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Research Article
Metabolic Stress Indicators in Ewes (*Ovis aries*) under Post-parturient and High Protein Diet Conditions

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Abstract

Background and Objective: Metabolic stress negatively impairs animal health and production. Investigating the changes in the behavioural and hemato-biochemical profile of metabolic stressed animals could serve as clinical markers of metabolic stress. Identification of such indicators could lead to the improvement of treatment success in metabolic disorders. The present study aimed to investigate the indicators of metabolic stress under post-parturient and high protein diet conditions in ewes via changes in behavioural, haematological, serum metabolites, urinary metabolites, serum and urine cortisol, metabolic hormones and electrolyte and enzymatic parameters. **Materials and Methods:** One hundred and twenty female cycling ewes of 2.5-3 years of age were selected and assigned to three groups, group-I: Control (n = 40), group-IIa: Post-parturient (n = 40) and group-IIb: High protein diet (n = 40). Whole blood was collected and used for haematological assay. Serum was used for estimation of biochemical, enzymatic, hormonal and electrolyte analysis. **Results:** Higher concentrations of Non-esterified fatty acids (NEFA), β-hydroxybutyric acid(β-OHB), cortisol and calcium in the serum of post-parturient ewes (group-IIa) compared to high protein diet (group-IIb) and control ewes (group-I). Changes in concentrations of biochemical parameters (glucose, cholesterol, total NEFA, β-OHB, T3, T4, insulin growth factor-1 (IGF1) insulin, calcium, magnesium, alkaline phosphatase and lactic dehydrogenase) indicated an energy insufficiency of ewes in metabolic stressed ewes (Group-II). Increase of White Blood Cells (WBC) count, hemoglobin and haematocrit values and behavioural changes were also observed in post-parturient ewes (group-IIa). **Conclusion:** Metabolic stress has a significant influence on serum levels of glucose, urea, ammonia and β-OHB, stress related hormones, calcium, magnesium, Alanine Aminotransferase (ALT) and Lactate Dehydrogenase (LDH).

Key words: Biochemical changes, behavior response, metabolic stress, post-parturient, high protein feeding

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Stress is characterized as inconvenient impact of collection of variables on well-being and generation of living organisms[1]. Stress results in declined reproductive and productive performance of an animal as it affects estrus cycle, increase percentage of abnormal ova production and higher incidence of embryonic and fetal mortality rate[2]. These physiological expressions had been linked to distorted endocrine functions[3]. Metabolic stress due to post-parturient conditions and imbalanced feeding might cause alterations in the biochemical composition of body fluids which negatively impact the reproductive performance of bovines and ewes[2,4,5]. Up to certain levels of metabolic stress, the animal attempts to overcome by behavioral and physiological responses. However, further increase in metabolic stress renders the animal unable to overcome the stress and leads to pathological responses[6]. Identification of potentially harmful metabolic imbalances could lead to the improvement of treatment success in metabolic disorders[7]. The indicators of nutritional stress in cattle include non-esterified fatty acids (NEFAs), β-hydroxybutyric acid (β-OHB), glucose, triglycerides and cholesterol[8]. Similarly, increased NEFA and β-OHB serum concentrations had been used as markers of excessive negative energy balance (NEB) and was associated with increased risk of developing postpartum disorders in bovines[9-11]. On the other hand, levels of certain metabolic hormones (insulin and thyroid hormones (T3:3,3',5-triiodothyronine and T4-thyroxin) were also found to vary with physiological status of animals[12].

Animal's adaptive response due to stress was hormone responsive that directly affects the health of animals. At the time of stress, adrenal gland plays key role in hormonal reactions and involves in both hypothalamic-pituitary-adrenocortical axis and the sympatho-adrenomedullary system functions. Heat stress has profound effect on haematological and biochemical parameters of ruminants[13]. Similarly, serum, follicular, oviductal and uterine fluid metabolite concentrations has been used as indicators of stress in ruminants[2,4,14]. Preferably, the assessment of stress should be based on a combination of biochemical, physiological responses, haematological and behavioural variables rather than a single measurement. Till date, no comprehensive study was reported to investigate the indicators of metabolic stress in ewes. Hence, the present study aimed to investigate serum metabolites, urinary metabolites, serum and urine cortisol, metabolic hormones, ions, enzymes, haematological parameters, clinical manifestations and behavioural changes as indicators of metabolic stress (post-parturient or fed with high protein diet in ewes (*Ovis aries*).

MATERIALS AND METHODS

Unless otherwise stated, media and chemicals were purchased from Sigma Chemicals (St Louis, MO, USA). The chemicals were of analytical grade. Selection of animals and their management were performed as per earlier study[5,14]. The experiments were conducted during February-April, 2016 under good laboratory conditions according to the guidelines of Organization for Economic Co-operation and Development (OECD)[15].

Sample collection: Investigations were carried out during moderate climate when the average temperature was 23.5°C. The blood was collected from the jugular vein (10 mL) into the vacuum tubes for further processing. Blood samples were taken in the morning before the first feeding of the day and again after 3 h of first feeding to negate the effect of diurnal variation, feed intake and comfort. Blood samples were kept at room temperature for 30-60 min and were centrifuged (1500×g at 4°C for 15 min). After centrifugation, serum was separated and stored in plastic vials at -80°C until analyzed. Urine samples were collected into 50 mL plain urine pots and placed on ice. Urine samples were stored in plastic vials at -80°C until analyzed.

Estimation of metabolites, ions, enzymes: Serum was subjected to biochemical analysis [metabolites: Glucose, cholesterol, triglycerides, urea, ammonia, total non-esterified fatty acids, β-hydroxybutyric acid, creatinine, uric acid and total protein, ions: Sodium, potassium, chloride, calcium, phosphorus and magnesium, enzymes: Acid phosphatase (AST), alkaline phosphatase (ALT) and lactate dehydrogenase (LDH)]. Metabolites and ions were analyzed by using a clinical analyzer (Photometer, Erba-Chem-5 Plus, Transasia, Mumbai, India). Reagent kits used for estimation of glucose, cholesterol, triglycerides and urea, uric acid, total protein were obtained from Span Diagnostics (Bangalore, India). Ammonia, NEFA and β-OHB kits were from Randox laboratories, UK. Estimation of enzymes (AST, ALT and LDH) was made using the Clinical chemistry analyzer, Trace-40 (Fosumed Tech Development Co. Ltd, Shanghai, China). Reagent kits for estimation of enzymes were from Human Gesellschaft fur Biochemica and DiagnosticambH (Wiesbaden, Germany). All measurements

were carried out according to the manufacturer's instructions. The intra- and inter assay coefficients of variation for all analyses were below 5%.

Haematological analyses: Haematological analyses were performed on the 2.5 mL individual blood samples collected into a vacuum plastic tube containing sodium heparin as an anticoagulant[16]. The blood samples were analyzed using an automatic photometer mentioned earlier for haematological assays [White Blood Cell (WBC) count, Red Blood Cell (RBC) count, Haemoglobin (Hb), hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and platelet count].

Estimation of metabolic hormones: Concentrations of total T3, T4, TSH, insulin and cortisol in serum and cortisol in urine were determined using commercial kits for clinical use in humans (AB Diachem System Pvt Ltd, New Delhi, India) by Enzyme Linked Immunosorbent Assay (ELISA). Concentrations of total IGF-I in serum were determined by using commercial kits for clinical use in humans (ABCAM, India) by ELISA. The kits were calibrated for ovine sample before used. All samples were assayed in the same ELISA kit to eliminate inter-assay variability.

Symptoms and behavioural changes: The signs and symptoms of the metabolic stress were recorded and the effect of stress was evaluated by scoring the number of vocalizations, foot pawings, circling attempts[17], the time spent with lying down and maintenance behaviours (eating and drinking)[18] were recorded. The behavioural tests lasted for 2 h in each session and were divided into two 1 h intervals. Total three sessions were performed.

Statistical analysis: Each sample was examined in quadruplicates and the mean values for the quadruplicates were calculated and used for analysis. Results were expressed as Mean±S.E.M. The data were analyzed by one way ANOVA followed by Tukey's multiple comparison tests[2]. For behavioral changes, non-parametric statistical tests (Friedman non-parametric test and Dunnett *post-hoc* test) were used[17-19]. A value of $p<0.05$ was considered statistically significant.

RESULTS

Serum metabolites: The serum levels of glucose and total cholesterol were significantly higher ($p<0.05$) in metabolic stressed [post-parturient (Group-IIa) and high protein diet fed ewes (Group-IIb)] compared to control (Group-I) ewes (Table 1). The β-OHB and total NEFA levels were significantly higher ($p<0.05$) in serum of metabolic stressed (post-parturient, Group-IIa) ewes compared to serum of high protein diet (Group-IIb) fed ewes and control ewes (Group-I). Ammonia and urea levels were significantly higher ($p<0.05$) in serum of high protein diet fed ewes (Group-IIb) compared to control and metabolic stressed (post-parturient, Group-IIa) ewes. No significant change was obtained in serum triglyceride, creatinine, uric acid and total protein levels in control (group-I) and metabolic stressed ewes (Group-IIa and Group-IIb).

Table 1: Concentrations of metabolites in control and metabolic stressed ewes serum

	Control	Metabolic stressed	
Metabolites	Group-I	PP (Group-IIa)	HP (Group-IIb)
Glucose (mM)	1.87±0.16[a]	1.14±0.11[b]	1.17±0.14[b]
Triglycerides (mM)	0.23±0.04	0.21±0.02	0.24±0.07
Cholesterol (mM)	3.57±0.25[a]	2.27±0.18[b]	2.25±0.31[b]
Total NEFA (μM)	81.40±3.80[a]	140.40±2.60[b]	84.50±3.20[b]
β-OHB (mM)	0.31±0.05[a]	0.53±0.01[b]	0.33±0.09[a]
Ammonia (μM)	102.30±8.60[a]	106.70±7.20[a]	157.40±10.2[b]
Urea (mM)	4.60±0.72[a]	4.70±0.53[a]	7.40±0.64[b]
Creatinine (μM)	42.70±5.20	40.70±6.10	43.70±4.80
Uric acid (μM)	4.20±0.22	4.40±0.16	4.60±0.27
Total protein (g L^{-1})	26.50±3.19	28.30±2.87	29.40±4.21

Superscripts bearing different letters in the same row differ significantly ($p<0.05$). NEFA: Non esterified fatty acids, β-OHB: Beta-hydroxybutyric acid, PP: Post-parturient ewes, HP: High protein diet fed ewes

Table 2: Concentrations of stress related hormones in serum of control and metabolic stressed ewes

	Control	Metabolic stressed	
Hormones	Group-I	PP (Group-IIa)	HP (Group-IIb)
T_3 (nmol L^{-1})	1.86±0.35[a]	1.44±0.26[b]	1.59±0.29[c]
T_4 (nmol L^{-1})	60.10±0.29[a]	42.21±0.31[b]	48.10±0.19[b]
TSH (ng mL^{-1})	0.67±0.09[a]	0.53±0.06[b]	0.65±0.02[a]
Cortisol (ng mL^{-1})	8.10±1.45[a]	31.40±1.24[b]	22.20±2.14[c]
IGF-1 (ng mL^{-1})	20.20±1.26[a]	14.30±1.19[b]	18.10±1.79[a]
Insulin (IU mL^{-1})	1.44±0.47[a]	1.19±0.39[b]	1.17±0.51[b]

Superscripts bearing different letters in the same row differ significantly ($p<0.05$). T3: 3-3'-5-triiodothyronine, T4-thyroxin, TSH: Thyroid stimulating hormone, IGF-I: Insulin like growth factor-I, PP: Post-parturient ewes, HP: High protein diet fed ewes

Metabolic hormones: The levels of T_3 and T_4 hormones were significantly lower in metabolic stressed (post-parturient, Group-IIa) and high protein diet fed (Group-IIb) compared to control (Group-I) ewes (Table 2) . Significantly high T_3 level was observed in high protein diet fed (Group-IIb) ewes compared to post-parturient (Group-IIa) ewes. The TSH and IGF-I levels were significantly lowered ($p>0.05$) in metabolic stressed ewes (post-parturient, Group-IIa) group compared to control group

(Group-I) and high protein diet fed (Group-IIb) ewes. The cortisol level was significantly higher ($p<0.05$) in post-parturient (Group-IIa) ewes compared to control (Group-I) and high protein diet fed (Group-IIb) ewes. Similarly, the cortisol level was significantly higher ($p<0.05$)in high protein diet fed (Group-IIb) ewes compared to control (Group-I). Insulin level was significantly higher ($p<0.05$) in metabolic stressed (post-parturient, Group-IIa) and high protein diet fed (Group-IIb) ewes compared to control ewes (Group-I).

Serum electrolytes: Calcium and magnesium levels were significantly higher ($p<0.05$) in metabolic stressed (post-parturient, Group-IIa) ewes compared to high protein diet fed (Group-IIb) group and control (Group-I) ewes (Table 3). No significant changes were observed in phosphorus, sodium, chloride and potassium levels between all the groups.

Table 3: Concentrations of serum electrolytes of control and metabolic stressed ewes

	Control	Metabolic stressed	
Serum electrolytes	Group-I	PP (Group-IIa)	HP (Group-IIb)
Calcium (mM)	7.4±0.260[a]	9.4±0.290[b]	7.6±0.340[a]
Phosphorous (mM)	5.6±0.640	5.5±0.580	5.6±0.520
Sodium (mM)	136.1±16.82	132.6±15.41	130.4±17.25
Chloride (mM)	128.4±10.25	126.5±9.980	126.8±12.12
Potassium (mM)	14.2±2.220	14.3±1.950	14.2±2.480
Magnesium (mM)	2.4±0.800[a]	1.6±1.240[b]	2.6±0.500[a]

Superscripts bearing different letters in the same row differ significantly ($p<0.05$). PP: Post-parturient ewes, HP: High protein diet fed ewes

Table 4: Concentrations of enzymes in serum of control and metabolic stressed ewes

	Control	Metabolic stressed	
Enzymes	Group-I	PP (Group-IIa)	HP (Group-IIb)
AST (U L^{-1})	104.20±9.680	106.3±8.890	109.4±8.830
ALT (U L^{-1})	22.62±6.850[a]	16.3±7.210[b]	15.4±5.540[b]
LDH (U L^{-1})	442.18±33.25[a]	390.4±30.42[b]	384.6±29.85[b]

Superscripts bearing different letters in the same row differ significantly ($p<0.05$). AST: Acid phosphatase, ALT: Alkaline phosphatase, LDH: Lactate dehydrogenase, PP: Post-parturient ewes, HP: High protein diet fed ewes

Serum enzymes: Significantly lower ($p>0.05$) ALT and LDH levels were observed in metabolic stressed ewes (post-parturient, Group-IIa) and high protein diet fed (Group-IIb) compared to control (Group-I) ewes (Table 4). No significant changes were observed, ALT and LDH levels in post-parturient (group-IIa) ewes compared to high protein diet fed (group-IIb) ewes and in any conditions in AST level.

Blood hematological parameters: The WBC count, hemoglobin and haematocrit values were significantly increased ($p<0.05$) in metabolic stressed (post-parturient, Group-IIa) ewes compared to control (Group-I) and metabolic stressed high protein diet fed (Group-IIb) groups (Table 5). No significant change was observed in RBC count, MCV, MCH, MCHC and platelet count between all the groups.

Urine metabolites and cortisol: β-OHB and cortisol levels were significantly higher ($p<0.05$) in urine of metabolic stressed (post-parturient, Group-IIa) ewes compared to that observed in urine of high protein diet fed (Group-IIb) and control (group-I) ewes (Table 6). Ammonia and urea levels were significantly higher ($p<0.05$) in urine of high protein diet fed (Group-IIb) ewes compared to control (Group-I) and metabolic stressed (post-parturient, Group-IIa) ewes. No significant change was obtained in urine uric acid and creatinine levels.

Behavioral patterns: The stressful responses like vocalizations, pawing, duration in maintenance and lying were significantly higher ($p<0.05$) in metabolic stressed (post-parturient, group-IIb) ewes compared to control (Group-I) and metabolic stressed (high protein diet fed, Group-IIb) ewes (Table 7). No significant difference was observed in behavioral pattern like circling attempts between all the groups.

Table 5: Concentrations of blood haematological parameters of control and metabolic stressed ewes

	Control	Metabolic stressed	
Blood parameters	Group-I	PP (Group-IIa)	HP (Group-IIb)
White blood cells (×10^9/L)	9.6±1.240[a]	16.6±0.980[b]	10.1±1.42[a]
Red blood cells (×10^{12}/L)	10.6±1.850	11.7±1.720	10.4±2.14
Haemoglobin (g L^{-1})	129.3±5.450[a]	168.6±4.820[b]	132.9±4.46[a]
Hematocrit (%)	42.2±1.860[a]	52.4±2.100[b]	41.7±1.90[a]
Mean corpuscular volume (fl)	44.1±3.540	40.3±2.840	43.6±3.910
Mean corpuscular hemoglobin (pg)	10.9±0.860	11.3±0.530	11.4±0.720
Mean corpuscular hemoglobin concentration (g L^{-1})	276.3±18.31	290.4±15.24	284.9±20.27
Platelet count ×10^3 mm^{-3}	451.6±79.54	446.4±83.21	439.3±72.91

Superscripts bearing different letters in the same row differ significantly ($p<0.05$). PP: Post-parturient ewes, HP: High protein diet fed ewes

Table 6: Concentration of metabolites and cortisol in control and metabolic stressed ewes urine

	Control	Metabolic stressed	
Metabolites	Group-I	PP (Group-IIa)	HP (Group-IIb)
β-OHB (mM)	0.37±0.03[a]	0.65±0.01[b]	0.41±0.06[a]
Ammonia (mM)	10.20±1.32[a]	10.80±0.98[a]	17.20±1.53[b]
Urea (mM)	34.60±6.23[a]	67.80±5.86[b]	100.60±6.72[c]
Uric acid (μM)	5.30±0.91	5.20±0.76	5.50±1.06
Creatinine (μM)	45.70±5.64	44.80±6.21	46.30±4.61
Cortisol (ng mL^{-1})	12.10±1.24[a]	42.40±1.86[b]	24.20±0.96[c]

Superscripts bearing different letters in the same row differ significantly ($p<0.05$). PP: Post-parturient ewes, HP: High protein diet fed ewes

Table 7: Behavioural patterns observed in ewes exposed to metabolic stress

		Control	Metabolic stressed	
Behavioural patterns		Group-I	PP (Group-IIa)	HP (Group-IIb)
Number	Vocalizations	3.4±2.01[a]	12.8±1.83[b]	5.0±1.57[b]
	Pawing	2.2±0.86[a]	9.4±0.42[b]	3.2±0.93[b]
	Circling attempt	1.0±0.13	2.1±0.16	1.0±0.19
Duration (s)	Maintenance	118.6±8.64[a]	43.4±6.49[b]	114.6±9.14[a]
	Lying	126.4±10.68[a]	64.69.3±8.16[b]	112.2±1.16[a]

Superscripts bearing different letters in the same row differ significantly ($p<0.05$). PP: Post-parturient ewes, HP: High protein diet fed ewes

Functional /clinical manifestations (symptoms) of metabolic stress: Anorexia, dull and depressed, isolation from rest of the herd, unable to walk long distances, searching for shadow area, avoid milk feeding to their lambs were the clinical manifestations seen in the metabolic stressed ewes of both the categories.

DISCUSSION

Change in body fluid metabolite profile causes a decline in productive and reproductive performance in ruminants. The response to stressors is context dependent and therefore, a combination of different measurements (e.g. physiological, biochemical and behavioural) for evaluating stress was beneficial. It is speculated that metabolic stress in ruminants leads to changes in the biochemical composition of serum and urine as well as changes in the behavioural patterns. This study suggested explanations for the serum/urine metabolic and biochemical profile as well as behavioural responses in the assessment of metabolic status in ewes and ensuring good health in metabolic stress due to post-parturient stress or high protein diet feeding in ruminants. This study will help to understand the physiological and behavioural changes in ewes under metabolic stress under tropical conditions.

Low reproductive performance in ruminants was one of the most critical issues faced by farmers. Many factors were responsible for the low reproductive performances. The reasons of reduced ovarian functions in recent times were imbalanced feeding, Neuroepithelial bodies (NEB) and the associated endocrine and metabolic signaling pathway alterations particularly in small ruminants where stall feeding was not much practiced. Fluctuations in serum metabolites occur because of imbalanced feeding behavior and/or NEB, these fluctuations cause ketonemia, elevated concentration of NEFA, hypoglycemia and other disorders which might lead to decline in fertility in ruminants[3]. During pre- and postpartum periods, there was high demand of energy and these periods might be associated and/or linked with under- and/or imbalanced nutrition resulting mobilization of store body fat and protein and as a result, it would create negative energy balance[20] leading to death of animals in some cases. Hence identification of metabolic changes in metabolic stress conditions and prediction of some indicators would be efficiently beneficial.

Serum glucose measurement was reflective of the energy status and glucose in animal body under tight homeostatic regulation[20]. Decreased blood glucose concentrations in metabolic stressed ewes in the present study might be because of energy requirement to meet the body demand which was in agreement with the previous findings[21]. In addition, similar findings were reported, where heat stress significantly decreased plasma glucose concentration in crossbred female calves[13]. Excess rumen degradable nitrogen in ewes diets elevated the urea and ammonia levels in serum[19], follicular fluid[2,19] and oviductal/uterine fluids[14]. Serum urea concentration was

significantly decreased in postpartum ewes compared to prepartum and control ewes[20]. The result of the present study was in conflict with the other finding[20], as no difference was found in serum urea concentration in control and postpartum ewes. The result of the present study was in agreement with an earlier study wherein the increment in the levels of ammonia and urea in blood were observed in ewes fed with rumen degradable protein or in an animal fed with energy deficit diet[19]. At postpartum, high serum NEFA concentration indicated increased rate of lipolysis or lipomobilization[22]. The serum concentrations of total NEFA in the present study were significantly higher in metabolic stressed (post-parturient) ewes compared to control and metabolic stressed (high protein diet fed) group which was in agreement with an earlier findings[2]. It was reported that measuring serum β-OHB concentration might serve as a useful method in monitoring the energy status of ewes under stress[20]. A study reported that β-OHB concentrations in serum and follicular fluid were similar and elevated β-OHB levels in serum (ketonemia) would appear in the follicular fluid as well.

A direct correlation was reported between serum thyroid hormone concentration and energy balance[23] was noticed where imbalanced feeding caused suppression of the TRH, as an outcome (TSH) production got reduced, thus, glycosylation pattern of newly synthesized TSH changed, so that newly synthesized TSH was biologically not much active. The postpartum negative energy balance induced similar changes in ruminants[24]. It was reported that plasma levels of T_4 concentration were found to be lower in post parturition animals compared to controls[23]. Similarly, significantly lower level of T_3 in the blood of post-parturient sheep than that of non-pregnant and pregnant sheep[25] was noticed. An increase in NEB was related with an increase in concentrations of IGF-I in serum during early lactation. This increased concentration of IGF-I was associated with increased P4 secretion during diestrus of the first and second postpartum estrous cycle[26]. However, a decrease in IGF-I level in post-parturient conditions was observed in the present study. Significantly lower concentrations of serum insulin were obtained in the serum of metabolic stressed ewes (post-parturient) and high protein diet fed ewes compared to control ewes in the present study. This was in agreement with the previous findings, who reported that stress due to parturition caused a greater concentration of insulin compared to non-lactating ones[20].

It was reported that the physiological state of an animal had a significant influence on serum levels of calcium, phosphates, sodium, potassium and magnesium[27].Changes in electrolyte concentration in serum of ewes, during stress were somewhat different from the reference values for ewes. This difference was linked with an increased requisite for energy demand. The lowest concentrations of Na and K were detected in the ewes during third trimester of pregnancy[27]. The lowest concentrations of Mg were detected in the ewes during high protein diet fed compared to post-parturient and control ewes in the present study. Changes in Na and K concentration was not found in all groups, however low concentrations of Na and K was reported during advanced stage of pregnancy in ewes[12].

Decreased ALT activity in the serum of metabolic stressed ewes was observed compared to control ewes which were in disagreement with previous findings[12]. Significantly lower LDH activity was observed in the serum of metabolic stressed ewes compared to control in the present study. Alteration in activities of AST, ALT and LDH enzymes might be because of low dry matter intake during metabolic stress, hence it cause hepatic lipidosis altering the normal functions of the liver.

Elevated WBC count in the present study, was observed in post-parturient ewes as postpartum leukocytosis was a physiological phenomenon. Elevated haemoglobin concentration in metabolic stressed ewes (post-parturient) in the present study might be due to elevated requirement of oxygen and higher metabolic rate. It was found that the higher levels of hemoglobin in metabolic stressed group (post-parturient) than in high protein diet fed ewes and in control group which was in agreement with one study[28], but was in conflict with the other findings, who reported the lower value of hemoglobin in stressed group compared to the control one[29]. Enhancement of hemoglobin levels might be because of attack of free radicals on the RBC membrane, which was rich in lipid content, and ultimate lysis of RBC or inadequate nutrient availability for hemoglobin synthesis as the animal consumed less feed or decreased voluntary intake under stress[28]. Basal haematological values observed in control and post-parturient ewes in the present study were in agreement with previous findings[19].

The physiological response of animals to stress includes increased respiratory rate, decreased feed intake, increased water intake and imbalances plasma and urine composition. The results of urinary metabolites in the present study were also in accordance with the previous reports[30]. High β-OHB during post-parturient stage in the present study might be due to the negative energy balance and development of hyperketonemia in ewes and also ruminant were equipped to

metabolize the butyrate by ruminal fermentation, mostly by using it as metabolic fuel for the ruminal musculature[31]. Measurement of urine cortisol was a potent non-invasive diagnostic test for measuring stress. The present study suggested that urine cortisol would be used in conjunction with other indicators to quantify the responses of an animal to management practices and to assess the effectiveness of remedial actions in welfare issues.

The behavioural responses of ewes with metabolic stress were in accordance with the reports where ewes exhibited stressful responses (significant lower levels of cortisol plasma, number of vocalizations, time spent with the head out of the cage and others) as compared to ewes infused with isolation stress[17,18]. Likewise in isolation stress[18], stressful responses increased and maintenance behaviours decreased in metabolic stress (post-parturient stress). The results of the present study also supported the view that plasma cortisol concentration was closely related with the behavioural responses[32]. An increase in blood cortisol concentrations during a stressful condition was reported as successful coping rather than a sign of distress. But, when a stressor was prolonged or when the animal was exposed to several stressors simultaneously, there were negative consequences for the welfare of animals. When the cortisol was under- or over-produced or dysregulated, a significant biological effect could be incurred, leading to detrimental effects on the animal[33].

High-producing dairy cows are in severe NEB during early postpartum period, which causes changes in biochemical, endocrinological and metabolic pathways leading to decline in reproductive performances[34]. Hence, interpretation of ovine profiles and comparing the results with cattle should be dealt with care. The characteristic changes observed in the present study may be compared with that resembling ketosis or keto-acidosis as seen in dairy cows in further study. More study could be planned to evaluate changes in blood metabolites in relation with changes in body weight[22]. Similarly, individual and breed variation as well as other challenges like low precision in estimating energy balance in individual animal[35] may be considered in future studies.

CONCLUSION

This study concluded that plasma glucose and cholesterol levels were lowered in metabolic stressed ewes. However, the plasma NEFA, β-OHB (post-parturient conditions) and urea (high protein diet fed) concentrations were higher in metabolic stressed ewes. In addition, significant decrease in stress related hormones (T3, T4, TSH, IGF-I, insulin) and increase in cortisol level were observed in metabolic stressed ewes. Some other changes like biochemical (calcium, magnesium, ALT and LDH), haematological profiles (haemoglobin, hematocrit and WBC values) as well as behavioural responses were observed in metabolic stressed ewes compared to control ones.

SIGNIFICANCE STATEMENT

Change in body fluid metabolite profile causes a decline in productive and reproductive performance in ruminants. Response to stressors is context dependent and therefore, a combination of different measurements (e.g. physiological, biochemical and behavioural) for evaluating stress is beneficial. This study suggests that metabolic stress in ruminants leads to changes in the biochemical composition of serum and urine as well as changes in the behavioural patterns. This study will develop to understand the physiological and behavioural changes in metabolic stress due to post-parturient stress or high ammonia generating diet feeding in ruminants.

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