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Effect of Seasonal Stress on Growth Performance, Blood Hemato-biochemical, Antioxidant, Thyroid Hormones and HSP 70 Gene Expression Profile of Beetal Does and Goat Kids

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted on adult beetal does and growing kids to analyze the effect of seasonal change on various physiological and molecular parameters. Blood samples was collected on 0, 15, 30, and 45th day during summer and winter season. The Average THI was found higher (83.08±0.35) during the summer season, while relative humidity remained high (63.77±2.32) during the winter season. As per ANOVA, values of rectal temperature, respiration rate, heart rate, packed cell volume, hemoglobin, total erythrocyte, and leukocyte count, lymphocyte, eosinophil, total bilirubin, creatinine, blood urea nitrogen, serum ALT, AST, ALP enzyme levels, erythrocytic lipid peroxidation, reduced glutathione, total antioxidant capacity (FRAP value) were found significantly higher, while the values of average daily gain, neutrophil %, glucose, total protein, albumin, globulin, total cholesterol, triglyceride, erythrocytic superoxide dismutase levels were found significantly lower during summer season compared to the winter season. Levels of all tested antioxidants parameters were found significantly lower in goat kids compared to adult does. HSP 70 gene expression levels were found significantly higher, while mitochondrial Cyt-B and COX-I gene expression levels were found significantly lower during the summer season relative to winter season in beetal goats. Overall effect of the summer season on HSP 70, CYTB, and COX-I gene expression relative to winter season was found higher in goat kids compared to adult does. Thus goat kids were found to be more susceptible to heat stress than an adult does. Overall study indicated that adaptability of goats to seasonal stress is governed by altered physiological state of goats in different seasons.

Keywords: Seasonal stress; beetal goats; antioxidant; gene expression; HSP; does; kids.

1. INTRODUCTION

Seasonal stress is a major abiotic stress factor affecting the sustainability of goat production. Seasonal stress causes direct and indirect losses to the livestock owners in terms of reduced growth, milk production, and reproductive performance of livestock [1]. High environmental temperature is the major concern in tropical and arid areas that challenges the animal's ability to maintain energy, thermal, water, and physiological balance. During cold stress, goats showed lower milk production and more body fat mobilization without affecting the insulin levels [2]. The temperature-humidity index (THI) is used as a predictor of seasonal stress and used to correlate the production losses of an animal exposed to a hot and humid environment [3]. High THI, influenced the growth performance as well as the physiological responses of animals [4]. THI value above 70 is considered as heat stress for a small ruminant.

Animals develop various adaptive mechanisms to cope with the harsh environment, which causes stress in them [5]. Adaptive changes to seasonal stress include physiological, hormonal, hematological, biochemical & molecular responses [6]. The most significant behavioral & physiological changes observed during exposure of animals to heat stress leads to dramatic changes, including a decrease in feed intake and utilization efficiency, peripheral vasodilation for dissipating heat, physiological disturbances in water, protein, energy and mineral balances, enzymatic reactions, hormonal release, and blood metabolites levels, reduction in fecal and urinary water losses and an increase in sweating, respiration and heart rates [7]. Heat shock proteins (HSPs) are considered as markers of thermotolerance of animals. HSP70 is known to be a highly inducible chaperon and has a cytoprotective role by stabilizing the native conformation of proteins during thermal stress. HSP90α form is also having inducible expression thus exerts a relevant role in cell homeostatic responses to stressful conditions [8]. Mitochondrial functional status is found to be an important marker in determining adaptive cellular response to heat stress. Mitochondrial DNA is highly sensitive to oxidative stress [9,10]. Mitochondria are the main source as well as the target of free radicals which are extensively produced under heat stress [11]. In the present work, an extensive study has been conducted to assess and correlate the effect of seasonal stress with various physiological, hematological, biochemical, antioxidant, hormonal, and molecular parameters of adult beetal does and goat kids.

2. MATERIALS AND METHODS

The study was conducted for 45 days during the winter (Dec-Jan)) and summer (May-June) season, on sixteen adult beetal does, and on

sixteen goat kids (3 to 5 months age) separately. Blood and serum samples were collected from two groups on 0, 15th, 30th, and 45th day of the study period. For progesterone estimation, blood samples were collected from does during the study period on 0, 10 and 20th day of the estrous cycle (day 0 being the day of estrous). Plasma was separated for hormonal and total antioxidant capacity estimation. RBC hemolysate was prepared as per [12] for antioxidant parameter testing. Environmental parameters viz temperature, relative humidity was recorded THI was calculated as per Tucker et al. [13]. Rectal temperature (°C) by a digital thermometer, respiration rate (breaths/Min) by flank method, and heart rate (Beats/Min) by placing a stethoscope over the heart between left 4th to 5th intercostal space was recorded fortnightly as a physiological indicator of heat stress. In growth parameters, body weight was recorded at fortnightly interval. Average daily gain (g/day) was calculated from body weight measurements. Hematological parameters testing viz PCV by microhematocrit method, Hemoglobin by sahli's hemoglobinometer, total erythrocyte count (TEC) & total leukocyte count (TLC) by hemocytometer, differential leukocyte count (DLC) by leishman's staining was carried out from collected blood samples. Metabolites viz glucose, total proteins, albumin, globulin, total cholesterol, triglyceride, creatinine, bilirubin, blood urea nitrogen (BUN), and enzymes ALT, AST, ALP, GGT were estimated in serum samples by a semi-automated analyzer. Erythrocyte lipid peroxidation level, reduced glutathione, superoxide dismutase were estimated in RBC hemolysate. Total antioxidant capacity (FRAP value) was measured in plasma samples. Hemoglobin in hemolysate was determined according to the method of Drabkin and Austin [14]. Erythrocytic lipid peroxidation levels were determined by thiobarbituric acid reactive substance (TBARS) by MDA assay as per Placer et al. [15]. GSH was assayed using the method of Ellman [16]. Erythrocytic superoxide dismutase activity (SOD) was determined as per Nishikimi et al. [17]. SOD activity was calculated using a correlation factor as per Zhang et al. [18]. Plasma total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay as per Benzie and Szeto [19]. T3 and Thyroxine (T4) hormones were estimated in 0 and 30th day plasma samples with commercially available ELISA kit (Calbiotech). The sensitivity of T3 and T4 assay kit was 0.066 ng/ml and 3.20 ng/ml, respectively. Quantitative estimation of plasma progesterone was done by DIAsource progesterone C-ELISA kit. The sensitivity of the assay kit was 0.057 ng/ml. Values of all the tested parameters were expressed as mean± SEM. Statistical analyses were performed using SPSS version 20.0 (IBM Corp., 2013). Variation between two seasons on various tested parameters was compared by repeated measures ANOVA.

Gene expression studies for the heat stress-related gene HSP 70, HSP 90, and mitochondrial functional marker genes Cytochrome B and Cytochrome oxidase -I was carried out 0 and 30th day on blood samples in does and kids during winter & summer Seasons. Gene-specific primers were designed using online genescript real-time PCR primer design software available online, and the specificity was checked using NCBI BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The details of the primer are described in Table 1.

Table 1. Primer sequence of target genes

Sr. No	Gene	F/R	Sequence	Product size	Accession No.
1)	HSP 70 (HSP70.3)	Forward	TCATCGGAGATGCAGCCAAGAA	210	JN833720.1
		Reverse	AGATCTCCTCGGGGAAGAAGGT		
2)	HSP 90 (HSP90AA1)	Forward	TCTGATGACAAGCCCGAGAT	145	AF548366.1
		Reverse	TGGTCCAGATAGGCTTCGTC		
3)	Cyt B	Forward	ATATTCCGCCCAATCAGCCA	169	MK341077.1
		Reverse	TGGTGCTAGCTGCTGGTATT		
4)	Cox I	Forward	GGCACCCTCTACCTTCTGTT	148	MK341077.1
		Reverse	TTACGAATGCGTGTGCAGTT		
5)	GADPH	Forward	GCAAGTTCCACGGCACAG	118	AJ431207
		Reverse	TCAGCACCAGCATCACCC		

Table 2. Real-time qPCR reaction

Segment	Remark	Thermal Profile	Time	No of cycles
1	Hot start PCR	95^{0}C	15 min	1
2	Denaturation	95^{0}C	10 sec	35
	Annealing	58^{0}C	30 Sec	
	Extension	72^{0}C	30 sec	
3	Dissociation curve analysis	95^{0}C	1 min	1
		65^{0}C	30 sec	
		65^{0}C -95^{0}C	2 degree per min	
		95^{0}C	30 sec	

Total RNA from the blood sample using trizol method. The total RNA concentration was determined, and purity was checked using the nanodrop spectrophotometer. The constant amount (6 µg) of isolated total RNA was reverse transcribed using cDNA Synthesis kit. Gene expression was studied using SYBR green chemistry. The GAPDH gene was used as an internal control. No template control (NTC) was put for gene quantification for checking the contamination in the reaction component other than the cDNA. Melt curve analysis was done to rule out non-specific amplification. The real-time qPCR reaction conditions were Table 2.

Cycle threshold (Ct) values and amplification plots for all were calculated once the run was completed. Mean ΔCt of each target gene with reference to internal control was calculated. The relative gene expression was calculated for the summer season relative to the winter season in does and goat kids and was expressed as fold (2^-ΔΔCt). Log2 fold value was calculated for symmetrical analysis. Significant differences in mRNA expressions of the examined factors were assessed by nonparametric Wilcoxon- Mann-Whitney U test with critical alpha level 0.05 was used for statistical significance using SPSS statistical tool.

3. RESULTS AND DISCUSSION

3.1 Environmental Parameters

Average Temperature (^{0}C), relative humidity % & THI recorded during the study period was 12.91±0.26, 78.66±1.28, 55.60±0.43 during the winter season and 37.10±0.68, 29.83± 1.39, 83.08±0.35 during the summer season, respectively. A climatic environment having an air temperature in the range of 13– 27 °C, relative humidity of 60–70 %, THI of less than 65% [20] is considered as a comfort zone. During summer and winter, animals were found exposed to an ambient temperature or humidity beyond their comfort zone, which indicates that they were experiencing seasonal stress in both seasons while pronounced thermal stress exhibited by animals during the summer season.

3.2 Growth Parameters

The growth rate is an age and developmental stage-dependent phenomenon. Also, the growth rate calculation depends on the initial body weight of the animal. Considering this, univariate ANCOVA was conducted on control group body weight data using the initial body weight of does and kids as a covariate. The results of the Univariate analysis have been represented in Table 3.

The result shown significantly lower final body weight (at 45 day) of does ($P<0.05$) and kids ($P<0.01$) in the summer season than in the winter season when the initial body weight difference of the goats was controlled.

Overall Average daily gain (ADG) recorded in adult does and goat kids during the winter season were 53.89 ±6.06 and 76.67±1.19 g/day respectively, while overall ADG during the summer season were 43.61 ±2.44and 54.17±1.62 day respectively. The average daily gain was considerably lower in the summer season compared to the winter season in goat kids ($P<0.001$), while the difference was non-significant in does. Indicating season stress adversely affects growth rate in beetal goat kids. While body condition scores were non-significant between does and kids. These results of growth parameters were similar to the earlier finding by Attia [21] in heat-stressed goats who reported the lower growth rate of goats during the summer season.

Table 3. effect of seasonal stress on body weight of does and goat kids

Goat	Covariate (0 day)	Winter (45th day)	Summer (45th day)	P values
Does	42.44	44.99	44.28	0.038
Kids	11.13	14.64	13.52	0.000

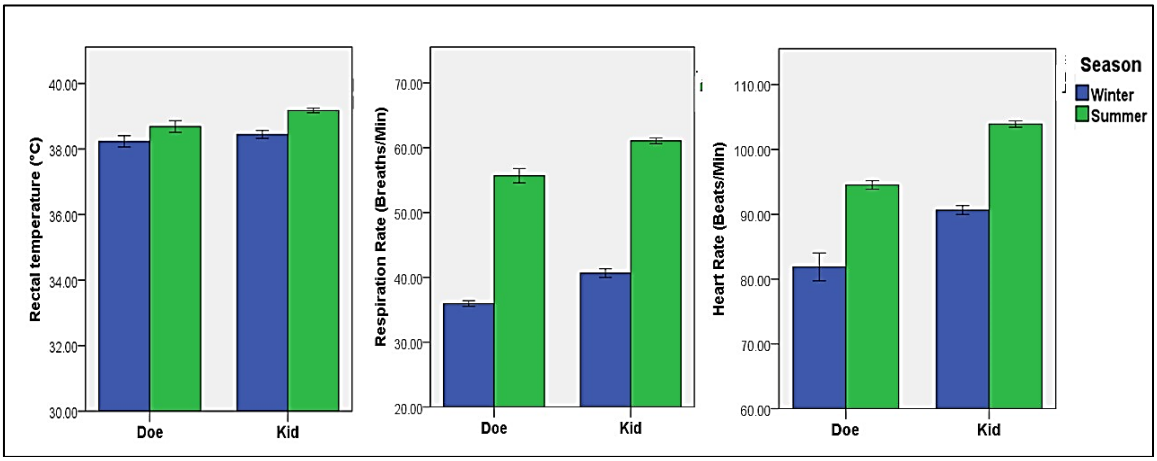

Fig. 1. Results of physiological parameters recorded in does and goat kids during winter and summer season

3.3 Effect of Season on Physiological Parameters

Results of physiological parameters recorded in goats during the study period have been graphically represented in Fig. 1. Rectal temperature, respiration rate and heart rate were significantly increased during the summer season compared to the winter season ($p<0.01$) in goats. The increased rectal temperature during the summer season indicates that animals experienced heat stress. Animal tries to maintain body temperature in thermal equilibrium by dispersion of excess heat from their bodies during heat stress, while animals try to conserve the metabolic heat during the lower environmental temperature.

The result of the respiration rate in the present study is in agreement with Phulia et al. [22] in Sirohi goats and Rahman and Nagpoul [23] in crossbred goats. Respiration rate increased during summer to increase heat loss through respiratory evaporation. While the lower respiration rate during winter can reduce the amount of heat going out with exhalation

Increase in heart rate during summer was mainly due to direct temperature effect on goats which causes an increase in blood flow from the core to periphery to maintain body temperature and as a result of this more heat is lost by sensible (loss by conduction, convection and radiation) and insensible (loss by diffusion water from the skin) means [6].

3.4 Hematological Parameters

The hematological parameters during winter and summer seasons in adult does and goat kids have been presented graphically in Fig. 2. Overall values of PCV (%), hemoglobin (g/dl), total erythrocyte count (x 10^6 cells/µl), total leukocyte count (x10^3 cells/µl), neutrophil % and lymphocyte % in beetal goats found during summer season were 31.53±0.31,10.15±0.21, 9.4±0.17,11.45±0.21,40.37±0.33 57.59±0.67 respectively and during winter season were 30.64±0.27, 9.48±0.22, 8.87±0.15, 10.90±0.19, 42.54±0.34, 55.78±0.55 respectively. Significantly higher (<0.05) levels PCV%, Hb, TEC, TLC, & lymphocyte % and significantly lower neutrophil % (<0.01) were found in summer compared to winter.

These hematological results were similar to the findings by Kour et al. [24], who reported significantly higher PCV %, Hb, ESR in the summer season compared to winter. When exposed to heat stress, goats showed an increased amount of red blood cells, packed cell

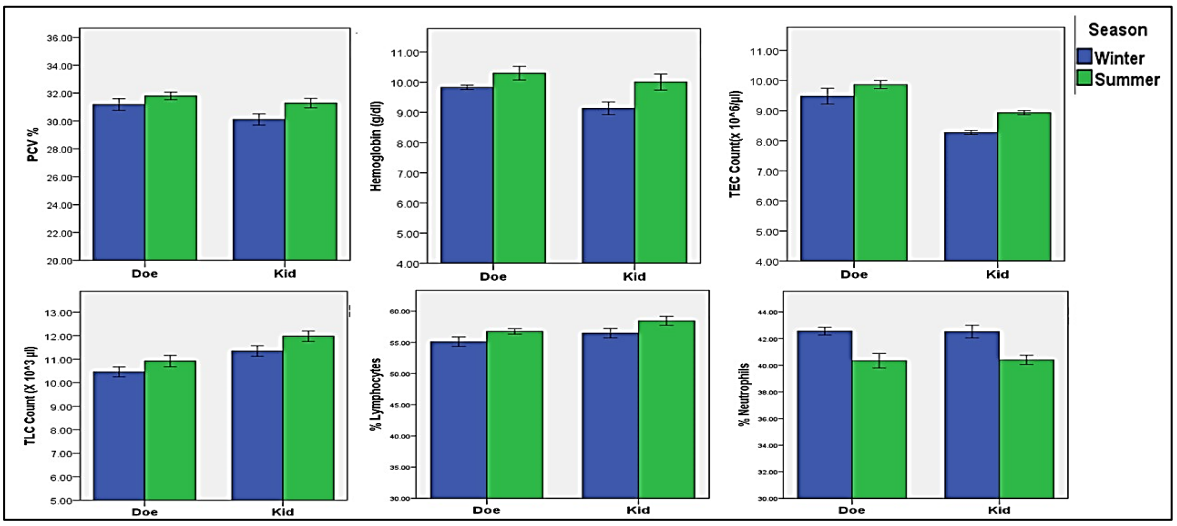

Fig. 2. Results of hematological parameters of does and goat kids during winter and summer season

volume, hemoglobin, white blood cells, neutrophil, eosinophil, lymphocyte, and monocyte Alam et al. [25] Increase in PCV, Hb & TEC during the summer season might be due to hemoconcentration due to dehydration during the summer season.

3.5 Serum Biochemical Parameters

Results of the tested serum biochemical profile has been tabulated in the Table 4. Overall mean values of all the tested biochemical parameters except AST were found significantly different (P<0.01) between winter & summer seasons.

Significantly lower values of glucose, total protein & cholesterol (P<0.01) along with considerably higher values of Creatinine and ALT (P<0.01) levels were found during the summer season compared to the winter season in both does and kids. Total bilirubin (P<0.01) and GGT(P<0.05) were found significantly higher (P<0.01) only in does during the summer season compared to the winter season. Significant decrease in albumin/globulin ratio and a significant increase in BUN level (P<0.05) was observed in goat kids only during the summer season compared to the winter season. Albumin, Globulin, Triglyceride, and ALP were found to significantly lower in the summer season (P<0.01) in the case of does only.

The decreased glucose level during summer is correlated closely to the decreased basal metabolism that will lead to reduced feed intake by the animals, decrease in gluconeogenesis and also be due to negative energy balance by an increase in blood glucose utilization to produce more energy for high respiratory and muscular activity while during winter season animals consume more feed due to increase in basal metabolic rate that fulfills the requirement of glucose in the body and also is associated with the inhibition of insulin secretion during low ambient temperature [26]. The lower blood glucose during hot condition might also be due to variation in thyroid activity [27].

A significant decrease in total protein concentration in goats has been reported during heat stress (Alam, 2011). Total plasma protein, albumin, and globulin levels decrease in goats subjected to heat stress. Decreased plasma protein synthesis by the liver may be due to negative effect of heat stress on metabolic rate. A decrease in cholesterol and triglyceride may be attributable to a decrease in their biosynthesis during heat stress.

An increase in BUN during heat stress may be due to an increase in protein catabolism. Creatinine concentration in the present study was higher during summer, which indicates increased muscle metabolism during hot conditions. Renal perfusion and glomerular filtration rate affects rate of excretion of creatinine. Reduction in blood flow to the kidney and decreased urinary output during heat stress may increase creatinine concentration. Cortisol-mediated creatinine metabolism during heat stress may cause higher creatinine concentration (Kataria, 2008).

Table 4. Seasonal variation in serum biochemical profile of Beetal does and goat kids

Sr. No	Parameter		Winter	Summer	Sr. No	Parameter		Winter	Summer
1)	Glucose	Does	57.84 ± 0.63	54.92 ± 0.28 **	8)	Total	Does	0.65 ± 0.01	0.72 ± 0.03
	(mg/dl)	Kids	62.17 ± 0.21	58.66 ± 0.96 **		Bilirubin	Kids	0.75 ± 0.03	0.84 ± 0.04
		Overall	60.00± 0.46	56.79± 0.71**		(mg/dl)	Overall	0.70± 0.03	0.77± 0.03*
2)	Total Protein	Does	6.45 ± 0.07	6.07 ± 0.05**	9)	Creatinine	Does	0.77 ± 0.02	0.95 ± 0.01**
	(g/dl)	Kids	6.48 ± 0.08	6.17 ± 0.08*		(mg/dl)	Kids	0.61 ± 0.02	0.71 ± 0.01**
		Overall	6.46± 0.08	6.12± 0.07 **			Overall	0.69± 0.02	0.82± 0.01**
3)	Albumin	Does	2.77 ± 0.05	2.47 ± 0.02**	10)	BUN	Does	20.29 ± 0.41	21.37 ± 0.49
	(g/dl)	Kids	2.49 ± 0.05	2.40 ± 0.05		(mg/dl)	Kids	17.65 ± 0.42	19.08 ± 0.48*
		Overall	2.62± 0.05	2.43± 0.04**			Overall	18.99± 0.38	20.22± 0.44
4)	Globulin	Does	3.89 ± 0.04	3.59 ± 0.05**	11)	ALT	Does	15.34 ± 0.25	19.11 ±0.29**
	(g/dl)	Kids	3.72 ± 0.05	3.77 ± 0.05		(U/L)	Kids	18.33 ± 0.81	23.62± 0.91**
		Overall	3.80± 0.05	3.68± 0.05*			Overall	16.83± 0.79	21.36± 0.84**
5)	A/G ratio	Does	0.71 ± 0.01	0.64 ± 0.02**	12)	AST	Does	56.56 ± 3.06	61.10 ± 3.02
		Kids	0.74 ± 0.02	0.69 ± 0.01**		(U/L)	Kids	65.31 ± 2.85	69.41 ± 3.06
		Overall	0.72± 0.02	0.66± 0.02**			Overall	60.93± 2.99	65.25± 2.85
6)	Total	Does	87.62 ± 0.55	84.14 ± 0.44**	13)	ALP	Does	90.18 ± 2.36	79.80 ± 2.50**
	Cholesterol	Kids	78.26 ± 0.60	75.39 ± 0.43**		(U/L)	Kids	110.02±2.60	103.05±2.61
	(mg/dl)	Overall	82.94± 0.43	79.76± 0.33*			Overall	100.10±2.64	91.42±2.66
7)	Total	Does	26.94 ± 0.88	23.47 ± 0.82*	14)	GGT	Does	26.69 ± 3.70	29.86 ± 2.27**
	Triglyceride	Kids	22.67 ± 0.89	20.41 ± 0.84		(U/L)	Kids	27.21 ± 0.53	28.92 ± 0.51*
	(mg/dl)	Overall	24.80± 0.77	21.93± 0.71*			Overall	26.95± 0.72	29.39± 0.53**

Means significant at < 0.05 level and means significant at <0.01 level in summer season than corresponding value during winter season*

Serum ALT value increases during heat stress in goats [28]. Elevated serum ALT levels may be attributed to increased heat adaptation. The increase in ALT activity may be due to an increase in gluconeogenesis. Heat stress reduces alkaline phosphatase and lactate dehydrogenase activity in goats [29].

3.6 Oxidative Stress Level and Antioxidant Parameters

Results of antioxidant parameters in beetal goats during two seasons have been shown in the Table 5. Overall levels of erythrocytic lipid peroxidation in beetal goats and total antioxidant capacity in beetal does were found significantly higher ($p<0.01$) during summer season than winter season. Values of the estimated parameters with respect to does and kids during winter and summer season in has been represented in the Table 5. Variations in SOD & GSH levels between the two seasons were not significant. Overall values of all four tested parameters were found significantly lower ($P<0.001$) in goat kids compared to adult does.

An increase in lipid peroxidation observed during the summer season is due to increased production of free radicals, which are responsible for oxidative damage to the plasma membrane, which is made up of phospholipid. Reactive oxygen species (ROS) produced during heat stress causes opening of Ca^{2+} channels, causing an influx of Ca^{2+} into the cell, raising intracellular Ca^{2+} levels, which are taken up by mitochondria resulting decrease in mitochondrial membrane potential, thus downsizing mitochondrial oxidative capacity and further increasing production of ROS through the respiratory chain which exacerbates oxidative stress. Both oxidative stress and aberrantly high Ca^{2+} levels can result in cytotoxicity induced by heat via activation of apoptotic cell death. Li et al. [30].

3.7 Thyroid Hormone Levels

Results of thyroid hormone profile in beetal goats during two seasons have been shown in the Table 6. The overall difference in T3 level during the two seasons was non-significant. T4 level ($P<0.01$) was found significantly higher in the winter season compared to the summer season.

Table 5. Seasonal variation in oxidative stress and antioxidant parameters in does and goat kids

Sr. No	Parameter		Winter	Summer	Overall
1)	MDA (nmol/g of Hb)	Does	164.94±4.56	192.62±4.12**	178.96±4.29
		Kids	126.23±4.19	145.05±4.43**	135.63±4.21
		Goat	145.77±5.32	168.83± 5.78*	157.30±2.15
2)	Reduced glutathione	Does	3.35 ± 0.20	3.57 ± 0.19	3.46 ±0.12
	(GSH) (µmol/g of Hb)	Kids	3.14 ± 0.21	3.27 ± 0.21	3.20 ±0.13
		Goat	3.24± 0.18	3.42± 0.24	3.33±0.10
3)	SOD(U/mg of Hb)	Does	7.4 ± 0.21	7.05 ± 0.19	7.22±0.12
		Kids	6.11 ± 0.22	5.92 ± 0.23	6.01 ±0.14
		Goat	6.75± 0.19	6.48± 0.21	6.62±0.10
4)	Total Antioxidant	Does	336.25±3.76	353.52±3.81**	344.88±3.72
	Capacity (FRAP	Kids	288.09±3.99	298.6 ± 4.13	293.34± 3.5
	value)(µmol/ml)	Goat	312.16±4.21	326.06± 4.81	319.11± 5.5

Means significant at < 0.05 level and means significant at <0.01 level in summer season than corresponding value during winter season*

Table 6. Seasonal variation in Thyroid hormone profile in does and goat kids

Sr. No	Parameter		Winter	Summer
1)	T3 (ng/ml)	Does	1.93 ± 0.07	1.68 ± 0.02
		Kids	2.42 ± 0.09	2.24 ± 0.02
		Overall	2.19± 0.08	1.97± 0.07
2)	Thyroxin (T4) (ng/ml)	Does	61.64 ± 0.41	59.47 ± 0.6*
		Kids	67.77 ± 0.75	63.15 ± 1.15**
		Overall	64.70±0.81	61.31± 0.84**

These results were in consonance with results obtained from many authors who reported that during thermal stress, blood serum concentrations of T3 and T4 were decreased in goats and Surti buffaloes [31], whereas T3 and T4 were increased in the cold season. Thyroid function declines as an acclimation response to alleviate heat stress. This reduction may be due to the effect of heat on hypothalamic-pituitary-adreno cortical axis to decrease the thyrotropin-releasing hormone, which enables the animal to reduce basal metabolism [32]. Similarly, T3 and T4 level increases during cold stress resulting in an increase in oxygen consumption and heat production by cells to increase BMR [33,34]. Additionally, Todini et al. (2007) reported there was an inverse relationship between temperature and activity of thyroid gland hormonal secretion in goats.

A major exogenous regulator of thyroid gland activity is environmental temperature. Free radical H202 serves as a substrate for the thyroperoxidase enzyme, which catalyzes the synthesis of thyroid hormone. Production of more H202 under stress conditions might reduce the activity of the thyroid hormone levels [35]. Moreover, 5' mono deiodinase, an enzyme that converts T4 to T3, is affected by free radicals under heat stress [36].

T3 and T4 serum concentrations were decreased during the summer season to reduce the metabolism, decrease heat production from the body amid so reducing the heat load on the animal. On the other hand, during the winter season, there was a need for increased heat production of the body to compensate for the decline in ambient temperature through increased heat metabolism by an increase in T3 and T4 serum concentrations.

3.8 Plasma Progesterone Levels

The pattern of progesterone concentration was the same, i.e., basal on day 0 of estrus and high level on day 10. On day ten, plasma progesterone concentrations were found higher due to the presence of CL irrespective of animal conceived or not. Overall (Mean ±SE) levels of estimated progesterone hormone level in adults do during the winter and summer seasons were 2.09± 0.02 and 1.96± 0.04 ng/ml, respectively. Data revealed significantly higher progesterone concentration during the winter season compared to the summer season ($P<0.05$)

3.9 HSP & Mitochondrial Gene Expression

Mean Gene expression fold (2^-ΔΔct) and Mean Log2 fold relative mRNA expression in summer season relative to winter of target genes have been presented in Table 7. Mean Log2 fold relative mRNA expression is graphically represented in Fig. 3. During the summer season, the mRNA expressions of HSP 70 and HSP 90 were found to be significantly higher ($P <0.01$), while COX-I and Cyt-B expression were found to be significantly lower than ($P <0.01$) winter season in both Does and Goat kids. The mRNA expressions of HSP 70 and HSP 90 were significantly higher ($P <0.01$), while COX-I and Cyt-B expression were found to be significantly lower ($P <0.01$) in the summer season than winter season in both Does and Goat kids.

Seasonal mRNA expression fold difference of HSP 70 was significantly higher ($P=0.01$) while COX-I and CYT B expression were significantly lower($P<0.05$) in goat kids compared to Does, indicating goat kids were more susceptible to seasonal stress compared to does. HSP 90 fold seasonal fold differences were non-significant between goat kids and does. HSP are molecular chaperones that protect cellular proteins from the adverse effect of heat stress & are responsible for the thermotolerance capacity of the animals. In adaptive mechanism increase in Ca^{2+} results in activation of calmodulin and calmodulin-dependent proteins, which mediates an increase in transcription of heat shock proteins, thus increasing thermotolerance [37].

The HSP70, along with HSP27, 90 proteins, is observed to be anti-apoptotic & cytoprotective in mammalian cells. Elevation in HSP 70 and 90 was observed in sheep, buffalo, cattle, broilers, and goats [38]. Banerjee et al. [39] reported that both heat and cold stress-induced the overexpression of HSP70 genes. Cold-adapted goats had higher HSP70 gene expression in the summer while heat-adapted goats had higher HSP70 gene expression in the winter. Overall, the findings suggest that variations in heat tolerance and adaptation to various climatic circumstances are responsible for HSP70 expression patterns.Our findings were also in accordance with previous studies where heat stress-induced HSP70 expression was observed in the bovine lymphocytes [40,41], in kidneys of goats [42], thereby indicating that heat shock proteins provide protection from toxic effects of thermal stress. Higher HSP expression under

Table 7. Gene expression in summer season relative to the winter season in does and goat kids of tested target genes

Genes	Does		Goat Kids		Fold Difference ratio	P-value
	2^-ΔΔct	Log 2 fold gene expression	2^-ΔΔct	Log 2 fold gene expression		
HSP 70	**34.42±5.55**	4.82±0.23**	**120.99±16.96**	6.69±0.21**	3.51	0.019
HSP 90	**10.16±1.34**	3.10±0.23**	**9.69±1.20**	3.04±0.23**	1.04	0.546
COX I	**0.24±0.01**	-2.09±0.10**	**0.10±0.03**	-3.92±0.34**	2.4	0.021
CYT B	**0.15±0.03**	-3.03±0.29**	**0.03±0.005**	-5.08±0.20**	5	0.001

***Significant at 0.01 level in summer season compared to winter*

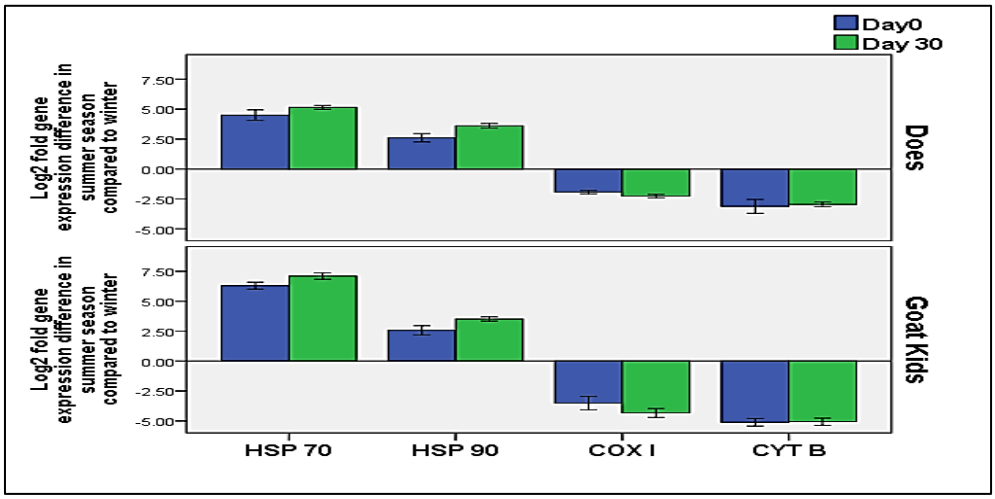

Fig. 3. Log fold 2 expression difference of summer season relative to the winter season

temperature stress helps goats preserve cellular integrity and homeostasis by reducing the negative effects of the stress. Lower expression of COX-I and Cyt B protein which are components of the respiratory chain during the summer season, is due to lower metabolic rate and lower level of thyroxin hormone during the summer season. It has been reported that extreme environmental heat stress in human subjects leads to downregulation of most of the genes involved in the respiratory chain, including genes encoding the components of complexes I (NADH dehydrogenase-ubiquinone), IV (ubiquinol-cytochrome c reductase), and V (cytochrome c oxidase), with a predicted decrease in ATP production, and increase in oxidative stress [43].

Correlation study of recorded THI values with various tested growth, physiological hematological, biochemical, antioxidant parameters of a control group of goats during winter and summer season revealed a significantly high correlation with THI. Values of rectal temperature, respiration rate, heart rate, PCV, Hb, TEC, TLC, lymphocyte, eosinophil, total bilirubin, creatinine, BUN, ALT, AST, ALP, erythrocytic lipid peroxidation, reduced glutathione, total antioxidant capacity, HSP 70 and HSP 90 were found positively correlated with THI, while the values of average daily gain, neutrophil %, glucose, total protein, albumin, globulin, A/G ratio, total cholesterol, triglyceride, SOD, Cytochrome B, and Cytochrome oxidase-II gene expression were found to be negatively correlated with THI.

4. CONCLUSION

Overall, a study has indicated that seasonal stress has a significant effect on the physiological characteristic of the goat, and variations in these parameters contribute to the adaptive capacity of animals during seasonal stress. Antioxidant and molecular gene expression study revealed that goat kids were more susceptible to heat stress than an adult does. Overall study indicated that adaptability of goats to seasonal stress is governed by altered physiological state of goats.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rojas-Downing MM, Nejadhashemi AP, Harrigan T, Woznicki S A. Climate change and livestock: Impacts, adaptation, and mitigation. Climate Risk Management. 2017;16:145-163.
2. Coloma-García W, Mehaba N, Such X, Caja G, Salama AA. Effects of cold exposure on some physiological, productive, and metabolic variables in lactating dairy goats. Animals. 2020; 10(12):2383.
3. Kohli S, Atheya UK, Thapliyal A. Assessment of optimum thermal humidity index for crossbred dairy cows in Dehradun district, Uttarakhand, India. Veterinary World. 2014;7(11).
4. Popoola MA, Bolarinwa MO, Yahaya MO, Adebisi GL, Saka AA. Thermal comfort effects on physiological adaptations and growth performance of West African dwarf goats raised in Nigeria. European Scientific Journal; 2014.
5. Sejian V, Bagath M, Manjunathareddy GB, Kurien EK, Varma G, Bhatta R. Summer season related heat and nutritional stresses on the adaptive capability of goats based on blood biochemical response and hepatic HSP70 gene expression. Biological Rhythm Research. 2017;48(1):65-83.
6. Ribeiro MN, Ribeiro NL, Bozzi R, Costa RG. Physiological and biochemical blood variables of goats subjected to heat stress–a review. Journal of Applied Animal Research. 2018;46(1):1036-1041.
7. Sejian V, Maurya VP, Naqvi SM. Adaptive capability as indicated by endocrine and biochemical responses of Malpura ewes subjected to combined stresses (thermal and nutritional) in a semi-arid tropical environment. International Journal of Biometeorology. 2010;54(6):653-661.
8. Singh K, Singh S, Ganguly I, Nachiappan R, Ganguly A, Venkataramanan R, Narula HK. Association of heat stress protein 90 and 70 gene polymorphism with adaptability traits in Indian sheep (Ovis aries). Cell Stress and Chaperones. 2017;22(5):675-684.
9. Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proceedings of the National Academy of Sciences. 1997;94(2):514-519.
10. Barja G, Herrero A. Oxidative damage to mitochondrial DNA is inversely related to the maximum life span in the heart and brain of mammals. The FASEB Journal. 2000;14(2):312-318.
11. Bernabucci U, Ronchi B, Lacetera N, Nardone A. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. Journal of Dairy Science. 2002;85(9):2173-2179.
12. Saleh MA. Erythrocytic oxidative damage in crossbred cattle naturally infected with Babesia bigemina. Research in Veterinary Science. 2009;86(1):43-48.
13. Tucker C, Rogers A, Schutz K. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. Applied Animal Behaviour Science. 2008;109:141-154.
14. Drabkin DL, Austin JH. Spectrophotometric studies. Journal Biological Chemistry. 1935;112:51-65.
15. Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analytical Biochemistry.1966;16(2):359-364.

16. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959;82(1):70–77.
17. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications.1972;46(2):849-854.
18. Zhang, Chen, Bruins, Marieke Yang, Zhi-qiang, Liu, Shu-Tao, Rao, Ping-Fan. A new formula to calculate activity of superoxide dismutase in indirect assays. Analytical Biochemistry. 2016;503.
DOI: 10.1016/j.ab.2016.03.014
19. Benzie IF, Szeto YT. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry. 1999;47(2):633-636.
20. Misra RP, Puneet K. Improved shelter management, feeding and watering devices for goats. Goat enterprises. CIRG, Mathura (UP), India. 2009;132.
21. Attia NE. Physiological, hematological, and biochemical alterations in heat-stressed goats. Benha Veterinary Medical Journal. 2016;31(2):56-62.
22. Phulia SK, Upadhyay RC, Jindal SK, Misra RP. Alteration in surface body temperature and physiological responses in Sirohi goats during day time in summer season. Indian Journal of Animal Sciences. 2010;80:340-342.
23. Rahman A, Nagpaul PK, Singh B. Effect of two different shelter systems on milk yield and composition, feed intake, feed conversion efficiency and physiological responses in lactating crossbred goats during winter season. Egyptian Journal of Sheep and Goat Sciences. 2013;8(1):89-94.
24. Kour S, Devi J, Kour K, Chakraborty D, Ganai AW, Khajuria P, Zargar R. Effect of thermal stress on haematological parameters and enzymatic activities in two breeds of goat after thermal stress. Journal of Animal Research. 2015;5(4):855-862.
25. Alam MM, Hashem MA, Rahman MM, Hossain MM, Haque MR, Sobhan Z, Islam MS. Effect of heat stress on behavior, physiological, and blood parameters of goat. Progressive Agriculture. 2011;22(1-2):37-45.
26. Nazifi S, Saeb M, Rowghani E, Kaveh K. The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T 3), thyroxine (T 4) and cortisol concentrations. Comparative Clinical Pathology. 2003;12(3):135-139.
27. Nath R. Seasonal variation of blood biochemical parameters in cross bred calves. Indian Veterinary Journal. 2006;83(7):800-801.
28. Kataria N, Kataria AK, Chaturvedi M, Sharma A. Changes in serum enzymes levels associated with liver functions in stressed Marwari goat. Journal of Stress Physiology and Biochemistry. 2011;7(1).
29. Helal A, Hashem ALS, Abdel-Fattah MS, El-Shaer HM. Effect of heat stress on coat characteristics and physiological responses of Balady and Damascus goats in Sinai, Egypt. American-Eurasian Journal of Agricultural and Environmental Science. 2010;7(1):60-69.
30. Li L, Tan H, Gu Z, Liu Z, Geng Y, Liu Y, Su L. Heat stress induces apoptosis through a Ca2+-mediated mitochondrial apoptotic pathway in human umbilical vein endothelial cells. Plos One. 2014;9(12):e111083.
31. Chaudhary SS, Singh VK, Upadhyay RC, Puri G, Odedara AB, Patel PA. Evaluation of physiological and biochemical responses in different seasons in Surti buffaloes. Veterinary World. 2015;8(6): 727.
32. Igono MO, Johnson HD, Steevens BJ, Krause GF, Shanklin MD. Physiological, productive, and economic benefits of shade, spray, and fan system versus shade for Holstein cows during summer heat. Journal of Dairy Science. 1987;70(5):1069-1079.
33. Todini L. Thyroid hormones in small ruminants: Effects of endogenous, environmental and nutritional factors. Animal. 2007;1(7):997-1008.
34. Bernabucci U, Lacetera N, Baumgard LH, Rhoads RP, Ronchi B, Nardone A. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal, 2010;4(7):1167-1183.
35. Deshpande Usha R, et al. Effect of antioxidants (Vitamin C, E and turmeric extract) on methimazole induced hypothyroidism in rats; 2002.
36. Brzezinska-Slebodzinska E. Fever induced oxidative stress: The effect on thyroid status and the 5'-monodeiodinase activity, the protective role of selenium and vitamin

E. Journal of Physiology and Pharmacology. 2001;52(2).
37. Pirkkala L, Nykänen P, Sistonen LEA. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. The FASEB Journal. 2001;15(7):1118-1131.
38. Archana PR, Aleena J, Pragna P, Vidya MK, Niyas APA, Bagath M, Bhatta R. Role of heat shock proteins in livestock adaptation to heat stress. Journal of Dairy Veterinary Animal Research. 2017; 5(1):00127.
39. Banerjee D, Upadhyay RC, Chaudhary UB, Kumar R, Singh S, Polley S, De S. Seasonal variation in the expression pattern of genes under HSP70. Cell Stress and Chaperones. 2014;19(3): 401-408.
40. Lacetera N, Bernabucci U, Scalia D, Basiricò L, Morera P, Nardone A. Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. Journal of Dairy Science. 2006;89(12):4606-4612.
41. Kumar A, Ashraf S, Goud TS, Grewal A, Singh SV, Yadav BR, Upadhyay RC. Expression profiling of major heat shock protein genes during different seasons in cattle (*Bos indicus*) and buffalo (Bubalus bubalis) under tropical climatic condition. Journal of Thermal Biology. 2015;51: 55-64.
42. Zulkifli I, Norbaiyah B, Cheah YW, Soleimani AF, Sazili AQ, Goh YM, Rajion MA. A note on heat shock protein 70 expression in goats subjected to road transportation under hot, humid tropical conditions. Animal. 2010;4(6):973-976.
43. Bouchama A, Aziz MA, Al Mahri S, Gabere MN, Al Dlamy M, Mohammad S, Hussein M. A model of exposure to extreme environmental heat uncovers the human transcriptome to heat stress. Scientific Reports. 2017;7(1):1-14.

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