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Seasonal Variations of Milk Composition and Its Association with Weather Parameters and Biological Indicators of Thermal Stress in Crossbred Dairy Cows Under Humid Tropical Climate

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Abstract

Thermal stress (TS) adversely affects the physiological processes and productivity of animals. The association of TS factors and weather parameters with seasonal variations in milk composition was studied in early postpartum cows. Only SNF and protein showed significant variation between seasons consistently for morning and afternoon samples. Despite ambient temperature (AbT), relative humidity (RH), and temperature-humidity-index (THI) being high enough to cause TS during all seasons, only RH showed consistent influence on milk parameters. HSP 70 was significantly (P<0.01) high during summer together with significant positive correlations with milk major components. Cortisol level was high during all the seasons, however, the variations between seasons and correlation with milk parameters were non-significant. It is concluded that seasonal variations of milk composition are regulated mainly by RH, rather than AbT and THI. Unlike cortisol, HSP 70 was strongly associated with major milk parameters indicating the influence of TS on milk composition.

Keywords: Cortisol, HSP 70, Milk composition, Thermal Stress; Weather.

Introduction

Thermal stress (TS) is one of the major adverse factors affecting health and productivity of animals especially in the tropical region (Nasr and El-Tarabany 2017; Pramod *et al.*, 2021). Increasing atmospheric temperature consequent to global warming and the continuous rise of productivity achieved by systematic breeding and management interventions have made dairy animals highly vulnerable to TS factors (Das *et al.,* 2016). Besides elevation of ambient temperature (AbT), relative humidity (RH) also contributes to TS mainly through inhibition of evaporative cooling (Kristyna *et al.,* 2017). To assess the combined influence of AbT and RH, the temperature humidity index (THI) is often used as a composite weather parameter for TS (Allen *et al.*, 2009; Amstrong, 1994). Besides reduction in the milk yield, TS has been found to alter the milk composition including a significant reduction of fat, lactose, and protein content (Das *et al.*, 2016; Nasr and El-Tharabany, 2017).

Elevation of cortisol forms one of the major indicators of stress's impact on biological processes. Also, there occurs an up-regulation of HSP expression (Archana *et al.*, 2017), intended for the repair of cellular damage (Gaughan *et al.,* 2013) and to perform important cellular roles to ensure tolerance and adaptation to TS (Kumar *et al.*, 2014). Thus, HSPs are considered more specific indicators of TS. However, there is a lack of studies reporting the association of TS factors on milk composition. Accordingly, the present study was carried out to compare the composition of milk across seasons, to assess the influence of TS factors, and to understand the association of stress indicators in serum such as cortisol and HSP 70 with milk components.

Materials and Methods

The study was carried out at the Livestock Research Station of Kerala Veterinary and Animal Sciences University in India. The dairy farm of the station having 300 heads of crossbred dairy cattle intensively managed as per standard recommendations (ICAR-NIANP, 2013) formed the study subjects. The study was carried out in eight cows each during the four seasons, belonging second to third months postpartum (PP). Around the middle of each season, five each morning (AM) and afternoon (PM) milk samples were collected from the eight cows at weekly intervals. The composition of the milk samples was assessed using an Ekomilk Ultra pro milk analyzer (Milkana KAM 98-2A), both separately and also after pooling the AM and PM samples. Serum samples of the study animals were also collected on the same day and stored for estimation of HSP 70 and cortisol using ELISA kits (Chongqing Biospes Co Ltd, China, and Neogen – USA respectively). Weather parameters were recorded using a data logger (HOBO Pro V2, Onset Computer Corporation, USA) installed inside the sheds and THI values were calculated using the formula (LPHSI, 1990).

$$THI\ (LPHSI) = T - \left(\left(0.55 - \frac{0.55 \times RH}{100}\right) \times (T - 58)\right)$$

Where T - Average temperature (in Degree Fahrenheit)

RH - Percent relative humidity

The period of study was divided into four seasons of three months each based on the day length and pattern of rainfall in the region (Kutty, 2013; Kutty *et al.*, 2019), such as North-east monsoon - September to November (SON), Post monsoon - December to February (DJF), Summer - March to May (MAM), and South-west monsoon - June to August (JJA) respectively (Kutty 2021). The data of milk composition, TS indicators, and weather parameters were analyzed using SPSS software for seasonal patterns and to find out the influence of weather parameters and stress-associated serum factors on the milk composition.

Results and Discussion

The composition of the 160 milk samples analyzed is shown in Table 1. The overall mean of total solids was 11.39 ± 0.08 % and the mean density was 25.46 ± 0.25. PM samples had significantly high (P<0.001) fat % (4.57 ± 0.09% versus 2.99 ± 0.08 %) contributing more total solids (P<0.001) and low (P<0.001) density (24.35 ± 0.35 versus 26.57± 0.29), while SNF and lactose did not vary significantly between PM and AM.

The composition of the pooled samples was at the lower range for crossbred cows with a mean fat % of 3.78 ± 0.06 attributable to the predominance of Holstein crossbreds and higher yield of early PP period under the study. Fat %, total solids %, and density of pooled samples were between the values for AM and PM samples, and the difference was significant (P<0.01) between each other. The SNF was slightly more (P<0.05) in AM milk attributable to low fat %, while protein and lactose % was more or less the same in both AM and PM samples.

Table 1: Composition of milk collected in the morning, and afternoon and the pooled samples

Sample type	Fat (%)	SNF (%)	Density	Lactose (%)	Protein (%)
Morning	2.99 ± 0.08	7.70 ± 0.29	26.57 ± 0.29	4.06 ± 0.03	3.04 ± 0.03
After noon	4.57 ± 0.09	7.45 ± 0.06	24.35 ± 0.35	3.98 ± 0.04	3.06 ± 0.04
Pooled	3.78 ± 0.06	7.60 ± 0.05	25.46 ± 0.25	4.02 ± 0.03	3.05 ± 0.02

Seasonal Pattern

Between seasons, density and percentages of SNF, protein, and sugar of AM milk, as well as percentages of SNF, protein, and total solids of PM milk showed significant variation (P<0.01), while none of the components in pooled milk samples varied significantly between seasons. The fat percentage of none of the three sets of milk samples varied significantly between seasons indicating no influence of TS factors on fat % and is similar to the earlier report by Rhoads *et al.*(2009). Smith *et al.* (2013) also reported that the milk fat % of more heat-tolerant cows did not differ significantly between periods of varying levels of TS exposure.

Components of milk samples having significant variation across seasons are shown in Table 2. AM-SNF was significantly high during DJFas against SON and JJA, and density was significantly lower during SON than in the other three seasons. Corresponding to the lowest SNF, lactose, and protein contents of AM milk were the lowest during SON and highest during DJF. SNF and protein in PM milk also varied significantly between seasons with a pattern almost similar to that of AM samples. However, despite an inverse relationship with density (having significant variation), fat % of PM milk did not vary significantly between seasons.

Table 2: Mean ± SE of milk quality parameters having significant seasonal variations

Milk parameters	Seasons				F value	p-value
	SON	DJF	MAM	JJA		
AM-SNF (%)	7.48 ± 0.21[a]	8.01 ± 0.06[b]	7.78 ± 0.06[ab]	7.57 ± 0.06[a]	4.044	0.008**
AM-Density	25.06 ± 0.91[a]	28.012 ± 0.25[b]	26.73 ± 0.50[b]	26.47 ± 0.36[ab]	4.649	0.003**
AM Lactose (%)	3.89 ± 0.11[a]	4.25 ± 0.03[c]	4.11 ± 0.04[bc]	4.01 ± 0.04[ab]	5.436	0.001**
AM-Protein (%)	2.95 ± 0.08[a]	3.16 ± 0.02[b]	3.05 ± 0.02[ab]	3.00 ± 0.03[a]	4.167	0.007**
PM-SNF (%)	7.53 ± 0.17[ab]	7.81 ± 0.09[b]	7.43 ± 0.08[a]	7.23 ± 0.13[a]	4.164	0.007**
PM-Protein (%)	3.13 ± 0.07[bc]	3.24 ± 0.13[c]	2.96 ± 0.03[ab]	2.90 ± 0.04[a]	4.312	0.006 **
PM-Total Solids	11.95 ± 0.23	12.43 ± 0.13	12.34 ± 0.16	11.56 ± 0.18	4.986	0.002**

***P <0.01 - Significant at 1 % level, Values with different superscripts in the same row vary significantly*

Stress Factors Across Seasons

Weather parameters such as AbT, RH, and THI showed significant variation across seasons as shown in Table 3. Even though the preferred temperature range for better milk production in dairy cows falls between 20.0 to 27.0 °C (Dikmen and Hansen, 2009), the daily mean AbT was beyond the range during three of the seasons, indicating the possibility of affecting the quality parameters of milk, concurring earlier reports (Bernabucci *et al.*, 2015; Forsback *et al.*, 2010). AbT and RH were lowest during DJF while the highest was in MAM and JJA respectively. THI had its highest and lowest figures during MAM and DJF respectively corresponding to the temperature variation. During JJA, even though RH was maximum, THI was not very high reflecting a comparatively lesser contribution of RH to THI.

Variation of HSP 70 and cortisol in serum samples collected on the days of milk sampling are shown in Table 3. HSP 70 level varied significantly between seasons with a major hike (6.48as against the mean 3.78 ± 0.34ng/Ml) during MAM and is attributable to the maximum AbTof the season and indicates its association with TS (Archana *et al.*, 2017; Gaughan *et al.*,2013). During other seasons, HSP 70 was low despite high THI and showed no significant variation between the three seasons, which might be due to the adaptation of these animals to moderate levels of TS as reported by Hansen (2009) and Archana *et al.* (2017), attributable to the continuous propagation of these animals at the locality.

Table 3: Mean ± SE of climatic stress factors and stress indicators in serum samples across seasons

Thermal stress factors/serum indicators	Seasonal averages					F value
	SON	DJF	MAM	JJA	Overall	
Average Temperature (º C)	28.50 ± 0.14[b]	26.91 ± 0.25[a]	32.02 ± 0.12[c]	27.30 ± 0.23[a]	28.68 ± 0.19	144.92**
Average Relative Humidity (%)	79.77 ± 0.96[c]	63.77 ± 0.71[a]	69.09 ± .52[b]	85.55 ± 0.76[d]	74.55 ± 0.78	173.28**
THI	80.46 ± 0.19[c]	76.00 ± 0.41[a]	84.24 ± 0.13[d]	79.24 ± 0.29[b]	79.99 ± 0.27	149.36**
HSP 70	2.41 ± 0.53[a]	3.00±0.10[a]	6.48 ± 1.02[b]	3.23 ± 0.56[a]	3.78 ± 0.34	8.18**
Cortisol	9.11 ± 0.49	9.02 ± 1.54	9.38 ± 0.40	9.20 ± 0.38	9.18 ± 0.42	0.33[NS]

***P<0.01 - Significant at 1 % level, Values with different superscripts in the same row vary significantly, NS- Non -significant*

The THI was high enough to cause mild stress (72 to 76) even during the season of lowest temperature (DJF) and within the range for moderate stress (78- 84) during JJA and SON (Allen et al, 2009; Pederson 2014). The highest THI to the extent of severe stress (84 to 90) might be the reason for the significant rise of HSP 70 during MAM evidenced in the study. Cortisol level is expected to increase with any form of stress (Torres-Junior *et al.*,2008), and the level was high (more than 9 ng/ml) throughout the year as against 1.92 to 8.91 ng/mL reported in Sahiwal cattle exposed to THI of62.0 to 79.0 (Bhan *et al.*, (2012). Harikumar (2017) also reported high cortisol levels in cross-bred cattle under similar management in Kerala. Also, there was no significant variation between seasons indicating the prevalence of acute stress throughout the year concurring with the reports by Prasad (2014) and Zarina (2016), reflecting the involvement of multiple stress factors besides TS, (Rees *et al.*, 2016) cortisol being a non-specific indicator of TS together with others.

Thermal Stress Influence on Milk Composition

Comparison of HSP 70 levels with quality parameters of milk showed a significant positive correlation with lactose content of PM milk (r = 0.234, P<0.01); and a negative correlation (r = -0.156, P<0.05) with protein % of AM samples. TS has been found to increase the utilization of amino acids for maintenance activities including gluconeogenesis and immune response, and the resultant decrease in amino acid availability was found to affect the synthesis of milk protein (Guo *et al.*, 2018; Kadzere *et al.*, 2002). The increase in lactose content of milk was found to be associated with feed intake (Kekana *et al.*, 2018). As reported earlier, concentrate intake was higher in the herd during the hotter months (Kutty *et al.*, 2020), which might have increased the availability of blood glucose and its conversion to lactose (Rhoads *et al.*, 2009) explaining the positive correlation of lactose with HSP 70 observed in the study.

Cortisol level as well as AbT did not show a significant correlation with any of the milk quality parameters studied, while THI showed a significant negative correlation (r = -0.176, P<0.05) with a density of PM milk alone. At the same time, RH had a significant negative correlation with most of the milk parameters of both AM and PM except fat % as shown in Table 4. The negative correlation of RH with all parameters of AM milk and SNF of PM milk in the study was highly significant. These findings indicate that RH was more influential on milk components than ambient temperature or THI.

Conclusion

The influence of TS factors on various components of milk was not consistent across AM, PM, and pooled samples. Between seasons, only SNF and protein %showed significant variation consistently for AM and PM samples. Among weather parameters, despite AbT, RH, and THI being high enough to cause TS during all seasons, only RH was found to have a consistent association with milk composition. Even though cortisol levels were high throughout the year, being a non-specific indicator of TS, the variation of the same was non-significant between seasons, and correlation with milk components was also non-significant. HSP 70 was significantly high during summer compared to other seasons, and there was a significant correlation with major components of milk indicating the influence of TS on milk composition.

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Contribution by Authors

Equal contribution. All authors declared that 'written informed' consent was obtained from the approved parties for the publication of this article and accompanying images.

Conflict of Interests

There is no conflict of interest.

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