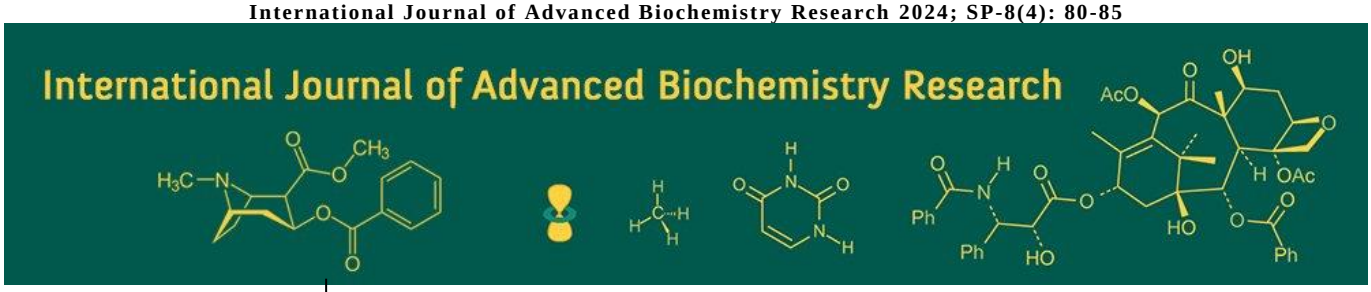

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Ameliorative effect of *Withania somnifera* on haemato-biochemical and hormonal parameters during heat stress in murrah buffalo calves

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Abstract
A study was carried out in order to investigate the effect of daily supplementation with *Withania somnifera* (Ashwagandha) roots in powder form on physiological, haematological, and hormonal indices in buffalo calves during heat stress. Twelve healthy Murrah buffalo calves were divided into control and supplemented groups were taken for the study. In supplementation to basal diet, *Withania somnifera* (25g/daily) was given at morning time from day 1 up to day 45. Throughout the experiment, both the control and supplemented groups had non-significant rectal temperatures at fortnightly intervals. Significant difference ($p<0.05$) was observed in pulse and respiration rate were observed at fortnightly interval in both control and supplemented groups. Blood glucose, protein, and cholesterol concentrations in the supplemented and control groups did not differ significantly ($p>0.05$) throughout the course of the study. Similarly, a non-significant difference ($p>0.05$) were observed in concentration of serum sodium, potassium and chloride in treatment groups as compared to control group. Non-significant difference ($p>0.05$) were observed in various haematological parameters, during the entire experimental period in both control and treatment group except RBC related parameters and platelets concentration. Non-significant difference ($p\geq0.05$) were found in concentration of serum cortisol, T_3, T_4 and TSH were observed during the entire experimental period in both control and supplemented groups. In conclusion, *Withania somnifera* affects thyroid hormone response and cortisol release under summer stress. It lowers T_3, T_4, and cortisol levels. It lowers rectal temperature, increases respiration and pulse rate in animals, and helps them cope with the detrimental impacts of summer stress.

Keywords: buffalo calves, *Withania somnifera*, serum TSH, T_3, T_4 and cortisol

Introduction
Our livestock industry depends on calves for its future, and the development of the nation's dairy industry depends on effective calf management. Successful calf management is essential to the dairy sector. The physiological manifestations of heat stress as a stress response have a substantial impact on the biological rhythm (diurnal and seasonal) of buffalo calves. Due to their darker skin tone and absence of sweat glands, buffalo calves experience these consequences more severely than cow calves do. Buffalo calves under heat stress experience numerous physiological changes, including reduced feed intake, alterations in hormonal and metabolic secretions and elevations in rectal temperature (RT), respiration rate (RR), and oxidative stress (Bombade *et al.*, 2018) [3].

Dairy farmers all around the world have begun providing their animals with herbal feed supplements to increase their health and performance when under heat stress (Jayasena and Jo, 2013) [13]. Alkaloid and flavonoides, which naturally possess antibacterial and antioxidant qualities, make up the majority of herbal feed supplements (Hirasa and Takemasa, 1998) [11]. Ashwagandha (*Withania somnifera*), an antioxidant herbal supplement, has been demonstrated to have antioxidant and immune-boosting properties. The roots are frequently employed as an adaptogen. It provides a refreshing and calming impact. It demonstrates antioxidant activity via the hypothalamic-pituitary-adrenal (HPA) axis regulation of anti-stress activities.

Materials and Methods

The experiment was carried out at the Department of Veterinary Physiology and Biochemistry and the Livestock Farm Complex Adhartal College of Veterinary Science and Animal Husbandry N. D. V. S.U. Jabalpur (M.P.), as well as an organised farm in Jabalpur. Twelve buffalo calves were divided into two groups at random. Group 1 was kept as the control. Group 2 supplied each animal with 25g of *Withania somnifera* root powder daily. The trials were designed in compliance with the institutional animal ethics committee. Rectal temperature (RT) (0F) was measured with a digital thermometer, pulse rate (per minute) was determined by palpating the middle coccygeal artery, and respiration rate (per minute) was determined by counting the movement of the flank at fortnightly intervals throughout the experiment.

Blood collection and hematological analysis

Blood sample ware collected from animal on day 0, 15, 30, 45 from the external jugular vein at 9.30 am. Blood samples were taken in heparin tubes (20 IU heparin/ml of blood) for plasma and non-heparin tubes for serum collection, maintained in an ice bucket, and transported back to the laboratory. In the laboratory, all blood samples were centrifuged at 3000 rpm for 30 minutes to separate the plasma. Whole blood samples were analysed using a blood autoanalyzer for various haematological parameters.

Temperature humidity index (THI)

It was calculated through recording the dry and wet bulb te mperatures in an open area each morning and evening durin g the study period (NRC, 1971).

THI = 0.72 (Tdb + Twb) + 40.6

Where, Tdb = dry bulb temperature Twb = wet bulb temperature

Biochemical parameters

Blood glucose (mg/dl), total protein (g/dl) and total cholesterol (mg/dl) concentrations were determined using diagnostic kits purchased from Erba Diagnostics, Mannheim GmbH, Germany, and the Star 21 automatic biochemistry auto-analyzer. Serum sodium (Na+), potassium (K+), and chloride (Cl-) concentrations will be measured using an electrolyte analyzer, while serum thyroid stimulating hormone (TSH), triiodothyronine (T_3), and thyroxine (T_4) concentrations will be determined using Elisa kits obtained from IVD (*in vitro* diagnostics), manufactured by Rapid Diagnostic Pvt. Ltd. Cortisol concentration was measured using a cortisol Elisa kit obtained from Calbiotech. The LISA plus Enzyme-linked Immunosorbent Assay (ELISA) reader and the Model LISA wash ELISA plate washer were used to measure hormone levels.

Data analysis

The data collected was statistically analysed using one way ANOVA on the R platform (R Team, 2013) with library Agricole one way ANOVA (between groups). Fisher's multiple comparison tests were used to compare means pair-wise, following the usual statistical procedure given by Snedecor and Cochran (1994) [22].

Results and Discussion

The current study intends to investigate variations in biological rhythms of physiological, biochemical and hormonal responses of buffalo calves to tropical climatic conditions in Jabalpur, as well as the effects of Ashwagandha supplements on the biological rhythms of the buffalo calves.

Physiological responses

Rectal temperature (^{0}F)

Rectal temperature is a vital indicator of body temperature that can help determine the severity of heat stress. An rise in rectal temperature of 1 °C or less is sufficient to decrease animal performance. The rectal temperature of buffalo calves is shown in Table 02. The statistical analysis revealed no significant difference (p>0.05) in rectal temperature across the treatment groups. In this study, the G1 group had a greater temperature than the G2 group.

Table 1: Mean Physiological responses of buffalo calves at fortnightly interval

Parameters	Groups	Day 0	Day 15	Day 30	Day 45
Rectal temperature (^{0}F)	G1	101.28±0.36	101.51±0.36	101.76±0.20	101.95±0.32
	G2	101.26±0.34	101.20±0.28	101.18±0.64	101.16±0.37
Pulse rate (per minute)	G1	70.66±1.02	72.00±0.77	71.83^a±0.83	73.0^a±1.30
	G2	72.66±3.60	69.83±1.28	59.83^b±2.44	60.5^b±2.43
Respiration rate(per minute)	G1	26.50±.1.05	32.0^a±0.68	33.7^a±0.88	35.0^a±0.73
	G2	26.33±0.98	28.16^b±1.58	25.30^b±0.61	26.80^b±0.74

Means bearing different superscripts within same column differ significantly (p<0.05)

Pulse rate

The mean pulse rate of buffalo calves has been presented in Table 02. In present study, higher pulse rate were observed in G1 group as compared to G2 group. Adaptogenic effects of Ashwagandha supplementation could have normalised the pulse rate during heat stress and keep the animal in homeostatic state.

Respiration rate (per minute)

The respiration rate is a practical and precise measure of heat stress. The mean respiration rate of buffalo calves is shown in Table 02. Significant differences (p<0.05) were seen among treatment groups on days 15, 30, and 45. The adaptogenic effects of Ashwagandha administration may have normalised breathing and maintained the animal's homeostatic state.

Haematological parameters

The mean haematological parameters of buffalo calves have been presented in Table 03.

Red blood cell (RBC)

On days 0 and 15, there was no significant difference (p>0.05) in the mean concentrations of RBC, haemoglobin, and haematocrit (HCT) values. However, on days 30 and 45, there was a significant difference (p<0.05) between all groups. The current study found a rise in RBC, haemoglobin, and HCT values in buffalo calves in the control group on days 30 and 45. When an animal is subjected to extreme heat stress, it attempts to keep its body temperature stable by evaporative water losses, resulting in haemoconcentration and plasma dehydration. Fagiolo *et al.* (2004) [8] similarly found that milking buffaloes had greater

haemoglobin concentrations in the summer (13.62 g/dl) than in the winter (11.37 g/dl). Similarly, an increase in HCT may be caused by hemoconcentration. The increase could also be attached to the fact that an animal requires more oxygen in any stressful situation, and so haemoglobin concentration may rise. These findings are comparable to those of El-Nouty *et al*. (1990) [7] and Broucek *et al*. (2009) [4].
Contrary to these findings (Srikandakumar and Johnson, 2004) [23] found reduce erythrocytes concentrations in blood during heat stress in buffaloes. Berian *et al*. (2019) [1] also reported lowered Hb concentration and RBC were observed in heat stressed cattle. Young animals, showing higher intensity of changes of hematological parameters indicates that they are more susceptible to heat stress. Therefore, these animals need greater attention towards protection against adverse climatic conditions.

White blood cell (WBC)
The mean WBC concentration showed no significant difference (p>0.05) across all groups for the whole trial period; however, the G2 group had a numerically higher concentration of WBC than the G1 group. The concentration of WBC in the control group decreased as the temperature increased. Bhan *et al*. (2012) [2] also observed that heat stress lowers leucocyte concentrations, while greater WBC were recorded under heat stress (Mazzullo *et al*., 2014) [14].
The increase in WBC with Ashwagandha administration could be attributable to the fact that leukocytes are generally involved in the immune system. So, when a buffalo calves is supplied with Ashwagandha, the immune system is engaged, and WBC levels may rise. Significantly (p<0.05) higher platelets were observed in G2 as compared to G1 group.

Table 2: Mean haematological parameters of buffalo calves at fortnightly interval

Parameters	Groups	Day 0	Day 15	Day 30	Day 45
RBC (milliions /µl)	G1	5.50±0.31	7.13±0.87	8.90^{a}±1.36	9.98^{a}±1.41
	G2	6.38±0.72	6.74±0.35	6.25^{b}±0.74	6.52^{b}±0.75
HGB (g/dl)	G1	8.18±0.56	8.28±0.24	9.23^{a} ±0.96	10.56^{a}±1.06
	G2	9.02±0.76	8.10±0.78	7.56^{b} ±0.78	7.91^{b}±0.87
HCT (%)	G1	22.65±1.50	22.25±0.47	27.11^{a}±4.47	29.00^{a}±3.94
	G2	23.35±2.60	20.76±3.91	19.95^{b}±1.66	22.26^{b}±2.08
WBC (10^3 / µl)	G1	9.69±0.74	9.31±1.04	9.04±0.86	9.03±0.64
	G2	9.04±0.90	9.23±1.01	9.55±1.12	9.70±1.19
Platelets (10^3/µl)	G1	199.12±10.9	184.80^{b}±22.6	201.50^{b}±23.3	214.0^{b}±24.0
	G2	201.66±23.6	288^{a}±22.6	308.83^{a}±15.6	327.83^{a}±5.76

Means bearing different superscripts within same column differ significantly (p<0.05)

Biochemical parameters
Physiologically, blood glucose and total serum cholesterol levels are an adaptation mechanism that can be affected by high ambient temperatures.

Biochemical parameters
The mean serum glucose, total protein, and cholesterol concentrations in buffalo calves are shown in Table 4. The statistical analysis indicated no significant difference (p>0.05) in serum glucose, total protein, and cholesterol levels between the control and supplemented groups. In the current investigation, buffalo calves' mean plasma glucose concentrations were within the physiological range. The rise in serum protein could reflect a natural attempt to maintain a larger plasma volume. However, heat exposure and dehydration during heat stress led in a large increase in ADH levels, which was related with a considerable decrease in urine output and an increase in plasma protein.
Rasooli *et al*. (2004) [16] reported a significant rise in plasma total protein in non-pregnant Holstein heifers during hot summers. H. M. Yousef (1997) [26] found that Egyptian buffalo calves had somewhat greater protein contents throughout the summer months. In contrast to our previous findings, serum protein concentration in buffaloes fell dramatically throughout the summer season (Verma *et al*., 2000) [24]. In the current investigation, the mean serum cholesterol values in buffalo calves are within normal limits.

Table 4: Mean serum biochemical parameters of buffalo calves at fortnightly interval

Parameters	Groups	Day 0	Day 15	Day 30	Day 45
Glucose concentration (mg/dl)	G1	66.50±3.43	65.83±3.12	63.33±3.53	62.33±3.75
	G2	67.70±1.05	68.83±0.91	65.16±3.46	64.16±4.90
Total protein concentration (g/dl)	G1	5.18±0.38	6.30±0.23	6.88±0.37	6.95±0.33
	G2	5.20±0.18	6.15±0.33	6.55±1.18	6.81±0.22
Total cholesterol concentration (mg/dl)	G1	87.30.±6.22	88.31±6.69	85.92±9.00	80.62±4.06
	G2	78.20±7.17	78.83±7.90	101.62±11.0	95.67±9.20

Serum electrolytes concentration
The mean serum sodium, potassium, and chloride concentrations of buffalo calves are shown in Table 5. The mean serum sodium, potassium, and chloride values were not significantly different (p>0.05) between the control and supplemented groups. Chaudhary *et al*. (2015) [6] reported elevated plasma sodium content during hot season in Surti buffaloes and related to dehydration. Singh *et al*. (2001) [20] found that buffalo calves housed in open areas had higher plasma sodium concentrations than those kept in shady areas, both during the summer season. The serum potassium concentration measured during the summer was higher in the supplemented group than in the control group. The serum potassium content during summer with hot environment was lower in the control group, which is similar to the findings of Chaiyabutr *et al*. (1997) [5] and Singh *et al*. (2012) [2]. Acute heat stress causes sodium and potassium ions to move between intracellular and extracellular components, whereas chronic heat stress causes an external and internal electrolyte balance,

predominantly by urinary potassium excretion (Chaiyabutr *et al*., 1997) [5]. The lower concentration of potassium in heat stressed Murrah baffaloes was due to potassium loss through sweating (Singh *et al*., 2012) [2]. Heat stress causes a shift in blood electrolyte balance (Hooda and Singh, 2010) [12]. The chloride concentrations did not differ substantially (p>0.05) between the control and supplemented groups, with the control group having the highest and lowest values, respectively. Yadav *et al*. (2016) [25] discovered no significant difference in serum chloride concentrations between exposed and cooled buffaloes.

Table 5: Mean serum electrolytes concentration of buffalo calves at fortnightly interval

Parameters	Groups	Day 0	Day 15	Day 30	Day 45
Sodium (mEq/L)	G1	136.70±2.43	143.69±2.65	143.41±0.78	148.94±2.72
	G2	138.54±0.88	143.23±1.23	145.18±3.11	146.40±2.42
Potassium (mEq/L)	G1	5.36±0.40	4.69±0.20	4.29±0.22	4.14±0.13
	G2	5.45±0.35	5.39±0.31	5.36±0.40	5.06±0.28
Chloride (mEq/L)	G1	95.40±4.25	100.09±3.32	99.80±3.28	99.86±3.50
	G2	94.54±3.71	98.34±2.28	98.60±3.15	98.66±3.37

Hormonal analysis

Thyroid-stimulating hormone, thyroxine, triiodothyroxine and cortisol are involved in adaptation to heat stress and could be important indicators for assessment of heat stress. These hormonal changes are mediated through HPA axis.

Serum thyroid-stimulating hormone (TSH) concentration: The mean serum thyroid-stimulating hormone (TSH)**,** triiodothyronine (T_3), thyroxine (T_4) and cortisol concentration of buffalo calves has been presented in Table 6. The mean values of above hormones showed non-significant difference (p>0.05) between the control and supplemented groups.

Table 6: Mean serum hormone concentration in buffalo calves at fortnightly interval

Parameters	Groups	Day 0	Day 15	Day 30	Day 45
TSH (µIU/ml)	G1	0.04±0.02	0.05±0.02	0.04±0.01	0.04±0.02
	G2	0.11±0.04	0.13±0.06	0.10±0.04	0.10±0.05
T_3 (ng/ml)	G1	3.44±0.34	3.35±0.54	3.30±0.32	3.26±0.40
	G2	3.17±0.16	3.16±0.14	3.10±0.05	3.02±0.12
T_4 (µg/dl)	G1	3.91±0.39	3.44±0.40	3.05±0.54	2.90±0.22
	G2	3.88±0.27	3.73±0.58	3.11±0.50	2.86±0.40
Cortisol (ng/ml)	G1	22.97±1.32	24.77±12.2	23.10±5.19	22.60±3.57
	G2	22.14±3.85	19.27±4.20	18.70±4.23	16.60±2.26

Serum triiodothyronine (T_3) concentration

Thyroid hormones are the principal regulators of an individual's metabolic rate. In this study, T_3 and T_4 levels did not differ substantially (p>0.05) between the supplemented and control groups of rats. Increased thyroid hormone secretion enhances body metabolism and, as a result, heat generation. As a result, the decrease in T_3 and T_4 in response to temperature stress represents an adaptive process. T_3 and T_4 concentrations showed a non-significant declining trend with increased temperature exposure (Sharma *et al*. 2013) [19].

Serum thyroxine (T_4) concentration

The T_4 values were numerically lower in summer during the entire experimental period. According to Habeeb *et al*. (2011) [9], T_3 and T_4 readings are much lower during the summer season. Thyroid hormone alterations are associated with decreased metabolic rate, feed intake, and development during heat stress. Animals exposed to high heat stress circumstances restrict the synthesis of hormone releasing factors from the hypothalamic centres, resulting in a drop in pituitary hormonal secretion and a decrease in thyroid stimulating hormone, which lowers thyroid hormone release. Furthermore, a reduction in thyroid function in an animal under heat stress is a result of adaptation to its surroundings. Thyroid hormone levels fall in reaction to heat stress, presumably in an attempt to limit metabolic heat output.

Serum cortisol concentration

Higher serum cortisol concentrations were found in the control group compared to the supplemented group, which is consistent with the findings of Haque *et al*. (2012) [10], who reported that serum cortisol concentrations were statistically higher at 40°, 42°, and 45 °C as compared to 22 °C temperature in young Murrah buffaloes exposed to acute heat stress. Group G2, which has a lower serum cortisol content, exhibits adaptogenic and antistress properties of Ashwagandha in buffalo calves under heat stress.

Body weight: The mean body weight of buffalo calves has been presented in Table 7. The mean body weight showed non-significant difference (p>0.05) between all the groups.

Table 7: Average body weight (Kg) in buffalo calves at fortnightly interval

Groups	Day 0	Day 15	Day 30	Day 45
G1	69.50±3.77	75.00±5.43	80.00±5.36	86.00±5.88
G2	70.80±2.30	78.00±2.12	84.80±2.36	93.00±4.88

Meteorological Parameters

The average meteorological parameters during the experimental study have been presented in Table 8.

Table 8: Average meteorological parameters during the experimental study

S. No.	Period	Temperature Maxi mum (Average) (^{0}C)	Temperature Minimum (Average) (^{0}C)	THI (Average) (%)	Solar radiation (Lux)
1	15-30March 2022	38.52±0.32	22.16±0.42	80.95±0.67	15.87±0.47
2	1-15 April 2022	41.24±0.14	24.13±0.54	82.30±0.68	16.83±0.05
3	15-30 April 2022	42.04±0.27	26.04±0.45	82.06±0.55	16.51±0.50
4	1-15 May 2022	41.96±0.26	27.72±0.36	84.13±1.21	15.43±0.29
5	15-30 May 2022	40.31±0.59	26.77±0.66	84.10±0.62	15.42±0.47
6	1-15 June 2022	42.44±0.41	29.54±0.21	85.37±0.68	14.71±0.49
7	15 -30 June 2022	33.52±0.48	24.24±0.43	81.58±0.45	12.89±0.53

Conclusion

In response to the increasing consumer demand for functional foods with probiotics, industries are actively seeking viable strains like Lactobacillus brevis. However, the challenge lies in maintaining probiotic viability during food processing and storage. The study discussed here tackles this challenge by methodically isolating and assessing LAB strains from fruits, paving the way for innovative probiotic beverages. This research signifies a pivotal advancement in crafting reliable functional foods that not only meet consumer expectations but also contribute significantly to promoting health and wellness.

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