methyl ester preparation

Milk fatty acids were trans-esterified with sodium methoxide prepared in our laboratory according to the method of Christie and Han (2012) [4] with modifications. The sodium methylate was obtained from Sigma Aldrich (India). Hexane (2 mL) was added to 40 mg of butter oil followed by 40 µl of methyl acetate. After the mixture was vortexed, 40 µl of methylation reagent (1.75 mL methanol: 0.4 mL of 5.4 mol/L sodium methylate) was added. The mixture was vortexed and allowed to react for 10 min; then 60 µl of termination reagent (1 g oxalic acid/30 mL diethyl ether) was added. The sample was then centrifuged for 5 min at 2400 x g at 5 °C leaving a clear layer of hexane; an aliquot of the hexane was taken and used directly for chromatographic determination (Chouinard *et al*., 1999) [3].

2.2.4. Gas Chromatography analysis

Methyl ester composition of fatty acids were analysed by Gas Chromatography (GCMS-QP2010 Ultra, Shimadzu, Japan) equipped with an auto sampler, a flame ionization detector (Plate 2). A capillary column (100 m length x 0.25 mm internal diameter, 0.20 µm; Rt-2560 Restek®) was used for analysis. The carrier gas used was high purity helium (99.9999 per cent) with a total flow rate of 106.7 ml/min and a column flow rate of 1 mL/min. The sample volume was 1 µL with a split ratio of 1:100. Oven temperature program was initially set at 100 °C which was held for 4 min, then ramped at 3 °C /min to 190 °C and held for 5.0 min, then ramped at 2 °C/min to 230 °C and held for 20 min. The total run time was 79 min. The injection port, and the flame ionization detector temperatures were 225 °C, and 245 °C, respectively. Standard mixture (C4-C24; Food Industry FAME Mix, Restek® USA, Cat no. 35077) and cis 9, trans 11 conjugated linoleic acid (CLA; Sigma Aldrich, India) were used as reference for quantification. Fatty acids were quantified as g fatty acid per 100 g of lipids. The obtained spectrum of the sample was matched with the retention time of the individual fatty acids in the FAME standard mixture. Similarly, the presence of cis9, trans11 conjugated linoleic acid (CLA) was confirmed by comparing the retention time with the Sigma analytical standard.

2.3. Statistical Analysis

SPSS V.24 was used to analyze the fatty acid profile in goat milk in the group on 14th, 28th, 42th, 56th day of the experiment.

3. Results and Discussion

The percentages of individual fatty acids in Malabari goat milk are given in Table 1. Chromatograph showing the peaks of individual fatty acid in the standard Food Industry Fatty Acid Methyl Ester FAME Mix, Restek® (FAME) is presented in Figure 1. Chromatograph showing the peaks of individual fatty acid of the group is depicted in Figure 2.

Among the individual fatty acids detected in Malabari goat milk, C14:0 (Methyl Myristate) showed a significant difference ($p<0.01$) between 14th day and 28th day. The fatty acid C15:0 (Methyl pentadecanoate) showed a significant difference ($p<0.01$) between the fortnights with the exception of 14th day and 42nd day. Similarly, C18:1(c9) (Methyl oleate) showed a significant difference ($p<0.05$) between the fortnights with the exception of 28th day and 56th day. The long chain fatty acid C18:3 (Methyl linoleate) also exhibited a significant difference ($p<0.05$) between the fortnights with the exception of 42nd day and 56th day.

Generally, the fatty acid detected in the milk samples of were categorised to short, medium and long chain fatty acids and are presented in Table 2. The short chain fatty acids (SCFA) in the milk did not exhibit any significant difference between the fortnights till 56th day. The medium chain fatty acids (MCFA) exhibited a significant difference ($p<0.05$) between the fortnights with the exception of 28th day and 56th day. The long chain fatty acids (LCFA) also exhibited a significant difference ($p<0.05$) between the fortnights with the exception of 28th day and 56th day.

Milk is a major source of essential nutrients in the form of dietary energy, proteins and fat. It also provides different physiologically active constituents like vitamins, bioactive peptides, antioxidants, minerals and nutritionally desirable fatty acids (FA) including alpha linoleic acid, conjugated linoleic acids and oleic acids (Ludmila *et al.*, 2017) [11]. The components of fat in milk are highly variable and is constituted with more than 400 fatty acids including the milk fat triacylglycerols (Newburg *et al*., 1995) [13]. The triacylglycerols comprising of fatty acids with chain lengths from C4-C18 accounted for 95 per cent of the milk fat content and 5 per cent was represented by phospholipids, cholesterol, diacylglycerols, monoacylglycerols and free fatty acids. In milk, the short (SCFA) and the medium chain fatty acids (up to C14) were produced *de novo;* while dietary lipids and lipolysis of adipose tissue triacylglycerols contributed half of

the C16 and all long chain fatty acids (Bauman and Griinari, 2001) [1]. Milk fat yield and its fatty acid composition are influenced by physiological, genetic and nutritional factors.

The relationship between lauric (C12:0) and capric (C10:0) acid is a distinctive feature that sets goat milk apart from cow milk (Haenlein and Wendorff, 2006) [7].

Table 1: Percentages of milk fatty acids of Malabari does

Name of the fatty acid	14th Day	28th Day	42nd day	56th day	P-Value
(C4 : 0) Methyl butyrate	2.91±0.08	2.67±0.61	2.27±0.56	2.65±0.30	0.616^{ns}
(C6: 0) Methyl caproate	3.00±0.03	2.32±0.51	1.99±0.51	2.37±0.18	0.123^{ns}
(C8:0) Methyl octanoate	3.51±0.05	2.13±0.69	3.33±0.86	2.49±0.19	0.297^{ns}
(C10:0) Methyl decanoate	10.99±0.30	7.39±1.70	7.66±1.38	7.33±0.80	0.072^{ns}
(C12:0) Methyl dodecanoate	5.25±0.24	3.69±0.89	3.08±0.74	3.48±0.37	0.065^{ns}
C14:0 Methyl Myristate	10.69±0.18^{a}	8.29±0.88^{b}	9.02±0.65^{b}	7.95±0.63^{b}	0.008**
C15:0 Methyl pentadecanoate	1.79±0.04^{ab}	0.86±0.39^{b}	1.69±0.37^{ab}	2.22±0.15^{a}	0.008**
C16 Methyl palmitate	16.58±0.19	15.97±0.87	17.78±1.01	16.19±0.25	0.208^{ns}
C16:1 (c9) Methyl palmitoleate	1.06±0.13	0.70±0.23	1.01±0.24	1.30±0.20	0.203^{ns}
C17:0 Methyl heptadecanoate	1.66±0.05	1.10±0.36	1.80±0.42	2.03±0.23	0.205^{ns}
C17:1 (c10) Methyl heptadecenoate	0.67±0.05	0.34±0.16	0.56±0.27	1.05±0.16	0.075^{ns}
C18:0 Methyl stearate	10.91±0.36	14.07±1.77	13.61±1.86	12.78±1.02	0.286^{ns}
C18:1 (t9) Methyl octadecenoate	0.94±0.36	2.34±1.61	1.42±0.87	1.15±0.41	0.701^{ns}
C18;1(c9) Methyl oleate	21.22±0.28^{b}	24.00±1.05^{a}	22.50±2.23^{ab}	26.86±1.61^{a}	0.045*
C18:2(t9, t12) Methyl linoleaidate	0.27±0.09	0.22±0.10	0.12±0.08	0.31±0.07	0.471^{ns}
C18:2 (c9, c12) Methyl linoleate	3.66±0.10	3.31±0.68	3.21±0.67	4.35±0.24	0.232^{ns}
C20:0 Methyl arachidate	0.29±0.01	0.22±0.07	0.35±0.08	0.40±0.04	0.207^{ns}
C18:3 Methyl linoleate	0.33±0.11^{b}	0.66±0.26^{ab}	1.01±0.23^{a}	0.92±0.08^{a}	0.020*
C21:0 Methyl heneicosanoate	1.87±0.13	4.03±2.33	1.02±0.35	1.95±0.21	0.353^{ns}
Misc.	0.13±0.02	0.32±0.24	0.37±0.26	0.12±0.01	0.547^{ns}

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant
Means having different letter as superscript differ significantly

Table 2: Profile of different category of fatty acids

Fatty acids	14th Day	28th Day	42nd day	56th day	P-Value
SCFA	3.11±0.06	2.52±0.54	2.56±0.32	2.56±0.16	0.422^{ns}
MCFA	4.61±0.19^{a}	3.68±0.42^{b}	4.08±0.53^{ab}	3.70±0.43^{b}	0.022*
LCFA	1.90±0.12^{b}	2.29±0.20^{a}	2.09±0.20^{ab}	2.26±0.14^{a}	0.043*

* Significant at 0.05 level; ns non-significant
Means having different letter as superscript differ significantly

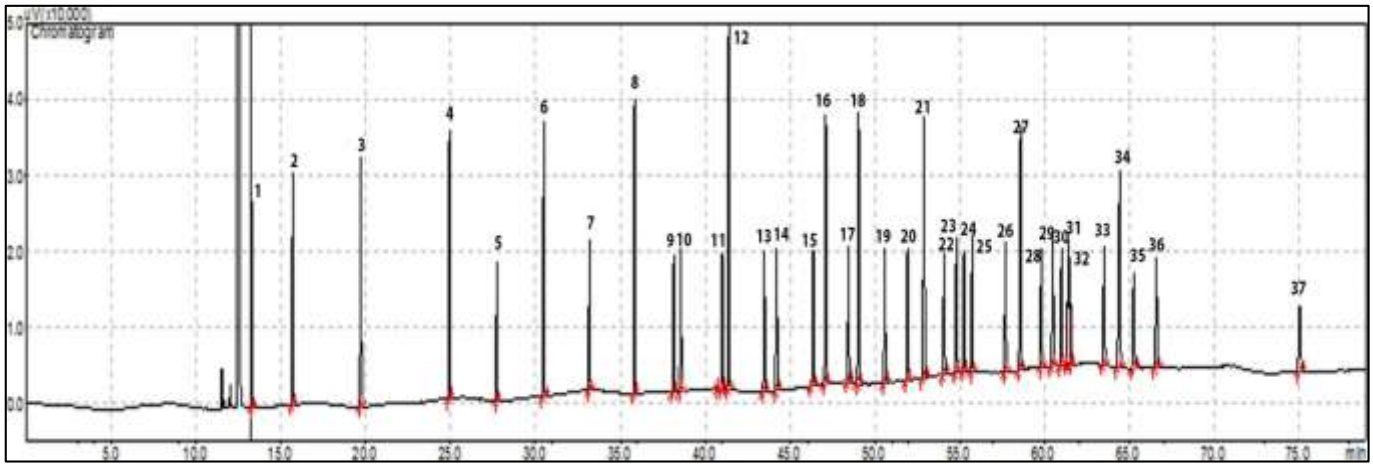

Fig 1: Chromatograph showing the peaks of individual fatty acid in the standard Food Industry Fatty Acid Methyl Ester FAME Mix, Restek® (FAME)

Names of individual fatty acid numbered against each peak in the Fig 1

1. C4: 0 Methyl butyrate
2. C6: 0 Methyl caproate
3. C8: 0 Methyl octanoate
4. C10:0 Methyl decanoate
5. C11:0 Methyl undecanoate
6. C12:0 Methyl dodecanoate
7. C13:0 Methyl tridecanoate
8. C14:0 Methyl Myristate
9. C14:1 (c9) Methyl myristoleate
10. C15:0 Methyl pentadecanoate
11. C15:1 (c10) Methyl pentadecenoate
12. C16 Methyl palmitate
13. C16:1 (c9) Methyl palmitoleate
14. C17:0 Methyl heptadecanoate
15. C17:1 (c10) Methyl heptadecenoate
16. C18:0 Methyl stearate

17. C18:1 (t9) Methyl octadecenoate
18. C18;1 (c9) Methyl oleate
19. C18:2(t9, t12) Methyl linoleaidate
20. C18:2 (c9, c12) Methyl linoleate
21. C20:0 Methyl arachidate
22. C18:3(c6, c9, c12) Methyl linolenate
23. C20:1(c11) Methyl eicosenoate
24. C18:3(c9, c12, c15) Methyl linoleate
25. Cis 9, trans 11 Conjugated Linoleic Acid
26. C20:2(c11, c14) Methyl eicosadienoate
27. C22:0 Methyl behenate
28. C20:3(c8, c11, c14)Methyl eicosatrienoate
29. C23:0 (c13)Methyl erucate
30. C20:3 (c11, c14, c17) Methyl eicosatrienoate
31. C23:0 Methyl tricosanoate
32. C20:4 (c5, c8, c11, c14) Methyl arachidonate
33. C22:2 (c13, c16) Methyl docosadienoate
34. C24:0 Methyl lignocerate
35. C20:5(c5, c8, c11, c14, c17) Methyl eicosapentaenoate
36. 36) C24:1(c15) Methyl nervonate 37) C22:6 Methyl docosahexaenoate

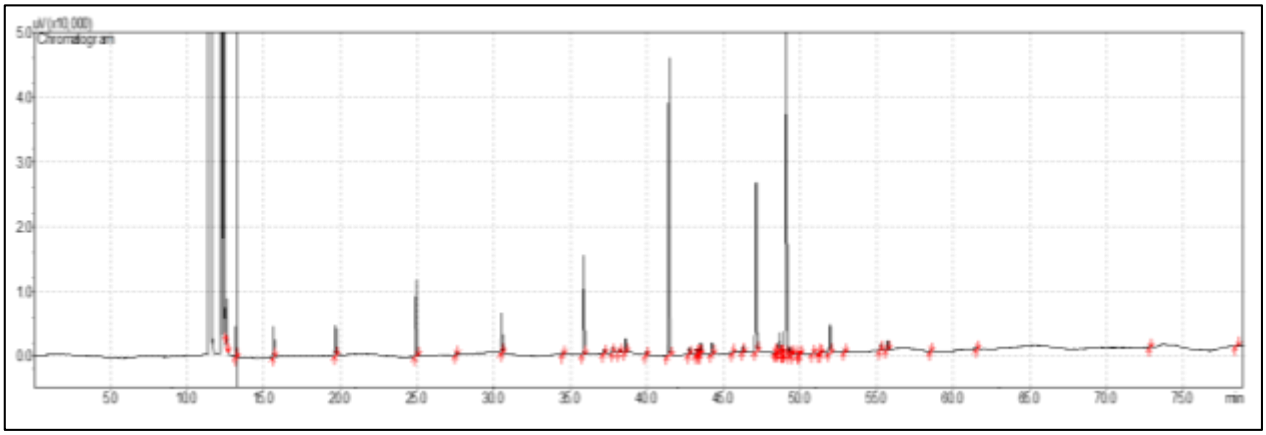

Fig 2: Chromatograph showing the peaks of individual fatty acid of the group

The goat milk fat is made up of several hundred fatty acids, whose proportions in the overall fatty acid pool vary greatly. Over 75% of the total FA in milk is made up of five of those acids (C10:0, C14:0, C16:0, C18:0, and C:18-1 cis) (Park *et al*., 2007) [14]. In the present study also, the medium chain fatty acids contributed the maximum percentage (47.92%) compared to other fatty acids. The second highest percentage (32.32%) was observed for SCFA in the present study. This was in accordance with the findings of Strzałkowska *et al*., 2009 [15] that the percentage of SCFA in the first stage of lactation was 29.22%. Goat milk is a rich source of short chain fatty acids (SCFA - C6:0, C8:0, and C10:0) which are synthesized *de novo* in the mammary gland (Chilliard *et al*., 2006) [2]. The SCFA content in goat milk is crucial because it determines the flavour and sensory qualities of the milk and dairy products that are produced from goat milk (Eknes *et al*., 2009) [5].

4. Conclusion

In the present study, the normal fatty acid profile of Malabari goat breed from 6th to 56th day of lactation was elucidated. There were significant differences in the percentages of a few fatty acids between the fortnights. Similarly, the profile of MCFA and LCFA varies significantly between the fortnights. The data on the normal fatty acid profile of Malabari goat breed would be useful in improving the quality of goat milk and other milk derived products.

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