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Effect of Lactation Stages on Biochemical Entities of Indigenous Badri Cattle of Uttarakhand

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ABSTRACT

Background: The indigenous cattle of Uttarakhand, Badri is the first registered cattle breed of the state, mainly reared in Kumaon and Garhwal regions. The present study was carried out in forty healthy cows to evaluate the effect of lactation stages on biochemical entities.

Methods: Forty healthy animals were selected and divided into five groups with eight animals in each group during different stages of lactation *viz.* 0 to 60 days, 61 to 120 days, 121 to 180 days, 181 to 240 days and non-lactating animals. Blood samples were collected during different lactation stages and non-lactation period and serum was harvested. On each serum sample total protein, albumin, globulin, A:G ratio, urea, creatinine, glucose, cholesterol, triglycerides, HDL, LDL, calcium, phosphorus, Ca:P, total bilirubin, ALT, AST, ALP, GGT and CK were determined spectrophotometrically.

Result: A significant effect of stages of lactation on serum total protein, albumin, glucose, cholesterol, triglycerides, phosphorus, Ca: P, ALT, ALP and CK was observed ($p<0.05$).

Key words: Badri, Biochemicals, Indigenous.

INTRODUCTION

The indigenous cattle of Uttarakhand is the first registered cattle breed of the state on 21st June 2016 and is named as Badri (ICAR-NBAGR, 2016). It is mainly reared in hilly areas of the Kumaon and Garhwal regions of the state. Unique physical characteristics of Badri cattle are small size, weighing about 200-250 kg, have bright and alert eyes with erected ears, neck is wide and small and have a prominent hump. The coat colour is varied as black, brown, red, white or grey, and mostly hooves and muzzle are black or brown in colour. Their legs are long and straight with hard foot pads for hilly and rugged terrains. Udder is small sized and tucked up within the body and milk yield is about 1.5 (0.5-2.0) kg per day. The average age at first calving is 3-5 years and have 8-10 calving in its fertility life. The breed have short lactation length (208 days) and long dry period 138 days (4-6 months), but well adapted to the hills under the prevailing climatic conditions and resistant to many diseases (Pundir *et al*., 2014). The livestock owners of Uttarakhand prefer Badri cattle over other animals due to salient importance in terms of religious value and others like better adaptability in hilly region, disease resistant, medicinal properties of milk and urine, good manure, requires less external inputs for its maintenance, more livelihood generation, docile temperament, milk flavour, excellent feed conversion efficiency and draught power (Joshi *et al.,* 2018).

Lactation period is more sensible period from metabolic point of view. Blood biochemical entities are important indicators of the metabolic activity in lactating dairy animals (Karapehlivan *et al.,* 2007). Lactation is one of the important physiological stage which can modify metabolism in animals and induce stress (Tanritanir *et al.,* 2009). The main problem of this period is the limited intake capacity for dry matter, as a consequence of which dairy cows are unable to meet their energy demands from feeds (Wathes *et al.,* 2009). Lactation has effect on the health and subsequent performance of dairy cattle as animals develop several metabolic and physiological changes (Tanaka *et al*., 2011). The fact that lactation stages effect the physiology of normal animals, the present study was undertaken to evaluate the effect of different stages of lactation on biochemical entities of Badri cattle.

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MATERIALS AND METHODS

This study was carried out in forty healthy lactating Badri cows maintained at instructional dairy farm (October, 2019 to February, 2020) in the Department of Veterinary Physiology and Biochemistry of GBPUAT, Pantnagar, Uttarakhand. Animal were devided into five groups with eight animals in each group during different stages of lactation

viz. group I (0-60 days), group II (61-120 days), group III (121-180 days), group IV (181-240 days) and group V (non-lactating animals). Blood samples were taken after taking all precautionary measures and serum was separated. Serum samples were analysed for total protein (biuret method), albumin (BCG dye method), globulin, A:G, urea (GLDH-Urease method), creatinine (Jaffe's method), glucose (GOD-POD method), cholesterol (CHOD-PAP method), triglycerides (GPO-Trinder method), HDL (HDL-C estimation method), LDL (modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classical precipitation method), calcium (Arsenazo III method), phosphorus (ammonium molybdate method), Ca:P, total bilirubin (Diazo method), ALT, AST, ALP, GGT and creatine kinase using biochemical estimation kit spectrophotometrically. Statistical analysis was performed using SPSS software and statistical significance was checked at p value 0.05.

RESULTS AND DISCUSSION

As shown in Table 1, serum total protein and albumin were significantly ($p<0.05$) higher in early lactation and lower in mid lactation and non-lactating animals. Serum globulin was non-significantly highest during early lactation stage compared to other lactation stages and non-lactating animals. Contrary to present findings Mohamed (2014) found non-significant relationship between serum globulin concentration in lactating and dry animals. Increased serum globulin level during early lactation could be due to synthesis of immunoglobulins in colostrum (Birgel Junior *et al.,* 2003) and to provide immunity to the animal against certain post-partum infection as this phase is most stressed physiological phase. Substantial decrease in serum total protein, albumin and globulin concentration from early to mid-lactation were agreeable with previous findings (Abd-El Naser *et al.*, 2014). This may be due to maternal requirement of proteins for milking and providing immunoglobulins (Roubies *et al*., 2006). Higher concentrate to forage ratio provided during lactation is associated with higher level of starch and lower level of fibre in the diet leading to increased production of propionic acid in the rumen and an increased microbial protein synthesis (Heck *et al*., 2009). This is reflected in our study, by an increase of serum total protein during initial phases of lactation than non-lactating animals. Total protein content is usually regarded as an appraisal of nutritive status of an animal which reflects food intake and metabolism. Such an increase in serum protein after calving might also be attributed to water losses and hemoconcentration following parturition. Serum A:G ratio was higher during all lactation stages compared to non-lactating animals, however, the difference was non-significant.

Serum urea levels were highest during late lactation as compared other lactation stages and non-lactating animals though non-significantly. Abd-El Naser *et al.* (2014) reported lower serum urea during early lactation stage than mid lactation stage which was contrary to present findings. Higher serum urea levels during early lactation than mid lactation, might be due to increased protein intake during the early lactation because of increased requirements of milk synthesis (Roubies *et al*., 2006). Increased levels of urea during late lactation could be due to either increased protein intake or increased deamination (Roche *et al.,* 2009). Increased urea and decreased serum proteins during late lactation stages confirm that urea was not completely utilized for protein synthesis, which is pointing towards energy deficiency conditions (Wathes *et al.,* 2009). Serum creatinine was significantly lower during early lactation and highest in non-lactating animals. Our findings were in accordance with Piccione *et al.* (2012) who reported significant ($p<0.05$) effect of lactation stages on serum creatinine values. The renal functions are principally represented by urea and creatinine concentrations. Stress has been shown to increase catabolism of amino acids for energy (Abeni, 2007) and some of these amino acids could be derived from the protein

Table 1: Mean±SE values of serum protein and metabolites during different stages of lactation in indigenous Badri cattle (n=8/group).

Serum protein and metabolites	Stages of lactation				
	Group I (0-60 days)	Group II (61-120 days)	Group III (121-180 days)	Group IV (181-240 days)	Group V (non-lactating)
Total protein (g/dl)	8.46±0.43^{b}	7.50±0.31^{ab}	6.76±0.38^{a}	7.61±0.24^{ab}	6.83±0.38^{a}
	(6.81-10.22)	(6.46-9.37)	(5.16-8.27)	(6.17-8.46)	(4.67-8.28)
Albumin (g/dl)	2.83±0.12^{b}	2.81±0.08^{b}	2.30±0.18^{ab}	2.57±0.09^{ab}	2.24±0.19^{a}
	(2.51-3.54)	(2.34-3.05)	(1.37-3.0)	(2.22-3.05)	(1.39-2.77)
Globulin (g/dl)	5.59±0.49	4.69±0.29	4.46±0.41	4.66±0.32	4.60±0.33
	(3.97-7.58)	(3.70-6.32)	(2.37-6.28)	(3.33-6.20)	(3.29-5.58)
Albumin:Globulin	0.54±0.06	0.65±0.05	0.57±0.10	0.57±0.050.	.50±0.06
	(0.35-0.83)	(0.46-0.95)	(0.32-1.17)	(0.36-0.78)	(0.27-0.83)
Urea (mg/dl)	38.58±3.61	32.69±2.25	35.34±4.85	40.98±3.91	37.87±6.80
	(26.92-58.65)	(22.12-43.27)	(18.27-61.53)	(19.23-58.65)	(17.30-50.96)
Creatinine (mg/dl)	1.73±0.09^{a}	2.09±0.09^{ab}	2.08±0.12^{ab}	2.05±0.12^{ab}	2.26±0.15^{b}
	(1.44-2.1)	(1.74-2.61)	(1.38-2.41)	(1.59-2.77)	(1.64-3.02)

Mean values with different alphabets (^{a,b}) in superscript differ significantly ($p<0.05$) along the row.

mobilization of muscle tissue which could increase serum level of creatinine.

The important indicators of energy status of ruminants are glucose, cholesterol and triglycerides (Pechova and Pavlata, 2005). As shown in Table 2, serum cholesterol was significantly lower during early lactation and highest during mid- lactation than other stages of lactation and non-lactating animals. Similarly, Piccione *et al.* (2012) and Abd-El Naser *et al*., 2014 reported lower serum cholesterol during early lactation and higher during mid-lactation. This might be due to an increase in the energy demands for regulatory mechanisms responsible for the processes involved in preparation of milking (Roche *et al*., 2009). Higher levels of serum cholesterol with progress of lactation could be a physiological adjustment to meet requirement of lactation such as increased gonadal steroid production during different stages of lactation in dairy cattle (Setty and Razdan, 1966). Serum triglycerides were significantly lower during early lactation, then progressively increased as stages of lactation advanced and highest in non-lactating animals. Serum HDL levels were lower during all lactation stages than non-lactating animals, whereas LDL levels were higher during all lactation stages as comparison to non-lactating animals, though non-significantly. Serum glucose levels were significantly ($p<0.05$) lower during early lactation, then progressively increased as lactation advanced and highest in non-lactating animals. The lowered values of glucose during early lactation stage might be due to large amount of glucose withdrawl by the mammary gland for the synthesis of lactose for milk secretion (Nale, 2003). During pregnancy endocrine profile changes, lipolysis and lipogenesis are regulated to increase lipid reserves and subsequently these reserves are utilized following parturition and initiation of lactation (Nafizi *et al.,* 2002 and Roche *et al*., 2009).

As shown in Table 3, serum calcium levels showed non-significant variation among difference stages of lactation, however phosphorus and calcium: phosphorus ratio showed significant variation among different stages of lactation ($p<0.05$). All animals require minerals for growth, reproduction and lactation (Samardzija *et al.,* 2011). Serum calcium levels were lower during early lactation stage, progressively increased with the progress of lactation which corroborates with the findings of Nale (2003) and Abd-El Naser *et al.* (2014) and highest during non-lactation phase. This could be due to impaired absorption of food metabolites from gastrointestinal precursors, excessive losses through urine, colostrum during excessive milking and due to insufficient mobilization from skeletal tissue (Liesegang, 2008).

Serum phosphorus levels were significantly ($p<0.05$) lower during mid-lactation and higher during non-lactation phase. Lower phosphorus levels during early lactation stage

Table 2: Mean±SE values of blood energy metabolites during different stages of lactation in indigenous Badri cattle (n=8/group).

Blood energy metabolites	Stages of lactation				
	Group I (0-60 days)	Group II (61-120 days)	Group III (121-180 days)	Group IV (181-240 days)	Group V (non-lactating)
Cholesterol (mg/dl)	91.90±9.06^{a} (65.24-137.61)	141.72±9.52^{b} (101.09-178.09)	103.03±6.25^{ab} (84.76-130.95)	106.66±14.85^{ab} (56.66-182.85)	94.93±13.02^{a} (46.66-151.9)
Triglycerides (mg/dl)	15.54±0.81^{a} (12.96-18.45)	20.87±2.18^{ab} (12.31-27.91)	22.56±2.70^{ab} (16.09-40.20)	23.67±1.61^{ab} (17.79-30.31)	26.97±3.24^{b} (18.78-44.15)
High density lipoprotein (HDL) (mg/dl)	13.90±1.62 (8.0-22.54)	19.91±3.05 (12.36-37.09)	20.64±1.81 (13.09-28.36)	21.0±2.92 (12.36-39.27)	22.98±2.36 (13.09-32.73)
Low density lipoprotein (LDL) (mg/dl)	26.22±4.46 (12.95-54.62)	28.01±3.53 (12.95-43.08)	22.42±1.41 (17.46-29.56)	25.09±2.27 (12.95-33.50)	20.48±2.65 (13.80-37.16)
Glucose (mg/dl)	38.17±3.61^{a} (29.22-59.59)	40.15±1.09^{a} (37.44-45.59)	41.90±1.97^{a} (37.21-54.23)	44.81±4.41^{ab} (34.93-73.52)	52.17±2.98^{b} (44.74-69.93)

Mean values with different alphabets (^{a,b}) in superscript differ significantly ($p<0.05$) along the row.

Table 3: Mean±SE values of blood electrolytes during different stages of lactation in indigenous Badri cattle (n=8/group).

Blood electrolytes	Stages of lactation				
	Group I (0-60 days)	Group II (61-120 days)	Group III (121-180 days)	Group IV (181-240 days)	Group V (non-lactating)
Calcium (mg/dl)	6.72±0.31 (5.68-8.31)	7.24±0.60 (5.77-11.03)	7.31±0.2 (6.29-8.63)	7.46±0.25 (6.54-8.69)	7.71±0.43 (5.51-9.34)
Phosphours (mg/dl)	5.39±0.63^{ab} (3.83-7.18)	6.16±0.36^{ab} (4.44-7.14)	4.57±0.38^{a} (3.18-6.57)	5.50±0.33^{ab} (4.09-7.17)	6.46±0.41^{b} (4.61-8.19)
Calcium: Phosphours	1.34±0.13^{ab} (0.80-2.04)	1.25±0.12^{a} (0.97-1.94)	1.68±0.15^{b} (1.09-2.29)	1.39±0.10^{ab} (0.95-1.76)	1.25±0.14^{a} (0.67-2.03)

Mean values with different alphabets (^{a,b}) in superscript differ significantly ($p<0.05$) along the row.

Table 4: Mean±SE values of serum liver enzymes during different stages of lactation in indigenous Badri cattle (n=8/group).

Liver enzymes activities	Stages of lactation				
	Group I (0-60 days)	Group II (61-120 days)	Group III (121-180 days)	Group IV (181-240 days)	Group V (non-lactating)
Total bilirubin (mg/dl)	0.49±0.06 (0.24-0.68)	0.47±0.08 (0.20-0.69)	0.39±0.06 (0.25-0.67)	0.39±0.05 (0.25-0.57)	0.38±0.09 (0.01- 0.70)
ALT (U/L)	23.35±2.35^{a} (15.32-36.54)	36.24±4.26^{b} (15.32-53.63)	21.73±2.60^{a} (14.73-37.13)	27.05±3.03^{ab} (17.68-38.31)	22.32±2.78^{a} (12.38-32.41)
AST (U/L)	50.61±6.90^{b} (35.36-91.94)	47.07±2.95^{ab} (35.36-60.11)	44.56±5.29^{ab} (24.43-65.42)	47.96±3.33^{ab} (33.59-65.42)	39.48±2.75^{a} (30.06-53.04)
ALP (U/L)	20.52±3.49^{a} (21.70-68.71)	36.21±5.61^{b} (10.85-27.12)	16.50±1.79^{a} (12.66-74.13)	38.87±8.23^{b} (23.50-59.66)	37.52±4.02^{b} (10.85-41.22)
GGT (U/L)	7.33±0.94 (5.40-13.12)	5.09±0.84 (2.32-8.49)	5.74±1.76 (1.93-13.51)	5.74±0.79 (2.32-8.49)	6.08±1.35 (2.32-14.63)
CK (U/L)	26.28±2.28^{ab} (16.51-36.03)	24.76±4.56^{ab} (12.38-48.15)	33.36±2.48^{b} (24.76-42.65)	18.92±2.78^{a} (11.0-31.64)	23.39±2.98^{ab} (12.38-35.77)

Mean values with different alphabets (^{a,b}) in superscript differ significantly ($p<0.05$) along the row.

ALT- Alanine aminotransferase, AST-Aspartate aminotransferase, ALP- Alkaline phosphatase, GGT- Gamma glutamyl transferase, CK- Creatine kinase.

might be due to requirement for the colostrum synthesis (Szenci *et al.,* 1994) and enhanced carbohydrate metabolism. Significantly higher Ca:P ratio was observed during mid-lactation and lower during non-lactation phase. The requirement of calcium and phosphorus depends up on the physiological status and animal's productivity (Brezezinska and Krawczyk, 2009). Milk phosphorus and calcium output is directly related to milk yield, as phosphorus concentration in milk is constant (Valk *et al.,* 2002). In our study, serum calcium and phosphorus levels were low during all lactation stages as compared to non-lactating animals. With increasing milk production, more phosphorus from ingested amount is transferred to milk and less is excreted in faeces (Valk *et al.,* 2002).

As shown in Table 4, total bilirubin concentration progressively decreased as lactation stages advanced, however, the difference was non-significant ($p>0.05$). Similarly, Piccione *et al*. (2012) reported non-significant effect of stage of lactation on total bilirubin values. Serum ALT activity was significantly higher during mid-lactation as compared to other lactation stages and non-lactating animals. Our findings were consistent with Yaylak *et al.* (2009), while Todorovic *et al.* (2012) reported non-significant effect of lactation stages on ALT activity. The increased serum ALT activity during mid lactation stage suggested an increased hepatic metabolism (Ashmawy, 2015). Serum AST activity was higher during early lactation, decreased as lactation stages progressed and lower in non-lactating animals. Our findings were agreeable with previous studies (Todorovic *et al.,* 2012 and Mohamed, 2014) with higher AST activity in early lactation than in mid-lactation. Higher serum AST activity during early lactation stage could probably be due to intense muscular activity involved in the process of parturition (Birgel Junior *et al.,* 2003) and higher probability of liver damage as a result of negative energy balance due to increased energy demands of lactation period.

Serum ALP activity was significantly ($p<0.05$) higher during late lactation stage and in non-lactating animals and lower during mid-lactation. Our study corroborates with the findings of Mohamed (2014), while Sato *et al.* (2005) observed higher serum ALP activity during lactation than in dry cows. In contrary to our findings, Rocha *et al.* (2019) reported high serum ALP activity after parturition which could be due to placental ALP released into blood stream at the time of parturition. Serum activity of CK was significantly higher during mid lactation and lower during late lactation stage. Serum GGT activity was higher during early lactation stage, decreased during subsequent lactation stages and again increased in non-lactating animals. These findings were in accordance with Mohamed (2014). Abdel-Raheem *et al*. (2010) reported higher GGT enzyme during lactation period in comparison to dry cows. In contrary, Todorovic *et al.*, (2012) found no significant change during different lactation stages in GGT levels. Increased serum GGT enzyme might reflect liver damages in dairy cattle (Moore, 1997).

CONCLUSION

It may be concluded that different stages of lactation effected the biochemical entities of indigenous Badri cattle. The data generated during the current investigation may be useful as reference values for the scientific community as this is the first study of its kind in this breed of cattle.

Conflict of interest

All authors declared that there is no conflict of inteest.

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