

## SERUM BIOCHEMICAL PARAMETERS DURING DIFFERENT STAGES OF PREGNANCY IN MURRAH BUFFALOES

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### ABSTRACT

The most significant and productive milk producer among Indian buffalo breeds is the Murrah. Determining reference values for the serum biochemical markers of Murrah buffaloes at different stages of pregnancy was the aim of the current study. This study included 28 Murrah buffaloes from the Indore district of Madhya Pradesh, India. They have been separated into 4 groups: Group I was non-pregnant, Group 2 was in “the 1<sup>st</sup> trimester of pregnancy, Group 3 was in the 2<sup>nd</sup> trimester of pregnancy, and Group 4 was in the 3<sup>rd</sup> trimester of pregnancy” till parturition. Every group had 7 animals. Blood samples were tested for serum biochemical measures, including total albumin, BUN (blood urea nitrogen), AST (aspartate aminotransferase), glucose, uric acid, globulin, HDL (higher density lipoprotein) cholesterol, A/G (albumin/globulin) ratio, LDL (lower density lipoprotein) cholesterol, creatinine, total cholesterol, ALT (alanine-aminotransferase), triglycerides, ALP (alkaline phosphatase), and protein. Late-pregnancy serum albumin levels in comparison to mid-pregnancy and non-pregnant animals; HDL cholesterol in early-, mid-, and late-pregnancy in animals as well as non-pregnant,

mid-, as well as late-pregnant animals; ALT as well as AST levels in late pregnancy in comparison to other cohorts; glucose levels in pregnant animals as opposed to those that are not; Urea levels in non-pregnant, early, middle, and late-pregnant animals; BUN between early-, middle-, and late-stage pregnancy and non-pregnant animals; early-pregnant with late pregnant animals-all displayed a greatly significant variance ( $P<0.01$ ). Serum “levels of total protein in the middle along with late phase of pregnancy has been significantly ( $P<0.05$ ) different from those of the non-pregnant” animal, total cholesterol in the 3<sup>rd</sup> trimester of pregnancy was different from other groups, and only uric acid was different between the non-pregnant and postpartum animals. The levels of globulin, LDL cholesterol, A/G ratio, ALP, triglycerides, as well as creatinine did not significantly differ ( $P>0.05$ ) across the groups. The current research concluded by establishing reference values that serve as a helpful guide for evaluating blood biochemical markers in Murrah buffaloes at various stages of pregnancy.

**Keywords:** *Bubalus bubalis*, buffaloes, Murrah buffalo, protein profile, lipid profile, pregnancy, enzymes

## INTRODUCTION

One of the most valuable animal resources in Asia, particularly India, is the buffalo (*Bubalus bubalis*). Despite being well suited to hot, humid climates, buffaloes' reproductive capacities are significantly diminished by heat stress (Marai and Haebe, 2010). India has 109.85 million buffalo overall, with Madhya Pradesh having the fourth-largest buffalo population in the country at 10.30 million (20<sup>th</sup> Livestock Census, 2019). The most significant and productive milk producer among Indian buffalo breeds is the Murrah. It is common practice to upgrade poor stock with bulls. Lactation produces 1,500 to 2,500 kg of milk on average (Khan *et al.*, 2009; Prakash *et al.*, 2005).

Standard levels of essential haematobiochemical elements are crucial for preserving the reproductive system's functional integrity. Livestock reproductive efficiency may be negatively impacted by any variation in hematological and biochemical markers (Sabasthin *et al.*, 2012). When assessing an animal's health, blood biochemical characteristics are thought to be significant. The diagnosis of various pathophysiological and metabolic problems in cattle requires the estimation of biochemical constituents (Mc Dowell, 1992).

In order to provide value references for blood biochemical characteristics in Murrah buffaloes at various phases of pregnancy, the current study was conducted with consideration for both experimental and economic utility.

## MATERIALS AND METHODS

### Selection of animals

In the current study, 28 Murrah buffaloes

from the Indore area of MP, India, which appeared to be in good health, were split up into four groups, each containing seven animals. For example, those who are not pregnant (Group 1), those "who are in their 1<sup>st</sup> trimester of pregnancy (Group 2), those who are in their 2<sup>nd</sup> trimester of pregnancy (Group 3), and those who are in their 3<sup>rd</sup> trimester of pregnancy till" parturition (Group 4).

### Collection of blood

Blood had been drawn from the jugular vein and placed in a sterile, anticoagulant-free vacutainer. Following clotting, serum was extracted and kept at -20°C for hormonal along with biochemical examination.

### Methodology

Using commercially available biochemical kits (Erba Mannheim, Germany), the following parameters were estimated: globulin, albumin, albumin:globulin (A:G) ratio, HDL cholesterol, triglycerides, ALT, ALP, BUN, creatinine, serum total protein, AST, glucose, uric acid, total cholesterol, and LDL cholesterol.

By deducting serum albumin from serum total protein, the globulin fraction in serum samples was calculated.

$$\text{Globulin (g/dl)} = \text{Total protein} - \text{Albumin}$$

The albumin: globulin ratio had been acquired by dividing serum albumin concentration by serum globulin concentration.

The concentration of LDL cholesterol has been computed based on Friedwald's formula defined "by Friedwald *et al.* (1972).

$$\text{LDL cholesterol (mg/dl)} = \text{Total cholesterol} - (\text{Triglyceride}/5) - \text{HDL cholesterol}$$

### Ethical approval

The Institutional Animal Ethical Committee at the College of Veterinary Science and Animal Husbandry in Mhow, Madhya Pradesh, has approved the current inquiry.

## RESULTS AND DISCUSSION

ANOVA (Analysis of variance) using a CRD (completely randomized design) was performed on the serum concentration of biochemical parameters in four groups of Murrah buffaloes (Snedecor and Cochran, 1994). At the 1% and 5% levels of significance, the concentration was displayed. Analysis was evaluated as follows:  $P < 0.01$  denotes a highly significant variance;  $P < 0.05$  denotes a significant variance, and  $P > 0.05$  denotes a non-significant variance.

### Serum protein profile

Comparing mid- and late-pregnant “animals to non-pregnant ones, a significant ( $P < 0.05$ ) drop in serum total” protein was noted (Table 1). The findings of Mir *et al.* (2008) in crossbred cows, Bamerny (2013) in Meriz does, and Piao *et al.* (2015) in Hanwoo cows are all consistent with the observations. Serum total protein levels may have dropped as a result of the fetus’s development, particularly the use of maternal circulation amino acids for protein synthesis in the fetus’s muscle. According to Aich *et al.* (2007) in Black Bengal goats and Abd Ellah *et al.* (2013) in buffaloes, there had been a “non-significant ( $P > 0.05$ ) change in protein concentration among non-pregnant and pregnant animals.

There is a substantial ( $P < 0.01$ ) difference in the albumin fraction among Groups 1 and 2; 1 and 4, Group 2 and Group 3 (Table 1). Significant

( $P < 0.05$ ) reduction in albumin concentration in late-pregnancy as compared to mid-pregnancy as well as primary lactation had been” observed by Aich *et al.* (2007) in Black Bengal goats and Al-Mujalli (2008) in cows are in accordance with present findings. On the contrary, increases in albumin concentration during pregnancy were observed by Mir *et al.* (2008) in crossbred cows, Piccione *et al.* (2009) in Comisana ewes, Abdulkareem (2013) in Iraqi Riverine buffaloes, Bamerny (2013) in Meriz does and Piao *et al.* (2015) in Hanwoo cows.

No-significant ( $P > 0.05$ ) variance in the concentration of globulin and A:G ratio has been reported among the groups (Table 1). Non-significant ( $P > 0.05$ ) higher globulin concentration was recorded in early pregnancy as compared to other groups. Decreasing order of serum globulin concentration was recorded in mid and late-pregnancy by Mir *et al.* (2008) in crossbred cows. The a:G ratio of the current studies is in accordance having Zvorc *et al.* (2000) in cows.

### Serum lipid profile

Compared to other groups, there was a significant ( $P < 0.05$ ) drop in serum total cholesterol levels in the 3<sup>rd</sup> trimester of pregnancy (Group 4) (Table 2). The concentrations stated by Hagawane *et al.* (2009) in buffaloes as well as Piccione *et al.* (2009) in Comisana ewes were consistent with the outcomes.

A greatly significant ( $P < 0.01$ ) decreasing trend of HDL cholesterol had been noted in the progress of pregnancy (Table 2). Results were comparable with the concentration obtained by Rawat (2010) in Sirohi goats.

Non-significant ( $P > 0.05$ ) elevated concentrations of LDL-cholesterol have been noted in the middle of pregnancy but, the lowest concentration had been recorded in late pregnancy

(Table 2). Similar outcomes have been observed by Rawat (2010) in Sirohi goats.

Non-significant ( $P>0.05$ ) elevated concentrations of triglycerides have been observed in middle and late pregnancy but, the lowest concentration was recorded at early pregnancy (Table 2). Decreased concentration of serum triglycerides during late pregnancy was informed by Piccione *et al.* (2009) in Comisana ewes, Rawat (2010) in Sirohi goats as well as Piccione *et al.* (2012) in dairy cows are in agreement with current findings.

### Serum enzyme activity

Significant ( $P<0.01$ ) variance had been detected in serum alanine aminotransferase (ALT) activity in Group 4 compared to other groups (Table 3). The values were decreased with the progression of pregnancy stages and were lowest in the 3<sup>rd</sup> trimester. Similar observations of decreased concentration of ALT during late pregnancy were reported by Aich *et al.* (2007) in Black Bengal goats, Rawat (2010) in Sirohi goats, and Abd Ellah *et al.* (2013) in buffaloes.

A highly significant ( $P<0.01$ ) difference in serum aspartate aminotransferase (AST) activity was detected in Group IV compared to different groups (Table 3). Decreased concentration of AST during late pregnancy was reported by Rawat (2010) in Sirohi goats, Abd Ellah *et al.* (2013) in buffaloes and Abdulkareem (2013) in Iraqi Riverine buffaloes in accordance with present findings.

Non-significant ( $P>0.05$ ) higher concentrations of serum alkaline phosphatase (ALP) activity were observed in non-pregnant and the lowest concentration in the middle of pregnancy (Table 3). A similar finding of the lowest ALP activity at mid-pregnancy has been stated by Piao *et al.* (2015) in Hanwoo cows. Although,

a great concentration of ALP activity at mid-pregnancy was observed by Abd Ellah *et al.* (2013) in buffaloes and Abdulkareem (2013) in Iraqi Riverine buffaloes.

### Serum glucose

Highly significant ( $P<0.01$ ) increased concentrations were observed when compared to animals that are not pregnant (Table 4). Serum glucose concentrations are gradually decreased in mid and late pregnancy from early pregnancy. A similar pattern had been stated by Mir *et al.* (2008) in cows that are crossbred, Waziri *et al.* (2010) in Sahel goats as well as Bamerny (2013) in Meriz does.

### Serum metabolites

A highly significant ( $P<0.01$ ) variance in blood urea nitrogen (BUN) concentrations was observed in Group 1 compared to other groups; Group 2 as well as Group 4 (Table 5). The gradually decreasing concentrations were recorded with the progression of pregnancy. Similar outcomes have been revealed by Piccione *et al.* (2009) in Comisana ewes.

Levels of serum uric acid have been significantly ( $P<0.05$ ) elevated in late-pregnancy mice than in non-pregnant, early-pregnant, along with mid-pregnant animals (Table 5). A similar finding of increased serum uric acid in pregnant animals than non-pregnant had been observed by Aich *et al.* (2007) in Black Bengal goats. However, a decreased concentration of uric acid during late pregnancy was seen by Rawat (2010) in Sirohi goats.

Early and late pregnancy have been associated with non-significantly ( $P>0.05$ ) greater serum creatinine concentrations, while non-pregnant animals had the lowest concentration

Table 1. The mean serum protein profile in Murrah buffaloes at various stages of pregnancy.

Parameters	Group 1	Group 2	Group 3	Group 4
Total protein(g/dl)*	7.45±0.21 <sup>b</sup>	6.95±0.57 <sup>ab</sup>	6.21±0.25 <sup>a</sup>	6.18±0.21 <sup>a</sup>
Albumin (g/dl)**	4.14±0.12 <sup>c</sup>	3.17±0.15 <sup>a</sup>	3.72±0.17 <sup>bc</sup>	3.54±0.16 <sup>ab</sup>
Globulin (g/dl) <sup>NS</sup>	3.13±0.26	3.78±0.48	2.50±0.33	2.64±0.32
A:G <sup>NS</sup>	1.32±0.14	0.95±0.14	1.82±0.37	1.54±0.25

<sup>NS</sup>Non significant (P>0.05); \*\*Highly significant (P<0.01); \*Significant (P<0.05).

Significant” differences exist among means with various superscripts within a row.

Table 2. The mean serum lipid profile in Murrah buffaloes at various stages of pregnancy.

Parameters	Group 1	Group 2	Group 3	Group 4
Total cholesterol (mg/dl)*	215.69±29.62 <sup>b</sup>	213.02±46.55 <sup>b</sup>	180.22±25.02 <sup>b</sup>	94.40±9.71 <sup>a</sup>
HDL cholesterol (mg/dl)**	119.01±18.57 <sup>c</sup>	99.72±19.17 <sup>bc</sup>	62.37±7.36 <sup>ab</sup>	29.06±4.24 <sup>a</sup>
LDL cholesterol (mg/dl) <sup>NS</sup>	95.04±17.62	111.75±29.83	113.33±20.18	62.49±8.04
Triglycerides (mg/dl) <sup>NS</sup>	8.16±1.72	7.71±3.35	22.57±9.11	14.21±5.65

<sup>NS</sup>Non significant (P>0.05); \*\*Highly significant (P<0.01); \*Significant (P<0.05).

Significant” differences exist among means with various superscripts within a row.

Table 3. The mean serum enzyme profile in Murrah buffaloes at various stages of pregnancy.

Parameters	Group 1	Group 2	Group 3	Group 4
ALT(IU/L)**	82.94±6.08 <sup>b</sup>	81.00±10.17 <sup>b</sup>	70.89±5.98 <sup>b</sup>	41.89±3.70 <sup>a</sup>
AST (IU/L)**	213.81±20.81 <sup>b</sup>	193.33±9.46 <sup>b</sup>	182.34±5.7 <sup>b</sup>	138.59±6.32 <sup>a</sup>
ALP (IU/L) <sup>NS</sup>	127.43±28.06	83.71±8.01	61.14±9.96	100.86±28.24

\*\*greatly significant (P<0.01); <sup>NS</sup>Non significant (P>0.05).

Significant differences exist between means with various superscripts within a row.

Table 4. Mean serum glucose concentration (mg/dl) when Murrah buffaloes are pregnant at various stages.

Parameter	Group 1	Group 2	Group 3	Group 4
Glucose(mg/dl)**	79.21±4.29 <sup>a</sup>	113.33±7.19 <sup>b</sup>	107.54±6.31 <sup>b</sup>	98.02±6.08 <sup>b</sup>

\*\*Highly significant (P<0.01).

Significant differences exist between means with various superscripts within a row.

Table 5. Mean concentration of serum metabolites when Murrah buffaloes are pregnant at various stages.

Parameters	Group 1	Group 2	Group 3	Group 4
BUN (mg/dl)**	39.44±1.79 <sup>c</sup>	26.37±5.03 <sup>b</sup>	18.94±2.39 <sup>ab</sup>	15.46±2.51 <sup>a</sup>
Uric acid (mg/dl)*	0.91±0.18 <sup>a</sup>	0.86±0.09 <sup>a</sup>	0.85±0.04 <sup>a</sup>	1.39±0.12 <sup>b</sup>
Creatinine(mg/dl) <sup>NS</sup>	0.81±0.08	1.25±0.08	1.16±0.06	1.25±0.19

\*\*Highly significant (P<0.01); \*Significant (P<0.05)<sup>NS</sup>; Non significant (P>0.05).

Significant differences exist between means with various superscripts within a row.

(Table 5). Similar to the current finding, Piao *et al.* (2015) detected an raise in concentration in Hanwoo cows in the late stages of pregnancy in contrast to animals that are mid-pregnant and not pregnant. Waziri *et al.* (2010), on the contrary, found nosignificant variations (P>0.05) in creatinine concentration in Sahel goats.

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