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Haematological and oxidative stress studies in various body condition score in Marwari sheep during cold ambience

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Abstract
A study was conducted to investigate the effects of cold stress on haematological and oxidative stress parameters under different body condition score (BCS). Apparently healthy 180 Marwari sheep were divided in 6 groups (2.0, 2.5, 3.0, 3.5, 4.0 and 4.5) as per their condition score. BCS was estimated by method as described by Jefferies (1961) and Russel (1969). Serum Catalase and Gamma-glutamyl transferase (GGT) were measured by colorimetric and spectrophotometric method, respectively. Haemoglobin, PCV and ESR were estimated by standard method (Jain, 1986). The month of October-November was considered as moderate ambience (control) and the data observed during cold ambience (December-January) were compared from these data. The overall mean values of serum catalase and serum gamma glutamyl transferase showed a significant ($p \leq 0.05$) increase during extreme cold ambience as compared to moderate ambience. The overall mean values of haemoglobin and packed cell volume showed a significant ($p \leq 0.05$) decrease whereas erythrocyte sedimentation rate showed a non-significant decrease during extreme cold ambience. Serum catalase activity was observed highest in BCS 2.5 and lowest in BCS 4.5. Higher catalase activity indicated the activation of the protection mechanism in animals. Maximum and minimum increase in serum GGT were observed in the mean values of 4.5 BCS and 2.5 BCS. Increased serum GGT activity reflected increased oxidative stress due to cold ambience. In the present study, the haematological parameters were minimum in sheep with low BCS and maximum in higher BCS group. Decreased Hb and PCV during extreme cold ambiance in low BCS group may be due to reduction in synthesis of Hb and RBCs.

Keywords: Cold ambience, oxidative stress, Marwari sheep, catalase, GGT, haemoglobin, packed cell volume and ESR

Introduction
Sheep plays a vital role in the arid tracts of Rajasthan by contributing nutritional and economical security to marginal and landless farmers. Due to scarcity of feed, water and adverse climatic conditions in arid areas sheep mostly have low body weight as well as low Body Condition Score (BCS). BCS is a useful managemental tool for assessing body reserves (quantity of fat and muscle) and enables the livestock owner to maintain optimum health and production. Scores are important for evaluating the health status of individual animals beside this they establish the condition of animals during routine welfare inspections. Oxidative stress is a divergence between increased level of reactive oxygen species (ROS) and decreased activity of antioxidant mechanism to detoxify the reactive oxygen species. An elevated oxidative stress can induce cellular damage. This dysregulation of oxidative stress makes the animal susceptible to diseases and is associated with many frequent physiological or pathological conditions (Preiser, 2012) [19]. Determination of the blood parameters profile is used for evaluating the individual's health conditions and monitoring the nutritional and metabolic conditions of the animals. It involves the laboratory facilities to arise at a conclusion. The levels of blood parameters are influenced by several factors such as sex, breed, age, stress, diet, handling, climate, physiological status and the laboratorial methodology (González and Silva, 2006 and Kaneko *et al*., 2008) [9, 13]. There is a dearth of literature concerning the relationship between BCS and oxidative stress in Marwari sheep. Thus, the aim of the present study is to investigate the oxidative stress biomarkers relationship with BCS for better prediction of health status and productivity of sheep. The haematological parameters like Hb, PCV and ESR are the tests that are used for quick screening the health and nutritional status of animals. Hence, these parameters are also taken under consideration to

find out possible relationship with Condition Scoring. Information provided in the present investigation will help the sheep owners to understand this basic technique for monitoring the health status and optimizing the returns from flock.

Material and Methods

Apparently healthy, 180 female Marwari sheep belonging to marginal farmers were screened from the in and around the areas of Bikaner district, Rajasthan. Sampling was completed in moderate and cold ambience during morning hours. Animals were categorised in 6 different groups according their Body condition score (BCS 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5). BCS was recorded as described by Jeffries (1961) [12] and Russel (1969) [21]. This system uses a six-point scale and it is the basis of the current condition scoring scale which was expressed from 1-5. Blood samples were collected in clean, dry test tubes with anticoagulant EDTA for haematological analysis and without anticoagulant for harvesting serum to estimate antioxidants. Total 90 samples were collected in each ambience from 6 months to 6 years aged animals. Serum Catalase activity was measured by colorimetric (Goth, 1991) [10] and serum gamma glutamyl transferase (GGT) was determined by spectrophotometric method (Wolf and Williams, 1973) [24]. Haemoglobin (Sahli's haemoglobinometer), PCV & ESR were determined by method as described by Jain (1986) [16].

Result and Discussion

1. Catalase and Gamma-Glutamyl Transferase

Table 1 represents the mean ± SEM values of serum catalase and gamma-glutamyl transferase in female Marwari sheep of different BCS i.e. 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 during moderate and extreme cold ambience. The overall mean values of serum catalase (kU L^{-1}) of female Marwari sheep during moderate and extreme cold ambience were 59.54 and 68.30, respectively. The overall mean values of serum catalase showed a significant ($p \leq 0.05$) increase during extreme cold ambience as compared to moderate ambience. The overall per cent increase during extreme cold ambience was 14.71 as compared to moderate ambience. The range of per cent increase was 6.56 to 24.56. According to the results cold conditions produced a stressful state resulting in excessive production of free radicals and oxidative stress, which is an imbalance between the oxidant and antioxidant systems (Nazifi *et al.*, 2009) [15]. The body's response to oxidative stress was almost entirely reflected by variations in the mean values of catalase in this study. Higher catalase activities were associated with the activation of the protection mechanism, whereas lower catalase activities were associated with a decrease in cellular resistance to oxidant-induced cell damage (Cam *et al.*, 2009) [5].

All the categories of BCS showed changes in mean values of serum catalase with progression of BCS in each ambience. Serum catalase activity was observed highest in BCS 2.5 and lowest in BCS 4.5. Increase activity in 2.5 BCS indicates increased cellular resistance to oxidant induced cell damage. The maximum per cent increase was observed in the mean values of BCS 3.0 (24.57) which indicated the shifting toward the defence mechanism to combat the harmful effect of free radicals. Minimum per cent increase was observed in the mean values in BCS 2.0 which indicates the inability of animals to raise their enzymatic antioxidant defence mechanism at sufficient level to escape from detrimental effect of free radicals.

The overall mean values of serum gamma glutamyl transferase (U L^{-1}) of female Marwari sheep during moderate and extreme cold ambience were 45.96 and 52.60, respectively. The overall mean values of serum gamma glutamyl transferase showed a significant ($p \leq 0.05$) increase during cold ambience (per cent increase 14.44) as compared to moderate ambience. The range of per cent increase from 2 to 4.5 BCS were 4.21 to 21.25. Maximum and minimum increases were observed in the mean values of 4.5 BCS and 2.5 BCS. According to Onat *et al.*, (2006) [17] increased serum GGT activity reflected increased oxidative stress, and therefore it can be used as a marker of both hepatic involvement and oxidative stress. Pareek (2020) [18] also observed elevated serum GGT levels due to superimposed stressors in Magra sheep. The thoroughness of the preceding discussion aided in concluding that elevated serum GGT levels in the current study indicated environmental stress culminating in to oxidative stress.

2. Haemoglobin, PCV & ESR

The mean ± SEM values of haemoglobin, PCV & ESR in female Marwari sheep of different BCS i.e. 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 during moderate and extreme cold ambience are presented in table 2. The overall mean values of haemoglobin showed a significant ($p \leq 0.05$) decrease during extreme cold ambience. The overall mean values of haemoglobin (g L^{-1}) in female Marwari sheep during moderate and extreme cold ambience were 104.14 and 99.27 respectively. The overall mean values were found to be decreased 4.67 per cent as compared to moderate ambience. The range of per cent decrease was1.57 to 12.02. The results of present study in cold ambience were similar to the earlier findings of Banerjee *et al.*, (2015) [3] in goat. Ferreira *et al.*, (2017) [8] observed decrease in Hb content from June to December from 109.5 to 88.2 in Morada nova sheep. The low Hb value is indicative of either decrease in the number of RBCs or increase in circulating plasma volume. All the categories of BCS showed an increase in haemoglobin level with increasing score. In the present study the haemoglobin level was low in the sheep of low body condition score and the mean values of haemoglobin were maximum in 4.5 BCS. Addass *et al.*, (2012) [2] also reported increase in Hb content with the advancement of BCS in bull. Yilmaz *et al.*, (2014) [25] reported similar trend of increase with advancement of body condition score in anaemic goat. The lower values may be due to the low body condition of the animals, advancement of BCS reflected their improved nutritional resources hence increase in values were observed. Maurya *et al.*, (2012) [14] also corelated the reason for greatest reduction in Hb in the lower BCS group of rams due to reduction in the synthesis of RBC and Hb.

The overall mean values of packed cell volume (%) of female Marwari sheep during moderate and extreme cold ambience were 30.75 and 28.79, respectively. The overall mean values of packed cell volume showed a significant ($p \leq 0.05$) decrease during extreme cold ambience. The PCV values were influenced not only by nutritional compromise conditions but also by season, age, parasitic infestation and physiological conditions. The overall mean values were found to be decreased 6.37 per cent as compared to moderate ambience. The range of per cent decrease was 4.83 to 8.30. The results of present study in cold ambience were more or less similar to the earlier findings of Abdelatif *et al.*, 2009 [1]; Rathwa, 2016

[20]; Sejian, 2013 [23] Indu *et al*., 2014 [11] and Pareek (2020) [18] in sheep and Banerjee *et al*., 2015 [3] in goat. The low PCV values indicate either decrease in the number of RBCs or increase in circulating plasma volume.

Table 1: Mean ± SEM values of Serum Catalase (U L^{-1}) and GGT (U L^{-1}) in Marwari sheep during moderate and extreme cold Environment temperature periods (ETPs)

Mean ± SEM values during moderate extreme cold Environmental temperature periods (ETPs)						
Categories	**Serum catalase (kU L^{-1})**			**GGT (U L^{-1})**		
	Moderate (90)	**Cold (90)**	**Overall (180)**	**Moderate (90)**	**Cold (90)**	**Overall (180)**
BCS 2.0 (15)	66.05 ± 5.91	70.69 ± 5.63	68.37^{ab} ± 4.03	49.88 ± 6.55	51.98 ± 5.38	50.93^{ab} ± 4.17
BCS 2.5 (15)	67.84 ± 5.57	80.00 ± 7.85	73.93^{b} ± 4.86	40.21 ± 3.03	44.93 ± 3.33	42.59^{a} ± 2.25
BCS 3.0 (15)	50.57 ± 3.41	67.04 ± 6.28	58.81^{a} ± 3.83	41.09 ± 3.76	45.95 ± 4.76	43.52^{a} ± 3.02
BCS 3.5 (15)	55.08 ± 5.19	62.84 ± 5.71	58.96^{a} ± 3.86	45.25 ± 5.08	54.74 ± 5.64	50.01^{ab} ± 3.83
BCS 4.0 (15)	59.63 ± 4.82	64.52 ± 5.61	62.08^{a} ± 3.66	48.48 ± 5.31	56.47 ± 5.72	52.48^{ab} ± 3.81
BCS 4.5(`15)	58.07 ± 4.83	64.76 ± 4.90	61.42^{a} ± 3.44	50.90 ± 5.31	61.50 ± 4.52	56.20^{b} ± 3.56
Overall (90)	59.54^{A} ± 5.91	68.30^{B} ± 5.57	-	45.96^{A} ± 2.09	52.60^{B} ± 5.39	-

Figures in the parentheses = number of Marwari sheep
ATP = ambient temperature period
BCS = body condition score
A, B = significant ($p \leq 0.05$) differences between overall mean values.
a, b, c = significant ($p \leq 0.05$) differences between mean values of a BCS in a row

Table 2: Mean ± SEM values of haemoglobin (g L^{-1}), PCV (%) and ESR (mm hr^{-1}) in Marwari sheep during moderate and extreme cold Environment temperature periods (ETPs)

Mean ± SEM values during moderate extreme cold Environmental temperature periods (ETPs)									
Categories	**Haemoglobin (gm/dl)**			**PCV (%)**			**ESR (mm/hr)**		
	Moderate (90)	**Cold (90)**	**Overall (180)**	**Moderate (90)**	**Cold (90)**	**Overall (180)**	**Moderate (90)**	**Cold (90)**	**Overall (180)**
BCS = 2.0 (15)	101.20 ± 2.46	89.04 ± 2.59	95.13^{a} ± 2.09	27.85 ± 0.80	26.04 ± 0.94	26.95^{a} ± 0.63	0.65 ± 0.12	0.64 ± 0.13	0.65 ± 0.08
BCS = 2.5 (15)	98.06 ± 3.80	93.36 ± 2.72	95.72^{a} ± 2.34	29.61 ± 0.72	27.15 ± 0.79	28.38^{ab} ± 0.57	0.65 ± 0.12	0.64 ± 0.12	0.65 ±0.08
BCS = 3.0 (15)	100.99 ± 4.48	94.35 ± 3.61	97.67^{ab} ± 2.89	30.15 ± 1.24	28.69 ± 0.91	29.42^{bc} ± 0.77	0.71 ± 0.10	0.67 ± 0.12	0.70 ± 0.07
BCS = 3.5 (15)	105.67 ± 4.72	104.01 ± 4.28	104.84^{bc} ± 3.13	31.08 ± 1.23	29.11 ± 1.07	30.10^{bc} ± 0.82	0.71 ± 0.10	0.71 ± 0.11	0.71 ±0.07
BCS = 4.0 (15)	108.49 ± 3.82	106.51 ± 3.26	107.50^{c} ± 2.47	32.25 ± 1.05	30.24 ± 1.03	31.25^{cd} ± 0.75	0.75 ± 0.10	0.71 ± 0.11	0.73 ± 0.07
BCS = 4.5 (15)	110.48 ± 3.66	108.33 ± 4.42	109.41^{c} ± 2.82	33.59 ± 1.08	31.54 ± 1.09	32.57^{d} ± 0.78	0.81 ± 0.09	0.78 ± 0.11	0.80 ± 0.07
Overall (90)	104.14^{A} ± 2.60	99.2^{B}± 2.72	-	30.75^{A} ± 0.94	28.7^{B} ± 0.79	-	0.71 ± 0.04	0.69 ± 0.05	-

Figures in the parentheses = number of Marwari sheep
ATP = ambient temperature period
BCS = body condition score
A, B = significant ($p \leq 0.05$) differences between overall mean values.
a, b, c = significant ($p \leq 0.05$) differences between mean values of a BCS in a row

All the categories of BCS showed a significant ($p \leq 0.05$) decrease in the values of packed cell volume. The findings of this study were similar to Maurya *et al.*, (2012) [14] and Carlos *et al.*, (2015) [6] in sheep. The decrease in PCV in low BCS may be attributed to reduction in synthesis of RBCs and Hb these findings were supported by the earlier work of Sejian *et al*., (2010) [22]. Addass *et al*., (2012) [2] and Yilmaz *et al*., (2014) [25] also reported increasing order of PCV with advancement of BCS. In a study positive correlation was observed between BCS and PCV, and it was claimed that BCS was a useful tool in estimating the energy and protein status of goats (Cabiddu *et al*., 1999) [4].

The overall mean values of erythrocyte sedimentation rate (mm hr^{-1}) of female Marwari sheep during moderate and extreme cold ambience were 0.71 and 0.69, respectively. The overall mean values of erythrocyte sedimentation rate showed a non-significant decrease during extreme cold ambience. The overall mean values were found to be decreased 2.81 per cent irrespective of BCS as compared to moderate ambience. The range of per cent decrease was 1.39 to 5.63. Lowering of ESR during extreme ambiences denoted lowering of plasma volume and heamoconcentration, and acts by decreasing blood viscosity (Pareek, 2020) [18]. ESR values are directly proportional to the temperature within physiological limits. The normal value of ESR in sheep is 0-1 (mm hr^{-1}). In the present investigation the value was found to be decreased. Charan (2002) [7] in goat also observed decrease in ESR during cold ambience.

All the categories of BCS showed a non-significant change in the values of erythrocyte sedimentation rate. In the present study the erythrocyte sedimentation rate was low in the sheep of low body condition score i.e. 2.0 and the mean values of erythrocyte sedimentation rate were maximum in 4.5 BCS. There is dearth of literature regarding the ESR and BCS in our study. It was observed increase in BCS resulted in increase in ESR in moderate as well as cold ambience. ESR is an important non-specific test that indicates an abnormal process and is useful in detecting occult disease. Therefore, it is used in screening of animals. There are physiological conditions associated with variations in ESR like anaemia, pregnancy and hydration status. Lowering of ESR during extreme ambiences denoted lowering of plasma volume and heamoconcentration, and acts by increasing blood viscosity.

Conclusion

The impact of cold environment was observed on oxidative stress parameters as well as on haematological parameters. The present investigation indicated that the optimum BCS to combat the oxidative stress were between 2.5- 3.0 hence the optimum score is necessary to avoid the untoward effect of climate

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