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Effect of Different Diet Level on the Physiological Adaptability, Biochemical and Endocrine Responses and Relative Hepatic HSP70 and HSP90 Genes Expression in Osmanabadi Kids

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Abstract: The study was conducted to investigate the impact of different levels of feed on the adaptive capability based on physiological, blood biochemical, endocrine and molecular mechanisms in growing Osmanabadi kids. The primary objective of the study was to identify if HSP70 and HSP90 can be a nutritional stress marker for goat. The study was conducted for a period of two months. The animals were randomly divided into three groups as GI (n = 6; *ad libitum* feeding), GII (n = 6; 20% less than *ad libitum*) and GIII (n = 6; 40% less than *ad libitum*). The animals were fed with feed consisting of 50% roughage and 50% concentrate. Blood collection was carried out at fortnightly intervals. Body weights were recorded at weekly interval. Physiological responses, biochemical responses, plasma tri-iodo-thyronine (T_3), thyroxin (T_4) and cortisol were recorded at fortnightly interval. At the end of study period, only GI and GIII animals were slaughtered and different organs were collected for histopathological studies as well as for hepatic HSP70 and HSP90 mRNA transcript expression. Body weight recorded showed significant ($P < 0.01$) differences between the groups. Physiological responses showed significant ($P < 0.01$) variation among the groups. Among the biochemical parameters, plasma glucose and total plasma protein and globulin showed significant ($P < 0.01$) differences between the groups. Plasma T3 ($P < 0.01$), T4 ($P < 0.01$) and cortisol ($P < 0.05$) also differed significantly between the groups. The relative hepatic HSP70 mRNA transcript expression was significantly ($P < 0.05$) higher in GIII (2.8 fold) as compared to GI (1 fold) kids. Similar result was obtained for hepatic HSP90 mRNA transcript expression. From the results, it can be concluded that Osmanabadi kids possessed the ability to alter their adaptive mechanisms to maintain homeostasis. Further, the study revealed the significance of providing the optimum nutrition for these animals to adapt to existing environmental conditions. The study also established that respiration rate (RR), rectal temperature (RT), T_3, T_4 and cortisol are considered as nutritional stress markers for goat. Further, the results revealed that probably this is the first study to establish the nutritional stress impact on heat shock protein (HSP) expression in goats. The study identified both HSP70 and HSP90 to be the ideal molecular markers for feed deficit in goats.

Key words: Adaptation, cortisol, goat, HSP70, HSP90, nutritional stress.

1. Introduction

While climate change is a global phenomenon, its negative impacts are more severely felt by poor people in developing countries who rely heavily on the natural resource base for their livelihoods. Climatic extremes and seasonal fluctuations in herbage quality

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and quantity are considered as imperative source of influence on the well-being of livestock in extensive production systems. This can result in impairing production and reproduction efficiency of grazing animals [1]. Therefore, grazing animals in extensive rearing can face nutritional imbalance during extreme summer months.

Goat as a source of supplementing household income is getting increasing attention especially among the landless agricultural laborers and small and marginal farmers [2]. Most breeds of tropical goats owe their existence to their ability to survive in periods of drought and undernutrition. During periods of surplus feed, energy can be stored, whereas during time of scarcity, energy may be used sparingly and efficiently [3]. In goats, it has been observed that a deficient nutrition induces lower weight gain [4, 5] apart from endocrine disorders especially in those hormones related with nutritional and sexual metabolism, such as insulin, growth hormone, thyroxin (T_4) and steroidal hormones in females [6, 7].

Even though undernutrition has negative effects on female and their progeny, there are few studies in goats reporting the genetic and endocrine mechanisms, by which nutrition stress impacts production and reproduction. Information currently available on the nutrient requirements of local or indigenous breeds of goat for optimum production is meager. Much of the available information on their requirements has been extrapolated from those of exotic farm animals with little adaptation for the additional considerations or requirements needed to cope with climatic conditions. A basic understanding of nutrition and dietary requirements under local condition is therefore required before one can consider and adopt possible improvements.

The application of meteorological and physiological principles to identify the optimum nutrition for goat, as well as employing ideal nutrient management practices will improve the economy of goat farming, especially in climate-limiting zones of the world [8, 9]. Dietary energy plays important role in nutrient utilization and thereby affects adaptive capability of goats [10, 11]. Animals fed on different energy levels in the diet significantly affect the total feed intake, metabolizable energy and the average daily gain, and thereby affect their adaptive capability [12, 13]. Varying dietary energy and protein levels also affects the adaptive capability of an animal [14].

Reports pertaining to different levels of diet on the ability of goat to adapt to a particular environment is very limited [10]. Most of the production losses as a result of climate change in livestock species arise either through non-availability or low quality of pastures available in tropical environment [15, 16]. Efforts are needed in the changing climate scenario to identify the appropriate nutrient requirement to adapt to the existing harsh climate in tropical environment. In addition, HSP70 has been considered ideal biological marker for heat stress in livestock [17, 18]. However, no reports are available in literature to consider HSP70 as marker for nutritional stress in livestock species. Hence, efforts are also needed to identify biological markers to quantify nutritional stress in livestock. The present study was thus initiated to investigate the impact of different levels of feed restriction on the adaptive capability based on physiological, blood biochemical, endocrine and molecular mechanisms during summer season in growing kids, and to identify if HSP70 can be a nutritional stress marker for goat.

2. Materials and Methods

2.1 Location

The experiment was carried out at the experimental livestock farm of ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India, which is located in Southern Deccan Plateau of the country at longitude 77°38′E, the latitude of 12°58′N and altitude of 920 m above mean sea level. The average annual ambient temperature ranges between 15 °C and 36 °C. The mean annual relative humidity ranges between

20% and 85%. The average annual minimum and maximum temperature ranges between 15-22 °C and 27-34 °C, respectively. The annual rainfall in this area ranges from 200 mm to 970 mm with an erratic distribution throughout the year. The average annual relative humidity (RH) ranges in 40%-85%. The experiment was carried out during April-May. The temperature and RH variations during the study period (April-May) ranged in 24-38 °C and 30%-38%, respectively.

2.2 Animals

Osmanabadi is a dual purpose (meat and milk) hardy goat breed, which originated in the semi-arid areas of central tropical India. The study was conducted in Osmanabadi kids (six months old, $n = 18$) weighing between 14 kg and 18 kg. The animals were housed in a well ventilated shed in Eastwest orientation with the dimensions of 13 m × 9 m × 3.1 m for length, width and height, respectively. The area allocated to each animal was 4.2 m^2. The roof of the shed is made up of galvalume sheet with two sides of the shed kept open with wire mesh, and the floor is made up of concrete. The shed has the stocking density of 36 animals. The shed was maintained under proper hygienic conditions. Prophylactic measures against goat diseases, like goat pox, peste des petits ruminants, enterotoxaemia, endo and ectoparasitic infestations, were carried out as prescribed by the health calendar of the institute to ensure that the animals were in healthy condition throughout the study.

2.3 Technical Details

The study was conducted for a period of two months during summer season (April-May). The animals were randomly divided into three groups as GI ($n = 6$; *ad libitum* feeding), GII ($n = 6$; 20% less than *ad libitum*) and GIII ($n = 6$; 40% less than *ad libitum*). Allocation of animals to each group was carried out to ensure there was no significant difference in average body weight between the groups. The animals were fed with feed consisting of 50% roughage and 50% concentrate. The animals had *ad libitum* access to clean water. The composition of the diet included roughage (hybrid Napier grass) and concentrates mixture (maize 36%, wheat bran 37%, soya bean meal 25 kg, mineral mixture 1.5% and common salt 0.5%). The roughage was provided as dry fodder after chopping. The diet was supplied as a total mixture of roughage and concentrate according to different feeding protocols for each group. Table 1 describes the chemical composition, energy and nutrient content of the diet. The animals were acclimatized to different feeding proportions as per the groups 15 d before starting the actual experiment. Body weights were recorded at weekly intervals. Physiological responses were recorded at fortnightly

Table 1 Ingredients and chemical composition of concentrate mixture and hybrid Napier hay fed to Osmanabadi kids.

Attribute	Concentrate mixture (kg/100 kg)	Napier hay (*Pennisetum purpureum*)
Ingredients		
Maize	36.00	-
Wheat bran	37.00	-
Soybean meal	25.00	-
Mineral mixture	1.50	-
Salt	0.50	-
Chemical composition (%)		
Dry matter	92.90 ± 0.079	94.00 ± 0.289
Organic matter	95.90 ± 0.190	95.40 ± 0.298
Crude protein	19.60 ± 0.176	6.21 ± 0.098
Ether extract	1.82 ± 0.183	1.49 ± 0.026
Total ash	4.10 ± 0.190	4.64 ± 0.298
Fibre fractions (%)		
Neutral detergent fibre	40.40 ± 1.400	82.90 ± 0.881
Acid detergent fibre	11.10 ± 0.239	64.60 ± 1.950
Acid detergent lignin	2.14 ± 0.029	12.30 ± 0.651
Nutritive value		
Total digestible nutrients (%)*	72.20	55.00
Digestible energy (kJ/kg)*	13.30	10.10
Metabolizable energy (kJ/kg)*	10.90	8.28

*Calculated values.

intervals. Blood collection was done at fortnightly interval for estimating biochemical and endocrine parameters. At the end of study period, only GI and GIII animals were slaughtered and different organs were collected for histopathological studies as well as relative gene expression studies. The study was conducted after obtaining approval from the Institute Animal Ethical Committee (IAEC) for subjecting the animals to different feeding protocol.

2.4 Blood Collection and Plasma Separation

Blood samples were collected from all three groups on day 0, day 15, day 30 and day 45 after feeding at 11:00 h using 20 gauge sterile needles and plastic syringes from external jugular vein in tubes with heparin anticoagulant. Plasma was separated from blood by centrifugation at 3,500 rpm at room temperature for 15 min. The plasma was then divided into aliquots in micro centrifuge tubes, and kept frozen at -20 °C till further analysis. Plasma samples were used to determine biochemical and endocrine variables.

2.5 Parameters Studied

Body weight was recorded at weekly interval. Physiological responses, such as respiration rate (RR), pulse rate (PR), rectal temperature (RT) were recorded twice daily (8:00 and 14:00) at fortnightly interval. RR was recorded based on the flank movements at the paralumbar fossa of the ewes using a stop watch, and represented by number of breaths per minute. PR was recorded based on the pulsations noted in the femoral artery per unit of time, and represented by number of beats per minute. RT was recorded using a clinical rectal thermometer and represented in °C/min.

Blood metabolites, such as plasma glucose, total protein, albumin, globulin, total cholesterol and high density lipoproteins (HDL) cholesterol were estimated at fortnightly intervals. All biochemical variables were estimated using Span diagnostic kits, India, as per standard method [19] using microplate reader (UV-160A; Thermo Scientific, Finland).

Endocrine variables, such as plasma tri-iodo-thyronine (T_3), thyroxin (T_4) and cortisol were estimated at fortnightly intervals. Plasma T_3 was of analytical sensitivity 0.16 ng/mL and intra-assay and inter-assay coefficient of variations were 11.7% and 9.7%, respectively; plasma T_4 was of analytical sensitivity 0.6 μg/dL and intra-assay and inter-assay coefficient of variations were 6.4% and 9.9%, respectively; plasma cortisol was of analytical sensitivity 0.4 μg/dL and intra-assay and inter-assay coefficient of variations were 9.4% and 8.1%, respectively. All hormonal variables were determined by enzyme linked immunosorbent assay (ELISA) using microplate reader (Thermo Scientific, Finland) by employing goat specific ELISA kits (Cusabio Biotech Co., LTD, China).

2.6 Histopathological Observation

At the end of study period, only GI and GIII animals were slaughtered and different organs were collected in buffered 10% formalin and processed to obtain hematoxylin and eosin (H&E) stained sections.

2.7 Hepatic HSP70 and Heat Shock Protein (HSP) Transcripts Expression

At the end of study period, only GI and GIII animals were slaughtered and their liver samples were collected for relative HSP70 and HSP90 mRNA transcript expression studies. The liver samples were collected from the animals (n = 6 each group) immediately after slaughter, cut into small pieces, washed in normal saline and immersed in RNAlater solution (Ambion, USA). All the samples were snap frozen in liquid nitrogen (LN_2) and stored at -80 °C.

2.7.1 RNA Isolation

After thawing, the samples were removed from RNAlater (Sigma-Aldrich, Bangalore) and immediately processed for RNA isolation. The total RNA was isolated using RNeasy mini kit (Qiagen, USA) as per manufacturer's instruction with slight

modifications. In brief, 30-40 mg of liver tissue was homogenized by grinding in LN_2 in diethylpyrocarbonate (DEPC) treated mortar and pestle. After homogenization, 600 μL of lysis buffer (RLT) with β-mercaptoethanol (10 μL/mL) was added and the content was transferred to 1.5 mL tube. The lysate was vortexed for 5 min and centrifuged at 20,000 ×g for 30 s to remove the debris. To the supernatant, 0.8 volume of 96%-100% ethanol (480 μL) was added and mixed well by pipette. Then the mixture was transferred to spin column membrane attached to a 2 mL collection tube and centrifuged at 8,000 ×g for 30 s. After discarding the flowthrough, 700 μL of wash buffer (RW1) was added and centrifuged for at 8,000 ×g for 30 s, followed by two time wash with 500 μL wash buffer (RPE). After washing and drying the membrane, warm (60 °C) nuclease free water was added to the membrane, incubated at room temperature for 1 min and then centrifuged at 10,000 ×g for 1 min. The total RNA was eluted three times (50, 30 and 30 μL), and finally all three elutions were pooled and added 2 μL of RNase inhibitor (20 U/μL, Invitrogen, USA) and dithiothreitol (DTT) to a final concentration of 10 mM. The RNA samples were stored at -80 °C until cDNA synthesis.

2.7.2 DNase Treatment

Total RNA was treated with DNase (TURBO DNA-free, Ambion, USA) in order to eliminate the gDNA contamination. During and after DNase treatment, 1 μL of RNase inhibitor (20 U/μL, Invitrogen, USA) was added. After DNase treatment, quality and quantity were analyzed using Spectrophotometer (Nanodrop-1000, Thermoscientific, USA).

2.7.3 cDNA Synthesis

The total RNA was reversely transcribed into cDNA using superscript II (Invitrogen, USA) as per manufacturer's protocol using oligo dT primers (Invitrogen, USA). The 2 μL of cDNA (50 ng/reaction) was used for qPCR experiments.

2.7.4 Primer Design and Synthesis

Gene specific primers were designed using online NCBI primer design software (Primer3, http://bioinfo.ut.ee/primer3/) and the specificity was checked using Primer3 and BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The preferences were given to the primers binding to the exon-exon junction. The primers were titrated with different concentrations (10, 5, 2.5 and 1 μM) for selecting the optimum concentration to be used for qPCR experiments. The details of primer are described in Table 2.

2.7.5 Real Time qPCR

The expression of selected genes was studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA). The 10 μL reaction was carried out in triplicates using 50 ng of template and 1 μM primer concentration. The real time qPCR reaction conditions were: enzyme activation at 95 °C for 10 min and amplification cycle (40 cycles; initial denaturation at 95 °C for 15 s, anealing at 61 °C for 30 s and extension at 72 °C for 30 s). The melt curve analysis was performed to check the non-specific

Table 2 Primers used for gene expression studies.

Gene ID	Primers	Primer sequence (5'-3')	Primer length (bp)	Product size (bp)	Accession No.
HSP70	F	TGGCTTTCACCGATACCGAG	20	167	NM_001285703.1
	R	GTCGTTGATCACGCGGAAAG	20		
HSP90	F	AAGAGCCTGACCAACGACTG	20	107	XM_005695418.1
	R	AAAGGAGCTCGTCTTGGGAC	20		
ACTB	F	GATCAAGATCATCGCTCCTCCC	22	167	XM_005694067.1
	R	TCTGCTCGCAGTCCGTTTAG	20		

ACTB: beta-actin used as reference gene to normalize the gene expression of target genes; HSP70: heat shock protein 70; HSP90: heat shock protein 90.

amplification. The beta-actin was used as an internal control and the relative expression was analyzed using the formula $2^{-\Delta\Delta CT}$. The results were expressed in fold change as compared to untreated control (control = 1 fold).

2.8 Data Analysis

The data were analyzed by general linear mode (SPSS 16.0) multivariate analysis of variance. The analysis takes into account the data from the same animals across time. Effect of fixed factors, namely treatment (G1, GII and GIII), days (longitudinal time over which experiment was carried out, i.e., at day 0, day 15, day 30 and day 45) and also interaction of treatment and days, was analyzed on the various parameters studied. Comparison of means of the different subgroups was made by Duncan's multiple range tests. The statistical significance of differences in mRNA expressions of the examined factors was assessed by paired *t*-test. The minimum significant range of confidence was evaluated at 0.05 level.

3. Results

3.1 Body Weight Changes

The effect of different levels of nutrition on body weight changes was described in Fig. 1. The body weight differed significantly ($P < 0.01$) between GI, GII and GIII group kids. The largest body weight was recorded in GI, and body weight in GII and GIII was significantly lower than GI kids. However, both the experimental days and interaction between treatment and experimental days did not influence body weight.

3.2 Physiological Responses

The effects of different levels of nutrition on the physiological responses are described in Table 3. The RR differed significantly both during morning ($P < 0.05$) and afternoon ($P < 0.01$). The highest RR in morning was recorded in GI and GII, while the lowest in GIII. The highest RR in afternoon was recorded in GI, followed by GIII and then GII. Further, the experimental days significantly ($P < 0.01$) influenced RR in the morning. PR also differed significantly ($P < 0.01$) between the groups. The highest PR was recorded in GI, while the low in both GII and GIII both during morning and afternoon. Further, the experimental days had no significant effect on the PR during morning, while it is highly significant ($P < 0.01$) for afternoon. In addition, the interaction

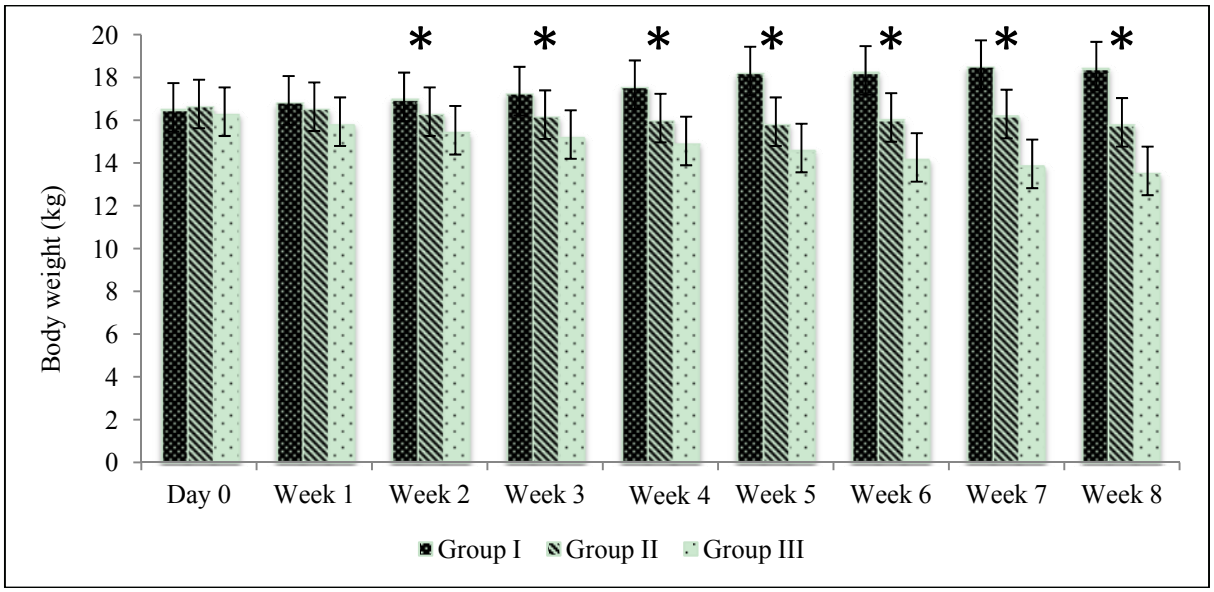

Fig. 1 Effect of different level of diet on the changes in body weight of Osmanabadi kids.
*Values differ significantly at $P < 0.05$.

Table 3 Effect of different levels of feed restriction on the physiological responses in Osmanabadi kids.

Items	Morning			Afternoon		
	RR	PR	RT	RR	PR	RT
μ ± SE	23.956 ± 0.390	59.822 ± 0.565	37.606 ± 0.050	34.333 ± 0.703	69.622 ± 0.745	38.951 ± 0.035
Group	*	**	**	**	**	**
I	24.800^{a}	62.533^{a}	37.737^{a}	37.933^{a}	76.333^{a}	39.020^{a}
II	24.600^{a}	58.533^{b}	37.820^{a}	31.733^{b}	67.067^{b}	39.047^{a}
III	22.467^{b}	58.400^{b}	37.260^{b}	33.333^{b}	65.467^{b}	38.787^{b}
Pooled SE for group	± 0.675	± 0.979	± 0.087	± 1.217	± 1.291	± 0.060
Week	**	NS	**	**	**	**
0	22.333^{b}	59.778^{a}	37.328^{c}	39.111^{a}	74.111^{a}	39.306^{a}
1	26.111^{a}	61.111^{a}	37.794^{b}	34.222^{b}	70.444^{ab}	38.733^{c}
2	24.889^{a}	59.778^{a}	38.211^{a}	40.000^{a}	64.667^{c}	39.106^{ab}
3	21.111^{b}	58.444^{a}	37.367^{c}	28.889^{c}	67.333^{bc}	38.911^{bc}
4	25.333^{a}	60.000^{a}	37.328^{c}	29.444^{c}	71.556^{ab}	38.700^{c}
Pooled SE for week	± 0.871	± 1.263	± 0.113	± 1.571	± 1.666	± 0.078
Group × week	NS	NS	NS	NS	NS	*

RR: respiration rate; PR: pulse rate; RT: rectal temperature.

μ indicates the overall mean for the parameter. *Indicates level of significance at $P < 0.05$; **indicates level of significance at $P < 0.01$; $^{a\text{-}c}$means with similar superscripts for a particular parameter do not differ significantly ($P > 0.05$) from each other; NS: non-significant.

Table 4 Effect of different levels of feed restriction on the biochemical responses in Osmanabadi kids.

Items	Glucose (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)
μ ± SE	44.716 ± 0.489	7.644 ± 0.110	3.701 ± 0.064	3.997 ± 0.132	65.674 ± 2.065	34.224 ± 1.105
Group	**	**	NS	**	NS	NS
I	45.934^{a}	8.387^{a}	3.780^{a}	4.769^{a}	69.601^{a}	34.967^{a}
II	45.760^{a}	7.560^{b}	3.569^{a}	3.992^{b}	62.300^{a}	32.934^{a}
III	42.455^{b}	6.986^{c}	3.756^{a}	3.230^{c}	65.120^{a}	34.770^{a}
Pooled SE for group	± 0.847	± 0.191	± 0.112	± 0.229	± 3.577	± 1.913
Week	**	NS	**	**	NS	*
0	43.634^{b}	8.070^{a}	3.337^{c}	5.003^{a}	66.597^{ab}	36.549^{ab}
1	45.892^{b}	7.358^{a}	3.391^{c}	3.968^{b}	76.660^{a}	39.996^{a}
2	51.573^{a}	7.619^{a}	3.942^{ab}	3.677^{b}	64.247^{ab}	34.068^{ab}
3	45.080^{b}	7.460^{a}	4.163^{a}	3.297^{b}	60.726^{b}	30.363^{b}
4	37.403^{c}	7.715^{a}	3.674^{bc}	4.041^{b}	60.140^{b}	30.142^{b}
Pooled SE for week	± 1.094	± 0.247	± 0.144	± 0.296	± 4.618	± 2.470
Group × week	*	NS	NS	NS	NS	NS

μ indicates the overall mean for the parameter. *Indicates level of significance at $P < 0.05$; **indicates level of significance at $P < 0.01$; $^{a\text{-}c}$means with similar superscripts for a particular parameter do not differ significantly ($P > 0.05$) from each other; NS: non-significant.

between group and experimental days also had no influence on PR both during morning and afternoon. RT also differed significantly ($P < 0.01$) both during morning and afternoon. The highest RT was recorded in GII, while the lowest in GIII both during morning and afternoon.

3.3 Blood Biochemical Responses

The effect of different levels of nutrition on the blood biochemical parameters is described in Table 4. Plasma glucose differed significantly ($P < 0.01$) between the groups. The highest level of plasma glucose was recorded in both GI and GII, while the lowest in GIII. Further, the experimental days significantly ($P < 0.01$) influenced the plasma glucose level. In addition, there was significant ($P < 0.05$) interaction between group and experimental days for plasma glucose. Total plasma protein significantly ($P < 0.01$) differed between the groups. The highest level of total protein was recorded in GI, while the lowest in GIII. Further, both experimental days and interaction between group and experimental days did not influence total plasma protein. The nutritional treatment had no significant influence on plasma albumin levels. Further, the experimental days significantly ($P < 0.01$) influenced albumin level. The globulin levels differed significantly ($P < 0.01$) between the groups. The highest level of globulin recorded in GI, while the lowest in GIII. Further, the experimental days had significant ($P < 0.01$) influence on globulin level. There were no significant influence of nutritional treatment on plasma total cholesterol. Further, the experimental days and interaction between treatment and experimental days also did not influence total plasma cholesterol. The HDL cholesterol also showed similar trend to that of total cholesterol for treatment and interaction. However, experimental days significantly ($P < 0.05$) influenced HDL cholesterol.

3.4 Endocrine Responses

The effect of different levels of nutrition on endocrine parameters is described in Table 5. The level of plasma T3 differed significantly ($P < 0.01$) between groups. The highest level of plasma T3 was recorded in both GI and GII, while the lowest in GIII. Further, the experimental days had no significant influence on plasma T3 level. Plasma T4 also showed similar trend ($P < 0.01$) to that of T3. The highest level of plasma T4 recorded in both GI and GII, while the lowest in GIII. Further, both experimental days and interaction between treatment and experimental days did not influence the thyroid hormones level. The level of plasma cortisol also differed significantly ($P < 0.05$) between groups. The highest level of plasma cortisol was recorded in GI, while the lowest in GIII. Further, both experimental days and interaction between treatment and experimental days did not influence the plasma cortisol level.

3.5 Hepatic HSP Expression

The relative hepatic HSP70 mRNA transcript expression was significantly ($P < 0.05$) higher in GIII (2.8 fold) as compared to GI (1 fold) kids (Fig. 2). Similar result was obtained for hepatic HSP90 mRNA transcript expression. The relative hepatic HSP90 mRNA transcript expression was significantly ($P < 0.05$) higher in GIII (2.3 fold) as compared to GI (1 fold) kids (Fig. 3).

Table 5 Effect of different levels of feed restriction on the endocrine responses in Osmanabadi kids.

Items	T_3 (ng/mL)	T_4 (μg/dL)	Cortisol (μg/dL)
μ ± SE	1.803 ± 0.047	10.527 ± 0.273	0.260 ± 0.010
Group	**	**	*
I	2.093^{a}	11.682^{a}	0.290^{a}
II	1.876^{a}	10.803^{a}	0.260^{ab}
III	1.440^{b}	9.095^{b}	0.230^{b}
Pooled SE for group	± 0.081	± 0.473	± 0.010
Week	NS	NS	NS
0	1.728^{a}	10.233^{a}	0.260^{a}
1	1.708^{a}	10.889^{a}	0.260^{a}
2	1.944^{a}	10.756^{a}	0.260^{a}
3	1.802^{a}	10.523^{a}	0.250^{a}
4	1.831^{a}	10.235^{a}	0.260^{a}
Pooled SE for week	± 0.105	± 0.611	± 0.020
Group × week	NS	NS	NS

T_3: tri-iodo-thyronine; T_4: thyronine.

μ indicates the overall mean for the parameter. *Indicates level of significance at $P < 0.05$; **indicates level of significance at $P < 0.01$; $^{a, b}$means with similar superscripts for a particular parameter do not differ significantly ($P > 0.05$) from each other; NS: non-significant.

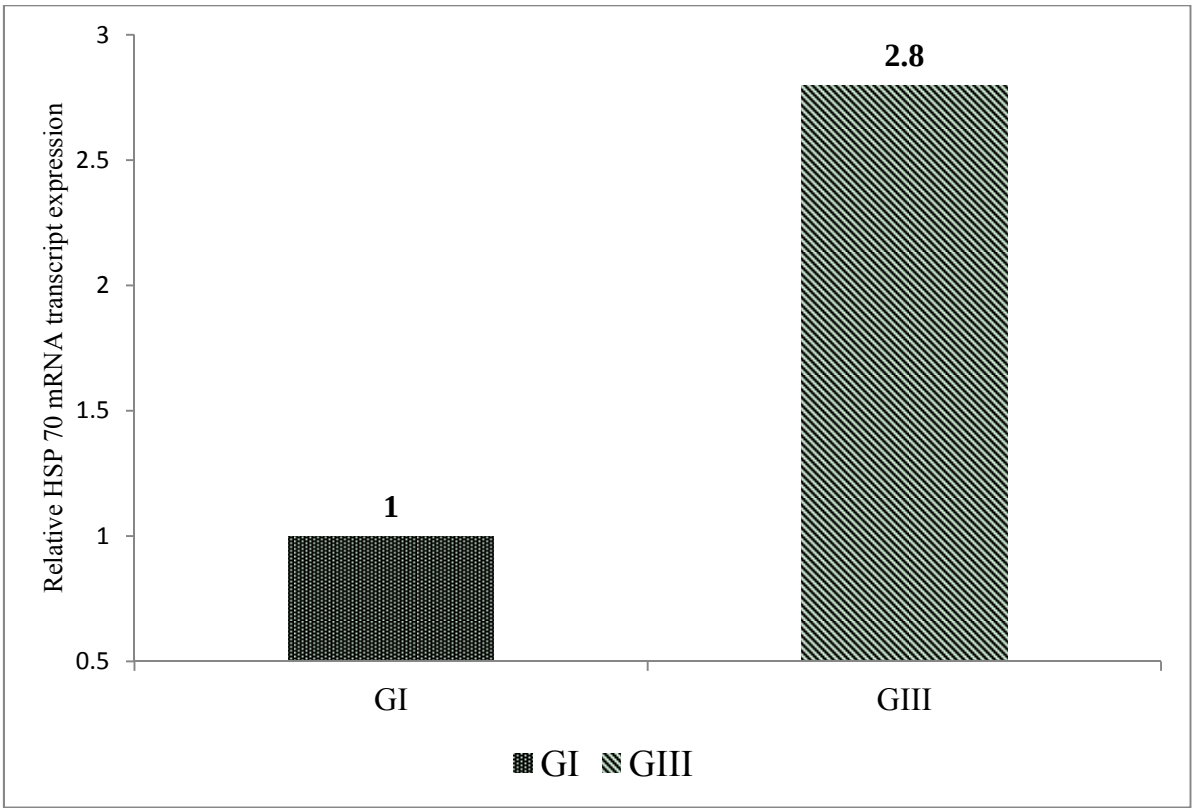

Fig. 2 Hepatic HSP70 mRNA transcript expression between the control and nutritional stress group of Osmanabadi kids.

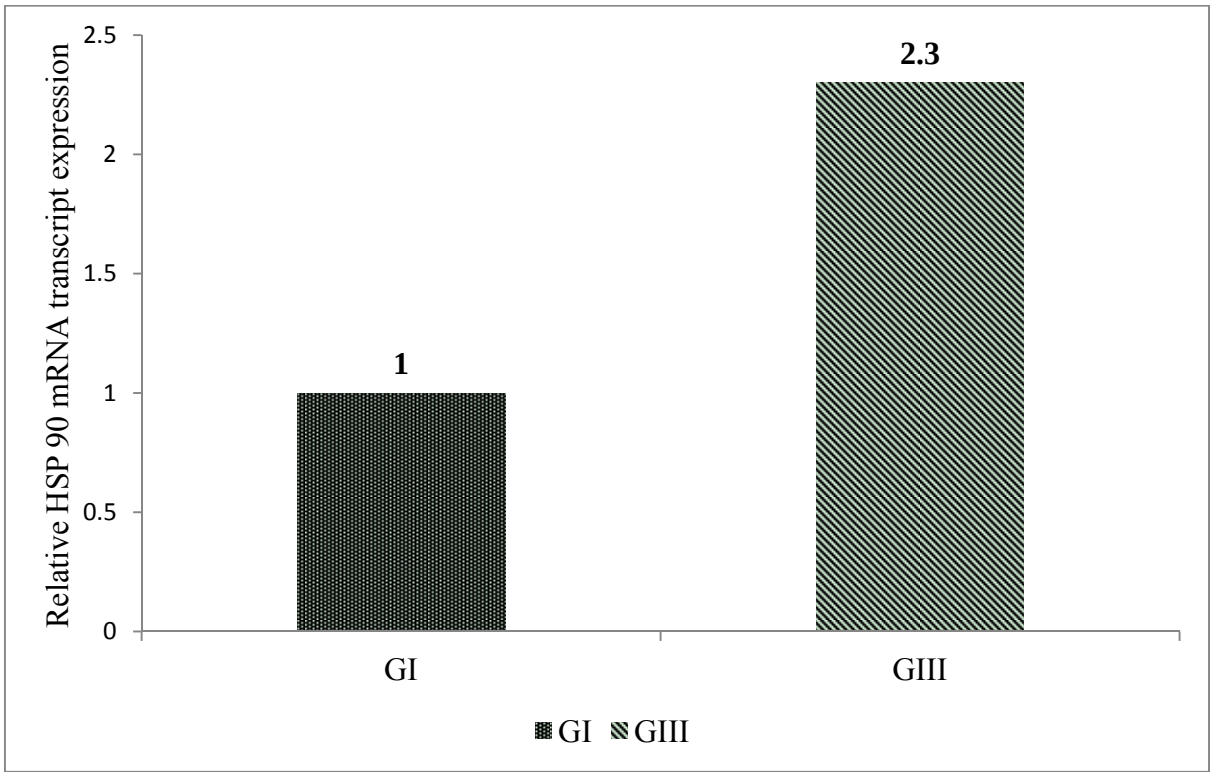

Fig. 3 Hepatic HSP90 mRNA transcript expression between the control and nutritional stress group of Osmanabadi kids.

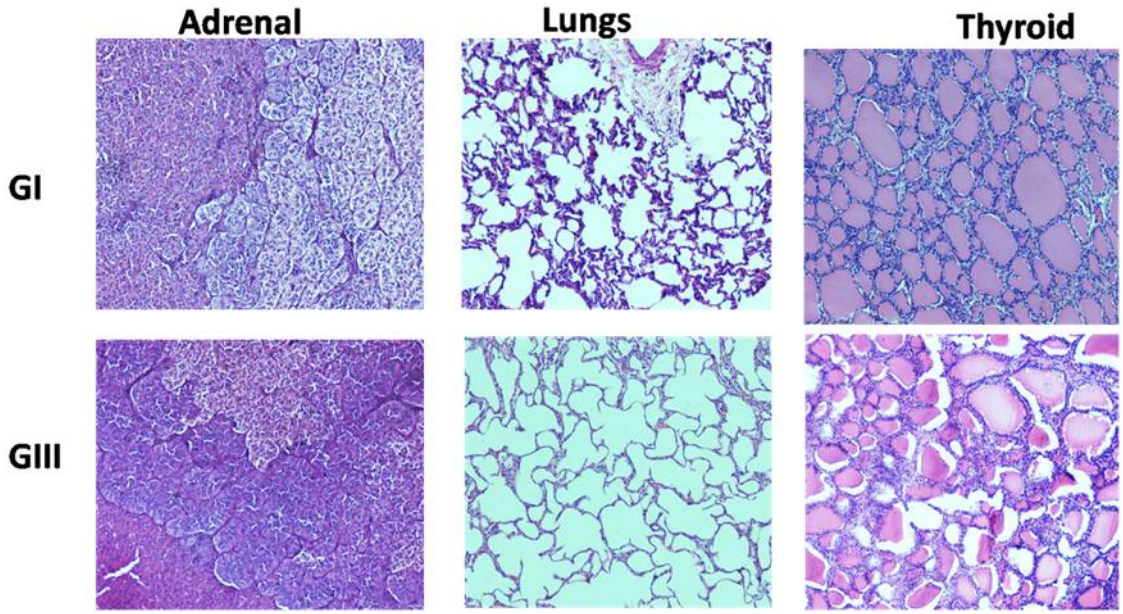

Fig. 4 Histopathological section showing changes in major adaptive organs between the control and nutritional stress group of Osmanabadi kids.

Histopathological observations in different groups of animals subjected to different stress: In GI (control) and GIII (nutritional stress) group kids, different organs pieces were collected immediately after sacrifice in buffered 10% formalin and processed to obtain H&E stained sections. The adrenal gland and thyroid sections in GIII kids showed changes in the histological sections as compared to GI kids.

3.6 Histopathological Sections

The histopathological sections of different organs were described in Fig. 4. The adrenal medulla and cortex in GIII animals showed more intensely stained hypertrophic cells than animals from GI, indicating more functional activity of adrenal gland. The lungs did not show many significant changes, expect dilation of alveoli in GIII. The thyroid gland of GIII animals showed less eosinophilic stained thyroglobulin in follicles, and the lining epithelial cells are less active than GI.

4. Discussion

Livestock production is hampered by the detrimental effects of extreme climates. Consequently, alleviation of the detrimental effects of extreme climates is important to maintain the productivity [20]. Most of the negative impact of climate change on livestock production is through reduction of pasture, which ultimately culminates in severe nutritional stress to the animals. The current experiment is one such attempt to study the different levels of diet on the adaptive capability of goat. The results obtained from the study signified the optimum nutrient requirement for goats' adaptation. The study also identified plasma T_3, T_4, cortisol and HSP70 and HSP90 genes as biological markers for nutritional stress in goat. To the best of our knowledge, this is the first report identified HSP gene expression as the marker for nutritional stress in goats.

The body weight showed significant difference between the groups. The reduced body weight could be attributed to the different levels of feed restriction in GII and GIII in the present study. Dashtizadeh et al. [21] indicated that restriction of dietary energy resulted in less body weight gain in goats. The lowest body weight in GIII kids could be attributed to the severe nutritional stress in these animals. Physiological responses showed significant ($P < 0.05$) differences between the groups. Physiological adaptation is defined as a modification in an animal's behavioral or metabolic response, resulting from an

experience that improves the ability of the animal to cope with a subsequent challenge [22]. The significant changes in physiological responses between the groups in the present study showed that the kids have developed a suitable adaptive strategy in terms of altering their physiological responses to cope with different levels of feeding. RR was significantly lower in GIII as compared to GI and GII both during morning and afternoon. This signifies the importance of the optimum nutrition for adaptation in goat. PR showed similar tends both during morning and afternoon with the lowest values recorded in both GIII and GII as compared to GI. This reduction could be achieved by the animal either by reduced intake or by activity reduction or both [23]. RT also was recorded the lowest in GIII as compared to GI and GII. This finding was similar to the finding reported by De Souza et al. [24] in dairy goat. This could be the adaptive mechanism exhibited by the kids of GIII in order to cope with the feed scarcity [25]. This apparently shows the ability of these kids to adjust their energetic expenditure through body temperature regulation. Further, as the exposure to nutritional stress progressed, the animals have tried their best to cope to feed restriction. This is evident from the significant effect of measurement time on different physiological responses.

Nutrition status of the animals reflects the level of blood cells and blood composition. Plasma glucose concentration was significantly lower in GIII as compared to both GI and GII kids. A possible reason for this could be the deprival of nutrition, which can lead to low levels of circulating glucose in GIII kids. Similar result of nutritional restriction induced plasma glucose reduction was reported in goat [26]. However, the level of plasma glucose did not differ between GI and GII kids. This shows the magnitude of nutritional stress in 80% *ad libitum* group (GII) is not severe enough, and the animals were able to cope with their body reserves. But in a similar study done by Hyder et al. [27] reported significant increase in blood glucose level after dietary energy restriction. In addition, Aboelmaaty et al. [28] did not find significant difference in plasma glucose between control and feed restricted goats. This shows the effect of nutritional restriction on blood glucose levels in ruminants is controversial. Further, both total plasma protein and plasma globulin levels were significantly lower in GIII as compared to GI and GII. Similar result of nutritional stress induced reduction in total plasma protein was recorded in goat [29]. The reduction in total plasma protein concentration could be to synthesize glucose by inducing hepatic gluconeogenesis in order to combat stress [19]. However, the blood cholesterol level did not differ between the groups. Aboelmaaty et al. [28] also reported non-significant effect on total cholesterol between *ad libtum* fed and restricted fed goats.

The different nutritional treatment influenced plasma thyroid hormone concentration. The non significant difference between GI and GII showed that 20% reduction of feed in GII did not bring any significant influence on the metabolic activity of these animals. This signifies the adaptive capability of GII animals to moderate nutritional deficiency. However, both plasma T3 and T4 significantly reduced in GIII as compared to both GI and GII kids, reflecting the severity of nutritional stress in GIII kids. It is an established fact that depression of thyroid function during stress was part of the process of metabolic adaptation, by which the heat production may consequently be maintained at low level [30, 31]. In addition, blood thyroid hormones are considered to be good indicators of the nutritional status of an animal [32]. Following feed restriction or food deprivation, plasma thyroid hormone concentrations were reduced in sheep [33]. These effects suggest that energy balance could play a major role in decreasing the plasma thyroid hormone levels [19]. Besides endogenous and environmental climatic factors, nutrition plays a primary role on thyroid gland activity and on blood thyroid hormone concentrations [19].

Furthermore, energy balance can also play a major role in affecting the decrease in plasma thyroid hormone (TH) levels in small ruminants [34], thus signifying the importance of the optimum nutrition for maintaining appropriate thyroid hormone levels in sheep. Consistent with this notion, plasma thyroid hormone concentrations are correlated with feed intake in several ruminant species [34, 35]. In addition, thyroid hormones decide the metabolic activity, and such metabolic activity are drastically affected by nutritional stress [19, 34]. This indicates that thyroid hormones could directly depict the nutritional status of the animals. Based on the above findings, it could be inferred that circulating thyroid hormone concentration can be a good indicator for nutritional stress in goat. The plasma cortisol concentration was also significantly lower in GIII as compared to GI. However, cortisol concentration did not differ between GI and GII, indicating that 20% reduction in feed in GII did not stress the animals much, even though the study was conducted during summer season. But the significant decrease in cortisol concentration in GIII as compared to GI shows that the energy reserves in these animals are not sufficient to synthesize sufficient cortisol. This again shows the severity of nutritional stress in GIII kids. This finding established that the additional nutrition restriction in the GIII kids proved detrimental for synthesis of sufficient cortisol for relieving the stress in these animals. Similar finding of low level of cortisol in nutrition stress group in sheep supports this argument [19]. This justifies the importance of the optimum nutrition to overcome stressful condition in ruminant species.

The histopathological section of adrenal gland in GIII animals showed more intensely stained hypertrophic cells than animals from GI, indicating more functional activity of adrenal gland. This indicated hyper activity of adrenal gland to cope up to the nutritional insufficiency and to counter the heat stress in summer season by initiating more secretion of cortisol. The lungs did not show much significant changes except dilation of alveoli in GIII. This indicates that the GIII animals are under stress and require more oxygen to favour respiratory evaporative cooling mechanisms. The thyroid gland of GIII animals showed less eosinophilic stained thyroglobulin in follicles, and the lining epithelial cells are less active than GI. Thyroid follicles, which is the iodine reserve containing less thyroglobulin, indicates less iodine and reflects indirectly the less production of thyroid hormones, which will in turn have effect on body growth as well as adaptive capability.

The results revealed that both HSP70 and HSP90 mRNA transcript expression levels in liver were higher in GIII as compared to GI samples. The higher expression of both HSP70 and HSP90 in liver of GIII as compared to GI could be the adaptive mechanism to counter the nutritional stress in goat. HSP70 is one of the most abundant and best characterized HSP family that consists of highly conserved stress proteins, expressed in response to stress and plays crucial roles in environmental stress tolerance and adaptation in goat [18]. Enhanced HSP70 expression may be a response to stressful environments and may improve cell survival by protecting proteins from degradation and facilitating their refolding [36]. In the current study, both HSP70 and HSP90 mRNA expression in liver was significantly higher in GIII as compared to GI. The increase in HSP70 and HSP90 expression in this study could be attributed to nutritional stress for GIII kids. Probably this is the first study to reveal HSP70 and HSP90 as suitable biological marker for nutritional stress in goat. In comparison to expression pattern, the level of expression was significantly higher for HSP70 (2.7 fold) as compared to HSP90 (2.3 fold), proving that HSP70 may act as ideal biological marker for nutritional stress in goat. However, HSP70 as well as HSP90 mRNA transcript expression was significantly higher in GIII group than the control group (GI). This finding indicates that both

HSP70 and HSP90 may act as suitable biological marker for nutritional stress in goat. This shows that HSP70 and HSP90 might play an important role in nutritional stress tolerance in goats.

5. Conclusions

The results from the study indicated that Osmanabadi kids possessed the ability to modify their adaptive mechanisms according to their nutritional status to maintain homeostasis. The results also revealed that RR, RT, T_3, T_4 and cortisol are considered as nutritional stress markers for goat. Probably this is the first study to establish the nutritional stress impact on HSP expression in goats. The study identified both HSP70 and HSP90 to be the ideal molecular markers for feed deficit in goats. The identification, through genetic selection possibly using systemic biomarkers, of such tolerant goats could be used as a strategy to counteract the detrimental effects of feed deficit during extreme weather conditions and thereby assist in increasing the overall production performance in goats.

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