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Body Condition Score Versus Metabolites in *Marwari* Sheep under Cold Stress

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ABSTRACT

Background: The present study investigates the association between plasma metabolites and body condition score (BCS) in *Marwari* sheep under cold stress. BCS is considered as an easy, rapid and non-invasive assessment method for determining an animal's state of health. Animals modify their body's metabolic state to adapt and combat cold ambient temperature conditions.

Methods: The present study was conducted on apparently healthy *Marwari* sheep by screening 180 animals from marginal farmers in two different ambiences from in and around Bikaner district of Rajasthan. The study included 6 months to 6 years age group animals which were divided in to six different categories as 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5. The technique outlined by Jefferies (1961) and Russel (1968) was used to determine the body condition score of the sheep. Blood samples were collected during moderate and extreme cold ambiences in the morning hours from the slaughter house and health status was monitored by recording rectal temperature, respiratory rate and body condition score. Estimated plasma metabolites were glucose, total proteins, urea, cholesterol and creatinine.

Result: The plasma glucose concentration increased significantly in extreme cold environment compared to a moderate environment (increasing by 9.21%), Whereas total plasma protein concentrations decreased significantly under cold conditions by 7.66%. Similarly, plasma urea and cholesterol levels increased significantly (8.81% and 13.81% increase, respectively). The results suggest that a particular BCS is not sufficient to represent the health state of sheep. However, animals with a BCS of 2.5 to 3.5 demonstrated strong physiological adaptability to their surroundings, indicating that *Marwari* sheep exposed to cold may have an ideal BCS.

Key words: BCS (Body condition score), Cold stress, *Marwari*, Metabolites.

INTRODUCTION

Sheep is a multipurpose species of livestock in India and plays an important role in animal production by wool, meat, milk, skin and manure. Sheep husbandry is one of the fundamental occupations of poor farmers because it may survive with minimum expenditure and may tolerate extreme cold environmental temperature. Sheep rearing is an important income source of small, marginal and landless farmers due to its all-around utility, particularly those farmers who depends on livestock for their livelihood. In temperate climates, sheep may face a variety of environmental factors as well as scarcity of food (Verbeek, 2010). Low intake of food can affect body condition score (BCS) and live weight which can affect sheep welfare by increasing hunger and changing their metabolic status.

A number of non-specific regulatory processes has been established which may arouse due to environmental stress resulting in general adaptation of animals. The variations in different metabolites and blood constituents are necessary for physiological adjustment to changing ambient conditions. Numerous studies have reported that environmental factors cause the alteration in morphological traits and biochemical markers in animals (Celi, 2010). Animals have better morphological and physiological adaptations to climatic conditions, making studies in specific regions unreliable. Blood parameters and BCS are indicators of nutritional and health status, preventing decline in productivity (Kaczmarowski *et al.,* 2006). Adipose tissue and intramuscular fat represents the pool of nutrients and reserve that can be mobilized if required (Rauw, 2008).

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Body condition score (BCS) is a non-invasive, quick assessment technique for evaluating subcutaneous fat and muscle reserve in sheep. It allows for daily evaluation of 200-300 sheep, allowing for a single day inspection of a large flock or 2-3 small flocks. However, it is not widely practiced on farms. This technique was first published by Jefferies (1961) and the basic concept of this technique was to control the nutrition of sheep so that there will be optimum use of available nutrients and prompt steps can be taken timely to minimize the losses if there is decline in condition. It is a useful managemental tool for assessing body reserves (quantity of fat and muscle) on a mature sheep and enables

the livestock owner to maintain optimum health and production.

MATERIALS AND METHODS

Experiment design

The experiment was conducted at Department of Veterinary Physiology, Bikaner. For this study 180 apparently healthy female *Marwari* sheep of different age groups were screened from unorganized sector encompassing marginal owners from in and around Bikaner district, Rajasthan. The observations were recorded in two different phases: moderate and cold. Months of October and November considered as moderate ambience. The observations recorded during the months of December and January served as extreme cold ambience values when ambient temperature was extremely low. The results of this phase parameters were compared with control phase parameters. The body condition score of female *Marwari* sheep ageing more than 6 months to 6 years of age was recorded. According to the body condition score the animals were categorized in to six groups (i) 2 BCS, (ii) 2.5 BCS, (iii) 3 BCS, (iv) 3.5 BCS, (v) 4 BCS and (vi) 4.5 BCS during moderate and cold ambiences. Each category of body score comprised of 15 animals in each ambience. In each ambience 90 blood samples were collected in the morning hours from clinically healthy animals. Health status was accessed by recording rectal temperature, pulse and respiration rate. Blood samples were collected during slaughtering from the government slaughter house. The age, body weight and body condition score of each animal was recorded before slaughtering.

Body condition score (BCS)

In present investigation Body Condition Score was done by method described by Jefferies (1961) it was based on six-point scale from 0 to 6 and it included only the whole units. Later Russel *et al.* (1969) introduced the concept of 0.5 and 0.25 units. In present study we had followed the score of Jefferies (1961) and the modifications suggested by Russel *et al.* (1969) for 0.5 increment which indicates a middle value of the upper and lower score.

Plasma metabolites

Plasma Glucose was estimated by Folin-Wu method described by Oser, (1976). Plasma total protein was estimated by Biuret method as described by Oser (1976). Plasma creatinine was measured by using protocol of Folin and Wu (Oser, 1976). Plasma urea was determined by the diacetyl monoxime method of Natelson (Varley, 1988). Plasma cholesterol was measured by the procedure of Abell *et al.* (1958) as described by Oser (1976).

Statistical analysis

Data were analysed for ANOVA (2×6 factorial design, Snedecor Cochran, 1989) and were compared by Duncan's new multiple range test (Duncan 1955). Statistical significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Plasma glucose

The overall mean values of plasma glucose (m mol L^{-1}) of female *Marwari* sheep during moderate ambience and cold ambience were 2.82 and 3.08 (Table 1). The overall mean values of plasma glucose showed a significant increase (9.21%) as compared to moderate ambience. The range of per cent increase was 3.95 to 11.48 (Fig 1a). Maximum and minimum increases were observed in the mean values of 2.5 BCS and 4.0 BCS. All the categories of BCS in the present study showed significant effects of ambience on plasma glucose, indicating that cold affected the animals regardless of body composition in relation to body muscle and fat. Cold conditions function as stressors to the animal and there is increased secretion of glucocorticoids in animals. Glucocorticoids are well known for their gluconeogenic effect thereby increasing the blood glucose level and decreasing its peripheral utilization in stressed animals. Small ruminants have been found to exhibit elevated plasma glucose concentrations in cold environments (Zhang *et al*., 2022; García *et al*., 2020). The remarkable influence of glucose levels on all categories of sheep and goats were probably caused by increased energy consumption during cold stress (AL-Musawi *et al*., 2017). Higher blood glucose levels to the increased feed intake as a result of increased metabolism during extreme cold ambience (Maurya *et al*., 2013). Increased blood glucose levels helped the animal to fight against cold stress. Increased cortisol activity leads higher blood glucose levels during superimposed stressors in sheep (Pareek and Kataria, 2020). Increased glucose levels were due to activation of sympathetic nervous system and an increase in adrenal cortical activity (Avci *et al*., 2008). Catecholamine and glucocorticoids increase the glycogenolysis and gluconeogenesis and secondarily glycemia (Ganong, 1998).

All the categories of BCS showed a significant increase in the values of plasma glucose. In the present study the glucose level was less in the sheep of low body condition score. The decrease in plasma glucose concentration was related to less availability of substrate for glucose formation in the body of energy depleted sheep. Cows in moderate-to-thin conditions require more glucose than cows in obese conditions due to their higher maintenance needs. Hence, the higher demand for glucose in low-condition groups' blood glucose levels reduces (Singh *et al.,* 2009). Animals that have extremely excellent body conditions (3 to 5) may lose up to a point of body condition where they are unable to maintain appropriate blood metabolites, physiological responses and growth (Maurya *et al.,* 2012).

Total plasma proteins

The overall mean values of total plasma proteins (g L^{-1}) of female *Marwari* sheep during moderate and cold ambience were 60.91 and 57.14, respectively (Table 1). The overall mean values of total plasma proteins showed a significant

decrease (6.18%) as compared to moderate ambience. The results indicated that there is excessive breakdown of proteins as an alternate source to meet the higher demand of energy during cold stress. Halliwell and Gutteridge (1990) opined that total serum proteins and albumin are the potential targets of oxidative injury. Animals' blood cortisol levels can be stimulated via the hypothalamic-pituitary-adrenal and sympathetic-adrenal medullary axis to maintain homeostasis in response to environmental changes (Sejian *et al.,* 2018). Cortisol enhances the proteolysis and decrease the level of proteins.

All the categories of BCS showed a decrease in the values of total plasma proteins with advancement of score. The range of per cent decrease was 3.46 to 10.54 (Fig 1b). Maximum and minimum decrease were observed in the mean values of BCS 4.5 (10.53) and BCS 2.5 (3.45). The minimum loss of total plasma proteins occurred in 2.5 BCS is suggestive of better sustainability and maintenance of protein reserve of animal. BCS 4.5 animals were most severely stricken by extreme cold ambience and lost their plasma pool of proteins.

Plasma creatinine

The overall mean values of plasma creatinine (μ mol L^{-1}) of female *Marwari* sheep during moderate and extreme cold ambience were 71.35 and 76.82, respectively (Table 2). The overall mean values of plasma creatinine showed a significant increase (7.66%) as compared to moderate ambience. The normal values of metabolites can be affected by age and sex of animals. Metabolic status of the animal is reflected by levels of creatinine. Most of the creatinine excreted by urine originates from endogenous creatine. Creatine circulates in plasma and taken up by the muscles, where it stores energy in the form of phosphocreatine. In muscles phosphocreatine breaks to form inorganic phosphate and creatinine to provide energy. Serum creatinine is proportional to muscle mass (Kreider, 2003; Cirillo, 2010; Samra and Abcar, 2012) and increasing level represents the development of animal. In present study maximum mean values of serum creatinine during extreme cold condition were 90.94. The phenomenon of increase in creatinine may be a consequence of increased skeletal muscle breakdown or due to increased protein metabolism.

All the categories of BCS showed a significant increase in the values during cold ambience. The range of per cent increase was 6.08 to 13.89 (Fig 1c). The present study indicates the increasing levels of plasma creatinine with increase in BCS from 2.0 to 4.5 except 3.0. There was a non-significant change in the mean values of plasma creatinine in BCS 2.0, 2.5, 3.0 and 3.5 where as significant changes in mean values were observed in 4.0 and 4.5 BCS in respect to 2 to 3.5 BCS during moderate ambience. In the present investigation the mean values of plasma creatinine were maximum in 4.5 BCS. The high BCS indicates the increase body reserve which can be utilized to meet the requirement when nutrient supply is limited. During extreme cold condition the body reserves are mobilized to yield energy. The breakdown of reserves and combined effect of cold stress may be the reason for higher values of creatinine in the plasma. Kazumasa *et al.* (1998) explained that creatinine was an intrinsic substance which played an important role in oxidative stress and its products can be used as markers of oxidative stress. The maximum per cent variations were observed in the mean values of 2.0 BCS indicating that there was no fat reserve and for energy purpose there was mobilization of intrinsic substance leading to increased plasma creatinine levels.

Plasma urea

The overall mean values of plasma urea (m mol L^{-1}) of female *Marwari* sheep during moderate and extreme cold ambience were 6.81 and 7.41, respectively (Table 2). The overall mean values of plasma urea showed a significant increase (8.81%) as compared to moderate ambience. The range of per cent increase was 4.28 to 21.83 (Fig 1d). Maximum and minimum increases were observed in the mean values of 4.5 BCS

Table 1: Mean±SEM values of plasma glucose and total plasma proteins in *Marwari* sheep during moderate and extreme cold environment temperature periods (ETPs).

	Mean±SEM values during moderate extreme cold environmental temperature periods (ETPs)					
Categories	Plasma glucose (m mol L^{-1})			Total plasma proteins (g L^{-1})		
	Moderate (90)	Cold (90)	Overall (180)	Moderate (90)	Cold (90)	Overall (180)
BCS= 2.0 (15)	2.48±0.10	2.71±0.15	2.59^{a}±0.09	67.35±2.64	63.08±3.02	65.22^{c}±2.01
BCS= 2.5 (15)	2.70±0.09	3.01±0.16	2.86^{b}±0.09	62.15±3.98	60.00±2.15	61.08^{bc}±2.23
BCS= 3.0 (15)	2.78±0.15	3.08±0.14	2.93^{b}±0.10	57.45±2.91	54.78±2.56	56.12^{ab}±1.92
BCS= 3.5 (15)	2.90±0.15	3.12±0.12	3.01^{bc}±0.10	55.60±3.31	51.38±2.84	53.49^{a}±2.17
BCS= 4.0 (15)	3.04±0.13	3.16±0.12	3.10^{bc}±0.09	60.80±2.85	57.99±3.49	59.40^{abc}±2.23
BCS= 4.5 (15)	3.06±0.15	3.39±0.11	3.23^{c}±0.09	62.15±3.98	55.60±3.31	58.88^{abc}±2.61
Overall mean (90)	2.82^{A}±0.15	3.08^{B}±0.16	-	60.91^{A}±3.021	57.14^{B}±2.15	-

Figures in the parentheses= Number of *Marwari* sheep.

BCS= Body condition score.

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

a, b, c= Significant ($p \leq 0.05$) differences between mean values of different BCS in a column.

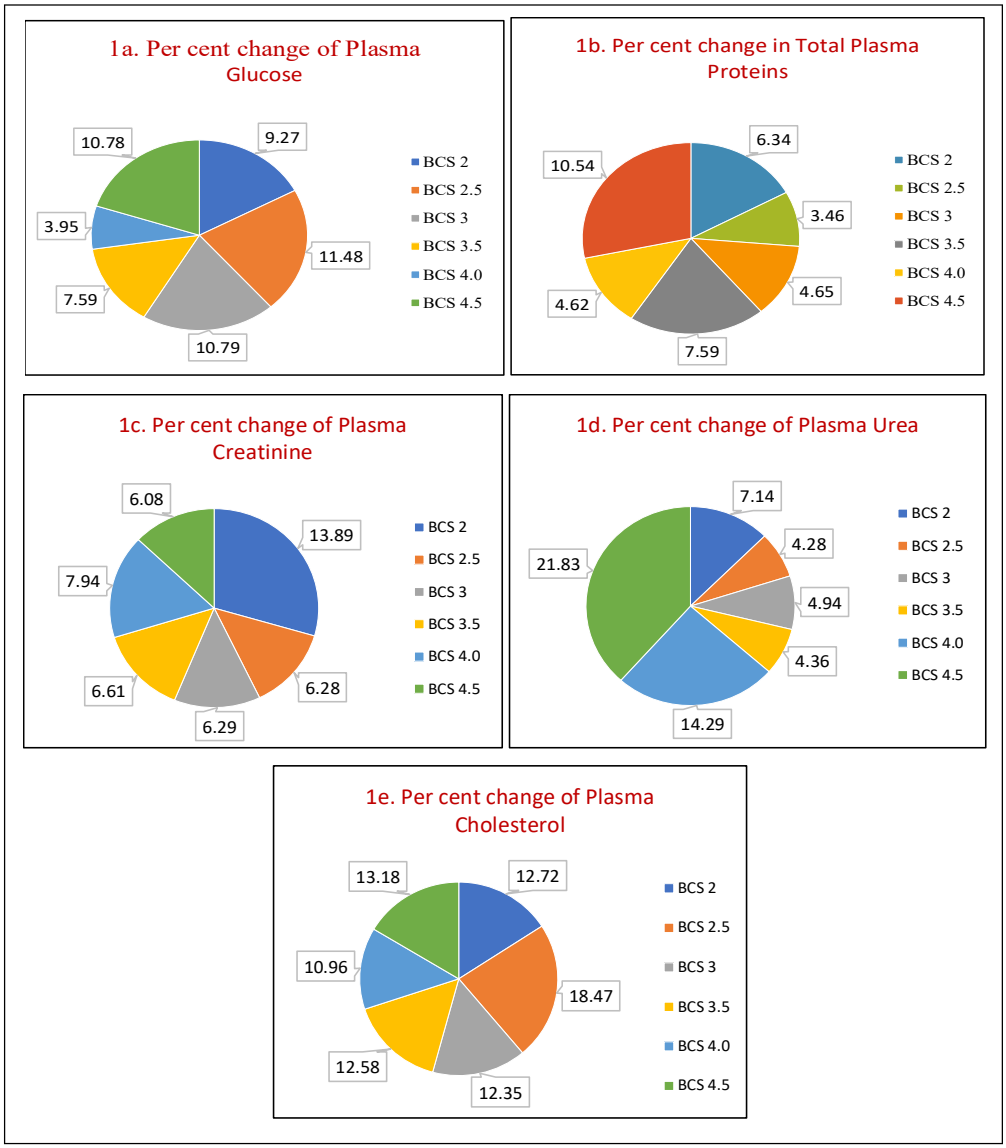

Fig 1: (1a to 1e) Per cent variations of plasma metabolites in *Marwari* sheep of different BCS represented by Piechart.

and 2.5 BCS. Stress from extreme environments can affect the urea cycle (Wotron,1971). Higher plasma urea showed increased hepatocyte activity, causing a profound effect during extreme cold conditions (Nazifi *et al.,* 2003). Earlier scientists also indicated the role of urea and possible development of oxidative stress due to extreme ambience. Chena *et al.* (2008) correlated the serum urea with the parameters of oxidative stress. This may be due to the fact that as a part of the oxidative stress mediated solute-signaling pathway in tissues, urea increases the expression of oxidative stress responsive transcription factors and is therefore associated with oxidative stress.

All of the categories of BCS showed a significant increase in the value of cold ambience. In the present investigation, the mean values of plasma urea were maximum in 2.0 BCS. The values fell progressively and was minimum in 4.5 BCS. However, Caldeira *et al.* (2007) and Carlos *et al.* (2015) observed highest plasma urea in 4 and 3 BCS, respectively and below that BCS there was a decrease in the plasma urea values were found. The maximum percentage change was observed in the mean values of 4.5 BCS. Similarly, total serum proteins showed maximum per cent change in 4.5 BCS. By comparing both the results, it can be interpreted that the breakdown of proteins resulted in increased plasma urea values.

Plasma cholesterol

The overall mean values of plasma cholesterol (m mol L^{-1}) of female *Marwari* sheep during moderate ambience were 1.52 and 1.73 respectively (Table 2). The overall mean values of plasma cholesterol showed a significant increase (13.81%) as compared to moderate ambience. The range of per cent increase was 10.96 to 18.47 (Fig 1e). Maximum and minimum increases were observed in the mean

Table 2: Mean ± SEM values of plasma creatinine, plasma urea and plasma cholesterol in *Marwari* sheep during moderate and extreme cold environment temperature periods (ETPs).

Categories	Mean±SEM values during moderate extreme cold environmental temperature periods (ETPs)									
	Plasma creatinine (μ mol L^{-1})			Plasma urea (m mol L^{-1})			Plasma cholesterol (m mol L^{-1})			
	Moderate (90)	Cold (90)	Overall (180)	Moderate (90)	Cold (90)	Overall (180)	Moderate (90)	Cold (90)	Overall (180)
BCS = 2.0 (15)	59.23±4.75	67.46±3.29	63.34^{a}±2.94	7.84±0.48	8.40±0.55	8.12^{d}±0.36	1.73±0.07	1.95±0.05	1.84^{d}±0.04
BCS = 2.5 (15)	68.18±4.48	72.46±5.20	70.32^{ab}±3.39	7.71±0.38	8.04±0.46	7.88^{cd}±0.30	1.57±0.07	1.86±0.04	1.72^{cd}±0.05
BCS = 3.0 (15)	64.25±4.18	68.29±4.55	66.22^{ab}±3.06	7.08±0.62	7.43±0.41	7.26^{bcd}±0.37	1.62±0.06	1.82±0.05	1.72^{cd}±0.04
BCS = 3.5 (15)	71.75±5.50	76.49±4.54	74.12^{bc}±3.53	6.88±0.42	7.18±0.49	7.04^{bc}±0.37	1.51±0.07	1.70±0.07	1.60^{bc}±0.05
BCS = 4.0 (15)	78.99±4.33	85.26±7.28	82.13^{cd}±4.20	6.02±0.61	6.88±0.42	6.45^{ab}±0.32	1.46±0.07	1.62±0.07	1.54^{b}±0.05
BCS = 4.5 (15)	85.73±4.15	90.94±3.12	88.34^{d}±2.60	5.36±0.41	6.53±0.56	5.95^{a}±0.35	1.29±0.7	1.46±0.07	1.37^{a}±0.05
Overall means (90)	71.35^{A}±3.29	76.82^{B}±5.20	-	6.81^{A}±0.55	7.41^{B}±0.46	-	1.52^{A}±0.05	1.73^{B}±0.04	-

Figures in the parentheses = Number of *Marwari* sheep.
BCS = Body condition score.
A, B = Significant (p≤0.05) differences between the overall mean values of both ambience.
a, b, c, d = Significant (p≤0.05) differences between the mean values of different BCS in a column.

values of 2.5 BCS and 4.0 BCS. Higher cholesterol levels can be attributed to higher thyroid activity in cold ambience as an increase in BMR is required with the need for extra production of heat to maintain body temperature (Kataria *et al.,* 2000) which increases turnover of basic fuel molecules in the blood. Baumgartner *et al.* (2022) reported that when newborn lambs are exposed to cold environments, their brown adipose tissues and plasma lipid undergo dramatic changes. This elevated lipid levels during cold stress is caused by the expression of genes (UCP2 and CPT1B) linked to fatty acid oxidation and thermogenesis.

All the categories of BCS showed a decrease in the value of plasma cholesterol in both the ambience. Significant differences in the overall mean values were observed in BCS 2.0, 3.5 and 4.5. In the present investigation, the mean values of plasma cholesterol were maximum in BCS 2.0. These animals represent the minimum amount of body reserve as fat. The higher BCS group represents a great amount of fat as body reserve and they moved their body reserve in cold stress. Caldeira and Portugal, (1991) observed significant changes in serum cholesterol only in increasing BCS state of animals, whereas in the other states, values remained unchanged, reflecting remarkable stability in concentrations because of structural roles. Increasing total cholesterol during the increase in BCS states may be due to greater availability of nutrients for their synthesis. Lower cholesterol values during food restriction may be explained by the sudden decrease in nutrient availability for synthesis and or a decrease in 3-hydroxy-3-methylglutaryl COA reductase by excess of hepatic ketone bodies (Vernon and Peaker, 1983) and reduction in hepatic synthesis of cholesterol transporter lipoproteins (Remesy *et al.,* 1983).

CONCLUSION

It is clear that a single BCS should not be considered ideal from a biological or economic standpoint based on the data collected for this study. These results suggest that more data is needed before a definitive conclusion can be made. However, in terms of their metabolic state, the animals with BCS 2.5 to 3.5 were better able to adapt and withstand the challenging environmental conditions.

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Conflict of interest

The authors declare no conflict of interest.

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