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Physiological responses of Attappady black and Malabari goats exposed to individual and multiple stresses

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Abstract
A study was conducted to compare the physiological responses of two native goat breeds of Kerala *viz.* Attappady Black and Malabari goats, when confronted with individual and multiple stresses. A total of 24 animals each, within two years of age, with a body weight of 20-30 kg and almost similar physical parameters were selected and randomly allocated into four treatment groups as heat stress (HS), nutritional stress (NS), combined stress (CS) and Control (C). The NS and C groups were maintained inside the shed and the HS and CS groups were exposed to summer heat by tethering them in an open paddock (11.30 a.m. to 2.30 p.m.) for 28 consecutive days. Feed restriction (40 percent of the control animals) was imposed in the NS and CS groups to impose nutritional stress. Climatological variables (ambient temperature and relative humidity) were monitored hourly during the period. Physiological responses like rectal temperature (RT), pulse rate (PR) and respiration rate (RR) were recorded before and after the stress to assess the variation. Water was provided *ad libitum* to all the groups. Breed difference was established for all the three parameters. Attappady goats had significantly low RT on days 0, 3, 7 and 14. Treatment had significant effect on RT only on days 14 and 28. PR was significantly ($p<0.01$) higher in Attappady goats on all days. Treatment groups of both the breeds differed in their pulse rate on day 7, where the heat exposed groups had higher PR than the control animals. Respiration rate was significantly ($p<0.01$) higher in Attappady goats on days 3 and 7 which later decreased upon chronic stress exposure. Treatment groups of Malabari goats differed in their RR on day 0. No significant effect was observed in Attappady goats.

Keywords: Indigenous goat breeds, multiple stresses, physiological responses

Introduction
Livestock sub-sector has a great significance in the Indian economy as it contributes 28.63 percent of total agriculture and allied sector GVA (Economic Survey, 2020-21). But variation in climatic variables like temperature, humidity and radiations pose potential risk to the growth and production of livestock. IPCC, (2014) [11] predicted an increase of 3.7–4.8 °C in global surface temperature by the year 2100 and this might possibly lead to the extinction of 20–30 percent of livestock (FAO, 2007) [6]. Though small ruminants are considered relatively thermotolerant, extremes of temperatures, either below or above their thermoneutral zone, further increase the energy required for subsistence and survival. Increased atmospheric temperature also leads to energy restriction or nutritional stress (NS) and production loss in livestock. This implies that the impact of heat stress is further aggravated by the simultaneous nutritional stress.

When in stress, animals elicit physical, physiological, hematological, behavioural, biochemical or molecular responses. But the degree of resistance varies with species and breeds due to the differences in these mechanisms. Native breeds are reported to be relatively thermotolerant. Among these responses, physiological responses are considered as the immediate cue to assess the welfare status of animals during heat stress. Understanding these tolerance mechanisms in goats and popularizing those stress resistant breeds have become the need of the hour to ensure global food security. Hence, the present study was envisaged to understand the physiological responses of Attappady Black and Malabari goats when exposed to multiple stresses.

Materials and Methods
A total of 48 non pregnant goats (24 animals/breed) of two years age, with an average body weight of 20-30 kg body weight and almost similar physical parameters were selected as

experimental animals. Animals were also scored on empty stomach as described by Ghosh *et al*. (2019) [7]. After pre-conditioning, animals of both the breeds were further assigned to four groups randomly and subjected to heat stress (HS), nutritional stress (NS) and combined stress (CS) for a period of 28 days from February 23rd to March 23rd, 2022 without any adjustment period. Control group (C) animals were maintained inside the shed and fed with concentrates and roughages as per package of practise recommendations of KVASU (2016) [12]. Drinking water was offered *ad libitum.* The HS groups were exposed to summer heat by tethering them in an open paddock for three hours between 11.30 a.m. and 2.30 p.m. for 28 consecutive days. Feed and water were provided similar to the control group animals. The NS group goats were maintained inside the shed and subjected to restricted feeding (40 percent of feed offered to control group) to induce nutritional stress. Nutritional stress protocol was adopted from published reports of Sejian *et al*. (2017) [18] with modifications. The CS groups were exposed to combined heat stress and nutritional stress.

Ambient temperature and relative humidity were recorded at hourly intervals on all days of the experiment using HOBO data logger (HOBO U 12 Temp/RH/Light/Ext.). Daily average THI during the experimental period of 28 days was calculated using the equation, THI = db°F - (0.55 – 0.55 x RH) x (db°F – 58), where db °F is the dry bulb temperature in Fahrenheit and RH is the relative humidity (RH%)/100 (LPHSI, 1990) [13]. Physiological parameters such as rectal temperature (RT), respiration rate (RR) and pulse rate (PR) were recorded on the day of start (0) and later on days 3, 7, 14 and 28 before and after stress exposure. Respiratory rate was recorded by counting the flank movements for one minute from a distance in order to avoid any disturbance to the animals. Rectal temperature was recorded by inserting a clinical thermometer into the rectum for one minute. Pulse rate was recorded by manual palpation of femoral artery for one minute.

Results and Discussion

1. Climatological parameters

Both exterior and interior climatological parameters *viz.* ambient temperature and relative humidity was recorded hourly during the entire study period and is given in table 1.

The average THI at the time of sampling was calculated and depicted in fig.1. Since the average THI (outside) during the period was above 82, the thermal stress and combined stress groups in the present study could be considered to be in extreme thermal stress (LPHSI, 1990; Marai *et al*., 2001) [13, 14]. But the nutritionally stressed animals and the control animals, housed inside the shed could be considered apparently thermal stress free as the average in-house THI value was around the threshold value 82.

Table 1: Average weather parameters inside and outside the shed during the experiment

	Time of recording	Temperature (°C)	RH (%)
Inside	Morning (8.30 h)	22.32±0.15	80.9±1.72
	Afternoon (2.30 h)	37.35±0.18	24.54±1.64
Outside	Morning (8.30 h)	25.34±0.30	82.52±2.13
	Afternoon (2.30 h)	41.78±0.93	27.78±1.72

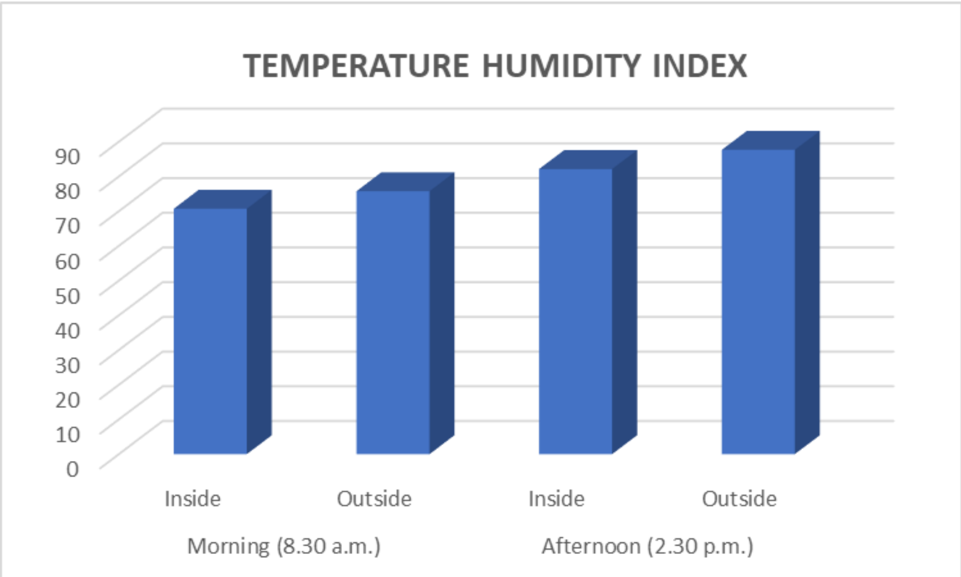

Fig 1: Average temperature humidity index (THI) inside and outside the shed

Physiological parameters

The physiological responses of Attappady Black and Malabari goats subjected to stress are presented in tables 2, 3 and 4.

1. Rectal temperature (RT)

Rectal temperature is the net result of the overall heat gained (metabolic and radiation) and lost from the body. Normal RT of goats ranged between 38.3 to 40 °C (Ayo *et al*., 1998) [4]. Comparing the breeds, the baseline temperature of Malabari goats itself was significantly higher than the Attappady Black goats and this breed difference was reflected in the subsequent days too. But the interaction between treatment and breed was non-significant ($p>0.05$). Physiological responses might vary between breeds. Attappady goats being more adapted to the hot-dry agroclimatic zones might have developed unique mechanisms to maintain their core body temperature lower than Malabari goats. No significant difference ($p>0.05$) was observed in RT of both Attappady Black and Malabari goats when subjected to HS, NS and CS except on days 14 and 28 in the afternoon. This shows the capability of indigenous goats to alter their metabolism in such a way so as to maintain their RT constant when exposed to short-term thermal stress. But by D14, RT varied significantly between the treatment groups with highest RT in

HS group followed by CS group and the lowest in NS and C groups. But on 28E, though HS goats recorded a higher RT, there was no significant difference (*p*>0.05) between NS, CS and C groups in both the breeds.

Generally, RT increase during thermal stress when both sweating and evaporative cooling mechanisms fail to maintain homeothermy, and this increase reflects the onset and magnitude of thermal stress. Also, the RT of all the treatment groups apparently increased (though inconsistent) by the end of 28 days of stress. Thus, from the present findings, it could be inferred that both these breeds are vulnerable to the deleterious effects of chronic heat stress. The higher RT observed in HS goats compared to CS group could be due to the additional metabolic heat produced by HS goats as there was no feed restriction. Shaji *et al*. (2015) [20] also had observed significantly lower RT in Osmanabadi bucks subjected to both heat and nutritional stress as compared to the heat stress alone group. It was attributed to the better adaptive capacity of indigenous goats when exposed to short term multiple stresses.

Table 2: Least square mean±SE of rectal temperature (RT) of Attappady Black (B1) and Malabari (B2) goats subjected to HS, NS, CS, °C, n=6.

Day	Breed (B)	Treatment group (T)				P-value		
		HS	NS	CS	C	B	T	B*T
Day 0 Morning	1	37.91±0.55	38.18±0.28	38.13±0.47	38.09±0.4	<0.01**	0.991^{ns}	0.488^{ns}
	2	39.01±0.2	38.7±0.17	38.74±0.21	38.73±0.24			
Day 0 Evening	1	39.54±0.14	39.36±0.18	39.51±0.16	39.42±0.25	0.609^{ns}	0.075^{ns}	0.386^{ns}
	2	39.69±0.4	39.22±0.21	39.44±0.18	39.67±0.19			
Day 3 Morning	1	38.22±0.18	38.03±0.17	38.06±0.16	38.10±0.46	0.010**	0.447^{ns}	0.435^{ns}
	2	38.36±0.28	38.43±0.38	38.20±0.3	38.71±0.36			
Day 3 Evening	1	39.05±0.34	39.41±0.36	39.3±0.26	38.81±0.23	0.402^{ns}	0.706^{ns}	0.066^{ns}
	2	39.11±0.46	39.00±0.16	38.86±0.41	39.19±0.13			
Day 7 Morning	1	38.04±0.21	38.10±0.34	38.07±0.18	38.16±0.18	<0.01**	0.069^{ns}	0.219
	2	38.15±0.4	38.55±0.26	38.75±0.27	38.78±0.16			
Day 7 Evening	1	38.85±0.33	38.90±0.19	38.75±0.07	39.10±0.19	0.938^{ns}	0.182^{ns}	0.340^{ns}
	2	38.76±0.37	39.13±0.26	38.80±0.18	38.87±0.19			
Day 14 Morning	1	38.46±0.14	38.38±0.3	38.09±0.5	38.75±0.08	0.232^{ns}	0.262^{ns}	0.568^{ns}
	2	38.51±0.21	38.55±0.36	38.58±0.56	38.71±0.39			
Day 14 Evening	1	39.80±0.15^{a}	39.12±0.26^{b}	39.42±0.32^{ab}	39.26±0.19^{b}	0.028*	<0.01**	0.643^{ns}
	2	40.23±0.24^{a}	39.48±0.54^{b}	39.87±0.55^{ab}	39.27±0.33^{b}			
Day 28 Morning	1	38.72±0.22	38.77±0.23	38.84±0.33	39.01±0.22	0.534^{ns}	0.072^{ns}	0.412^{ns}
	2	38.57±0.2	38.97±0.37	38.57±0.2	38.98±0.21			
Day 28 Evening	1	39.71±0.33^{a}	39.41±0.24^{b}	39.40±0.32^{b}	39.40±0.22^{b}	0.161^{ns}	<0.01**	0.150^{ns}
	2	40.12±0.27^{a}	39.46±0.21^{b}	39.71±0.22^{b}	39.21±0.17^{b}			

2. Pulse rate (PR)

Pulse rate of an animal primarily reflects the homeostasis of circulation along with their general metabolic status. Under normal health and environmental conditions, the HR of small ruminants ranges from 70-90 beats/min. According to Gupta *et al.* (2013) [9], the most noticeable effect of heat stress on heart and the associated vessels are evident from increased PR in goats during summer. This was evident in both Attappady Black and Malabari goats on D3M and D7E, where HS and CS groups had a higher PR than the C group. But the PR of CS group was lower than the HS alone group on both these days. Increased pulse rate is an adaptive response in HS goats to dissipate excess heat by increasing the peripheral circulation to facilitate evaporative cooling (Rashid *et al*., 2013) [17]. But contradictory to the expected results, PR was low in the CS group animals. Al-Haidary, (2004) [3] had reported that when animals were extremely stressed, they either reduce feed intake or decrease their metabolic activities. Since the CS group was subjected to restricted feeding, the reduced metabolic activities might have in turn resulted in reduced HR in CS group compared to C group (Barkai *et al*., 2002) [5]. No difference was noticed between the treatment groups on other days. Variation in PR between the goat breeds was significant (*p*<0.05) on almost all days where, Attappady Black goats recorded significantly higher PR compared to Malabari goats. Treatment-breed interaction had no significant effect on PR of goats except on day 28. Popoola *et al.* (2014) [16] had established a direct correlation between the pulse rate and the general metabolic status of goats. The non-significant change in PR of Attappady Black and Malabari goats compared to their control animals also establishes their higher adaptive capacity to the heat stress challenges by maintaining their metabolic and circulatory status even during intense stress.

3. Respiratory rate (RR)

Under thermoneutral conditions, respiration helps in evaporative cooling through the respiratory tract to maintain the thermal balance in animals. Normal RR in goats ranges from 15 to 30 breaths/min under thermoneutral conditions (Abdisa, 2017) [1]. RR could be considered as an early warning signal during thermal stress as it always precedes the other cardinal physiological changes. In Attappady Black breed, the treatment had no significant effect on RR of goats. But in Malabari goats, the treatment groups differed significantly in RR on the day of exposure (day 0 afternoon) where, HS group (56.67±2.35) recorded the highest RR followed by CS (53.33±2.17) and NS (34.67±4.22) when compared to C (45.00±5.11). Sejian *et al.* (2010) [19], also reported higher RR in HS Malpura ewes when compared to the CS group. But no difference was observed on any other days of treatment. Elevated respiratory rate in HS and CS groups could be either due to the increased evaporative cooling mechanism to restore thermal balance or an adaptive mechanism to meet the oxygen demand of the vital organs when suddenly exposed to stress (Habibu *et al.*, 2016) [10]. Both the breeds significantly (*p*<0.05) differed in their respiratory rate on all days except D0. But the pattern was inconsistent since Attappady Black goats had significantly (*p*<0.01) high RR on D3 and D7, whereas, Malabari goats recorded higher RR on D14 and D28. It could be inferred that Attappady goats adapted to the thermal stress provided within 14 days, but Malabari goats, though they resisted the short-term stress succumbed upon long-term stress exposure. Also, Attappady goats could maintain their RR lower than Malabari goats during morning hours. Breeds with higher adaptive capacity when exposed to extreme stress during day, alter their metabolism in such a way that they remain cool during early the morning hours (Al-Haidary, 2004; Marai *et al*., 2007) [3, 15]. According to Aleena *et al*. (2018) [2], RR could be considered as a reliable indicator of the thermotolerance of indigenous goat breeds. This gradual reduction in the RR of Attappady goats over treatment period could serve to indicate their superior adaptability to cope with hotter environments as they have been already primed in severe tropical climate at their place of origin.

Table 3: Least square mean±SE of PR of Attappady Black (B1) and Malabari (B2) goats subjected to HS, NS and CS, beats/ min, n=6.

Time	**Breed (B)**	**Treatment group (T)**				**P-value**		
		HS	**NS**	**CS**	**C**	**B**	**T**	**B*T**
Day 0 Morning	1	83.83±6.52	67.33±4.06	76±5.75	84.33±3.03	0.220^{ns}	0.359^{ns}	0.345^{ns}
	2	70.00±4.93	69.67±7.87	78.00±4.7	70.67±5.23			
Day 0 Evening	1	101.00±9.23	89.5±6.96	114.83±4.87	105.00±5.24	<0.01**	0.083^{ns}	0.408^{ns}
	2	76.50±2.70	80.33±5.23	86.00±3.22	81.33±8.16			
Day 3 Morning	1	97.67±8.80^{a}	65.67±6.16^{b}	73.67±5.90^{ab}	87.33±4.55^{a}	0.223^{ns}	0.029*	0.161^{ns}
	2	75.00±5.46^{a}	72.00±3.72^{b}	73.33±7.06^{ab}	71.17±8.73^{a}			
Day 3 Evening	1	93.17±6.54	87.67±8.40	92.33±4.80	107.33±7.19	0.008**	0.185^{ns}	0.455^{ns}
	2	76.00±2.48	84.67±7.76	85.00±4.81	87.00±4.52			
Day 7 Morning	1	76.00±5.96	77.33±5.72	76.17±4.87	81.67±4.08	0.003**	0.717^{ns}	0.192^{ns}
	2	71.00±4.78	64.67±5.10	68.67±11.47	52.17±2.26			
Day 7 Evening	1	102.67±3.21^{a}	84.83±5.74^{c}	98.5±1.67^{ab}	85.67±4.27^{bc}	<0.01**	0.001**	0.076^{ns}
	2	77.00±3.79^{a}	79.00±4.73^{a}	77.00±8.39^{a}	56.00±2.37^{b}			
Day 14 Morning	1	72.83±5.48	66.33±6.50	59.33±5.13	78.67±5.97	0.953^{ns}	0.127^{ns}	0.284^{ns}
	2	73.83±4.25	68.33±4.46	68.00±3.61	68.83±3.92			
Day 14 Evening	1	86.5±4.03	86.5±4.36	82.5±5.56	89.33±4.06	0.01**	0.256^{ns}	0.618^{ns}
	2	79.00±5.88	68.33±3.56	73.00±4.02	80.33±2.99			
Day 28 Morning	1	71.00±3.79	75.00±2.18	65.67±3.32	78.67±2.51	0.020*	0.286^{ns}	0.051^{ns}
	2	66.67±2.81	69.00±2.91	69.33±2.46	65.67±2.75			
Day 28 Evening	1	85.33±3.00^{A}	86.00±2.42^{A}	77.00±4.02^{B}	88.17±3.29^{A}	0.722^{ns}	0.196^{ns}	0.004**
	2	94.67±5.51^{aA}	80.67±5.72^{bA}	94.67±8.60^{aA}	71.33±1.69^{bB}			

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant
Means having different small letters as superscripts differ significantly within a row
Means having different capital letters as superscripts differ significantly within a column for each time

Table 4: Least square mean±SE of RR of Attappady Black (B1) and Malabari (B2) goats subjected to HS, NS and CS, breaths/min, n=6.

Time	Breed (B)	Treatment group (T)				P-value		
		HS	NS	CS	C	B	T	B*T
Day 0 Morning	1	28.67±3.78	26.00±2.88	28.50±3.54	35.67±2.09	0.172^{ns}	0.210^{ns}	0.893^{ns}
	2	30.67±4.81	29.5±3.79	38.33±8.11	41.33±9.22			
Day 0 Evening	1^{ns}	52.83±6.73	49.67±4.45	46.67±3.53	44.5±1.20	0.731^{ns}	0.018*	0.052^{ns}
	2	56.67±2.35^{a}	34.67±4.22^{c}	53.33±2.17^{ab}	45.00±5.11^{bc}			
Day 3 Morning	1	48.33±2.33	46.67±2.86	42.67±3.37	47.67±4.08	0.005**	0.444^{ns}	0.964^{ns}
	2	40.67±4.31	40.00±4.62	33.83±2.07	37.00±6.61			
Day 3 Evening	1	60.17±4.05	59.33±4.89	53.83±3.53	49.00±3.79	0.564^{ns}	0.529^{ns}	0.184^{ns}
	2	50.00±6.83	47.67±7.80	49.33±0.99	47.33±3.68			
Day 7 Morning	1	39.67±1.50	36.00±1.79	40.67±1.69	44.33±2.09	0.073^{ns}	0.070^{ns}	0.170^{ns}
	2	41.33±3.53	31.17±2.07	39.67±5.67	33.67±1.89			
Day 7 Evening	1	45.33±3.37	50.67±4.37	48.33±1.09	52.00±3.72	0.002**	0.976^{ns}	0.170^{ns}
	2	44.33±3.52	38.67±3.04	43.33±4.78	36.67±2.51			
Day 14 Morning	1	33.00±2.85^{B}	28.67±4.02^{B}	32.00±2.58^{B}	28.83±3.19^{B}	0.007**	0.751^{ns}	0.752^{ns}
	2	41.00±4.73^{A}	36.17±6.13^{A}	37.83±5.75^{A}	43.83±5.39^{A}			
Day 14 Evening	1	58.33±6.31	56.33±5.07	59.67±6.5	62.00±3.9	0.111^{ns}	0.948^{ns}	0.660^{ns}
	2	57.5±5.56	53.67±5.81	51.67±5.04	49.00±3.53			
Day 28 Morning	1	39.33±3.17	40.67±2.35	38.67±2.17	40.67±0.67	0.317^{ns}	0.878^{ns}	0.993^{ns}
	2	41.67±5.07	45.17±4.37	41.00±5.16	44.67±8.74			
Day 28 Evening	1	52.67±2.81^{B}	48.67±1.91^{B}	46.67±3.25^{B}	49±1.34^{B}	<0.01**	0.188^{ns}	0.169^{ns}
	2	73.00±10.77^{A}	64.33±6.96^{A}	78.33±6.05^{A}	55.67±4.45^{A}			

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant
Means having different small letters as superscripts differ significantly within a row
Means having different capital letters as superscripts differ significantly within a column for each time

Conclusions

Thermal, nutritional and combined stresses altered the physiological parameters of Attappady Black and Malabari goats. Significant effect of breed was also observed in all the parameters measured. The responses highlight the capacity of Attappady goats to adapt to long term stress by evaporative cooling mechanisms as evidenced by their lower rectal temperature, higher pulse rate and the gradual reduction in respiratory rate over time compared to.

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