Effect of Ramie and Mango seed kernel supplementation on the physiological and metabolic status of cattle under seasonal heat stress

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Abstract

This study evaluated the effects of seasonal climatic variation and dietary treatment on physiological and blood biochemical parameters in cattle exposed to heat stress. Sixteen healthy Sahiwal heifers were selected for this study. All animals were divided into two groups control and treatment. The control group was fed with a normal farm diet according to ICAR standard 2013, whereas the treatment group received a modified diet in which 50% of the seasonal fodder was replaced with ramie grass, and mango seed kernel powder was supplemented at 3% of DMI. The study was completed in three different seasons that were hot dry, hot humid and thermoneutral. Ambient temperature and relative humidity were recorded twice daily for calculating daily THI in all seasons. Physiological responses and blood samples were collected for 4 fortnights in each season to observe overall seasonal changes in physiological and biochemical parameters. Biochemical parameters were estimated in plasma samples by spectrophotometric kits. Findings revealed that physiological responses such as rectal temperature, respiration rate, and pulse rate decreased significantly ($p<0.05$) in the treatment group. Metabolic parameters such as plasma glucose were increased significantly ($p<0.05$), whereas total plasma proteins, albumin, globulin, total cholesterol, and triglycerides decreased significantly ($p<0.05$) in the treatment group across all seasons. The result indicates that ramie and mango seed kernel as antioxidants are beneficial in alleviating heat stress and make animals more thermally adaptable by enhancing their physiological and metabolic status.

Keywords: Seasonal variations; Heat stress; Tannin; Glucose; Proteins; Cholesterol

Introduction

One of the most important environmental concerns of the twenty-first century is climate change, which has a significant impact on animal health, agricultural productivity, and global ecosystems. Climate change is a significant driver of heat stress in cattle, with wide-ranging implications for animal health, welfare, and production. Elevated ambient temperature (AT), when combined with relative humidity (RH), impairs the animals' capacity to dissipate heat to the environment, leading to heat stress (Aziz *et al*., 2016). Hot dry weather increases evaporative cooling, which increases the degree of heat stress in animals, whereas hot humid weather reduces the air's ability to absorb moisture (Kaliber *et al*., 2016). Heat stress affects cattle's metabolism, immune systems, and general health, making it a significant global issue for cattle production (Nardone *et al*., 2010). Under heat stress, animals demonstrate a range of behavioral, physiological, hematological, and biochemical adaptations to mitigate the imposed strain. Physiological responses are reliable markers of an animal's health and nutritional state. The immediate, ultimate reactions of animals to climatic stress are measured by physiological measures such as rectal temperature (RT), respiration rate, and pulse rate (PR); these measurements ultimately determine how comfortable or uncomfortable an animal is in an environment (Silanikove 2000). HSP 70 and HSP-72 genes are major molecular markers of heat stress in cattle (Vaidya et al., 2023; Biradar et al., 2024). Blood biochemical profiles are key indicators of animal health, physiology, and metabolism. Blood biochemical markers, including glucose, total protein, albumin, and globulin, can be used to monitor changes in energy consumption, protein turnover, and biological responses to heat stress (Bernabucci *et al*., 2014). Dietary measures for minimizing the effects of heat stress are growing more popular, especially the use of antioxidants to prevent oxidative stress and high-protein forages to maintain metabolic needs (Silanikove *et al*., 2009). Dietary supplementations have been successfully used for amelioration of heat stress in poultry (Maddahian et al., 2017; Rafiei-Tari et al., 2018).

Nutritional approaches using bioactive plant substances like tannins and polyphenols have attracted substantial interest for alleviating heat stress by lowering oxidative damage and modifying rumen fermentation (Jayanegara *et al*., 2022). Certain plants include polyphenolic substances called tannins, which have antioxidant qualities and may influence ruminal fermentation to enhance immune system response and nutrient utilization (Patra and Saxena 2010). Among them, fruit and fiber by-products like mango seed kernel (MSK) and ramie have attracted concern due to their sustainability and nutritional potential. The agro-industrial waste product known as mango seed kernel has potential as a partial replacement in bovine diets due to its high energy content and polyphenol content (Riad *et al*., 2025). Mango seed kernels (MSK) are rich source of natural antioxidant compounds due to the presence of polyphenols (mangiferin, hesperidin, vanillin, etc.) and phenolic acid (tannin, gallic acid, caffeic acid, etc.) (Choudhary *et al*., 2023). Ramie (*Boehmeria nivea*), a forage high in fiber, has been observed to improve rumen fermentation and ruminant feed utilization. Ramie may be utilized as superior green grass in animal feed (Tuyen *et al*., 2007). Ramie grass contains phenolic compounds, which include a high antioxidant capacity and promote physiological resilience during heat stress (He *et al*., 2019). Similarly, tannins and polyphenols can modify rumen fermentation and metabolic efficiency, resulting in less metabolic heat generation and better adaptability to high temperatures (Wang *et al*., 2022).

Thus, the purpose of this study was to understand whether these natural compounds help cattle better tolerate heat stress and maintain health, cattle performance, physiological responses, and welfare during periods of heat stress.

Materials and methods

Location

The study was conducted at the Animal Nutrition shed of ICAR-NDRI, National Dairy Research Institute, Karnal, which is situated at an altitude of 250 meters above mean sea level with latitude and longitude positions of 29° 42" N and 79° 54" E, respectively.

Ethical approval

The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of ICAR-NDRI, constituted under Article 13 of the CPCSEA and established by the Government of India (Reg. No. 51-IAEC-24-16, dated 16-03-2024). All ethical guidelines were followed during the course of the experiment.

Selection and management of experimental animals

The study was conducted in three different seasons, such as hot-dry (May, June), hot-humid (mid-July to mid-September), and thermoneutral (October, November). The same animals were used in all three seasons by providing 15 days of adaptation before each season. For this study 16 healthy Sahiwal heifers were selected and randomly divided into two groups: control (n=8) and treatment (n=8). Both groups were fed as per ICAR standards (2013). The control group was fed with a normal farm diet, whereas the treatment group was fed with a modification in farm animal diet that is 50% seasonal fodder replacement with ramie grass and also supplemented with 3% of DMI with mango seed kernel powder. Isonitrogenous and isocaloric diets for both groups were used in the present study for the physio-biochemical changes in Sahiwal cattle.

Table 1. Average Temperature Humidity Index (THI) during different seasons

SEASON	THI morning	THI afternoon	THI average
Hot Dry	78.96±0.53	87.79±0.44	83.35±0.46
Hot Humid	80.09±0.25	83.71±0.35	81.90±0.26
Thermoneutral	66.63±0.76	78.03±0.57	72.29±0.65

Table 2. Effect of Ramie and Mango Seed Kernel Supplementation on Physiological Parameters of Sahiwal Heifer Cattle During Different Seasons

Parameters	Season	Control	Treatment	Overall	p-value		
					Season	Group	Season × Group
Rectal Temperature (°C)	Hot Dry	38.55±0.09	38.11±0.1	38.33^{B}±0.08	0.00	0.00	0.00
	Hot Humid	39.11±0.08	38.35±0.05	38.73^{C}±0.07			
	Thermoneutral	37.8±0.07	37.71±0.09	37.76^{A}±0.06			
	Overall	38.49^{Y}±0.08	38.05^{X}±0.06				
Respiration rate (breath/min)	Hot Dry	25.82±0.38	23.44±0.38	24.63^{B}±0.31	0.00	0.00	0.236
	Hot Humid	27.57±0.27	26.32±0.37	26.94^{C}±0.24			
	Thermoneutral	23.5±0.33	22.07±0.4	22.79^{A}±0.28			
	Overall	25.63^{Y}±0.26	23.94^{X}±0.29				
Pulse rate (Pulse/min)	Hot Dry	68.66±0.55	67.1±0.43	67.88^{B}±0.36	0.00	0.00	0.69801
	Hot Humid	71.75±0.79	69.79±0.23	70.77^{C}±0.43			
	Thermoneutral	66.82±0.34	65.66±0.35	66.24^{A}±0.25			
	Overall	69.08^{Y}±0.4	67.52^{X}±0.27				

Values are expressed as mean ± standard error (SE). Superscripts (A, B, C) denote significant differences within overall seasons ($p < 0.05$). Superscripts (X, Y) denote significant differences between overall groups (control vs. treatment). p-values represent the significance of the effects of season, group (treatment), and their interaction (Season × Group).

Recording of climatic variables

Throughout the experiment, microclimatic data, including dry and wet bulb temperature, was recorded in the morning (9:00 AM) and afternoon (3:00 PM) using a Zeal thermometer [Zeal Masons Pattern Hygrometer P2506 (UK)]. The Temperature Humidity Index (THI) was calculated using the National Research Council (NRC, 1971) formula:

$$THI = 0.72 \times (T_{db} + T_{wb}) + 40.6$$

where Tdb = dry bulb temperature (°C) ,

Twb = wet bulb temperature (°C)

Measurement of physiological responses

In each season, physiological parameters, which were rectal temperature, respiration rate, and pulse rate, were recorded fortnightly from each animal between 09:00h and 10:00h. A digital thermometer was used to measure rectal temperature (°C) by placing it in contact with the rectal mucosa for around two minutes. The respiration rate was measured through observing flank movements. Each inward and outward movement was considered as one respiration (breath/min.). The pulse rate of the animals was determined by monitoring the pulsing of the main coccygeal artery for one minute (pulse/min) at the tail's base.

Blood sampling and serum biochemical assay

Blood samples were taken at fortnightly intervals before feeding in all seasons. Blood samples were collected via jugular venipuncture from each animal, using two sets of sterile vials treated with Ethylene Diamine Tetraacetic Acid (EDTA). Samples were kept in an icebox and brought to the lab for analysis immediately after collection. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at -20°C for further biochemical analysis. Plasma glucose concentration was determined by the glucose oxidase-peroxidase (GOD-POD) end point method. Total plasma proteins were determined using the modified Biuret technique, albumin concentration using the bromocresol green dye-binding method, total cholesterol using the cholesterol oxidase-peroxidase (CHOD-PAP) assay, and triglycerides using the glycerol-3-phosphate oxidase-peroxidase (GPO-PAP) end point assay. All tests were conducted using Autospan kits (Arkray Healthcare Pvt. Ltd.) according to the manufacturer's instructions.

Statistical analysis

The average of four fortnights of both groups in each season was obtained, and a season comparison was made between groups and within seasons. The whole data were analyzed using the computerized software program SPSS Ver. 20.0. Data were analyzed by two-way ANOVA to assess the effects of season, treatment, and their interaction on each parameter. Post hoc comparisons were performed using Tukey's HSD test. Differences were considered significant at $p<0.05$. Data are presented as mean ± standard error (S.E.). A correlation study of various parameters was conducted using the Pearson correlation method.

Results

Meteorological observations

THI was calculated for each season and presented in Table 1. In the two-tailed correlation analysis, THI showed significant positive ($p<0.05$) correlations with rectal temperature, respiration rate, pulse rate, total proteins, and albumin, whereas it showed significant negative ($p<0.05$) correlations with glucose, total cholesterol, and triglycerides.

Physiological Parameters

Mean±S.E. values of ramie and mango seed kernel supplementation on physiological parameters in Sahiwal heifers during different seasons in cattle are presented in Table 2. Rectal temperature, respiration rate, and pulse rate were significantly influenced by both season and group ($p=0.000$ for season and group). Rectal temperature was highest in the hot humid season (control: 39.11±0.08 °C; treatment: 38.35±0.05°C), whereas it was lowest in the thermoneutral season (control: 37.80±0.07 °C; treatment: 37.71±0.09 °C). Overall rectal temperature was significantly higher ($P<0.05$) in the control group (38.49±0.08 °C) than in the treatment group (38.05±0.06 °C). Interaction for rectal temperature was also significant ($P<0.05$). Similar trends were observed for respiration rate and pulse rate. Respiration rate and pulse rate are also influenced significantly by season and group. Overall respiration rate and pulse rate were significantly higher ($P<0.05$) in the control group as compared to the treatment group along all seasons.

Biochemical parameters

Blood biochemical profiles are crucial markers of an animal's physiology, metabolic function, and overall health. Mean±S.E. values of ramie and mango seed kernel supplementation on biochemical profile during different seasons in Sahiwal heifer cattle are presented in Table 3. Plasma glucose concentrations were observed to be significantly lowered ($p<0.05$) during the hot dry and hot humid seasons in both the control and treatment groups, compared to the thermoneutral season. However, the treatment group exhibited significantly higher ($p<0.05$) plasma glucose levels than the control group across all seasons. Plasma total protein concentrations exhibited significant seasonal variations in both the control and treatment groups, whereas the treatment group showed significantly reduced ($p<0.05$) total protein levels compared to the control group over all three seasons. Overall

Table 3. Effect of Ramie and Mango Seed Kernel Supplementation on Blood Biochemical Parameters of Sahiwal heifer cattle during Different Seasons

Parameters	Season	Control	Treatment	Overall	p-Value		
					Season	Group	Season*Group
Glucose (mg/dL)	Hot Dry	47.97±1.16	49.82±0.59	48.9^{B}±0.66	.000	.003	.437
	Hot Humid	44.36±0.6	48.27±1.41	46.31^{A}±0.8			
	Thermoneutral	51.95±0.79	53.5±1.13	52.73^{C}±0.69			
	Overall	48.1^{X}±0.6	50.53^{Y}±0.67				
Total Proteins (g/dL)	Hot Dry	7.05±0.09	6.69±0.16	6.87^{B}±0.09	.000	.000	.871
	Hot Humid	7.45±0.09	6.98±0.13	7.22^{C}±0.08			
	Thermoneutral	6.76±0.14	6.4±0.07	6.58^{A}±0.08			
	Overall	7.09^{Y}±0.07	6.69^{X}±0.07				
Albumin (g/dL)	Hot Dry	4.1±0.11	3.97±0.11	4.04±0.08	.277	.077	.817
	Hot Humid	4.13±0.11	3.88±0.1	4.00±0.07			
	Thermoneutral	3.92±0.14	3.8±0.12	3.86±0.09			
	Overall	4.05±0.07	3.88±0.06				
Globulin (g/dL)	Hot Dry	2.96±0.15	2.72±0.2	2.84±0.12	.006	.076	.997
	Hot Humid	3.32±0.12	3.1±0.13	3.21±0.09			
	Thermoneutral	2.84±0.2	2.6±0.13	2.72±0.12			
	Overall	3.04±0.09	2.81±0.09				
Albumin : Globulin	Hot Dry	1.67±0.21	2.02±0.32	1.85^{B}±0.19	.046	.614	.519
	Hot Humid	1.33±0.08	1.38±0.12	1.35^{A}±0.07			
	Thermoneutral	1.86±0.26	1.72±0.2	1.79^{B}±0.16			
	Overall	1.62±0.11	1.71±0.13				
Total Cholesterol (mg/dL)	Hot Dry	98.79±1	96.43±0.95	97.61^{B}±0.7	.000	.020	.965
	Hot Humid	94.33±0.96	91.63±1.11	92.98^{A}±0.75			
	Thermoneutral	107.76±1.65	105.72±1.53	106.74^{C}±1.12			
	Overall	100.29^{Y}±0.91	97.93^{X}±0.92				
Triglycerides (mg/dL)	Hot Dry	41.76±0.42	39.58±0.45	40.67^{A}±0.34	.000	.000	.319
	Hot Humid	40.57±0.56	39.06±0.45	39.82^{A}±0.37			
	Thermoneutral	45.16±0.45	42.22±0.47	43.69^{B}±0.37			
	Overall	42.5^{Y}±0.34	40.29^{X}±0.3				

Values are expressed as mean ± standard error (SE) Superscripts (A, B, C) denote significant differences within seasons ($p < 0.05$). Superscripts (X, Y) denote significant differences overall between groups (control vs. treatment).p-values represent the significance of the effects of season, group (treatment), and their interaction (Season × Group).

plasma total protein concentration was significantly higher (p<0.05) in the control group (7.09±0.07 g/dl) than in the treatment group (6.69±0.07 g/dl). Albumin and globulin were both highest in hot humid and lowest in thermoneutral, although there were no significant variations in albumin, whereas globulin showed significant variations in all seasons. The A:G ratio was significantly affected by season, with the highest values in the hot dry season, particularly in the treatment group. Total cholesterol concentration was significantly lower (p<0.05) in the treatment group compared with the control group. Total cholesterol and triglycerides were significantly (P<0.05) lower in summer than during the thermoneutral period, with the lowest values observed in the hot-humid season. Triglycerides were also significantly lower (p<0.05) in the treatment group than in the control group.

Discussion

The THI combines relative humidity and ambient temperature; it is a crucial and essential metric that has been effectively used to evaluate heat stress levels and how they affect dairy cow productivity (Macías-Cruz *et al*., 2016). When the THI rises above 72, a setting is often deemed stressful for dairy animals. The THI of summer months tended towards stressful times, according to the climatic parameter data gathered during our investigation. Indian hot-dry and hot-humid seasons are accompanied by protracted periods of high THI, which is linked to various physiological, hematological, biochemical, and associated alterations in animals (Bhan *et al*., 2013).

Rectal temperature increased significantly during the high THI months of summer in Sahiwal cattle, indicating pronounced heat stress and corroborating earlier reports (Gujar *et al.,* 2022). Rectal temperature is the most effective physiological measure for evaluating an animal's discomfort in a hot environment (Silanikove 2000). There was a significant increase in respiration rate as ambient temperature and relative humidity increased throughout the hot dry and hot humid seasons, respectively. Increased respiration is one of the earliest obvious indicators of heat stress in animals (Baena *et al*., 2018). This response is mediated by activation of cutaneous thermoreceptors, which stimulate the hypothalamus and enhance respiratory evaporative heat loss (Haidary and Ahmed, 2004). Besides rectal temperature and respiration rate, pulse rate is a useful predictor of heat stress and is highly associated with circulating corticoid levels (Sejian *et al*., 2010). The pulse rate reported in the present investigation during higher THI months is consistent with Gujar *et al*., 2022 and Das *et al*.,2016. The enhanced pulse rate increases blood flow to the body surface, facilitating heat dissipation (Marai *et al*., 2007). Physiological responses such as rectal temperature, respiration rate, and pulse rate were profoundly lowered in cattle fed diets enriched with tannin in ramie grass and mango seed kernel. Tannins have antioxidant and anti-inflammatory effects that reduce oxidative stress produced by heat stress in ruminants (Jayanegara *et al*., 2022).

Blood glucose offers a quick evaluation of the many types of stress that animals endure and is a useful indicator of nutritional health (Lakhani *et al*., 2018). In conditions of heat stress, baseline insulin levels were elevated, but basal glucose levels showed a tendency to decline (Wheelock *et al*., 2010). The diminished glucose levels seen during the pre-monsoon and monsoon periods, in contrast to the post-monsoon phase, may be attributed to heightened energy requirements for thermoregulation or to decreased dry matter intake due to heat stress (Bhatta *et al*., 2014). This increased plasma glucose level in cattle in the treatment group under hot conditions points to heat stress-related metabolic adaptations in the animals, which might be a result of treatment-induced metabolic adaptation and increased energy availability. The observed increase in serum protein in summer may indicate a physiological attempt to maintain extended plasma volume. The treatment group showed a significant decrease in total proteins, albumin, and globulin, which are similar to previous findings (Zhang *et al*., 2019). Decreased total protein in treatment groups probably indicates increased plasma volume or enhanced protein metabolic efficiency caused by antioxidant supplementation (Siddiqui *et al*., 2021). In accordance with earlier research, consistent albumin concentrations throughout seasons and groups support the use of it as a reliable marker of long-term nutritional and health condition (Kadzere *et al*., 2002). The levels of albumin and globulin in dairy cow blood have been identified as indicators for inflammation (Zhang *et al*., 2019). A seasonal increase in globulin, particularly during the hot humid season, suggests that environmental stresses have activated the immune system. As a protective mechanism against inflammation driven by stress and exposure to pathogens, increased immunoglobulin synthesis has been linked to elevated globulin concentrations under heat stress (Bernabucci *et al*., 2014). The modest decrease in globulin following treatment could be due to tannin's anti-inflammatory qualities, which have been demonstrated to influence ruminant immune responses (Patra and Saxena 2010). The albumin-to-globulin ratio (A:G) acts as an indicator of the balance between immunological and nutritional condition and exhibits a significant seasonal change that supports the physiological adaptations under heat stress. In the treatment group, an elevated A:G ratio indicates better protein metabolism which may indicate the efficacy of tannin present in ramie and mango seed kernel in the alleviation of heat stress impacts. With regard to preserving metabolic and immunological balance, our results highlight the intricate physiological adaptations cattle make to seasonal heat stress and the possible advantages of focused feed supplements. Cholesterol and triglycerides may be significantly lower (p<0.05) in the summer due to animals' reduced voluntary feed intake in warmer climates. The current findings of lower total cholesterol on heat exposure are consistent with the findings of Shehab-El-Deen *et al*., (2010). Plasma cholesterol levels were considerably lower throughout the pre- and monsoon periods compared to the post-monsoon phase (Nikhil *et al*., 2018). Elevated cortisol stimulates cholesterol catabolism to boost gluconeogenesis, giving additional energy to heat-stressed animals. This metabolic modification enables animals to deal with energy demands under heat stress (Nazifi *et al*., 2003). Total cholesterol and triglycerides were considerably lower in the treatment group across all seasons, suggesting that the cattle's health has improved. Ramie grass contains chlorogenic acid, a potent antibacterial, antiviral, antioxidant, and DNA damage inhibitor (Xu *et al*., 2012). Chlorogenic acid improves lipid metabolism and may reduce total cholesterol and triglyceride levels in goats (Wei *et al*., 2019).

Conclusion

Seasonal heat stress disrupts the physiological and metabolic status of cattle. This study demonstrated that tannin-containing ramie and mango seed kernel supplementation alleviated the adverse effects of thermal stress by lowering physiological markers of heat load and regulating energy, protein, and lipid metabolism. These modifications facilitated improved adaptive responses and thereby increased thermotolerance. As a source of natural antioxidants, ramie and mango seed kernel are a promising nutritional strategy to enhance resilience, maintain physiological function, and promote metabolic adaptability, ultimately improving cattle health and productivity under heat stress conditions.

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Conflict of interest

The authors have declared no possible conflicts of interest.

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