RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.1.135-141

Effect of heat stress on milk fatty acid profile in crossbred and vechur cattle#

K. Roshna[1], M.V. Chinnu[2], R. Uma[3], M. Shynu[4] and V. Beena[5]
Department of Veterinary Biochemistry
College of Veterinary and Animal Sciences
Mannuthy, Thrissur- 680651
Kerala Veterinary and Animal Sciences University
Kerala, India

Citation: Roshna K., Chinnu M.V., Uma R., Shynu M. and Beena V. 2024. Effect of heat stress on milk fatty acid profile in crossbred and vechur cattle. *J. Vet. Anim. Sci.* **55**(1):135-141
DOI: https://doi.org/10.51966/jvas.2024.55.1.135-141

Received: 12.10.2023 *Accepted:* *Published: 31.03.2024*

Abstract

A study was performed to investigate the impact of heat stress on milk composition and fatty acid profile in crossbred and Vechur cattle. The study was organized in two periods, period 1 with minimum Temperature Humidity Index (THI) (December 2022 to January 2023) and period 2 with maximum THI (March 2023 to April 2023). Six milk samples collected from lactating crossbred and Vechur cows were subjected to analysis. Milk composition analysis revealed no significant difference (p>0.05) between breeds and periods. The milk fatty acid analysis was accomplished using Gas chromatography-mass spectrometry (GC-MS). Six saturated, two monounsaturated and one polyunsaturated fatty acids were analysed. Among the analysed saturated fatty acids, the palmitic and caprylic acids showed a significant difference in milk samples of crossbred cattle with a surge in mean value during period 2 (p <0.05). However, in Vechur milk only stearic acid showed a significant difference with an increased mean in period 2. Monounsaturated fatty acids showed a significant difference in crossbred milk with an increased mean during period 2 (p <0.05). The only polyunsaturated fatty acid analysed in the work was linolenic acid. Compared to crossbred cattle, Vechur milk showed a decrease in the quantum of linolenic acid which is statistically significant (p <0.05) during period 2.

Keywords: *Temperature Humidity Index, fatty acid, heat stress*

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

1. *MVSc Scholar*
2. *Assistant Professor*
3. *Associate Professor and Head*
4. *Professor*
5. *Professor, Department of Veterinary Physiology*

**Corresponding author: chinnu@kvasu.ac.in , Ph. 9562968495*

Heat stress has adverse effects on the physiological, metabolic, immune and production performance of livestock (Das, 2018). Rise in temperature of 2-3º C and associated rise in humidity can be expected to exacerbate heat stress in dairy cows, which would impair its growth and milk production. The imbalance between heat production inside the animal body and its dissipation can result in increased core body temperature which consequently leads to heat stress (Kadzere *et al*., 2002). Temperature humidity index (THI) indicates the potential harm or risk of the animals resulting from climatic fluctuations (Lallo *et al.*, 2018). Milk is a thriving source of several nutrients like fat, protein, carbohydrates, minerals and vitamins. Milk fat is composed of triacylglycerides which contain different types of fatty acids. Fatty acid composition is influenced by genetic and environmental factors. The common environmental factors that affect milk composition include diet, climate and disease conditions (Djordjevic *et al*., 2019). Vechur cattle, the indigenous breed of Kerala is generally known for their adaptability to hot, humid tropical climatic conditions of its native tract. The milk of Vechur cow has high fat content and smaller fat globules than crossbred cattle, making it more digestible and palatable to children and geriatric people (Venkatachalapathy and Iype, 1996).

A perusal of the literature disclosed that the data on heat stress and its effect on the fatty acid profile of indigenous and crossbred cattle of Kerala are rare. The study is expected to throw light on the modifications in fatty acid profile in heat stressed crossbred and Vechur cattle.

Materials and methods

Meteorological data

Meteorological data (December 2021- November 2022) were collected from the Centre for Animal Adaptation to Environment and Climate Change Studies (CAADECCS), KVASU, Mannuthy, Thrissur. The monthly average Temperature Humidity Index (THI) was calculated using the following formula.

THI = db°F – ((0.55-0.55×RH) (db°F – 58)), where db°F is dry bulb temperature (Fahrenheit) and RH is relative humidity (%)

The month with the lowest (December 2022-January 2023) and highest average THI (March 2023-April 2023) was chosen as study period 1 and period 2 respectively.

Sample collection

Sample size of the present study was 12. The selected animals were of same parity and stage of lactation. Six milk samples were collected from lactating crossbred and Vechur cattle maintained at the University Livestock Farm and Fodder Research Development Scheme and Vechur Cattle Conservation Unit, Mannuthy respectively during period 1 and period 2. Morning milk sample of each animal per day was collected and the collected samples were subjected for milk composition analysis and fatty acid profiling.

Milk composition analysis

Milk samples of individual cows were collected and milk constituents such as fat, protein and solid-not-fat were estimated using an EKOMILK ultra milk analyser (Milkana kam98-2A, India).

Fatty acid profiling

Fifty milliliters of milk samples were collected, subjected to lipid separation and then converted to fatty acid methyl esters (FAME) for GC analysis. Analysis of fatty acid composition in milk samples was carried out using GC-TRACE 1300 (Thermo Scientific) equipped with autosampler and Flame Ionization Detector. Chromatographic separations were accomplished in 5 MS columns (5% Phenyl 95% dimethyl arylene siloxane, nonpolar) having a temperature of 320-360ºC.

Preparation of Working Standard Solutions

Four different standard solutions of FAME mix C14-C22 (CRM47885 - USA) were prepared. The stock standard of 400µg/mL (400 µg in 1 mL dichloromethane) was used to prepare the remaining working standards (200µg/mL, 100µg/mL, 50µg/mL) using dichloromethane.

Sample preparation

Fifty milliliters of fresh milk sample were centrifuged at 12,000 rpm for 30 minutes at 4ºC and collected 1 g of fat from the top and placed into a 15mL solvent resistant tube which was rinsed with hexane. A solution of hexane : isopropanol (3:2 v/v) containing 50 mg of butylated hydroxytoluene at the rate of 18 mL/g of fat was added and vortexed. To this mixture, 6.7 per cent sodium sulfate solution at the rate of 12 mL/g of fat was added, vortexed for one minute and allowed to stand for layer separation to complete. The upper hexane layer was transferred into a 15mL test tube containing one gram anhydrous sodium sulfate, vortexed for one minute and allowed to stand for 45 min. The upper hexane layer was transferred into a vial and then subjected to evaporation by placing it in a vacuum concentrator for two hours at 2000 rpm. Leftover unevaporated lipid was transferred into a 50 mL standing tube and 5 mL of 0.25 mol/ L sodium methoxide in methanol: diethyl ether (1: 1 v/v) was added and vortexed for one minute. Three millilitres of iso-octane and 15 mL saturated NaCl were added and vortexed for phase separation. The top layer containing fatty acids was collected and stored in the refrigerator, until analysis.

Chromatographic condition

One microlitre of blank (hexane), standards and samples were injected separately into the 5 MS column. The components were separated by elution using carrier gas helium at a flow rate of 30 mL/min. The flame ionisation detector (FID) and injection temperature were set at temperature 250ºC. Air and H_2 gas flow rate of FID were 300 mL/min and 30 mL/min respectively. Helium gas was used as makeup gas with a flow rate of 30 mL/min. The eluted fatty acids were detected by flame ionisation detector.

Statistical analysis

The data obtained from milk composition analysis and fatty acid profiling were statistically analysed using repeated measures ANOVA.

Results and discussion

Temperature humidity index (THI) is used to measure the impact of heat stress on milk yield, composition and reproductive efficiency of dairy cows (Ghavi *et al.,* 2013). Daily meteorological data during the research period from December 2022 to April 2023 were taken from CAADECCS. Calculated average THIs of previous year data and monthly average THIs during study periods are illustrated in Table 1 and Table 2 respectively. According to the previous year data the monthly average THI observed was 76.90 and 75.29 during December -January whereas during March and April it was 79.62 and 82.35.

Analysis of milk composition

Ten millilitres of milk samples were subjected for the study using EKOMILK

Table 1. THI calculated from the weather data of the previous year

Sl. No.	Month	Average THI	Range
1	**December 2021**	**77.21**	**69.76 - 84.67**
2	**January 2022**	**76.56**	**68.34 - 84.78**
3	February 2022	79.58	70.07 - 89.02
4	**March 2022**	**81.99**	**72.46 - 91.57**
5	**April 2022**	**81.58**	**73.71 - 89.93**
6	May 2022	79.00	72.51 - 85.65
7	June 2022	78.74	71.69 - 85.88
8	July 2022	77.48	71.84 - 83.45
9	August 2022	77.54	71.76 - 83.72
10	September 2022	78.42	71.40 - 85.54
11	October 2022	79.38	71.26 - 87.73
12	November 2022	78.23	69.77 - 86.80

Rows in bold denote the period of minimum and maximum average THI

Table 2. Calculated average THI during study period

Sl. No	Month	Average THI	Range
1	December 2022	76.90	68.67 - 85.25
2	January 2023	75.29	66.46 - 84.25
3	February 2023	75.89	66.05 - 86.96
4	March 2023	79.62	70.25 - 88.99
5	April 2023	82.35	73.12 - 91.58

Table 3. Recorded data of milk composition

		PERIOD 1		PERIOD 2	
		Fat	**Protein**	**Fat**	**Protein**
Crossbred	1	3.4	3.5	2.37	2.73
	2	3.38	3.3	3.06	3.77
	3	3.54	3.52	2.55	2.37
	4	3.31	3.47	2.48	2.56
	5	3.46	3.16	4.76	3.34
	6	4.03	2.65	2.46	2.72
Vechur	1	3.01	3.62	2.11	3.62
	2	4.52	3.38	3.16	3.57
	3	5.01	3.64	3.28	3.63
	4	2.72	3.60	4.4	3.63
	5	3.4	3.5	2.37	2.73
	6	3.38	3.3	3.06	3.77

ultra milk analyser. The recorded data of fat and protein content of milk and its statistical analysis by repeated measures ANOVA are given in Table 3 and Table 4, respectively. Even though it showed a decrease in mean value of fat and protein in period 2, there was no significant difference between groups ($p > 0.05$). In the milk samples of 160 crossbred cattle, Ozrenk and Inci (2008) observed a decrease in the amount of fat and protein in the summer period. The current study revealed no changes in the fat and protein content of milk, probably as a result of the management strategies practised during summer period to reduce heat stress. The results of Abraham *et al*. (2014), who reported that Vechur cattle maintained their milk protein, SNF, and lactose content across various seasons, are consistent with the findings of current study that there is no significant difference in the content of milk fat and protein of Vechur. Vechur cattle are known for their high adaptability to the hot and humid conditions of Kerala.

Fatty acid profiling by gas chromatography

The study analysed six saturated, two monounsaturated and one polyunsaturated fatty acids. The total concentration of each fatty acids in crossbred and Vechur cattle and its statistical analysis during period 1 and period 2 are combined in Table 5. Among the fatty acids analysed palmitic and oleic acids were found to be the most abundant saturated and unsaturated fatty acids respectively, in accordance with Santin *et al.* (2019).

Among saturated fatty acids, caprylic and palmitic acid showed a significant difference in milk samples of crossbred cattle with an increase in mean value during period 2 compared to period 1 ($p < 0.05$). Whereas, in Vechur milk only stearic acid showed a significant difference with an increased mean in period 2. The finding of the present study was similar to the results of Saroj *et al*. (2017), who described an increased amount of saturated fatty acids during summer compared to the winter season and stated that feeding silage during the summer period could increase the saturated fatty acid content in cow milk. The feeding practices adopted during the summer period like feeding of bypass fat and yeast to reduce heat stress may impart to the change in fatty acid profile in crossbred cattle. Djordjevic *et al*. (2019) reported that the variation in milk fatty acid profile could be due to differences in breed and feeding practices adopted in different farms. Monounsaturated fatty acids analysed were myristoleic and oleic acids. Both fatty acids showed significant increase in crossbred milk during period 2 ($p < 0.05$). Compared to crossbred cattle, Vechur milk showed a significant increase in the amount of oleic acid ($p < 0.05$) during period 1. The

Table 4. Milk fat and protein during period 1 and 2 (Mean ± S.E)

Parameter	Breed	Period 1 (Mean ± S.E)	Period 2 (Mean ± S.E)
Fat	Crossbred	$3.52^{Aa} \pm 0.11$	$2.95^{Aa} \pm 0.38$
	Vechur	$3.67^{Aa} \pm 0.37$	$3.06^{Aa} \pm 0.33$
Protein	Crossbred	$3.27^{Aa} \pm 0.14$	$2.92^{Aa} \pm 0.22$
	Vechur	$3.51^{Aa} \pm 0.06$	$3.49^{Aa} \pm 0.15$

Means having same superscripts (capital letters A-B within rows, small letters a-b within columns) do not differ significantly at 5% level

Table 5. Fatty acid composition of milk fat during period 1 and 2 (Mean±S.E)

Sl. No.	Fatty acid	Breed	Period 1 (Mean ± S.E) (mg/g)	Period 2 (Mean± S.E) (mg/g)
Saturated fatty acids				
1	Caprylic acid (C8:0)	Crossbred	24.7^{Aa} ±0.3	25.9^{Ba} ±0.2
		Vechur	25.2^{Aa} ±0.5	24.7^{Ab} ±0.3
2	Capric acid (C10:0)	Crossbred	27.6^{Aa} ±0.3	28.9^{Aa} ±0.3
		Vechur	28.3^{Aa} ±0.7	28.1^{Aa} ±0.2
3	Lauric acid (C12:0)	Crossbred	33.0^{Aa} ±0.3	33.3^{Aa} ±0.3
		Vechur	33.9^{Aa} ±0.8	32.7^{Aa} ±0.1
4	Myristic acid (C14:0)	Crossbred	15.2^{Aa} ±0.5	16.9^{Aa} ±0.2
		Vechur	15.6^{Aa} ±1.1	16.0^{Aa} ±0.5
5	Palmitic acid (C16:0)	Crossbred	39.4^{Aa} ±0.6	42.1^{Ba} ±0.4
		Vechur	39.9^{Aa} ±1.1	41.2^{Aa} ±1.3
6	Stearic acid (C18:0)	Crossbred	30.3^{Aa} ±0.2	31.0^{Aa} ±0.4
		Vechur	29.7^{Ab} ±0.1	30.6^{Ba} ±0.5
Monounsaturated fatty acids				
7	Myristoleic acid (C14:1)	Crossbred	8.7^{Aa} ±0.2	9.6^{Ba} ±0.1
		Vechur	9.0^{Aa} ±0.5	9.2^{Aa} ±0.2
8	Oleic acid (C18:1)	Crossbred	27.8^{Aa} ±0.2	29.5^{Ba} ±0.2
		Vechur	28.1^{Ab} ±0.1	28.6^{Aa} ±0.2
Poly unsaturated fatty acids				
9	Linolenic acid (C18:3)	Crossbred	15.24^{Aa} ±0.02	15.27^{Aa} ±0.01
		Vechur	15.28^{Aa} ±0.03	15.22^{Ab} ±0.01

concentration of total monounsaturated fatty acids was increased in crossbred during period 2. The findings were similar to that of Narongsak (2012) and Kumar *et al.* (2020) who reported a rise in unsaturated fatty acids during the summer period. Supplementing the diet with polyunsaturated and conjugated linoleic acid precursors during summer promoted increased activity of rumen bacterium *Butirivibrio fibrisolvens* and linoleic isomerase resulting in the production of monounsaturated and polyunsaturated fatty acids throughout summer (Narongsak, 2012). Supplementing cow and buffalo diets with bypass fat during summer months increased the quantity of unsaturated and long-chain fatty acids in milk fat (Garg *et al.,* 2008; Purushothaman *et al.,* 2008). Bypass fat modifies the fatty acid level of milk by increasing the content of monounsaturated, long-chain and overall unsaturated fatty acids by resisting the rumen bacterial biohydrogenation (Garg *et al*., 2008). Supplementing yeast and products made from the yeast cell wall in feed could be able to reduce the adverse effects of heat stress (Broadway *et al*., 2020) and it can increase the total fatty acids with 16C atoms and C18:3(n-3) fatty acids in milk (Yalcin *et al*., 2011). The only polyunsaturated fatty acid analysed in the study was linolenic acid. Compared to crossbred cattle, Vechur milk showed a decrease in the concentration of linoleic acid which was statistically significant ($p < 0.05$) during period 2. Numerous factors including diet, differences in breeds, individual variations and hormonal regulation of lipid metabolism influence the production of fatty acids (Mauvoisin and Mounier, 2011).

Conclusion

It was learned that the animals maintained in the farm were provided with bypass fat and yeast with a rate of 100-150g/ cow/day and 10-15g/cow/day, respectively, during summer season as a part of heat stress mitigation during this period. Bypass fat comprises 80-84% of fat, primarily palmitic and oleic acid. The variations observed in this study might be due to modifications in the diet during summer month. The increased levels of monounsaturated fatty acid in crossbred cattle might be due to the feeding practices adopted in the farm in order to mitigate heat stress which are different from those in Vechur farm. So, in order to get a clear idea about the effect of heat stress the study has to be conducted in a temperature-controlled climatic chamber along with a formulated diet.

Acknowledgements

The authors gratefully thank the Dean, College of Veterinary and Animal Sciences, Mannuthy and Central Instrumentation Laboratory for providing facilities for conducting the study.

Conflict of interest

The authors declare that they have no conflict of interest

References

Abraham, B.L. and Gayathri, S.L. 2015. Milk composition of crossbred and desi cattle maintained in the sub-tropical high ranges of Kerala. *Indian. J. Vet. Anim. Sci.* **44:** 53-55.

Broadway, P.R., Carroll, J.A., Burdick Sanchez, N.C., Cravey, M.D. and Corley, J.R. 2020. Some negative effects of heat stress in feedlot heifers may be mitigated via yeast probiotic supplementation. *Front. Vet. Sci.* ***6***: 515.

Das, S.K. 2018. Impact of climate change (heat stress) on livestock: adaptation and mitigation strategies for sustainable production. *Agric. Rev*. **39**: 130-136.

Djordjevic, J., Ledina, T., Baltic, M.Z., Trbovic, D., Babic, M. and Bulajic, S. 2019. Fatty acid profile of milk. In: *International Meat Industry Conference - Earth and Environmental Science;* 22th -25th September 2019, Mt. Kopaonik, Serbia. **333**: 012057.

Garg, M.R., Sherasia, P.L., Bhanderi, B.M., Gulati, S.K. and Scott, T.W. 2008. Effect of feeding bypass fat supplement on milk production and characteristics of butter fat. *Indian J. Dairy Sci.* **61**: 56-61.

Ghavi Hossein-Zadeh, N., Mohit, A. and Azad, N. 2013. Effect of temperature-humidity index on productive and reproductive performances of Iranian Holstein cows. *Iran. J. Vet. Res*. **14:** 106-112.

Kadzere, C.T., Murphy, M.R., Silanikove, N. and Maltz, E. 2002. Heat stress in lactating dairy cows: a review. Livest. Prod. Sci. **77**: 59-91.

Kumar, T.N., Varma, G.G. and Karthiayini, K. 2020 Physiological response of crossbred calves to pre-monsoon, monsoon and post-monsoon seasons of kerala. *J. Vet. Anim. Sci.* 47: 74-77.

Lallo, C.H., Cohen, J., Rankine, D., Taylor, M., Cambell, J. and Stephenson, T. 2018. Characterizing heat stress on livestock using the temperature humidity index (THI) prospects for a warmer Caribbean. *Regional Environ. Change*. **18**: 2329-2340.

Mauvoisin, D. and Mounier, C. 2011. Hormonal and nutritional regulation of SCD1 gene expression. *Biochimie*, *93*: 78-86.

Narongsak Chaiyabutr. 2012. *Milk Production – An Up-to Date Overview of Animal Nutrition, Management And Health.* InTech, Croatia, 394p.

Ozrenk, E. and Inci, S.S. 2008. The effect of seasonal variation on the composition of cow milk in Van Province. *Pakist. J. Nutr*. **7**: 161-164.

Purushothaman, S., Kumar, A. and Tiwari, D.P. 2008. Effect of feeding calcium salts of palm oil fatty acids on performance of lactating crossbred cows. *Asian-Australasian J. Ani. Sci.* **21**: 376-385.

Santin Junior, I.A., Silva, K.C.C. and Cucco, D.C. 2019. Milk fatty acids profile and the impact on human health. *Dairy Vet. Sci. J.* **10**: 555779.

Saroj, B., Tran, L., Sharma, A., Kumar, S. and Tyagi, A. 2017. Seasonal variation in fatty acid profile in the milk of different species under popularly followed feeding system in India. *Indian J. Anim. Sci.* **87:** 484-489.

Venkatachalapathy, R.T. and Iype, S. 1996. Fat globule size and distribution in milk and iodine value of milk fat of Vechur cattle of Kerala. *Ind. J. Anim. Sci.* **67:** 10-12.

Yalcin, S., Can, P., Gurdal, A.O., Bagci, C. and Eltan, O. 2011. The nutritive value of live yeast culture (Saccharomyces cerevisiae) and its effect on milk yield, milk composition and some blood parameters of dairy cows. *Asian-Australasian J. Anim. Sci.* **24**: 1377-1385. ■