J Endocrinol Reprod 17 (2013) 2 : 113-122 **Original Article**

Adaptive and ecological significance of the seasonal changes in hematological, biochemical and hormonal parameters in the tropical goat *Capra hircus*

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Summary

The neuroendocrine system, through which animals integrate environmental changes and decide when to reproduce, to grow and to store energy, plays a major role in adaptation to the environment. Adaptation of any vertebrate in general and ruminants in particular are influenced by climatic changes being maximally exposed to nature. Elaborating adaptive significance of ruminants in response to season-dependent ecological stresses, we selected the best window i.e., study of variations in blood biochemistry which is totally lacking for goats. Our objective was to find the season- and gender-dependent variations of blood biochemistry at (i) metabolic (glucose, cholesterol, protein, %hemoglobin) (ii) hormonal (testosterone, estrogen, progesterone, melatonin) (iii) hematological (total leucocytes count- TLC; differential leukocyte count- DLC) and, finally, (iv) oxidative load of blood (superoxide dismutase-SOD; catalase, malondialdihyde- MDA) in the goat *Capra hircus* during three different seasons (summer, winter and monsoon). Compared to summer significant changes were noted at metabolic level during monsoon and winter as those seasons provide for inflammatory and cold stress. Cholesterol and glucose levels were high in females than males during all three seasons. Irrespective of sexes, serum protein was highest during winter while testosterone was high irrespective of seasons; hence, males were sexually active throughout the year. Estrogen was high only during the onset of winter (October, heat phase) making the female goats short-day breeders. Melatonin, a neurohormone, regulating reproduction and immunity, was highest in winter (short days) and low during summer and monsoon. Hematological parameters were lowest during summer (long days). Blood oxidative load was high during monsoon and winter due to season-bound infections that induce oxidative stress. High metabolic and immune parameters were noted during winter and monsoon which suggest an adaptive significance in tropical goats against ecological stress induced by low temperature of winter and pathogenic invasion occurring while grazing during monsoon.

Key words: Adaptation, blood-biochemistry, goat, season, sex.

Introduction

Goats are economically important short-day breeder ruminants (MacHugh and Bradley, 2001) which experience a variety of ecological challenges like wide variation in temperature, humidity and pathogenic invasions (Kaushalendra and Haldar, 2012), but never served as a favorable model and, hence, remained experimentally ignored. The tropical climate of Indian sub-continent provides various environmental signals to different animal species to develop adaptive strategies to cope up with ecological stress. Being short-day breeder, goats are mostly affected by temperature- and humidity-induced pathogenic infections. Because of seasonality in reproduction in female goats and their high tolerance level (high temperature of summer and low temperature of winter) attracted our attention to study their adaptive strategies in a season- and sex-dependent manner.

Unlike the goats of temperate zone, those of tropical zone are capable enough to tolerate heat stresses, but during the months of monsoon and winter they get infected with several season-dependent diseases and may even succumb to death (Kumar et al., 2010). On the other hand, adaptation of animals for ecological stress makes them better survivors for which the metabolic strength of blood is highly important. Blood, being the most important specialized tissue of the body, creates an open channel system to provide the equal amount of nutrients, hormones and other important factors to different organs. Thus, blood biochemistry is not only the important marker for the general health and basic metabolic pattern of an animal but it may also throw light on the adaptive modifications to different geographical distributions/conditions. Recent literatures on serum biochemistry of different species of goats under normal condition (Okonkwo et al., 2010), in relation to circulatory hormones (Nazifi et al., 2002) or under certain pathological conditions (Kiran et al., 2011) were reported. Serum level of glucose and lipid of Indian Osmanabadi (More et al., 2008) goats under normal and pathological conditions (Sharma et al., 2001) were also

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reported. But none of the reports suggest a sex- or season-dependent variation that might throw light on adaptive modifications. Therefore, we set as objectives to investigate the blood biochemistry of goat, *Capra hircus,* encompassing (i) metabolic (%Hb, protein, cholesterol, glucose, urea, BUN, creatinine), (ii) hormonal (testosterone, estrogen, progesterone, melatonin), (iii) immune (TLC, DLC), and (iv) oxidative stress parameters (SOD, catalase, MDA) as features of adaptive strategy in season- and/or sex-dependent manner. We included the importance of sex in this study as the male goats are reproductively active throughout the year while females are cyclic in nature breeding mostly during short days (winter).

Materials and methods

Animals and maintenance

Ten male and ten female goats of approximately same age (~ 1 yr) and weight (~20 ± 2 Kg) were procured from a commercial goat raiser with ensured consistency in food and hygiene. The age of the goats was determined from their dentition pattern, which is related with food (Fandos et al., 1993) and also by the procedure as availed from elsewhere (http://fiascofarm.com/goats/age.htm). They were acclimatized to animal house conditions (Varanasi; $25^0 18'$N, $83^0 01'$E, India). The male and female goats were kept separately in different animal rooms (to avoid any chances of mating or pheromone effects) with 8 hours outdoor and 16 hours indoor conditions. Goats generally require 4-5 kg of fodder/day and were fed with usual ration made up of roughages (dry and green) and concentrate. Dry roughages contained crushed barley (*Hordeum vulgare*, 1 part), crushed maize (*Zea mays*, 2 parts), linseed (*Linum usitatissimum*) or mustard seed cake (*Brassica juncea*, 2.25 parts), rice bran (*Oryza sativa*, 2 parts) along with small amount of molasses or a pinch of salt when required. Green roughages contained maize (*Zea mays*), elephant grass (*Pennisetum purpureum), pearl millet (Pennisetum glaucum*), sorghum (*Sorghum* sp.) and oat (*Avena sativa*). The concentrate contained oilseed cakes and soaked gram (*Cicer arietinum*). The ration was prescribed by Central Institute for Research on Goats (CIRG), Mathura, India. Animals were fed on the required amount of ration and had access to water *ad libitum*. Health of the goats was monitored by noting down the body temperature (normal rectal temperature: 39.16^0C - 39.44^0C) and rumen movement by an authorized veterinarian. During the entire study period the goats were neither pregnant nor lactating. Goats were treated with helminthicides twice per year and 0.5% solution of malathion (acaricidal baths) as described by Chowdhury et al., (2002). All the experiments were conducted in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional practice under approval of Institutional Animal Ethics Committee.

Blood sampling

Twenty goats (10 males and 10 females) were numbered on the ear and the same goats were used for assessment of blood immune, metabolic, free radical parameters and hormonal profiles during three seasons i.e., summer [April-June, temperature 43.87 ± 1.02^0C, relative humidity (%RH) 36.74 ± 4.28%, day length, L:D-13.42 hr:10.18hr), monsoon (July-September., temperature 28.68 ± 2.76^0C, RH 87.04 ± 3.50%, day length, L:D-12 hr:12 hr), and winter (November-January, temperature 10.76 ± 3.63ºC, RH 64.12 ± 3.05%, day length, L:D-10.35 hr: 13.25 hr). Blood was collected from the jugular vein in a heparinized hypodermic syringe under total aseptic condition at day time (14:00 hr) and for melatonin at night (22:00 hr) under red light (Philips, intensity < 100 lux). The blood was centrifuged at 3000×g at 4ºC and the plasma was stored at -20ºC until analysis of different hormonal and metabolic parameters. Freshly collected blood was used immediately for determination of anti-oxidants, %Hb, TLC and DLC. The blood was collected during every month and the data were represented season-wise (i.e., summer, April-June; monsoon, July to September; winter, November to January). Thus, the sample size (N) was 10 for male (N=10) and 10 for females (N=10) for each month and N=30 for each sex and season.

Hematological parameters

TLC was performed following the standardized method (Haldar et al., 2004). WBCs were diluted 20 times with Turk's fluid and counted in Neubauer's chamber (Spencer, USA) under oil immersion lens of microscope (Nikon E100, Japan). For DLC, a thin blood film was stained with Leishman's stain and lymphocyte sub-population was counted under oil immersion lens of microscope (Nikon E100, Japan).

Metabolic parameters

Plasma cholesterol was determined adopting the protocol of Sackett (1925) with modifications (i.e., instead of tissue homogenate, plasma was used). Commercially available kits were used for plasma glucose (Beacon Diagnostics Pvt. Ltd., India), urea and creatinine (Span

Diagnostics Ltd., India) determinations following manufacturer's protocol. The estimated value for urea was multiplied with a conversion factor (provided in the kit) to measure BUN level. Plasma protein was determined following the protocol of Bradford (1976). The percentage hemoglobin was measured using Haldane's hemo-meter following the standard protocol.

Blood anti-oxidant enzymes

Superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and MDA in blood were determined adopting the protocols of Das et al. (2000), Sinha (1972) and Ohkawa et al. (1979) with a modifications where instead of tissue homogenate 100 µL of blood was used.

Hormonal parameters

Testosterone was assayed using ELISA kit (Dia Metra, Italy) according to manufacturer's protocol. The coefficient of intra- and inter-assay variation was less than 9% and 15%, respectively. Estradiol was assayed using ELISA kit (Biotron Diagnostics Inc., USA) according to manufacturer's protocol. The coefficient of intra- and inter-assay variation was less than 4.1% and 6.4%, respectively. The analytical sensitivity was 10 pg/mL. Melatonin was measured in the night time blood sample using a commercial kit (Biosource, NIVELLES, Belgium) according to manufacturer's protocol. The analytic sensitivity for melatonin was 2 pg/mL. Inter- and intra-assay variations were between 9.0% and 15%, respectively. Progesterone was measured using a commercial EIA kit (Biotron Diagnostics Inc., USA). The lowest detectable (analytical sensitivity) limit was 0.05 ng/mL. All hormonal assays were carried out in triplicate.

Statistical analysis

The values for testosterone, estradiol and progesterone were analyzed by one way ANOVA, followed by Dunnett Test (Post-Hoc Test). The remaining values were analyzed by two-way ANOVA on Microsoft office Excel Work-Sheet (2007). The significance was tested between sexes, between seasons and among the sexes and seasons at 0.05 (P<0.05) and 0.01 (P<0.01) levels. In the post hoc test the males and females of summer season were treated as control and were compared with all other groups. The P - values less than 0.05 (P<0.05) and 0.01 (P<0.01) were considered as significant and highly significant, respectively. Correlation study was performed to determine the possible linear relationship between different parameters and expressed as Pearson coefficient (r). The r value ≥ 0.5 was regarded as significant correlation among the parameters. The data were analyzed on Statistics Package for Social Sciences (SPSS) version 16.0.

Results

Hematological parameters

Two way ANOVA revealed that the TLC had sex- (P<0.001) and season-dependent variation (P<0.001). TLC increased during monsoon (July to September), reaching the peak in winter (November to January) in female. Minimum variation was noted in males. But the interactive effect of sex and season was not significant (P>0.05; Table 1). In DLC, %basophil was high in males during monsoon and winter but high in females only during monsoon. But significant variation was absent between sexes (P>0.05), seasons (P>0.05) and among sex*season interactions (P>0.05; Table 1). The %eosinophil was significantly high during winter months in both males and females. The variations were significantly high between seasons (P<0.001). However, significant variations were absent between the sexes (P>0.05) and sex*season interactions (P>0.05; Table 1). The %neutrophil was highest during winter in both the sexes. The variations were significantly high in both males and females (P<0.001) during all three seasons (P<0.001). However, interactive effect of sexes and seasons was not significant (P>0.05; Table 1).

The %lymphocyte in males was almost similar throughout the year but in females it was significantly low during winter. %Lymphocyte presented significant variations among seasons (P<0.01) and sexes (P<0.001); however, the sex*season interactive effect was not significant (P>0.05). %Monocyte showed decreasing trend in males during monsoon and winter. But, in females the level was high in monsoon and significantly low during winter. The %monocyte showed significant variation in both the sexes (P<0.001) during the three seasons (P<0.001) and the sex*season interactive effect was also significant (P<0.01; Table 1). In both sexes, TLC and melatonin showed a positive correlation, being stronger in males in comparison to females (Table 5). Gonadal steroids had non-significant correlation with TLC in both sexes (Table 5).

Metabolic parameters

Two way ANOVA showed that blood level of glucose in females was high throughout the year than males which fluctuated little. In females the value was highest during monsoon. The glucose level showed

Table 1 Effect of sex and season on variations in hematological parameters of goats

Season	Summer		Monsoon		Winter		† p values		
Sex	Male	Female	Male	Female	Male	Female	Sex	Season	Sex* Season
Total Leukocyte Count (TLC) Cells/mm^3	4250 ± 5.41	5246 ± 3.99	4740±4.69	6745±5.49	5264 ± 3.81	7756±9.23	<0.001	<0.001	0.100
% Eosinophil	5.82 ± 0.89	4.85 ± 0.85	3.95±1.13	5.07±0.71	8.90 ± 0.52	10.30±1.61	0.548	<0.001	0.498
% Neutrophil	18.41 ± 1.33	33.55 ±1.08	22.07±2.42	34.04±1.43	26.31 ±1.01	44.10±0.83	<0.001	<0.001	0.331
% Basophil	0.25 ± 0.01	0.25 ± 0.02	0.75 ± 0.01	0.50 ± 0.01	10.01	0.25 ± 0.03	0.304	0.546	0.623
% Monocyte	12.54 ± 1.51	4.95 ± 1.53	7.95 ± 0.32	9.30 ± 0.33	5.06 ± 1.34	2.95 ± 0.10	<0.001	<0.001	0.002
%Lymphocyte	63.05 ± 1.81	57.02 ± 0.73	65.47±4.87	51.11±4.81	59.11±1.44	42.52±0.73	<0.001	0.005	0.151

* Data represents mean values with standard error of mean (Mean ± SEM); sample size; N = 20 (10 males and 10 females)/season.

† Significance of difference (p values where indicated <); male *vs* female; summer *vs* monsoon and winter; sex x season.

Table 2 Effect of sex and season on variations in metabolic parameters of goats

Season	Summer		Monsoon		Winter		† p values		
Sex	Male	Female	Male	Female	Male	Female	Sex	Season	Sex* Season
Cholesterol (µg/mL)	62.92±6.45	68.78±9.15	85.53 ± 8.12	128.76±6.85	60.25±6.59	186.74±3.19	<0.001	<0.001	0.001
Glucose (mg/mL)	40.75±1.71	46.25±1.97	43.75± 4.72	68.69 ± 4.33	37.67±5.65	50.75 ± 1.91	<0.001	<0.001	<0.002
Protein (µg/µL)	4.89 ± 0.87	5.19 ± 0.89	6.61 ± 1.17	7.48±1.43	7.26±0.86	8.46 ± 1.37	0.579	0.023	0.737
BUN (mg/dL)	14.23±0.95	14.43± 1.11	15.36 ± 0.94	14.69±1.25	13.30±0.71	14.04 ± 0.86	0.954	0.271	0.618
Urea (mg/dL)	26.58±1.25	27.93±0.81	31.87 ± 2.31	32.12±2.44	28.68±0.80	33.71 ± 1.40	0.026	<0.001	0.170
Creatinine (mg/dL)	0.90 ± 0.05	0.88 ± 0.04	0.97 ± 0.05	1.02±0.04	1.01±0.05	0.98 ± 0.06	0.965	0.397	0.855
% Hemoglobin	54.25±1.63	38.25±1.73	66.25 ± 2.39	43.25±1.88	78.68±1.63	77.50 ± 2.13	<0.001	<0.001	0.002

* Data represents mean values with standard error of mean (Mean ± SEM); sample size; N = 20 (10 males and 10 females)/season.

† Significance of difference (p values where indicated <); male *vs* female; summer *vs* monsoon and winter; sex x season.

Table 3 Effect of sex and season on variations in free radical parameters of goats

Season	Summer		Monsoon		Winter		† p values		
Sex	Male	Female	Male	Female	Male	Female	Sex	Season	Sex* Season
SOD Activity (I.U./mL)	6.42 ± 0.19	6.41±0.68	10.73±0.30	9.01± 0.26	4.87± 0.62	5.57 ± 0.75	0.941	0.308	0.807
Catalase Activity (μ moles of H_2O_2 depleted/ min)	47.21±3.38	52.15 ± 7.01	59.35 ± 5.38	55.34 ± 5.01	63.66 ± 4.98	57.66 ± 5.46	0.172	<0.001	0.756
MDA (μ moles of TEP hydrolyzed/ mL)	76.34±4.34	73.56 ± 3.81	36.07 ± 3.51	26.52 ± 2.27	47.89 ± 4.61	45.72 ± 4.81	0.035	<0.001	0.157

* Data represents mean values with standard error of mean (Mean ± SEM); sample size; N = 20 (10 males and 10 females)/season.

† Significance of difference (p values where indicated <); male *vs* female; summer *vs* monsoon and winter; sex x season.

Table 4 Effect of sex and season on variations in hormonal parameters of goats

Season	Summer		Monsoon		Winter		† p values		
Sex	Male	Female	Male	Female	Male	Female	ex	Season	Sex* season
Testosterone conc. (ng/mL)	13.13± 0.84	--	15.42±0.81	--	12.11±0.96	--	--	0.034	N/A
Estrogen conc. (pg/mL)	--	175.65±1.68	--	187.51±3.38	--	168.72±2.96	--	0.028	N/A
Progesterone conc. (μg/mL)	--	1.21 ± 0.05	--	1.34 ± 0.06	--	1.28 ± 0.06	--	0.148	N/A
Melatonin conc. (pg/mL)	125.11±1.15	55.12±0.64	122.53±2.75	56.87 ± 2.15	192.23±1.65	110.33±1.66	<0.001	0.003	0.878

* Data represents mean values with standard error of mean (Mean ± SEM.); sample size; N = 20 (10 males and 10 females)/ season.

† Significance of difference (p values where indicated <); male *vs* female; summer *vs* monsoon and winter; sex x season.

Table 5 Correlation between free radical parameters (SOD, catalase), sex steroids (testosterone, estrogen and progesterone) with melatonin and TLC with melatonin and sex steroids (testosterone and estrogen) in male and female goats. Correlation was expressed in terms of Pearson coefficient (*r*)

Hormone	Group of goats	Parameters	Correlation (*r*)*
Melatonin	Male	SOD	0.61
	Female	SOD	0.61
Melatonin	Male	Catalase	0.39 (NS)
	Female	Catalase	0.37 (NS)
Melatonin	Male	Testosterone	0.62
Melatonin	Female	Estrogen	0.60
Melatonin	Female	Progesterone	0.08 (NS)
Melatonin	Male	TLC	0.77
	Female	TLC	0.63
Testosterone	Male	TLC	0.15 (NS)
Estrogen	Female	TLC	0.16 (NS)

* NS Not Significant

significant variations in both the sexes (P<0.001) during the three seasons (*P*<0.001) and interactive effect of sexes and seasons were also significant (*P*<0.01). The circulatory level of cholesterol in females was high throughout the year in comparison to males. The variation was significantly high during the three seasons in both the sexes even in sex*season interactive manner (*P*<0.001; Table 2). There was no significant variation in BUN and creatinine level between both sexes and seasons (*P*>0.05, Table 2) but urea level showed significant variations between the sexes (*P*<0.05) during the three seasons (*P*<0.001); however, the interactive effect was not significant (*P*>0.05). Season-dependent significant variation was observed in plasma protein level, it being low in summer and high during winter (*P*<0.05). But sex-dependent variation and interactive effects were not significant (*P*>0.05; Table 2). %Hemoglobin had a season-dependent variation (P<0.001), it being significantly high in both sexes during winter. % Hemoglobin also presented a sex-dependent significant variation (*P*<0.001) with sex*season interactive effect (*P*<0.01, Table 2).

Anti-oxidant parameters

Two way ANOVA showed that the blood SOD level was highest during monsoon in comparison to other seasons. But, the effect of seasons, sexes and sex*season interactive effect was not significant (*P*>0.05). Blood catalase level was high in males during monsoon and winter in comparison to summer month. Females showed a static pattern of catalase activity throughout the year. Non-significant effect of sex and sex*season interactions was observed (*P*>0.05); however, the effect of seasons on catalase activity was highly significant (*P*<0.001; Table 3). Blood MDA level was low during monsoon in both sexes. The effects of sex (*P*<0.05) and seasons (*P*<0.001) were significantly high but the interactive effect was not significant (*P*>0.05; Table 3). Melatonin level showed a significant positive correlation with SOD in both sexes (Table 5). It was not significant when tested with catalase (Table 5).

Hormonal parameters

One way ANOVA showed that the testosterone level was lowest during summer. The peak value of testosterone was obtained during monsoon and, hence, the effect of seasons was significantly high (*P*<0.05; Table 4). The estrogen level varied throughout the year presenting peak value during monsoon and the level was decreased during winter. The effect of season on estrogen level was significantly high (*P*<0.05; Table 4).

Progesterone did not show any year around variation (P>0.05) but a small peak was observed during winter which is the gestational period for the females (Table 4).

Two way ANOVA showed that, in males night time melatonin level was higher than females. In all the groups, the melatonin level started increasing from monsoon, reaching the peak during winter in both females and males. Low value of melatonin was recorded during summer (April to June). The effects of sexes and seasons on melatonin secretion were significantly high (P<0.001); however, the sex*season interactive effect was not significant (P>0.05; Table 4). The melatonin showed a significant positive correlation with testosterone and estrogen but progesterone did not show any correlation with melatonin (Table 5).

Discussion

We recorded the season- and sex-dependent variations of hematological, metabolic, free radical and hormonal parameters of Indian goat *Capra hircus* to infer the adaptive significance. Earlier reports are available on the hematological profile of sheep (Abouzeid et al., 2010) and goat under clinical conditions like bacterial enteritis (Meshram et al., 2009), mycoplasma infection (DaMassa et al., 1992), diarrhea (Zaki et al., 2010) or in an age-dependent manner (Zumbo et al., 2011). Some hormonal profiling in parts were recorded in respect of testosterone (Todini et al., 2007), progesterone (Estrada-Cortésa et al., 2009), estrogen (Paula et al., 2005), prolactin (Brackel-Bodenhause et al., 1994), and melatonin (Zarazaga et al., 2010) in goats. Some molecular approaches for separation of major and minor protein fractions in goat serum including IgG were also reported (Jain and Gupta, 2005). All these studies on goats were partial and till date no comprehensive report which deals with hormonal, hematological and metabolic parameters of any tropical goat that can give an idea about adaptive significance for their survival under stressful seasonal or ecological conditions. Our study on the serum biochemistry of goats in seasone- and sex-dependent manner may provide some key feature for immunity and energy allocation in male and female goats under one roof of adaptive significance.

The hematological parameters chosen for the present study were of clinical importance to provide first line information about immune status of goat, *Capra hircus*. During winter (November to January), summer (April to June) and monsoon (July to September) seasons the immune status (TLC & DLC) of females was higher than male and was in agreement with the report of others (Hotchkiss and Nelson, 2002) that experimental short day enhances lymphocyte proliferation in species ranging from mice to primates. Increased immune function under short days could be due to longer duration of melatonin secretion which acts as an immune stimulator has already been reported in rodents (Champney et al., 1998) including human beings (Carrillo-Vico et al., 2005). Melatonin is known to act directly or indirectly on target tissue within the immune system (Haldar and Ahmad, 2010). Recent studies (Auchtung et al., 2004) suggest that photoperiodic exposure can influence immune function in cattle due to increased circulatory level of melatonin. In goats, TLC has a positive correlation with the level of melatonin and gonadal steroids in both the sexes year round, suggesting that the immune regulation in goats is effected by both these hormones.

To delineate the role of melatonin in immune regulation of goats, we measured night time (22:00 h) melatonin and found it high in both sexes during short days of winter and low during long days of summer and monsoon. Our previous report (Kaushalendra and Haldar, 2012) suggests that high level of melatonin in winter was responsible for immune enhancement in goats. Male goats, being reproductively active throughout the year, showed least responses to melatonin and had little fluctuation in immune parameters even during the winter months when both melatonin and testosterone were high since *C. hircus* is a short-day breeder. Though having opposite physiological properties, melatonin and gonadal steroid (testosterone) maintained immunity and reproduction in a positive manner in male goats, suggesting an adaptive strategy. The melatonin level was high in males during winter season but might be lacking the threshold capacity to suppress testosterone and, hence, immunity was maintained by the basal level of melatonin. Hence, the interplay of both the hormones (melatonin and testosterone) is responsible for health and reproductive status of males.

A positive correlation was observed between the circulatory levels of estrogen and melatonin during winter months as female goats are cyclic and short-day breeder. Estrogen is reported to be a pro-inflammatory gonadal steroid (Calippe et al., 2008) while melatonin is pro-gonadotrophic (Arendt et al., 1981). No correlation between progesterone and melatonin was noted in female goats during different seasons suggesting that progesterone does not interfere much and permits melatonin to act as an immune stimulator to protect the females from infection or gestational stress. Simply, there

may be a mutual synergism between the gonadal steroid and melatonin as an adaptive strategy in both the sexes to maintain the complex immune – reproductive crosstalk in goats. This suggestion gets further support from the results of metabolic parameters.

Goats, being short-day breeders, are best model to study the path of energy distribution for immunity and reproduction that is occurring simultaneously during winter. We measured circulatory levels of glucose, protein and cholesterol as these are markers of main energy sources and some metabolic derivatives of protein such as urea, creatinine and BUN. In all seasons, the circulatory levels of glucose and cholesterol in females were significantly higher than in males. The serum protein levels in both the sexes was low in summer and highest during winter, but the protein derivatives did not change between seasons. This may suggest that in males the energy required for maintenance of various functions might be provided by the high protein level as suggested by Machen (1981). Another important metabolic parameter and marker for health, i.e., %Hb, was significantly high in both sexes during winter months as winter is a crucial period for goats in terms of reproduction and immunity. On the other hand, the levels of %Hb was significantly higher in males than in females throughout the year which suggests a testosterone-dependent increase in %Hb (Coviello et al., 2008).

Though there are many variables associated with the immune system, we studied TLC and DLC as peripheral immunological parameters. In males TLC, %neutrophil, %basophil, %eosinophil, %lymphocyte and %monocyte were high throughout the year; hence, the high energy requirement was balanced by high non-fluctuating level of glucose. Serum glucose, a ready source of energy, might be utilized by the males to modulate immunity and reproduction simultaneously. The females being cyclic and seasonal in nature (manifested by their circulatory estrogen level), their energy requirement was counter-balanced by elevated circulatory glucose (by glycolysis) and cholesterol (by gluconeogenesis) levels specifically during winter. The elevated cholesterol in winter suggests that the animals are switching to fat metabolism and need the cholesterol to form lipoproteins to transport the fat. Further, in females, both peripheral immune parameters and energy demand are high during winter, it being met by high levels of cholesterol (by gluconeogenesis). In females, circulatory cholesterol, therefore, performs two functions, steroidogenesis and immune maintenance. During winter and monsoon both male and female goats are highly susceptible to some diseases or pathogenic invasions and, thus, experience inflammatory stress, which causes increased blood glucose level in comparison to summer.

Melatonin, either as a free molecule or in a receptor-mediated pathway, scavenges free radicals (Ahmad et al., 2012). During the seasons of stress (inflammatory stress during monsoon; cold during winter) the anti-oxidant enzyme levels in circulation become high as suggested by a positive correlation between melatonin and SOD levels. On the other hand the high melatonin level of winter was sufficient enough to scavenge the free radicals generated in the body and thus anti-oxidant enzymes in goats were low in winter as melatonin itself is a free radical scavenger (Tan et al., 2000). During monsoon, anti-oxidant enzyme levels in blood were higher than summer (as they were synthesized at a high rate to scavenge free radicals generated in the body). During monsoon and winter, glucose level was high because of high energy consumption of organs that also increased ROS in circulation. Therefore, most of the increased free radicals or their derivatives get scavenged by melatonin during winter, thus favoring immune modulation. Overall a multi-factorial physiological adaptation exists for survival of goat through different seasons.

Thus, we propose a sex-dependent variation in metabolic parameters and melatonin level while a season-dependent variation in hematologic parameters exist in the tropical goat. High gonadal steroid and melatonin levels caused maintain a basal level of immunity in males throughout the year. In females gonadal steroid and melatonin were high during winter which provided for maintenance of a high immunity to balance gestational stress. Hence, in this tropical goat melatonin is pro-gonadotrophic and, interestingly, immune stimulator as well. Antioxidant enzymes were highest in goats during monsoon in view of inflammatory stress and also due to the low level of melatonin. Thus, a synergism between melatonin and gonadal steroids maintains immunity, reproduction and energy balance as an adaptive strategy in tropical goats unlike the goats of temperate zone.

Acknowledgments

The grant from Department of Biotechnology (DBT) New-Delhi, India, to Prof. C. Haldar, and financial assistance from Council of Scientific and Industrial Research (CSIR-SRF), New-Delhi, India, to S. Ghosh are gratefully acknowledged. We are thankful to Dr. P.K. Raut, Senior Scientist, CIRG, Mathura, India, for help and suggestions during the study.

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