

Estimation of blood biochemical parameters of Banni buffalo (*Bubalus bubalis*) at different age, sex and physiological stages

M.D. Patel^{1*}, A. Lateef¹, H. Das², M.V. Prajapati³, P. Kakati³ and H.R. Savani³

¹Department of Physiology and Biochemistry, College of Veterinary Science & Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Dantiwada-385506, Gujarat, ²Department of Physiology and Biochemistry, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih- 796014, Mizoram, ³Kankuba Pashupalan Vidyapith, Institute of Dairy Sciences, Ganpat University, Ganpat Vidyanagar-384012, Gujarat, India.
Corresponding author: Email: mekul.pt11@gmail.com

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Abstract

Blood biochemical profile forms the data base for most diagnostic investigations. Present study reports the reference values for different blood metabolites, minerals, electrolytes and some clinically important enzymes as well as their variation in serum concentration at different age, sex, physiological stages viz. pregnancy, dry and lactation of Banni buffalo (*Bubalus bubalis*). It was observed that the concentrations of glucose, total protein, creatinine, BUN, inorganic phosphorus, ALP, ALT, LDH and GGT showed significant variation ($p < 0.05$) with age, sex and/or physiological status unlike the other biochemical analytes. The values of glucose, total protein, albumin, globulin, total bilirubin, total cholesterol and creatinine were significantly higher in bulls than that of male calves. The levels of albumin, A/G ratio and creatinine were apparently lower in lactating buffaloes than dry buffaloes. Conversely, BUN, cholesterol and uric acid were higher in lactating buffaloes than dry buffaloes. The concentrations of calcium, magnesium, sodium and potassium varied non significantly between male calves and bulls. Similarly, in case of calves, the variation observed in the levels of calcium, magnesium, inorganic phosphorus, potassium and chloride were also non-significant between male and female. The data generated may be useful as reference values for the scientific community. Further, it would be helpful to assess the metabolic health status of Banni buffaloes as well as to identify dietary causes of disease and low productivity.

Key words: metabolite; enzyme; mineral; electrolyte; Banni buffalo, *Bubalus bubalis*

Introduction

Blood biochemical profile of animals is imperative to confirm clinical diagnosis and estimate the severity of diseases (Piccione *et al.*, 2010). Further, biochemical parameters are commonly employed as useful indicators of health as well as nutritional status of many species, and thus help in diagnosis of metabolic diseases and management of infertility as well as low productivity in farm animals (Amle *et al.*, 2014; Kaminski *et al.*, 2014). It is unequivocal that several factors viz. age, sex, pregnancy and lactation affect the metabolism and thereby the blood biochemical profile of animals (Jain *et al.*, 2009).

However, no study could be traced investigating the blood biochemical indices of Banni buffalo (*Bubalus bubalis*), a milch breed originated from the Banni area of Kutch district, Gujarat, India. The breed is the “*sui-gensis*” buffalo germplasm of Kutch maintained by maldharis (animal owners) under typically and locally adapted extensive production system in its breeding tract. It is very hardy breed and well adapted to harsh climatic conditions. The genetic makeup of these unique animal gives them the ability to free graze in the night to avoid harsh high temperatures of the day; and handle the stress of temperature difference and little fodder in droughts and yet when fed well can produce twice the milk than any other indigenous animal in the country (Jadav *et al.*, 2013). Genotyping carried out by National Bureau of Animal Genetic Research (NBAGR), Karnal and Sardarkrushinagar Dantiwada Agricultural University (SDAU) confirms the Banni buffalo as a distinct breed. Accordingly, it was recognized as 11th buffalo breed of India by Breed Registration Committee, ICAR, New Delhi. Previous report describes the physiological baseline values for hematological profile of Banni buffalo (*Bubalus bubalis*) as well as their alteration due to age, sex and physiological stages (Patel *et al.*, 2016). Nonetheless, combined with haematology, the blood biochemical parameters provide the data base for most diagnostic investigations (Akbar, 2014).

In view of the above, the present study was undertaken to determine reference values for different blood metabolites, minerals and electrolytes as well as to study their variation in serum concentration at different age, sex, physiological stages viz. pregnancy, dry and lactation of Banni buffalo (*Bubalus bubalis*).

Materials and methods

Ethical approval

The current study was conducted as a part of PG research work, which was approved by Director of Research, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat.

Place of study

The study was carried out between April, 2014 to March, 2015 in the department of Physiology and Biochemistry at College of Veterinary Science & A.H, Sardarkrushinagar, Gujarat, India.

Experimental animals

For the investigation, the experimental animals were selected from Cattle Breeding Farm, Bhuj. The region of the farm stretches between 22° to 24° N and 68° to 71° E and falls in the arid tract of Gujarat with the maximum annual average temperature of 39 to 45° C and 63% relative humidity.

Forty two clinically healthy animals of the farm were randomly categorized into six groups (n=6): group-I (male calves ≤1 year), group- II (bulls >1 year), group-III (female calves ≤1 year), group-IV (pregnant lactating buffaloes), group-V (non-pregnant lactating buffaloes), group-VI (pregnant dry buffaloes) and group-VII (non-pregnant dry buffaloes). All the buffaloes of the experimental groups were reared under standard feeding and husbandry conditions. The health of the selected animals was regularly examined based on behavior, rectal temperature, pulse rate, respiratory rate and fecal consistency. It is to be noted that since seven experimental groups were to be made at the same time as per age, sex, pregnancy and lactation, the sample size was to be restricted to 6 due to unavailability of more numbers of animals of each group in the farm during the experimental period.

Sample collection and parameters studied

About 10 ml of blood sample was collected aseptically from each animal of all the experimental groups by jugular vein puncture into serum clot activator tubes (Greiner Bio-One GmbH, Austria) to separate out the serum. Subsequently, the clear serum samples were collected in sterilized vials and stored in refrigerated condition till analyzed.

The biochemical parameters viz. (1) blood metabolites (glucose, total protein, albumin, globulin, albumin/globulin (A/G) ratio, total bilirubin, total cholesterol, creatinine, blood urea nitrogen, uric acid), (2) minerals and electrolytes (calcium, magnesium, inorganic phosphorus, sodium, potassium, chloride) and (3) enzymes (alkaline phosphatase, aspartate aminotransferase, alanine transaminase, lactate dehydrogenase, gamma glutamyltransferase, creatine kinase) were estimated using Clinical Analyzer-635 (Systronics India Ltd., India).

Statistical analysis

The data generated on biochemical estimation were analyzed statistically using two-way ANOVA as per method of Snedecor and Cochran (1994). $p < 0.05$ were considered to be statistically significant.

Results and Discussion

The Mean±S.E. values of blood metabolites in different experimental groups of Banni buffaloes are presented in table 1.

It was observed that levels of glucose, total protein, total cholesterol, creatinine and BUN showed significant ($p < 0.05$) variation with age, sex and/or physiological status. However, in case of calves, the variations were not significant between male and female, which was in agreement with the findings of Jacob (2012) in Jaffarabadi buffalo calves aging 3 and 6 months. Similarly, Cenesiz *et al.* (2011) also found non-significant variation for total protein, albumin, creatinine and BUN between male and female water buffaloes. The concentrations of total protein, total cholesterol and creatinine were recorded to be significantly ($p < 0.05$) higher in bulls as compared to that of male calves. Higher total protein in bulls than the male calves in current study confirms the increasing demand of proteins for the tissues of growing animals, which exhibit optimum metabolism as they grow older (Singh and Choudhary, 1988). The glucose concentration in current study was found to be significantly ($p < 0.05$) lower in lactating buffaloes than dry buffaloes, which corroborate the report of Hagawane *et al.* (2009). This could be indicative of a greater demand for glucose by mammary gland for the synthesis of lactose, which in turn control milk volume Collier (1985). According to Nale (2003), hypoglycemia in lactating buffaloes is due to heavy drain of glucose for lactose synthesis. However, apparently lower levels of the albumin, albumin/globulin ratio and creatinine were recorded in lactating buffaloes than dry buffaloes. Conversely, BUN value was significantly higher ($p < 0.05$) in lactating buffaloes than dry buffaloes. Similarly, cholesterol and uric acid were found to be slightly higher ($p > 0.05$) in lactating buffaloes compared to dry buffaloes. Doornenbal *et al.* (1988) also measured higher BUN level in lactating cows than the dry ones. Nonetheless, non-significantly higher cholesterol level in lactating buffaloes than dry buffaloes explains the increased concentration of cholesterol during lactation as a physiological adjustment to meet lactation requirements (Rawat *et al.*, 2006). This finding was in line with the study of Nath *et al.* (2005) and Hagawane *et al.* (2009). Similarly, pregnant buffaloes were found to have non significantly higher level of total bilirubin than that of non-pregnant buffaloes. This could be a consequence of additional bilirubin derived from degradation of fetal haemoglobin (Gurgoze *et al.*, 2009).

Table 2 depicts the Mean±S.E. values of serum minerals and electrolytes of Banni buffaloes and their variation with age, sex and physiological status. The level of inorganic phosphorus was observed to be significantly ($p < 0.05$) higher in male calves than that of the bulls. Similar variation was also observed by Doornenbal *et al.* (1988) in beef cattle. In contrast, the concentrations of calcium, magnesium, sodium and potassium varied non significantly between male calves and bulls, which is in agreement with the observations of Mikniene *et al.* (2014). Except sodium, the levels of calcium, magnesium, inorganic phosphorus, potassium and chloride did not vary significantly between male and female calves. The significantly ($p < 0.05$) higher level of sodium in male buffaloes than that of female buffaloes is consistent with the findings of Mikniene *et al.* (2014), who also found significantly higher sodium level in stallions than that of mares. The levels of calcium and chloride were noted to be numerically lower in lactating buffaloes as compared to dry ones. Paul *et al.* (2011) also found lower calcium levels in lactating Surti buffaloes than the dry buffaloes. Liesegang *et al.* (2006) have suggested that calcium requirement for the milk production has a significant effect on maternal mineral and skeletal homeostasis during lactation. The lower calcium level in lactating group might be due to excessive drainage of blood calcium in milk and insufficient adjustment by parathormone through mobilization of bone calcium (Paul *et al.*, 2011). Nonetheless, the variations observed pertaining to all the minerals and electrolytes in the current study between lactating and dry buffaloes were non-significant. Similarly, Kulkarni *et al.* (1984) reported that sodium, potassium, calcium and inorganic phosphorus were comparable in lactating and dry Indian buffaloes. Further, Hagawane *et al.* (2009) also found non-significant variation of calcium, magnesium and phosphorus between lactating and dry buffaloes. Moreover, calcium and inorganic phosphorus were also observed to be non-significantly lower in pregnant buffaloes than non-pregnant buffaloes, whereas magnesium, sodium, potassium and chloride were numerically higher in pregnant buffaloes.

The Mean±S.E. of serum enzymes in different experimental groups are summarized in table 3. Similar to other biochemical parameters, serum enzymes also found to be varied with age, sex and physiological status. The activity of alkaline phosphatase (ALP) was measured to be significantly ($p < 0.05$) higher in male calves than that of the bulls. Whereas, alanine transaminase (ALT), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) were significantly ($p < 0.05$) lower in male calves than the bulls. Higher ALP level in male calves as compared to bulls was also recorded by Elitok *et al.* (2004) in Anatolian water buffaloes and Jacob (2012) in Jaffarabadi buffaloes. The higher levels of ALP obtained in male calves than bulls might be helpful for better growth of long bones and overall growth of calves (Devaraj *et al.*, 1984). However, Cenesiz *et al.* (2011) also found non-significant variation in AST levels between male calves and female calves, which is similar to our findings. Moreover, LDH and GGT activities were significantly ($p < 0.05$) higher in adult buffaloes than that of calves. LDH mainly present in the myocardial cells is also widespread in body cells (Chattaerjea and Shinde2008). The rise in its activity as age increased in both sexes could be pointer to its relationship with body weight.

Table 1: Blood metabolites in Banni buffaloes at different age, sex and physiological status

Parameters	Male calves	Bulls	Female calves	Pregnant lactating	Non pregnant lactating	Pregnant dry	Non pregnant dry
Glucose (mg/dl)	52.94±1.92 ^{bc}	60.01±4.86 ^c	53.39±3.36 ^c	42.24±1.62 ^a	44.18±1.71 ^a	51.67±2.07 ^b	53.65±2.66 ^c
Total protein (g/dl)	6.82±0.32 ^a	7.96±0.18 ^b	6.77±0.23 ^a	7.37±0.47 ^{ab}	7.43±0.49 ^{ab}	7.56±0.26 ^{ab}	7.48±0.35 ^{ab}
Albumin (g/dl)	2.94±0.27	3.89±0.36	2.76±0.23	3.48±0.25	3.41±0.35	3.82±0.33	3.73±0.29
Globulin (g/dl)	3.58±0.32	4.29±0.21	3.80±0.18	3.52±0.10	3.73±0.33	3.67±0.20	3.84±0.37
A/G ratio	0.89±0.11 ^{ab}	0.80±0.17 ^{ab}	0.71±0.08 ^a	0.99±0.20 ^{ab}	0.97±0.18 ^{ab}	1.08±0.22 ^{ab}	1.15±0.16 ^b
Total bilirubin (mg/dl)	0.49±0.59 ^a	0.61±0.08 ^{abc}	0.50±0.05 ^{ab}	0.68±0.18 ^{bc}	0.63±0.17 ^{abc}	0.75±0.13 ^c	0.62±0.12 ^{abc}
Total cholesterol (mg/dl)	52.93±5.53 ^a	69.74±5.28 ^{bc}	50.08±5.36 ^a	78.76±7.99 ^c	72.32±5.41 ^{bc}	66.02±7.45 ^{bc}	65.17±3.43 ^b
Creatinine (mg/dl)	0.68±0.13 ^a	1.24±0.18 ^b	0.66±0.07 ^a	0.73±0.05 ^a	0.74±0.93 ^a	0.98±0.69 ^{ab}	1.05±0.17 ^{ab}
Blood urea nitrogen (mg/dl)	20.85±0.51 ^a	22.42±1.02 ^a	21.94±1.48 ^a	30.45±0.97 ^c	31.17±1.10 ^c	22.83±0.88 ^a	26.76±1.45 ^b
Uric acid (mg/dl)	0.89±0.73	0.92±0.09	0.66±0.12	0.87±0.12	0.94±0.15	0.71±0.91	0.70±0.08

Means with same superscript within a row do not differ significantly from each other.

Table 2: Minerals and electrolytes in Banni buffaloes at different age, sex and physiological status

Parameters	Male calves	Bulls	Female calves	Pregnant lactating	Non pregnant lactating	Pregnant dry	Non pregnant dry
Calcium (mg/dl)	8.23±0.66 ^{ab}	9.72±0.59 ^{bc}	8.11±0.36 ^a	8.54±0.60 ^{abc}	8.96±0.24 ^{abc}	9.53±0.19 ^{abc}	10.50±0.34 ^c
Magnesium (mg/dl)	2.95±0.32 ^{ab}	3.63±0.21 ^{abc}	2.77±0.30 ^a	4.09±0.19 ^c	3.81±0.37 ^{bc}	3.85±0.21 ^{bc}	3.38±0.31 ^{abc}
Inorganic phosphorus (mg/dl)	6.67±0.50 ^b	5.81±0.22 ^a	6.16±0.53 ^{ab}	6.31±0.36 ^b	6.49±0.24 ^b	6.67±0.26 ^b	6.94±0.32 ^b
Sodium (mEq/L)	128.88± 3.59 ^b	149.01±7.13 ^b	115.16±7.01 ^a	122.52±9.61 ^a	120.56±3.33 ^a	126.90±12.49 ^a	122.48±6.27 ^a
Potassium (mEq/L)	2.77±0.23	3.50±0.13	2.74±0.22	3.19±0.28	2.88±0.37	3.05±0.95	2.71±0.60
Chloride (mEq/L)	88.17±3.38	91.74±3.52	89.58±3.57	83.49±4.29	81.86±2.76	92.26±5.44	89.11±10.06

Means with same superscript within a row do not differ significantly from each other.

Table 3: Serum enzymes in relation to different age, sex and physiological stages of Banni buffaloes

Parameters (U/L)	Male calves	Bulls	Female calves	Pregnant lactating	Non pregnant lactating	Pregnant dry	Non pregnant dry
ALP	217.17±21.90 ^b	156.82±13.29 ^a	294.16±12.20 ^c	171.26±20.67 ^{ab}	158.39±10.37 ^a	167.13±14.87 ^{ab}	161.26±16.20 ^a
AST	112.64±8.69 ^{ab}	90.96±8.43 ^a	98.35±3.83 ^a	108.59±9.92 ^{ab}	97.50±5.22 ^a	124.33±9.89 ^b	113.53±4.89 ^{ab}
ALT	31.27±2.52 ^a	39.19±3.96 ^b	36.48±5.01 ^b	39.39±3.71 ^b	38.13±2.78 ^b	36.05±2.40 ^b	35.15±3.20 ^b
LDH	642.01±31.61 ^a	763.66±39.97 ^b	613.33±43.19 ^a	758.07±38.71 ^b	765.23±55.54 ^b	734.73±22.01 ^b	689.83±40.75 ^b
GGT	21.36±0.95 ^a	28.18±1.27 ^{bc}	21.58±0.86 ^a	26.97±1.60 ^b	26.72±1.98 ^b	32.82±2.54 ^c	31.29±1.78 ^{bc}
CK	91.25±10.51	89.43±11.25	88.45±8.55	76.07±4.53	75.04±7.68	72.89±4.38	78.72±3.15

Means with same superscript within a row do not differ significantly from each other.

The significantly ($p < 0.05$) higher GGT activities in adult buffaloes than the calves were in agreement with the findings of Canfield *et al.* (1984). ALP and ALT activities were significantly higher ($p < 0.05$) in female calves than that of the male calves. Devaraj *et al.* (1984) also recorded higher ALP in female Surti buffalo calves than those of male calves. Nevertheless, the levels of AST, LDH, GGT and creatinine kinase (CK) did not vary significantly between male and female calves. Study of Cenesiz *et al.* (2011) also revealed non-significant alteration in AST and LDH level between male calves and female calves. The levels of AST and GGT were noted to be numerically lower in lactating than that of dry buffaloes. This is supported by the study of Stojevic *et al.* (2005) in dairy cows. Higher metabolic rates in lactating buffaloes due to higher cellular reactions could account for high ALT levels in lactating animals (Prava *et al.*, 2012). Whereas, non-significantly higher ALT and LDH activities were measured in lactating buffaloes. Stojevic *et al.* (2005) found higher ALT activity in 46-90 days of lactation than that of dry cows. Moreover, ALP and AST were apparently higher in groups of pregnant buffaloes than non-pregnant animals. Non-significant lower levels of CK observed in pregnant dry buffaloes as compared to non-pregnant dry buffaloes were similar to the findings of Antunovic *et al.* (2011) in ewes.

Conclusion

The present investigation is the first study of its kind in case of Banni buffalo. The data generated may be useful as reference values for the scientific community. It also gives an insight about the variation of the biochemical parameters during different physiological stages, which may be used to assess the metabolic health status of animals. Further, the blood-biochemical analytes have been widely used to identify dietary causes of metabolic disorders and low productivity; hence the current study may be helpful in this aspect.

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