Biological Forum – An International Journal **15(10): 1671-1674(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Association of Some Haematological and Metabolic Parameters with Cold Stress in Female *Marwari* Sheep

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(Received: 25 August 2023; Revised: 25 September 2023; Accepted: 08 October 2023; Published: 15 October 2023)
(Published by Research Trend)

ABSTRACT: The study aimed to investigate the relationship between hematological and metabolic parameters in *Marwari* sheep in cold stress. A total of 180 animals were screened for sampling, 90 in moderate and 90 in cold environment. Blood samples were collected from healthy female *Marwari* sheep. Blood metabolites and hematological parameters were evaluated by their standard method. The parameters include metabolites (glucose, total protein, urea, creatinine, cholesterol) and hematological indicators (hemoglobin, PCV, ESR). The higher average values of metabolites (except total plasma proteins) cold environmental temperatures, suggesting that cold stress positively correlates to the metabolites. The lower average mean values of hematological parameters were observed in cold environmental temperatures, suggesting that cold stress negatively correlates to the hematological parameters. The findings from this study highlight that metabolic and haematological parameters behave differently as a result of changing seasons. Therefore, this study provided helpful insights into physiological adaptations towards environmental cold stress.

Keywords: Metabolites, Sheep, Haematological, Glucose, Cholesterol, Cold Stress, Correlation.

INTRODUCTION

Sheep are vital to the Indian agricultural economy because of their many uses, including meat, wool, skin, dung, and, to some extent, milk. India's sheep are among the most important livestock species that contribute significantly to the country's GDP, food supply, fiber production, and rural jobs. Sheep are an important livestock species that contribute significantly to the provision of food, fiber, rural jobs, and income in arid and semi-arid areas because of their many uses as supplies of milk, meat, wool, skin, and dung (Naqvi *et al*., 2013). Because of their many benefits, such as their capacity to survive in extremely severe settings, droughts, and famines, sheep have a well-established global relevance (Marino *et al*., 2015). Stress can lower an animal's resistance to infectious illnesses and output, leading to economic losses for owners (Kataria and Kataria 2006). Physiological and metabolic changes brought on by temperature stress are crucial for an animal's survival and ability to adapt (Sunwasiya *et al*., 2023). One of the main possible winter stresses for cattle is cold stress, which causes physiological reactions in homeotherms that are highly important and energy-demanding. In cold environments, homoeothermic animals must produce more heat from their food and bodies to deal with the stress caused by the cold, which increases the animals' energy requirements (Liang *et al*., 2018). Animals eat more during the winter because their basal metabolic rates are higher, which fulfills their body's need for glucose (Nazifi *et al*., 2003; Suhair and Abdalla 2013). When animals are under stress, both heat and cold trigger gluconeogenesis by secreting glucocorticoids, which helps to maintain blood glucose levels (Weber *et al*., 1965). In extreme cold conditions, sheep face multiple difficulties that induce a diverged response resulting in adaptive modifications that help them maintain bodily temperature. These metabolic adaptations include modifications to essential substances that support the synthesis of energy and the use of nutrients. Furthermore, hematological reactions, which include changes in hemoglobin levels and packed cell volume, are essential for sustaining the general health and wellbeing of the sheep.

MATERIAL AND METHODS

The study was carried out in 180 apparently healthy *Marwari* female sheep. 6 month to 6 yrs aged sheep were screened for sampling. The study was conducted in two different seasons, moderate and cold. Months of October and November are considered moderate ambience whereas months of November and December are considered as cold ambience. In each ambience, 90 blood samples were collected and the animals were categorized according to their body condition score.

Blood samples were collected through jugular veins during slaughter from private slaughter houses (Bikaner, Rajasthan, India). Blood samples were collected in different clean, dry test tubes with anticoagulants such as EDTA. A blood sample was centrifuged to separate the plasma for the purpose of estimating plasma metabolites. The following plasma metabolites and haematological parameters were measured in this study: total plasma proteins, plasma glucose, plasma urea, creatinine, cholesterol, haemoglobin, PCV and ESR. Plasma Glucose, Total Protein, creatinine and cholesterol were estimated by the standard method described by Oser (1976). Plasma urea was determined by the diacetyl monoxime method of Natelson (Varley, 1988). Haemoglobin (Sahli's haemoglobinometer), PCV & ESR were determined by method as described by Jain (1986).

Statistical Analysis. Average mean values and correlation between different parameters and significant levels were estimated by IBM SPSS v20.

RESULTS AND DISCUSSION

Results are represented in tabular form Table 1 represents the average mean values of metabolic and haematological parameters and Table 2 represents the correlation of different parameters in moderate and cold ambience. The findings therefore explain the relationship between metabolic indicators and haematological parameters in *Marwari* sheep during extreme cold environmental temperature periods (ETPs) as compared moderate values.

Table 1: Average Mean ± SEM values during moderate and extreme cold environmental temperature periods (ETPs).

Sr. No.	Parameters	Moderate	Cold
1.	Plasma Glucose	$2.82^{A} \pm 0.15$	$3.08^{B} \pm 0.16$
2.	Total Plasma Proteins	$60.91^{A} \pm 3.021$	$57.14^{B} \pm 2.15$
3.	Plasma creatinine	$71.35^{A} \pm 3.29$	$76.82^{B} \pm 5.20$
4.	Plasma Urea	$6.81^{A} \pm 0.55$	$7.41^{B} \pm 0.46$
5.	Plasma Cholesterol	$1.52^{A} \pm 0.05$	$1.73^{B} \pm 0.04$
6.	Hemoglobin	$104.14^{A} \pm 2.60$	$99.27^{B} \pm 2.72$
7.	PCV	$30.75^{A} \pm 0.94$	$28.79^{B} \pm 0.79$
8.	ESR	0.71 ± 0.04	0.69 ± 0.05

A, B = Significant ($p \leq 0.05$) differences between the overall mean values of both ambience

Plasma Glucose: In an extremely cold environment, the mean average plasma glucose levels increased significantly (Table 1). The percentage increase was 9.21. Higher glucose levels may be attributed to a greater need for energy to combat cold stress, which is met by consuming more food to increase the basal metabolic rate (Maurya *et al.*, 2013). Similar observations were obtained by Weber *et al.* (1965) and increase was credited to gluconeogenesis in stressed animals by secreting glucocorticoids, which act on the liver to increase blood glucose levels.

Plasma glucose shows highly significant positive correlation with haemoglobin (0.445**) and PCV (0.547**) (Table 2). Correlation between serum glucose and haemoglobin was highly significant, also reported by Bhartendu *et al.* (2016). Plasma glucose shows negative correlation with Urea(-0 .213*). It may be concluded that there is a change in glucose-rea relations between seasons, which indicates metabolic adaptations occur due to cold stress.

Total Plasma Proteins:

Table 2: Correlations between plasma metabolites and haematological parameters in cold stress.

	Glucose1	Protein1	Creatinine1	Urea1	Cholesterol 1	Haemoglobin 1	PCV1	ESR1
Glucose2	0.158	0.089	0.186	-.213*	-0.123	0.445**	0.547**	0.092
Protein2	-0.128	0.106	0.047	-0.028	-0.067	-0.122	-0.252*	-0.286**
Creatinine2	0.231*	-0.01	0.128	-.208*	-0.276**	0.210*	0.088	0.237*
Urea2	-0.015	0.107	-0.281**	.325**	0.004	0.17	-0.091	-0.129
Cholesterol2	-0.17	0.004	-.338**	0.17	0.124	0.043	-0.246*	-0.168
Haemoglobin2	.531**	-0.01	0.226*	-0.306**	-0.193	0.14	0.471**	0.169
PCV2	.591**	-0.154	0.167	-0.227*	-0323**	0.208*	0.251*	0.201
ESR2	-0.199	0.165	0.072	-0.224*	-0.183	0.085	0.039	0.047

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
1 moderate values
2 cold values

The average mean value of total plasma proteins was significantly lower in cold ambience as compared to moderate ambience (Table 1). The per cent decline in mean values was 0 per cent. Studies indicated that there was an excessive breakdown of proteins to fulfill the increased energy demand under cold stress.

Total plasma proteins shows significant negative correlation with PCV (-0.252*) and ESR (0-.286**) (Table 2) whereas weak negative correlation with plasma glucose. This negative correlation with PCV in cold stress suggests haematological adjustments.

Plasma Creatinine: Creatinine is the breakdown product of creatinine phosphate which is an energy source for muscles and protein metabolism. In the present investigation, the average mean plasma creatinine values were significantly higher (Table 1) in cold ambience as compared to moderate ambience.

Plasma creatinine showed significant negative correlation with plasma urea (-0 .208*) and plasma cholesterol (-0.276**) whereas significant positive correlation with plasma glucose (0.231*) and ESR (.237*). Plasma creatinine was negatively correlated with protein levels, although the effect was not significant. The results suggested that ample nutrition was provided to the animals, hence, limited break down of the proteins occurred as energy resources. Negative correlation with urea suggests that kidneys respond differently depending on whether they are functioning at normal or reduced levels.

Plasma Urea: Ureaisthe end product of protein metabolism. In ruminants, its value is also reflected by fermentation. Rumen protein metabolism is influenced by ruminal microorganisms' metabolic activity (Bach *et al.,* 2005). The excess ammonia that is released during the breakdown of amino-acids is converted to urea by the ornithine cycle in the liver. The urea forms, then enters the blood stream and some of it reaches to the rumen and is degraded by microbes.

In the present study, average mean values of plasma urea were significantly higher in cold ambience compared to moderate ambience (Table 1). Indu *et al*. (2014) postulate that the elevated blood urea nitrogen levels might be attributed to either ineffective absorption of rumen ammonia into microbial protein or hepatic deamination of amino acids released from the skeletal muscle. Cold temperatures had a significant impact on hepatocyte function, which has been shown by higher plasma urea (Nazifi *et al*., 2003).

Plasma urea shows significant negative correlation with plasma creatinine (-0.281**) and significant positive correlation with plasma cholesterol (0.325**) presented in Table 2. These findings suggest a complex interplay between urea, glucose, and creatinine that might signify changes in metabolic adjustments in response to temperature changes.

Plasma Cholesterol: Average mean values of plasma cholesterol were significantly high (Table 1) in cold ambience. The plasma cholesterol levels were higher in the winter than moderate ambience, and the high level of glucose results in less cholesterol being catabolized for energy production. Additionally, animals consume more feed in the winter than they do in the summer, which increases the possibility of elevated cholesterol levels during the winter (Rathwa *et al*., 2017). This increase in cholesterol could be related to increased thyroid activity in cold ambient temperatures because higher BMR is needed to maintain body temperature (Kataria *et al*., 2000), which increases the turnover of basic fuel molecules in blood.

Cholesterol shows significantly negative correlation with plasma creatinine (-0.338**) and PCV (-0.246*) (Table 2). An inverse association between cholesterol and packed cell volume is suggested by the negative correlation between the two, which may be caused by variations in blood volume or viscosity that impact PCV and cholesterol levels.

Haemoglobin: Average mean values of haemoglobin were significantly low in cold ambience as compare moderate ambience. The low haemoglobin readings suggest a reduction in red blood cells or an increase in the amount of plasma in circulation.

Haemoglobin shows highly significant positive correlation with plasma glucose (0.531**) and PCV (0.471**) (Table 2). The positive correlation with PCV reflects the interconnectedness of these haematological parameters. Haemoglobin and glucose levels have a highly significant positive connection that might be caused by glycation, haematological alterations, dehydration, or underlying medical diseases like diabetes mellitus, among other physiological and pathological processes.

Packed Cells Volume (PCV): Average mean values of PCV were significantly low in cold ambience as compare moderate ambience. The low PCV values suggest a reduction in red blood cells or an increase in the amount of plasma in circulation.

PCV has significant positive correlations with plasma glucose (0.591**), haemoglobin (0.208*), PCV (0.251*) and negative correlation with plasma urea (-0.227*) and cholesterol (-0.323*) presented in table 2. This relationship indicates that these indicators are influenced by common physiological processes, such as hemoconcentration, dehydration, or circumstances that impact red blood cell volume. Increased PCV and Hb values were also observed by Pareek and Kataria (2004) during complete water restriction of nine days in *Marwari* goat.

Erythrocyte Sedimentation Rate (ESR): Average mean values of ESR are non-significantly lower in cold ambience as compare to moderate ambience. ESR shows significant negative correlation with plasma urea (-0.224*). Erythrocyte sedimentation rate (ESR) and plasma urea have a negative connection, meaning that as ESR levels rise, plasma urea levels tend to fall and vice versa.

CONCLUSIONS

According to this study we concluded that sheep adapt to cold environments by altering their body's metabolic and haematological condition in order to manage stress.

Acknowledgements. The authors are greatly thankful to the Dean, College of Veterinary and Animal Science, Bikaner for providing facilities during this research work in Department of Veterinary Physiology.

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How to cite this article: Babita Kumari, Sunita Pareek, Ruchi Maan and Mohit Jain (2023). Association of Some Hematolgy and Metabolic Parameters with Cold Stress in Female *Marwari* Sheep. *Biological Forum – An International Journal, 15*(10): 1671-1674.