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**Vinod Kumar**  
Department of Veterinary  
Medicine, Lala Lajpat Rai  
University of Veterinary and  
Animal Sciences, Hisar,  
Haryana, India

**YS Rana**  
Department of Veterinary  
Medicine, Lala Lajpat Rai  
University of Veterinary and  
Animal Sciences, Hisar,  
Haryana, India

**Praveen Kumar**  
Department of Veterinary  
Medicine, Lala Lajpat Rai  
University of Veterinary and  
Animal Sciences, Hisar,  
Haryana, India

**Ricky Jhambh**  
Department of Veterinary  
Medicine, Lala Lajpat Rai  
University of Veterinary and  
Animal Sciences, Hisar,  
Haryana, India

**VK Jain**  
Department of Veterinary  
Medicine, Lala Lajpat Rai  
University of Veterinary and  
Animal Sciences, Hisar,  
Haryana, India

**Correspondence**  
**Vinod Kumar**  
Department of Veterinary  
Medicine, Lala Lajpat Rai  
University of Veterinary and  
Animal Sciences, Hisar,  
Haryana, India

## Assessment of metabolic parameters of dairy buffaloes from an organized dairy farm during different phases of lactation

**Vinod Kumar, YS Rana, Praveen Kumar, Ricky Jhambh and VK Jain**

### Abstract

Present investigation was carried out to assess the haemato-biochemical profile of healthy *Murrah* buffaloes ( $n=30$ ) in different phases of lactation *viz.* (I) early lactation (II) mid lactation (III) dry animals ( $n=10$  in each group) maintained at organized dairy farm under the standard nutritional and managerial practices. Haemato-biochemical alterations showed decreased glucose levels in group-III, lowest levels in group-I and highest levels in group-II. The levels of ketone bodies were significantly ( $p<0.05$ ) increased in early and mid-lactation animals. Significantly ( $p<0.05$ ) decreased levels of serum calcium were recorded in animals during early lactation. Animals during early and mid-lactation showed decreased levels of serum triglycerides. Higher cholesterol levels were recorded in animals during mid-lactation than early lactation but levels again decreased in late pregnant animals. Significantly ( $p<0.05$ ) lower values of haemoglobin in group-II were recorded. Significantly ( $p<0.05$ ) higher levels of blood urea levels were recorded in group-I and II, than the other groups. Serum creatinine levels were significantly increased in group III. Significantly ( $p<0.05$ ) higher activity of serum enzymes were recorded during mid-lactation. These findings may be helpful in clinical diagnosis of metabolic disorders as well as to formulate the strategy for their prevention in dairy herds.

**Keywords:** Dairy, lactation phase, metabolic parameters, murrah buffaloes

### 1. Introduction

Buffalo (*Bubalus bubalis*) is a long-time ruminant animal which has contributed to the integrated farming systems as a source of draught power, transportation, farm manure, meat, milk and livelihood of the farmers (Wanapat and Kang, 2013) [53]. Buffaloes find pre-eminent position in India's livestock economy as it has about 57.77 per cent of world buffalo population (FAO, 2015) [24] and constitutes about 21.22 per cent of India's total livestock population (BAHS, 2016) [10]. Buffaloes contributed about 49 per cent of the total milk production during the year 2015-16 (BAHS, 2016) [10].

The blood compton metabolic profile test (MPT) has been widely used for assessing metabolic status and diagnosis of metabolic disorders in dairy herds (Radostits *et al.*, 2006) [44]. Blood biochemical profile of farm animals is essential to confirm clinical diagnosis of disease, to assess the severity of disease, nutritional status of farm animals, management of infertility and low productivity in farm animals (Amle *et al.*, 2014 and Kaminski *et al.*, 2014) [8, 33].

Blood metabolic profile of the animals varies due to many genetic and non-genetic factors like age, sex, and physiological status like pregnancy and lactation, nutrition, managerial system such as housing and microclimate can also affect the level of different blood parameters (Jain *et al.*, 2009 and Roubies *et al.*, 2006) [32, 46].

In view of these facts, the present study was planned to assess the haemato-biochemical profile of *Murrah* buffaloes in different physiological stages maintained in organized dairy farm under the standard nutritional and managerial practices.

### 2. Materials and Methods

The present study was undertaken on 30 apparently healthy *Murrah* buffaloes in different stages of lactation. The animals included in the study were between 2<sup>nd</sup>- 6<sup>th</sup> lactation and 5-14 years of age.

#### 2.1 Nutrition and Management

Concentrate mixture was prepared at the farm itself by mixing wheat, maize, gram churi, wheat bran, ground nut cake and cotton seed cake and mineral mixture.

## 2.2 Selection criterion for the animals under the study group

Healthy buffaloes in good body condition and at different phases of lactation were selected randomly. General attitude of the animals were alert and active with normal mucous membrane. There was no history of diarrhoea in previous seven days as well as no history of urogenital abnormalities

during parturition. No medication was given in previous seven days. There was no skin lesions/alopecia as well as no gastro-intestinal or blood parasites which were examined by fecal and blood smear examination.

The selected animals were grouped according to lactation as follows:

**Table 1:** Details of study group and animals during various physiological phases

Group	No. of animals	Physiological Phase
Group I	10	Early lactation (between day of parturition and 30 days of lactation)
Group II	10	Mid lactation (between 50 and 120 days of lactation)
Group III	10	Dry animals (in last 30 days of pregnancy)

## 2.3 Deworming and vaccination

All the animals were regularly dewormed and vaccinated for different viral diseases like FMD and bacterial diseases like HS, BQ and Brucellosis (calf hood vaccine).

## 2.4 Collection of blood samples

Blood samples (~10 ml) were collected aseptically from jugular vein in EDTA vial for hematological analysis, while plasma and serum separation were done by collecting in heparin and vial without anti-coagulant, respectively for lab analysis.

## 2.5 Parasitological examination

Thin blood smears from the micro-capillary circulation (ear tip) stained with Giemsa stain were used to detect haemoprotozoan parasite (Coles, 1986) [18]. Parasitological analyses of fecal samples were done using sedimentation and floatation techniques (Soulsby, 1982) [50] and animals harbouring parasites were excluded from this study.

## 2.6 Lab estimation of various parameters

The blood or serum sample were analyzed for estimation of various haematological and biochemical parameters as described below:

Haematological functions like haemoglobin (Hb) and hematocrit (Hct%) were estimated by using Haematology cell counter (MS4s, Melet Schlosing Lab.); while biochemical parameters like glucose, triglycerides, total cholesterol, ketone bodies, total protein (TP), albumin (Alb), globulins, A:G ratio, urea, total calcium, inorganic phosphorus, magnesium, aspartate aminotransferase (AST) and gamma-glutamyl trans peptidase (GGT) by fully automated random access clinical chemistry analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH). Sodium, potassium and chloride were estimated by using fully automated Easylyte® expand Na/K/Cl/Ca/Li analyser using kits procured from same company.

## 2.7 Ketone bodies estimation

Ketone bodies levels were estimated in plasma by using method of Nadeau as cited by Henry (1966) [30].

## 2.8 Statistical analysis

The data was analyzed statistically by using statistical software (SPSS 16.0). For analysis of various parameters recorded between the groups were analyzed statistically two-way analysis of variance (ANOVA) was applied.

## 3. Result and Discussion

Hb levels recorded in our study were within reference limits reported by Jain *et al.* (1982) [31] in Indian buffalo, Abd Allah *et al.* (2014) [3] in adult water buffaloes. Ciaramella *et al.* (2005) [16] reported slightly higher mean Hb values in primipara buffaloes. Haematocrit (Hct) values varied non significantly in all groups of lactating animals. Our results were higher than reference values reported by Jain *et al.* (1982) [31] in Indian buffalo, Fagiolo *et al.* (2004) [23] and Abd Allah *et al.* (2014) [3] in adult water buffaloes. Differences might be due to stage of pregnancy, climatic conditions or breed of buffaloes. Under different physiological conditions, variations in haematological values can be due to a variety of factors such as the environmental temperature, muscular activity, quality of nutrition and water balance (Ciaramella *et al.*, 2005) [16].

The blood glucose level is regarded as one of the indicators of energy status in ruminants. Blood glucose concentration decreased significantly ( $p \leq 0.05$ ) during early stage of lactation and highest values were observed in the mid lactation stage as shown in Table 2. Similar findings were also reported by Grasso *et al.* (2004) [27]; Bhullar *et al.* (2009) [13] and Hagawane *et al.* (2009) [28] in lactating animals. During lactation glucose level lowered as secretory cells of mammary gland utilized 80% of the circulating metabolites in the blood for milk synthesis (Karapehliyan *et al.*, 2007) [35]. The hypoglycemia after parturition might be due to heavy drainage of glucose for lactose synthesis (Nale, 2003) [41]. In contrast, Borghese (2005) [15] reported higher glucose levels during lactation stages than present study.

Moreover, after parturition, there is a decrease in insulin production by the pancreas (Drackley *et al.*, 2001 and Accorsi *et al.*, 2005) [22, 5] which results in decreased glucose utilization by insulin-sensitive organs (e.g. adipose tissue and muscle) and glucose might be deviated to non-insulin-dependent tissues such as the mammary gland for milk production. Although insulin level decreased in late pregnancy, but increased level of thyroid hormones and increased demands of foetus are responsible factors for lowering of glucose levels in the late pregnant and dry animals.

The concentrations of triglycerides (TG) in dry animals were significantly ( $p \leq 0.05$ ) higher than the animals in early and mid lactation. Our results were similar to findings of Ghanem and El-Deeb (2010) [25] and Monteiro *et al.* (2012) [40]. Sevinc *et al.* (2003) [48] stated that low triglyceride and cholesterol levels near the time of parturition can be due to an influx of

free fatty acids from adipose tissues and a low output of lipoprotein by the liver. Limited amounts of VLDL (very low density lipoprotein) physiologically produced by ruminants (Kaneko 2008) [34], presence of negative energy balance

(Bonnet *et al.*, 2004) [14] and the greater concentrations of blood stream glucagon hormone (Gibbons, 1990) [26], as observed in the lactation peak, leads to the reduction of its serum contents.

**Table 2:** Haemato-biochemical parameters (Mean±S.E.) of animals during early (Group I), mid lactation (Group II) and dry period (Group III)

Parameters	Group I	Group II	Group III
Haemoglobin (g/dl)	12.20 <sup>a</sup> ±0.36	10.44 <sup>b</sup> ±0.28	11.80 <sup>a</sup> ±0.40
Haematocrit (%)	43.42±0.91	41.90±0.97	45.95±1.79
Glucose (mg/dl)	36.91 <sup>a</sup> ±1.02	54.11 <sup>b</sup> ±1.27	42.54 <sup>c</sup> ±1.26
Triglycerides (mg/dl)	16.13 <sup>a</sup> ±0.76	15.81 <sup>a</sup> ±0.70	26.49 <sup>b</sup> ±1.61
Cholesterol (mg/dl)	54.40 <sup>a</sup> ±4.19	100.50 <sup>b</sup> ±4.31	60.20 <sup>a</sup> ±3.95
Ketone bodies (mg/dl)	2.23 <sup>a</sup> ±0.15	1.85 <sup>b</sup> ±0.09	0.85 <sup>c</sup> ±0.15
Protein(g/dl)	7.56±0.15	7.83±0.09	7.79±0.15
Albumin(g/dl)	3.28 <sup>a</sup> ±0.05	3.49 <sup>b</sup> ±0.05	3.44 <sup>ab</sup> ±0.07
Globulin(g/dl)	4.23±0.14	4.28±0.09	4.36±0.13
A:G ratio	0.78±0.04	0.80±0.02	0.80±0.03
Calcium (mg/dl)	8.70 <sup>a</sup> ±0.23	9.27 <sup>b</sup> ±0.21	9.85 <sup>c</sup> ±0.16
Phosphorus (mg/dl)	5.12±0.14	5.14±0.30	5.20±0.24
Magnesium (mg/dl)	2.50±0.11	2.25±0.17	2.45±0.12
Sodium (mmol/l)	132.88 <sup>a</sup> ±1.81	132.53 <sup>a</sup> ±1.32	136.16 <sup>b</sup> ±1.18
Potassium (mmol/l)	4.23 <sup>a</sup> ±0.19	4.71 <sup>b</sup> ±0.11	4.43 <sup>b</sup> ±0.18
Chloride (mmol/l)	104.09 <sup>a</sup> ±1.00	103.30 <sup>a</sup> ±0.83	106.83 <sup>b</sup> ±0.86
AST (IU/L)	114.77 <sup>a</sup> ±2.49	130.59 <sup>b</sup> ±5.98	88.45 <sup>c</sup> ±2.51
GGT(IU/L)	19.83 <sup>a</sup> ±3.05	29.88 <sup>b</sup> ±2.74	20.95 <sup>a</sup> ±1.53
Urea (mg/dl)	27.60 <sup>a</sup> ±1.33	44.90 <sup>b</sup> ±3.18	23.18 <sup>a</sup> ±1.82
Creatinine (mg/dl)	1.65 <sup>a</sup> ±0.12	1.47 <sup>a</sup> ±0.06	2.34 <sup>b</sup> ±0.13

(n= 10 animals in each group)

**Note:** Means bearing different superscripts (a, b, c) differ significantly ( $p < 0.05$ ) in rows for each parameter.

In present study, concentration of cholesterol in mid-lactation animals were significantly ( $p \leq 0.05$ ) higher than in early lactation and dry animals. Similar reports were also reported by Monteiro *et al.* (2012) [40], Lone *et al.* (2003) [38] and Ashmawy (2015) [9] stated that the higher level of cholesterol with advancement of lactation is a physiological adjustment to meet the lactation requirements. Abayawansa *et al.* (2013) [1] noticed significantly higher serum cholesterol concentration during the pre-calving period and the low concentration at calving could be due to high demand for adrenal corticosteroid and placental steroid synthesis as well as colostrum and milk synthesis. The high cholesterol levels reported in the mid lactation might be due to lowered levels of thyroxin in the blood. In contrast, Tripathi *et al.* (2010) [52] reported higher total cholesterol than our study in early, mid and late stage of lactation.

Significant higher levels of ketone bodies during early lactation (2.23±0.15 mg/dl) might be due to negative energy balance because of energy expenditure due to milk production and limited feed intake (Macajova *et al.*, 2004) [39]. Lower glucose level leads to increased fat mobilization from the body reserves which leads to increased ketogenesis by hepatocytes (Djoković *et al.*, 2011) [21].

During early lactation, serum total protein values were slightly lower than mid-lactation and dry animals. Similar values were reported by Sharma and Sridhar (2007) [49] in apparently healthy buffaloes. In contrast, Hagawane *et al.* (2009) [28] reported slightly elevated serum protein in early lactation than normal healthy control which might be due to the water losses occurred postpartum. Protein catabolism increases around parturition to support milk protein synthesis and hepatic gluconeogenesis (Reynolds *et al.*, 2003) [45] occur after parturition.

Serum albumin is a very sensitive and an early indicator of protein status of the dairy animals (Agenas *et al.*, 2006) [6]

because its turnover is only 16 days. Lower serum albumin levels were recorded in animals during early lactation which differed non-significantly with the dry animals but differed significantly ( $p < 0.05$ ) with mid lactation animals. Similarly, Tainturier (1984) [51] also reported that the levels of albumin and globulin remain unchanged in Friesian dairy cows during lactation. Serum globulin levels and A:G ratio in all the groups of lactating animals varied non-significantly.

Urea concentration is an indicator of energy protein balance and is typically increased in animals deficient in energy. In present investigation, significantly ( $p < 0.05$ ) lower values were recorded in dry animals and non-significantly low in early lactation animals than the mid lactation ones. Similar findings were also reported by Hagawane *et al.* (2009) [28] and Pande *et al.* (2016) [42]. Higher urea levels in the first three months of lactation might be due to both increased food intake and higher tissue mobilization (Bertoni *et al.*, 1994) [11]. Serum levels are affected by renal and other physiological functions, external factors like days in milk (Serdaru *et al.*, 2011) [47], dietary protein supply relative to the requirements, season (Dhali *et al.*, 2006) [19], amount of crude protein in the diet as well as by degradable intake of protein (DIP) and undegradable intake of protein (UIP).

Reduced concentrations of creatinine indicate prolonged active tissue protein catabolism (Agenas *et al.*, 2006) [6]. It's concentrations vary due to animal diet, breed, muscle mass and sex (Hammond, 2006) [29]. In contrast to our findings, Ashmawy (2015) [9] and Abayawansa *et al.* (2013) [1] reported higher concentrations of plasma creatinine during lactation and at the time of calving in buffaloes.

Highest AST enzymatic activity during mid lactation might be due to increase in hepatic metabolism (Ashmawy, 2015) [9]. Similar results were also found by Djokovic *et al.* (2013). On contrary, Abdulkareem (2013) [4] reported higher AST values than our results in buffaloes which indicated that hepatic

metabolism and tissue catabolism might be more than the normal range. Ghanem and El-Deeb (2010)<sup>[25]</sup> and Abd Ellah *et al.* (2013)<sup>[2]</sup> reported lower mean values of GGT than our results, whereas Bertoni *et al.* (1994b)<sup>[12]</sup> reported almost equal mean values of GGT level.

Significantly ( $p < 0.05$ ) lower serum calcium levels (Table 2) were recorded in early lactation than mid lactating and dry animals. Similar findings were also reported by Hagawane *et al.* (2009)<sup>[28]</sup>; Piccione *et al.* (2012)<sup>[43]</sup> and Ashmawy (2015)<sup>[9]</sup>. Serum phosphorus (P) and magnesium concentration varied non-significantly in all groups of animals. Serdaru *et al.* (2011)<sup>[47]</sup> reported lower calcium, phosphorus and magnesium concentrations in lactating buffaloes than the pregnant ones which might be due to the necessity of it for the colostrum synthesis and enhanced carbohydrate metabolism.

In accordance to this study, Ashmawy (2015)<sup>[9]</sup> also reported higher concentrations of sodium and potassium during pregnant period than in lactation period. Significantly ( $p < 0.05$ ) low values of serum potassium during early lactation were also reported by Kulkarni *et al.* (1984). On contrary, Kuhn *et al.* (2006) reported highest sodium and potassium plasma levels at 7th day with respect to 30<sup>th</sup> day both in lactation and dry period. The concentrations of chloride in dry animals were significantly higher than early and mid-lactation. On contrary, Kulkarni *et al.* (1984)<sup>[37]</sup> reported lower mean values of chloride in lactating buffaloes than the present study. These differences might be due to different feeding and other managerial practices. Yokus and Cakir (2006)<sup>[54]</sup> and Akhtar *et al.* (2010)<sup>[7]</sup> stated that the mean serum sodium, potassium, and chloride concentrations varied non-significantly during lactation in cross-bred cows and Nili-Ravi buffaloes, respectively.

#### 4. Summary and conclusions

Haemato-biochemical profile of healthy *Murrah* buffaloes revealed that lower concentrations of glucose, triglycerides, albumin, Ca, P and higher levels of ketone bodies during early lactation might be due to negative energy balance or heavy drainage in colostrums. In mid-lactation, there was high concentrations of cholesterol, urea, AST, GGT and low concentration of triglycerides. Accordingly, the present findings might be utilized in clinical diagnosis as well as to plan the preventive strategy of metabolic disorders.

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