

COMPARATIVE ANALYSIS OF SERUM BIOCHEMICAL PARAMETERS DURING DIFFERENT PHYSIOLOGICAL CONDITIONS WITHIN HASSAN BREED OF SHEEP IN KARNATAKA

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ABSTRACT

Serum from Hassan sheep in different physiological conditions like pregnancy and lactation were collected and analyzed in order to establish the normal basal reference values for thirteen biochemical parameters. The results of present study revealed no significance difference in the values of total protein, albumin, urea, sodium, ALT, creatinine, total bilirubin, direct bilirubin, AST and total cholesterol between pregnant and lactating ewes, Whereas significantly higher values of serum calcium and plasma potassium was observed in pregnant ewes compared to lactating ewes and significantly higher serum glucose was observed in lactation ewes compared to pregnant ewes. The outcome of the present study clearly shows that the physiological status significantly affects the blood metabolic profile in Hassan breed of sheep.

Key words: Hassan Sheep, Lactating, Pregnant, Serum biochemical parameters

Small ruminants like sheep and goats are considered to be as poor man's cow meaning that it gives handy and easy economic incentives to the farmers who are the backbone of the Indian agriculture. India ranks 3rd in sheep population, there are about 60 sheep breeds in India including well recognized, lesser known and some wild species. Hassan sheep is one of the well recognized sheep breeds by NBAGR (National Bureau of Animal Genetic Resources) widely distributed in and around Hassan district of Karnataka. Health status of animals is easily accessed by looking into the serum biochemical parameters, good healthy animals are said to be the better breeding stock for future generation. Serum biochemical values are an important tool for assessment of the health status this has been shown to vary in healthy animals depending on physiological status of animals. During the physiological status like pregnancy and lactation, these parameters are known to vary a lot. Because there is very limited data available on basal biochemical parameters of pregnant and lactating animals of this breed, present work was conducted for the first time in order to establish the normal baseline values for various serum biochemical parameters under different physiological status in Hassan sheep.

MATERIALS AND METHODS

Pure bred Hassan sheep flocks were identified by evaluating the sheep flocks located in and around Hassan for breed specific morphological characteristics as given by NBAGR. Only flocks where in vaccination and

deworming were done on regular basis animals, no histories of disease outbreak in past three years were selected.

From each flock, 2 groups of animals i.e., pregnant and lactating animals of >2 years of age were selected, in order to get reliable and detailed information on the changing biochemical parameters under different physiological conditions. However, for healthy adult animals (< 2 years of age), non-lactating and non-pregnant animals, biochemical parameters have been analyzed recently from our lab (<http://ijlr.org/issue/study-on-serum-biochemical-profile-in-hassan-sheep-of-karnataka/>).

Blood samples, each of 5-10 ml, were collected from jugular vein of each animal into sterile vacutainer with and without anticoagulant. Samples were kept immediately after clotting, at about 4° C (ice pack box) and brought to the laboratory and the tubes were centrifuged at 4000 RCF for 10-15 min (REMI centrifuge India pvt. Ltd). Separated serum and plasma samples were collected into fresh sterile, microfuge tubes, stored at -20° C until analyzed.

All the serum samples were analyzed using automated serum analyzer. All reagents and kits for analysis were procured from SWAMED India Pvt. Ltd. On the day of blood collection, parameters like serum glucose and creatinine were analyzed and samples were preserved at 4-8° C for other parameters. Plasma sample was specifically used to estimate the potassium concentration in order to avoid the hemolysis. Within 48 hours, all other serum parameters were analyzed as per the manufacturer's instruction and serum samples were stored at -20° C.

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The data obtained in the present study were subjected to statistical analysis by using the GraphPad Prism version 5.01 (2007), by applying Student t test to analyse statistically significant difference between groups. The values were expressed as mean \pm standard error and the level of significant difference was determined at P value of 0.05.

RESULTS AND DISCUSSION

The present study revealed no significant difference in the values of total protein, urea, albumin, sodium, ALT, creatinine, total bilirubin, direct bilirubin, AST and cholesterol between pregnant and lactating ewes (Table 1). Whereas, significantly higher value of serum calcium and potassium was observed in pregnant ewes than in lactating ewes and significantly higher serum glucose was observed in lactation ewes compared to pregnant ewes.

Hassan sheep belongs to the southern peninsular region of the Indian sheep breeds. Several serum biochemical parameters are known to vary parallel to physiological change in ruminants and sheep after and before parturition (Salem, 2017) during which the nutritional requirements of ewes were much greater than the normal conditions. In the present study (Table 1), glucose concentrations were found to be in accordance with the previous experimental studies that recorded serum glucose levels to be higher in lactation than pregnancy in ewes (Bahman *et al.*, 2010; Moallem *et al.*, 2012; Mohammadi *et al.*, 2016). The increased glucose

level may reflect the recovery of feed intake and improving energy status of the ewe after lambing. The pregnancy and lactation in sheep lead to altered metabolism (Krajnicakova *et al.*, 2003) and during lactation, the mammary gland secretory cells use about 80% of blood components for milk synthesis (Bonev *et al.*, 2012). Reduced glucose level during pregnancy is attributed to glucose demands of the fetal-placental unit in pregnant ewes. Approximately 60% of fetal growth is known to take place during the last six weeks of pregnancy and within this period fetal growth is rapid and the demand for energy in terms of glucose increases remarkably (Moallem *et al.*, 2012).

In the present study (Table 1), the value of plasma potassium was significantly lower in lactating ewes as compared to pregnant animals as seen in an earlier study (Faith *et al.*, 2017). This might be attributed to the secretion of the increased aldosterone level which plays major role in maintaining the serum sodium and plasma potassium level. Also, similar information is perceived non-significantly in Buchi sheep (Akhtar *et al.*, 2015). This could be attributed to the variation pertaining to the breeds. Serum sodium concentration in the present study (Table 1) is comparable with the earlier reports by Sharma *et al.* (2015) for both pregnant and lactating ewes. Though the values reported by Faith *et al.* (2017) are found to be little higher than the present study but significant difference was noticed between pregnant and lactating ewes. This could be attributed to seasonal variation. Other studies on Buchi ewes reported no significant difference in the serum sodium, potassium concentrations during different physiological conditions (Akhtar *et al.*, 2015) which might indicate that regional and climatic conditions may play role in the serum parameters.

In the present study (Table 1), serum calcium level were found to be significantly higher in pregnant ewes as compared to lactating ewes; similar findings are also reported on Nari Suwarna breed of sheep (Talawar *et al.*, 2016). However, the result of present study suggest that calcium-levels depend on physiological status of ewes and the increased calcium level during pregnancy is probably attributed to increase of intestinal absorption of Ca and bone resorption because of hormonal changes during pregnancy (Abdelrahman *et al.*, 2002). In addition, significantly lower serum calcium during lactation clearly indicates the excessive drain of calcium through milk. Hypocalcemia in cows during lactation is a serious condition but in lactating ewes though it does not impose much mortality and economic loss, this condition can be reversed back with sufficient dietary supplements during pregnancy and also after parturition. Changes in the

Table 1
Serum biochemical values (Mean \pm S.E.) of pregnant and lactating ewes

Biochemical parameters	Pregnant ewes (n=26)	Lactating ewes (n=26)
Total protein (g/dl)	6.99 \pm 0.46	7.23 \pm 0.49
Albumin (g/dl)	3.23 \pm 0.18	2.98 \pm 0.2
Glucose (mg/dl)	49.31 \pm 4.38 ^a	58.65 \pm 4.12 ^b
Creatinine (mg/dl)	0.78 \pm 0.05	0.79 \pm 0.11
ALT (U/L)	20.28 \pm 1.38	19.88 \pm 2.3
AST (U/L)	98.16 \pm 3.43	96.72 \pm 7.48
Sodium (mEq/L)	149.1 \pm 0.46	147.82 \pm 0.2
Calcium (mg/dl)	7.53 \pm 1.64 ^a	3.47 \pm 0.24 ^b
Potassium (mEq/L)	5.11 \pm 0.76 ^a	3.1 \pm 0.4 ^b
Cholesterol (mg/dl)	67.53 \pm 8.3	64.25 \pm 7.34
Total Bilirubin (mg/dl)	0.09 \pm 0.01	0.07 \pm 0.02
Urea (mg/dl)	47.7 \pm 4.76	49.83 \pm 4.86
Direct Bilirubin/conjugated Bilirubin (mg/dl)	0.12 \pm 0.03	0.09 \pm 0.04

Values with different superscript within a row differ significantly at p<0.05

concentrations of electrolytes in blood of ewes, particularly during the lactation were mainly associated with increased requirements for both milk production and maintenance. Lactation in many species is associated with marked loss of energy through the milk which needs to be fully compensated by feed intake.

Serum albumin concentration in the present study is similar to the findings of Sharma *et al.* (2015), higher serum values are noticed in pregnant ewes compared to the lactating ewes (Whitney *et al.*, 2009).

Findings of concentration of total protein level in pregnant ewes are similar to the findings of Sharma *et al.* (2015). Earlier reports of Talawar *et al.* (2016) on Nari-Suwarna breed of sheep depicted that values of total protein are significantly higher in lactating than pregnant ewes, similar findings are also noticed in the present study.

Values of serum cholesterol concentration in the present study (Table 1) is comparable with that of the findings of Ercan *et al.* (2016), however slightly higher values were noticed in pregnant ewes than lactating which is similar to the reports of earlier studies (Balikci *et al.*, 2007). This increase in values during pregnancy appears to be related to energy demands of the fetal-placental unit in pregnant ewes (Schlumbohm *et al.*, 1997).

Serum creatinine level (Table 1) is comparable to the values reported by Ercan *et al.* (2016) and found to show no significant difference between pregnant and lactating ewes in the present study, on the other hand significant difference in serum creatinine level was noticed in the recent studies (Faith *et al.*, 2017). This could be attributed to the quantity of creatinine formed each day depends on the total body content of creatine, which in turn depends on dietary intake, rate of synthesis of creatine, and muscle mass of the animal. Values of serum urea reported in the current study (Table 1) for pregnant ewes are comparable with the reports of Mohammadi *et al.*, 2016; however, the values are higher than the values reported on Nari Suwarna breed of sheep (Talawar *et al.*, 2016). Moreover, greater urea concentration in the current study is observed in lactating ewes compared to pregnant is considered to be as a result of catabolizing muscles protein when large amount of body reserves is mobilized during lactation (El-sherif and Assad, 2001).

The values of total and conjugated bilirubin in the present study (Table 1) are found comparable and to be slightly lower than the values reported recently in cross-bred goats (Cepeda-Palacios *et al.*, 2017). Further, it is also noticed that serum concentration of total and conjugated bilirubin did not varied significantly between the groups.

The concentration of serum enzymes like ALT and AST in the present study (Table 1) is comparable to that of studies of Antunovic *et al.* (2011). In addition, earlier studies revealed that there is significant variation in the concentration of ALT between pregnant and lactating ewes (Antunovic *et al.*, 2011). However, these serum enzymes did not show any significant variation between the groups in the current study.

CONCLUSION

From outcome of the present study, it can be concluded that serum biochemical parameters vary due to increased metabolic activities in different physiological status like pregnancy and lactation in Hassan breed of sheep. This study also paves the way for further well-planned intensive investigation of hormonal/endocrine profile during different physiological stages in Hassan sheep.

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