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Haemato-biochemical profile of Corriedale ewes in relation to winter housing and feeding interventions in Kashmir region

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Abstract
A study was conducted to evaluate haematological and biochemical profile of pregnant ewes under different feeding and housing interventions during winter in Kashmir. Forty healthy Corriedale ewes in their third month of gestation were selected randomly and allotted to four groups (G_1, G_2, G_3 and G_4) of 10 animals each such that the parity and average body weight differences between the groups were statistically non-significant. Ewes of G_1 and G_3 were kept inside the shed while as G_2 and G_4 were kept in an open area which was wire fenced from four sides and provided with a roof shelter. G_3 and G_4 were supplemented additional pelleted concentrate @ 100g/head/day in addition to the basic ration. The blood samples were collected on the first day of the trial, post-parturition and at the end of the trial. Among the haematological parameters, haemoglobin and packed cell volume were significantly ($P< 0.05$) higher in G_2 and G_4. G_2 and G_4 had significantly ($P< 0.05$) higher neutrophil but lower lymphocyte count. Among the bio-chemical parameters, plasma glucose, total protein, triglyceride, triiodothyronine, thyroxine and cortisol levels were significantly ($P< 0.05$) higher in G_2 and G_4 while as cholesterol levels were non-significant among the groups. It was concluded that the experiment did not result in any adverse effect on the health state of the ewes as evidenced by the results which can provide an economic advantage for producers by reducing the cost associated with the construction of elaborate housing.

Keywords: Corriedale, Housing, Kashmir, Nutrition, Winter

Introduction
Sheep rearing has remained one of the main occupations of the farmers of Kashmir valley keeping in tune with the availability of the resources and socio-economic values associated with this enterprise. Over the years, there has been a constant decline in this valuable resource owing to better opportunities in other sectors, constant neglect of this sector and other unforeseen causes. This sector in the region continues to be in the hands of lower strata of the population who undertake low input-low output production system or complete transhumance production system. Winter management remains the major constraint for taking up sheep rearing as an enterprise. In order to boost the economy of this sector, commercial farming needs to be put forth. Further, this sector, keeping in view the traditions and demand of meat in the Kashmir region can serve the employment generation as it is one of the sustainable opportunities. Both these initiatives require such a setup wherein costs involved are reduced without compromising the performance of the animals. Geographically, Kashmir valley is a temperate zone lying in between the outer and inner range of Western Himalayas. Minimum temperature range of the region being -0.83°C to -1.5°C and maximum being 10.55°C during winter months (Salam *et al*.,) [1]. Sheep with its multifaceted utility (for meat, wool, skin, manure) production represents the source of income to a reasonable proportion of population of Jammu and Kashmir. Rearing of livestock during these months in the valley is considered a challenge owing to harsh winter climate where animals are subjected to extreme cold. However, sheep are considered probably most tolerant of environmental extremes compared with swine, cattle, and poultry. They are unique because of the potentially large insulatory value of the fleece and the thermal zones for sheep depend largely on amount of external insulation provided by the fleece.
Hemato-biochemical tests are important tools for evaluation of physiological and health status of farm animals. Winter-induced cold and nutritional stress leave their imprints on the blood metabolic profile of the animal.

Productivity and reproductive efficiency have been found to be correlated with the blood parameters (Abdel-Fattah *et al*.,) [2] in sheep. In view of the above-mentioned fact, the present study was planned to investigate the effect of cold on blood profile of the ewes and about management interventions needed to cope up with cold stress during winter season for the development of sheep industry in Kashmir.

Materials and Method

The study was conducted at the Mountain Research Centre for Sheep and Goat (MRCSG), Sher-e-Kashmir University of Agricultural Sciences and Technology, Shuhama, Kashmir, during the winter (January-March 2017). Forty pregnant ewes in their last trimester of gestation were randomly divided into four groups (G_1, G_2, G_3 and G_4) with ten ewes in each group based on their body weight and parity. Ewes of G_1 and G_3 were kept inside the sheds all the time while as the ewes of G_2 and G_4 were kept in an open area which was wire fenced from four sides and was provided with a roof shelter from direct rain/snow. The basic ration of all the four groups of animals consisted of 500 gram of concentrate/head/day (89% DM, 20% CP, 72% TDN) in addition to 1.25 kg of Oats hay/head/day (88% DM, 5% CP, 55% TDN). Animals belonging to groups G_3 and G_4 received 100 gram of additional concentrate/head/day respectively.

Blood samples, drawn from the jugular vein of the test animals, were taken at the start of the trial, post-parturition and at the end of the trial. Haemoglobin concentration was estimated by acid-haematin method using Hellige-Sahli haemoglobinometer. The packed cell volume (PCV) was measured by micro-haematocrit method as per International council for standardization of Haematology (ICSH, 1978). Immediately after collection of blood, the smears were prepared for differentiating and counting of each type of leukocytes afterwards. Differential count of leukocytes was determined by using Giemsa stain. Plasma glucose and plasma total protein were estimated by glucose oxidase-peroxidase method and biuret method respectively. Plasma cholesterol and triglyceride were estimated by Cholesterol oxidase- Peroxidase method and Glycerol-3-phosphate-oxidase – Peroxiadse respectively. Triiodothyronine (T3), thyroxine (T4) and cortisol were estimated by ELISA kit (Calbiotech). The data on meteorological variables (temperature, relative humidity) were recorded daily by sling hygrometer and used to calculate temperature humidity index (THI) using the formula (Buffington *et al*.,) [3]:

$$THI = [(0.8 \times \text{ambient temperature}) + \{(\% \text{ relative humidity} \div 100) \times (\text{ambient temperature} - 14.4)\} + 46.4]$$

Statistical Analysis

The data so obtained was statistically analyzed by analysis of variance (ANOVA) technique as per Snedecor and Cochran [4] (SPSS Software products, Marketing Department, SPSS Inc. Chicago, USA).

Results and Discussion

Environmental parameters

During the study period, minimum ambient temperature ranged from -2.73±0.67°C to 3.03±0.78°C in the open shed and from 5.13±0.25°C to 11.58±0.72°C in closed shed. The lowest THI was recorded in January in open shed (Table 1). Hahn *et al*. [5] stated that a THI of 74 and below is considered as normal, 75–78 as moderate, 79–83 as severe and ≥ 84 as very severe (emergency) for ruminants. While Khalifa [6], based on changes in body temperature, heat production and heat loss of sheep, reported that the comfort zone ranged between 60-70 THI while moderate heat stress ranged from 70 – 85 THI and severe heat stress occurred THI ≥85.

Table 1: Temperature, relative humidity and temperature humidity index (THI) of open and closed shed during the experimental period

Climatic variable	Housing	January		February		March	
		Min	Max	Min	Max	Min	Max
Temperature (°C)	Outside	-2.73±0.67	13.06±0.76	0.35±0.72	9.71±0.86	3.03±0.78	15.19±1.17
	Inside	5.13±0.25	10.74±0.89	8.83±0.47	12.78±0.13	11.58±0.72	16±0.39
Relative humidity (%)	Outside	77.35±2.53	93.42±1.16	67.10±2.06	88.07±2.84	50.62±2.99	79.37±4.02
	Inside	53.85±1.32	83.6±0.53	61.8±2.75	83.75±1.31	54.44±4.21	77.64±1.70
THI	Outside	30.97±1.25	55.60±0.90	37.25±0.80	50.04±1.58	43.07±1.69	59.18±2.39
	Inside	45.51±1.08	51.93±1.44	50.02±1.22	55.27±1.22	54.13±1.42	60.44±2.85

Haematological parameters

Haemoglobin (Hb) and PCV concentration were found significantly ($P< 0.05$) higher in the ewes that were reared outside (G_2 and G_4) though the increase was within the normal limits. This might be due to increase in the synthesis of RBC and Hb to maintain the homeostasis (Maurya *et al.*,) [7] and as a sequel to compensatory mechanism. Maurya *et al.* [7], Banerjee *et al.* [8] Mohamed and Mohamed [9] and Bhat *et al.* [10] also reported similar findings. Ewes of G_2 and G_4 showed significantly higher ($P< 0.05$) neutrophil count but lower lymphocyte count. While as eosinophil and monocyte count was almost similar in all the four groups. Leucocyte profiles are useful in the field of adaptation physiology because they are altered by stress and can be directly related to stress hormone levels (Banerjee *et al.,*) [8]. Slight increase in the neutrophil count in the animals kept outside in the present study might be due to the adaptation process on exposure to cold. Cold is known to affect leukocyte mobilization and can suppress lymphocyte functional activities. An increased glucocorticoid (cortisol) level leads to increase in numbers of neutrophils (neutrophilia) and decrease in lymphocyte numbers (Banerjee *et al.,*) [8]. This could be a possible explanation for increase in neutrophil and decrease in lymphocyte count in the present study.

Table 2: Hb and PCV under different management during winter in Corriedale ewes (Mean ± SE)

Parameters	Stage	G_1	G_2	G_3	G_4
Hb	Day -45	6.81±0.21^{ab}	7.00±0.28^{a}	7.04±0.21^{a}	6.30±0.22^{b}
	Day0	7.91±0.39^{b}	9.19±0.38^{a}	9.20±0.37^{a}	10.07±0.52^{a}
	Day +45	8.20±0.32^{b}	10.30±0.37^{a}	8.66±0.25^{b}	9.60±0.37^{a}
PCV	Day -45	20.20±0.57^{a}	21.00±0.81^{a}	21.30±0.59^{a}	18.4±0.74^{b}
	Day 0	23.60±1.16^{b}	27.90±1.03^{a}	27.66±1.14^{a}	29.60±1.45^{a}
	Day +45	25.80±0.98^{b}	30.80±1.13^{a}	26.00±0.77^{b}	28.00±1.11^{a}

[ab] Means with different letters within a trait in a row are significant at $P< 0.05$. Day -45=105 day of gestation, Day 0=day of parturition; Day +45= corresponding day post-partum

Table 3: Differential leucocyte count (%) under different management during winter in Corriedale ewes (Mean ± SE)

STAGE	Parameter	G_1	G_2	G_3	G_4
Day -45 (pre-partum)	Neutrophil	37.3±80.12	37.25±0.12	37.23±0.13	37.10±0.10
	Eosinophil	4.42±0.10	4.52±0.11	4.26±0.07	4.38±0.06
	Lymphocyte	54.75±0.23	55.12±0.20	54.86±0.21	55.16±0.18
	Monocyte	2.83±0.06	2.78±0.02	2.86±0.04	2.82±0.04
Day 0 (partum)	Neutrophil	37.42±0.13^{b}	38.57±0.1^{a}	37.01±0.1^{b}	38.34±0.19^{a}
	Eosinophil	4.88±0.06	4.98±0.08	4.83±0.05	4.95±0.06
	Lymphocyte	53.71±0.14^{a}	52.32±0.2^{b}	53.75±0.1^{a}	52.60±0.10^{b}
	Monocyte	2.66±0.04	2.63±0.02	2.77±0.05	2.71±0.04
Day 45 (post-partum)	Neutrophil	36.30±0.05^{b}	37.22±0.1^{a}	36.52±0.0^{b}	37.21±0.12^{a}
	Eosinophil	4.42±0.10	4.33±0.11	4.20±0.10	4.31±0.09
	Lymphocyte	54.92±0.15^{ab}	54.52±0.1^{b}	55.18±0.0^{a}	54.78±0.15^{ab}
	Monocyte	2.81±0.04	2.78±0.04	2.85±0.06	2.78±0.05

[ab] Means with different letters within a trait in a row are significant at $P< 0.05$. Day -45=105 day of gestation, Day 0=day of parturition; Day +45= corresponding day post-partum.

Biochemical parameters

A significant ($P< 0.05$) increase in plasma glucose levels was observed in G_2 and G_4 (values being within the normal limits in all the groups). This increased glucose level in cold exposed ewes could be due to increasing energy demand to keep them warm. The mobilization of lipid and glycogen to provide energy precursors is needed for thermogenesis which in turn elevates glucose level in the cold exposed animals (Godfrey *et al.* [17]; Himms-Hagen [18]). The results were in agreement with Nazifi *et al.*, [11]; Sano *et al.*, [12]; Can *et al.*, [13]; Pandey *et al.*, [14]; Maurya *et al.*, [7]; Mohamed and Abdelatif, [15] and Al-Mamun *et al.*, [16]. There was a significant ($P< 0.05$) increase in plasma total protein in G_4 compared to G_1 on the day of parturition. On day 45 post-partum, G_4, G_2 and G_3 attained significantly ($P< 0.05$) higher total protein levels than G_1 with highest mean value in G_4. This could be because of the adaptation of experimental animals to environmental stress and increased basic metabolism (Nazifi *et al.*,) [11]. It may further be potentiated by the response of the animals to cold winter to maintain homeostasis (Bhat *et al.*) [10]. Earlier studies in this regard revealed results similar to present findings in Iranian fat-tailed sheep (Nazifi *et al.*) [11] and Vrindavani calves (Bhat *et al.*,) [10]. G_2 showed significantly ($P< 0.05$) higher triglyceride mean value than G_1 on the day of parturition and on day 45 post-partum, levels were significantly ($P< 0.05$) increased in G_2 and G_4, increment being maximum in G_4. The present findings were in affirmative with those of Olson *et al.*, [19]; Doubek *et al.*, [20] and Al-Musawi *et al.*, [21]. Increased triglyceride level during winter could possibly be due to higher intensity of lipolytic thermogenesis (Doubek *et al.*, [20]). No significant difference in cholesterol levels was observed amongst the groups. However, numerically higher value was found in G_2 and G_4. The results were in accord with Antunovica *et al.*, [22] and Al-Musawi *et al.*, [21].

T3 and T4 levels were significantly ($P< 0.05$) higher in the ewes of group G_2 and G_4. The increased level of thyroid hormones may be in response to low ambient temperature during winter to increase metabolic rate and increased body heat production to maintain core body temperature (Banerjee *et al.*, [8]; Bhat *et al.*,) [10]. Results similar to present findings have been reported earlier in rams (Taha *et al.*, [23]; Souza *et al.*,) [24], lambs (Ekpe and Christopherson, [25]; Doubek *et al.*,) [20] and Indian goats (Banerjee *et al.*) [8]. Same trend was observed in cortisol levels with significantly ($P< 0.05$) higher value in G_2 and G_4. This was in good agreement with the findings of Doubek *et al.* [20] in Romney Marsh lambs, Nazifi *et al.* [11] in Iranian fat-tailed sheep, Gavojidian *et al.* [26] in lactating ewes, Maurya *et al.* [7], Snoj *et al.* [27] in ewes and Bhat *et al.* [10] in Vrindavani calves. This increased level during winter may be due to increase in lipolysis and utilization of brown adipose tissue for heat production (Maurya *et al.*, [7] and Bhat *et al.*,) [10]. In this respect, Ekpe and Christopherson have reported that cold temperature exposure and feed restriction increased plasma cortisol concentrations in lambs.

Table 4: Biochemical parameters in Corriedale ewes under different management during winter (Mean ± SE)

Parameters	Stage	G_1	G_2	G_3	G_4
Total protein (g/dl)	Day -45	6.17±0.02	6.20±0.01	6.13±0.02	6.19±0.02
	Day 0	6.05±0.02^{b}	6.11±0.02^{ab}	6.06±0.02^{ab}	6.14±0.02^{a}
	Day +45	6.20±0.04^{b}	6.36±0.03^{a}	6.35±0.03^{a}	6.41±0.03^{a}
Triglyceride (mg/dl)	Day -45	16.4±0.54	16.66±0.42	16.11±0.23	16.70±0.36
	Day 0	15.20±0.38^{b}	16.22±0.19^{a}	15.44±0.15^{ab}	15.90±0.31^{ab}
	Day +45	15.70±0.39^{b}	16.66±0.21^{a}	16.11±0.17^{b}	17.10±0.27^{a}

Cholesterol (mg/dl)	Day -45	55.9±1.25	57.22±1.07	57.11±0.82	57.80±0.91
	Day 0	55.10±1.11	56.22±0.97	54.77±0.32	56.20±1.09
	Day +45	56.70±1.00	58.11±0.80	56.77±0.24	58.40±0.87
T3 (ng/ml)	Day -45	1.62±0.02^{a}	1.62±0.02^{a}	1.58±0.02^{ab}	1.53±0.02^{b}
	Day 0	1.98±0.01^{b}	2.37±0.03^{a}	2.00±0.03^{b}	2.36±0.02^{a}
	Day +45	2.11±0.03^{c}	2.78±0.03^{a}	2.16±0.02^{c}	2.66±0.02^{b}
T4 (µg/dl)	Day -45	5.23±0.02	5.25±0.01	5.23±0.02	5.25±0.02
	Day 0	6.19±0.01^{b}	6.55±0.01^{a}	6.17±0.01^{b}	6.54±0.02^{a}
	Day +45	6.68±0.01^{c}	6.99±0.01^{a}	6.60±0.01^{d}	6.89±0.01^{b}
Cortisol (ng/ml)	Day -45	14.91±0.19	15.09±0.15	15.10±0.15	15.12±0.17
	Day 0	18.72±0.19^{b}	21.80±0.21^{a}	18.90±0.14^{b}	21.65±0.20^{a}
	Day +45	23.30±0.14^{b}	27.61±0.21^{a}	22.85±0.12^{b}	27.21±0.21^{a}

^{abcd} Means with different letters within a trait in a row are significant at $P< 0.05$. Day -45=105 day of gestation, Day 0=day of parturition; Day +45= corresponding day post-partum.

Conclusion

It can be concluded that the cold stress induced by harsh winter did not result in any deterioration of health state of the ewes as evidenced by the results, values of blood constituents being within the normal limits of the sheep. Thus, simple housing involving less cost of construction with or without supplementation can provide an economic advantage for producers by reducing the elaborate housing construction cost.

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