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Effect of stage of lactation on hematological and serum biochemical profile in Murrah buffalo (*Bubalus bubalis*)

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Abstract
The present study was carried out to investigate haemato-biochemical profile of Murrah buffaloes, a native milk breed of India with the intent of analyzing the physiological alteration under the influence of different lactation stages in terms of determining possible biomarkers to monitor the energetic balance and the metabolic adequacy the during lactation. In the present investigation, twenty-one clinically healthy lactating Murrah buffaloes of age 5 to 7 years were divided into three equal groups: Group I (Early lactation stage), Group II (Mid lactation stage) and Group III (Late lactation stage). The selected herd of buffaloes are maintained hygienically with similar feeding pattern and managerial practices under same housing system. Blood samples were collected in the jugular vein of the grouped animal's, blood was analysed for haematology and serum for biochemical analysis. Non-significant variations were found in the hematological parameters between the three groups of animals. The mean corpuscular volume (MCV), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) Blood Urea-nitrogen (BUN), creatinine and glucose were statistically significant. Glucose levels were significantly lowest in the early phase of lactation; In contrast, concentrations of cholesterol, globulin, MCV, MCH, WBC, creatinine, and BUN were higher at this stage. Hemoglobin (Hb), total red blood cell count (TEC), mean corpuscular haemoglobin concentration (MCHC), and total protein were highest in the middle of milk production and lowest in the early phase. The data obtained in the current study may be useful as reference values for the scientific community, as this is the first study of its kind in Murrah buffalo, an indigenous dairy breed in India.

Keywords: Murrah buffalo, lactation, haematology, serum, biochemical profile

1. Introduction
Blood metabolic profile of the animal is commonly used as indicators of health as well as nutritional status (Amle *et al*., 2014) [4]. The blood biochemical profiles are often considered important in evaluating the health status of the animals. The estimates of biochemical constituents are the prerequisites to diagnose several pathophysiological and metabolic disorders in buffaloes (Mc Dowell, 1992; Chaffe, 1976) [26, 6]. The present study was undertaken to study the hematological and some of the blood biochemical alterations according to stages of lactation in Murrah buffaloes. Amongst, the various factors affecting metabolic profile, the physiological status of animals is the predominant one (Ahmad *et al*., 2003) [3]. Pregnancy and lactation are two major stages in the life of dairy animals, which leads to major metabolic alterations (Krajnicakova *et al*., 2003; Iriadam, 2007) [20, 18]. Lactation is a very critical period for the animal with increased nutritional requirements. The increased need for energy, fats, protein and minerals for milk synthesis also leads to certain metabolic disorders. The milk components are directly or indirectly synthesized from blood that affect blood metabolites and enzymes biochemical estimation. It is also well known that lactation is associated with a physiologically increased rate of metabolic processes, that is characterized by a high energy requirement, especially in the early lactation, when milk yield is higher (Piccione *et al*., 2009; Cigliano *et al*., 2014) [30, 7]. Therefore, the level of blood biochemical parameters like glucose, total protein, A/G ratio, cholesterol, urea and uric acid changes throughout the course of lactation and are important indicators of the metabolic activity in lactating animals (Fernandez and Hoeffler, 1998; Karapehlivan *et al*., 2007) [14, 19]. Since the milk yield and composition varies across the length of lactation, it is crucial to study the blood

metabolites during different stages i.e., early, mid and late stage of lactation. In view of the above, the present study was designed to investigate the changes in metabolic profile during different stages of lactation in Murrah buffalo, a native milk breed of India.

2. Materials and methods

2.1 Study location and experimental animals

The present study was conducted at Buffalo Research Station (BRS), Venkataramannagudem of Sri Venkateswara Veterinary University (SVVU) located at an altitude of 44.7 meter above sea level on 16.8831°N latitude and 81.4513°E longitudes. The institute was located at the upland area where the water source is mainly dependent on rainfall and bore well, where temperature raises up to 37 ºC in summer and comes closer to 19 ºC during the winter season. Murrah breed is considered to be one of the superior breeds of Indian milk buffaloes with an average lactation yield and lactation length of 1752 kg and 310 days, respectively. Twenty-one (21) clinically healthy lactating Murrah buffaloes of age 5 to 7 years were selected for the present study and maintained at Buffalo Research Station of SVVU, Venkataramannagudem under loose housing system in lactating animal shed. The Murrah buffaloes were in various stages of lactation and based on the length of their lactation length, the animals were identified as in early (7 to 105 days), mid (106 to 210 days) and late (211 to 315 days) lactational stage. Accordingly, they were categorized into three different groups of seven animals each viz. group I (early lactation), group II (mid lactation) and group III (late lactation).

2.2 Collection of blood samples

Blood samples were collected aseptically from each buffalo of all the three groups by jugular venipuncture into collection tubes containing anticoagulant K3 EDTA (Bene Sphera) for hematological analysis and vacutainer containing clot activator tubes (Bene Sphera) for biochemical analysis.

2.3 Hematological analysis

The collected blood samples were analyzed for different hematological parameters including hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total erythrocyte count (TEC), total leucocyte count (TLC) and platelet count (PLT) using Automated Hematology Analyzer (BeneSphera 3-part differential hematology analyzer-H31).

2.4 Biochemical analysis

The collected blood samples were centrifuged at 3000 rpm for 15 minutes and by using a clean pipette technique, 2 mL serum was aliquoted and were analyzed for different biochemical analytes including Blood Glucose (GL)-GOD-POD method, Total protein (TP) -Biuret Method, Albumin (ALB)-BCG Method, Globulin (GLB), Blood Urea Nitrogen (BUN)-UREAS Method, Creatinine (Cr)-JAFFE'S method, Alkaline phosphatase (AP)-AMP method, Alanine aminotransferase (ALT)-IFCC method, Aspartate aminotransferase (AST)-IFCC method.

2.5 Statistical analysis

The data obtained from the present study were analyzed statistically using a Microsoft Excel-based data analysis tool pack for descriptive statistics and also the graphical presentations. In all analyses, significant differences were considered at values of $P<0.05$ (significant) or $P<0.01$ (highly significant).

3. Results and discussion

The results of the current study were presented in table 1, 2, 3, which includes descriptive measures of hematological and biochemical profile of lactating Murrah buffaloes. Mean±SE values of blood and biochemical parameters were represented in table 2.

Table 1: Descriptive measures of hematological and biochemical profile in Group I lactating Murrah buffaloes

Parameters	Hb	TLC	TEC	PLT	PCV	MCV	MCH	MCHC	BUN	Cr	ALT	AST	AP	TP	ALB	GLB	A/G	CL	GL
Mean	9.0	52171.4	5.3	393428.6	28.8	54.7	16.9	31.0	39.0	1.5	61.3	61.6	81.7	7.2	2.9	4.8	0.7	126.1	41.1
Standard Deviation	0.7	68752.7	0.5	79239.8	2.3	2.5	0.9	0.6	4.7	0.2	20.8	25.3	24.7	0.3	0.2	1.0	0.1	28.8	11.1
Standard Error	0.3	25986.1	0.2	29949.8	0.9	0.9	0.4	0.2	1.8	0.1	7.8	9.6	9.3	0.1	0.1	0.4	0.0	10.9	4.2
Range	2.1	151000.0	1.5	219000.0	6.9	6.4	2.5	1.7	14.0	0.3	50.0	62.0	62.0	0.7	0.6	3.0	0.1	82.0	28.0
Median	8.9	11700.0	5.3	394000.0	28.9	54.1	16.5	31.1	41.0	1.5	63.0	64.0	92.0	7.3	2.9	4.5	0.7	117.0	45.0
Minimum	7.7	8000.0	4.4	297000.0	24.6	52.6	15.7	30.4	29.0	1.4	37.0	31.0	53.0	6.8	2.6	4.0	0.6	87.0	41.0
Maximum	9.8	159000.0	5.9	516000.0	31.5	59.0	18.2	32.1	43.0	1.7	87.0	93.0	115.0	7.5	3.2	7.0	0.7	169.0	69.0
Count	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7

Table 2: Descriptive measures of hematological and biochemical profile in Group II lactating Murrah buffaloes

Parameters	Hb	TLC	TEC	PLT	PCV	MCV	MCH	MCHC	BUN	Cr	ALT	AST	AP	TP	ALB	GLB	A/G	CL	GL
Mean	9.2	11428.6	5.7	398714.3	29.1	51.1	16.1	31.6	16.3	1.4	33.9	40.6	136.7	7.5	2.9	4.5	0.7	117.9	46.1
Standard Deviation	0.3	3660.2	0.4	121652.1	1.2	2.6	0.7	0.6	5.8	0.1	10.3	10.7	72.3	0.5	0.2	0.4	0.1	33.0	5.1
Standard Error	0.1	1383.4	0.1	45980.2	0.5	1.0	0.3	0.2	2.2	0.0	3.9	4.1	27.3	0.2	0.1	0.2	0.0	9.1	1.9
Range	0.8	8000	1	346000	3.6	7.7	2.2	1.6	16	0.3	30	25	205	1.6	0.6	1.4	0.1	80	13
Median	9.3	10800	5.6	343000	28.8	50.8	16.2	39.5	14	1.4	29	36	108	7.3	3	4.6	0.7	117	44
Minimum	8.8	7400	5.3	296000	27.5	47.9	15	30.8	11	1.3	21	31	91	6.7	2.6	3.9	0.6	83	41
Maximum	9.6	15400	6.3	642000	31.1	55.6	17.2	32.4	27	1.6	51	56	296	8.3	3.2	5.3	0.7	163	54
Count	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7

Table 3: Descriptive measures of hematological and biochemical profile in Group III lactating Murrah buffaloes

PARAMETERS	Hb	TLC	TEC	PLT	PCV	MCV	MCH	MCHC	BUN	Cr	ALT	AST	AP	TP	ALB	GLB	A/G	CL	GL
Mean	9.0	13971.4	5.4	347428.6	28.7	52.9	16.5	31.4	31.8	1.7	83.7	74.3	157.6	7.3	2.9	4.4	0.6	102.0	58.6
Standard Deviation	0.0	9393.9	0.6	117927.7	2.9	4.1	1.1	1.0	10.4	0.1	49.0	38.6	80.6	0.3	0.1	0.4	0.1	17.3	11.7
Standard Error	0.3	3550.6	0.2	44572.5	1.1	1.5	0.4	0.4	3.9	0.0	18.5	14.6	30.4	0.1	0.0	0.1	0.0	6.5	4.4
Range	2.1	27500.0	2.1	371000.0	7.4	11.5	3.2	2.5	27.0	0.3	146.0	109.0	241.0	1.0	0.3	1.1	0.2	57.0	34.0
Median	9.2	10700.0	5.5	37.3	29.4	51.7	16.6	30.9	30.0	1.7	84.0	83.0	131.0	7.2	2.9	4.4	0.7	104.0	44.0
Minimum	7.7	6000.0	4.1	135000.0	24.5	46.5	15.2	30.3	19.0	1.5	32.0	24.0	72.0	6.9	2.7	4.0	0.5	73.0	49.0
Maximum	9.8	33500.0	6.2	506000.0	31.9	58.0	18.4	32.8	46.0	1.8	178.0	133.0	313.0	7.9	3.0	5.1	0.7	130.0	83.0
Count	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7

In all these hematological parameters, there were no significant differences between the groups of lactating Murrah buffaloes, as shown in Table 4. The limited sensitivity of the blood parameters to lactation stage in clinically normal dairy animals is not surprising, as most of these parameters are subjected to homeostatic control systems (Cozzi *et al*., 2011) [9]. In the early phase of lactation, the mean hemoglobin concentration was 8.971±0.26 fL which is the lowest and showed a higher side in mid and late lactation (Hagawane *et al*. 2012) [16]. Packed cell volume (PCV) and total erythrocyte count (TEC) were lowest in Murrah buffaloes at the early stage of lactation and these results were corroborating with the results of (Esievo and Moore 1979) [13] which concluded that the concentrations of PCV and total RBC decreased in early lactation and increased in mid-lactation and pre-lactation levels. The mean value of Total WBC in the early lactation group of Murrah buffaloes was 52171.42±25986.08, which showed a slightly higher trend than in the normal healthy Murrah buffaloes. The platelet concentration determined in this study remained constant throughout the lactation period, indicating that there is no much in the lactation phase. The erythrocytic indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were within the normal values established for the buffalo. The mean corpuscular volume levels of the different groups of lactating Murrah buffaloes differ significantly (Fig. 1). Similar to the present study, non-significant differences of various hematological indices were also found in Mehshani buffaloes during early and late lactation phases (Das *et al*. 2016) [10], might be due to the animal variation.

Table. 2: Mean±SE values of blood and biochemical parameters in Murrah buffaloes

Parameters	Group-I	Group-II	Group-III
Haemoglobin	8.971±0.269	9.1857±0.101	9.028±0.28
Total WBC	52171.42±25986.08	11428.57±1383.355	13971.42±3550.56
Total RBC	5.257±0.193	5.671±0.134	5.428±0.242
Platelet Count	393428.57±29949.84	398714.29±45980.179	347428.57±44572.49
Packed Cell Volume	28.814±0.877	29.085±0.452	28.742±1.082
MCV	54.657±0.937^{a}	51.085±0.967^{b}	52.928±1.546
MCH	16.914±0.35	16.057±0.263	16.514±0.411
MCHC	31.042±0.235	31.571±0.218	31.385±0.373
Blood Urea Nitrogen	39±1.786^{a}	16.285±2.179^{b}	31.817±3.924^{a}
Creatinine	1.457±0.084^{a}	1.414±0.045^{b}	1.7±0.037^{a}
ALT	61.285±7.845^{a}	33.857±3.875^{b}	83.714±18.5^{a}
AST	61.571±9.581	40.571±4.052^{a}	74.285±14.595^{b}
Alkaline Phosphate	81.714±9.334^{a}	136.714±27.34	157.57±30.447^{b}
Total Protein	7.214±0.107	7.457±0.198	7.285±0.118
Albumin	2.885±0.067	2.914±0.073	2.885±0.045
Globulin	4.757±0.381	4.542±0.161	4.4±0.139
A/G Ratio	0.657±0.02	0.657±0.02	0.642±0.029
Total Cholesterol	126.14±10.883	117.857±9.069	102±6.542
Total Glucose	51.142±4.177	41.142±1.944^{a}	58.571±4.433^{b}

Values bearing different superscripts within a row differed significantly.

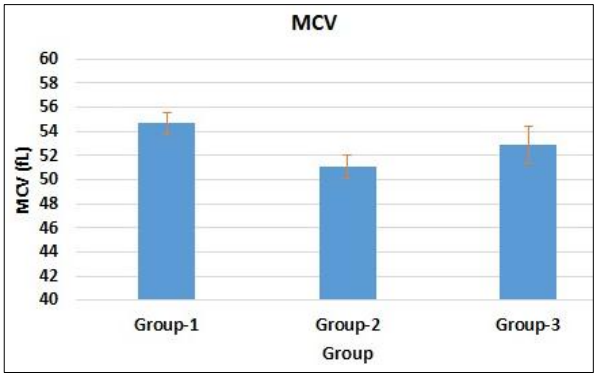

Fig 1: Graphical representation of mean corpuscular volume in three lactation groups

The Blood Urea-nitrogen levels are significantly different ($P < 0.05$) between the different groups of lactating Murrah buffaloes (Fig. 2). The mean value of BUN was recorded to be apparently higher 39±1.786 mg/dL in the initial phase of lactation and decreased as lactation progressed. It is found that the efficiency of utilization of metabolizable protein for milk production (0.68) is lower than that of maintenance (1.00) (McDonald *et al*., 1995) [25]. Thus, as milk production increases, the overall efficiency of protein utilization decreases, which consequently leads to higher excretion of nitrogen in the form of urea via urine and milk (Roy *et al*., 2003) [33]. It was also found that serum urea concentration was significantly affected by lactation stage (Reinartz and Hofmann, 1989) [31]. It was reported that there is no

relationship between lactation stage and urea levels, except shortly after calving (Coustumier, 1996) [8]. Whitaker and his co-workers (1995) were reported that cows in early lactation often had much lower milk urea levels. In contrast, it was also found there no relationship between urea concentration in milk and stage of lactation (Erbersdobler *et al*, 1990) [12]. Therefore, considering the different observations of the different researchers, a systematic and critical investigation on this aspect can be carried out.

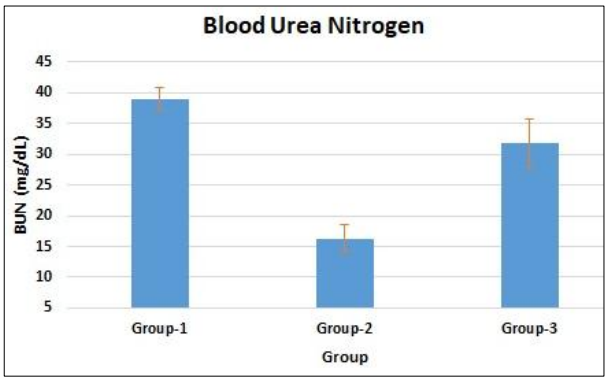

Fig 2: Graphical representation of blood urea nitrogen levels in three lactation groups

The present study showed that blood creatinine levels were lower in group I compared with Murrah buffaloes of groups II and III (Fig. 3). Nevertheless, it was found that there are no differences in creatinine concentration between the different stages of lactation (Peterson and Waldern, 1981) [29], but creatinine levels in dry cows increased with the increasing days of gestation. On working with 21 Holstein herds, reported the highest serum creatinine levels during peak lactation (Kronfeld, 1982) [22].

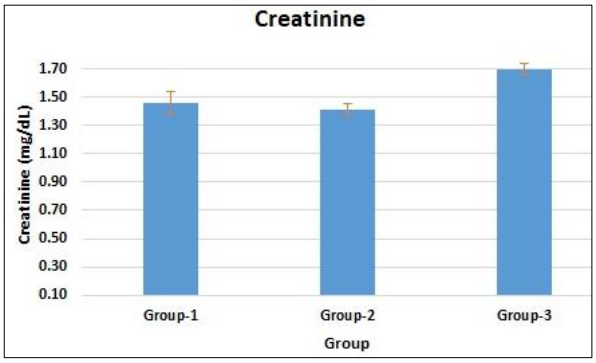

Fig 3: Graphical representation of Creatinine in three lactation groups

Measurement of serum activities of liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline Phosphate is considered a reliable indicator of liver function in lactating Murrah buffaloes. No significant change in the concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) was detected in the three buffalo groups in the current study. Increased levels of ALT with mean 61.285±7.845 U/L were found in early lactating Murrah buffaloes (Fig. 5). The stage of lactation significantly affects the activities of AST and ALT (Yaylak *et al*., 2009) [37]. Alterations in the activity of these enzymes may also be related to reduced dry matter intake around birth, leading to hepatic lipidosis and altering normal liver function (Greenfield *et al*., 2000) [15], AST (Fig. 4) and Alkaline Phosphate (Fig. 6) are considered as effective biomarkers for detecting energetic and mineral imbalance in Saanen dairy goats (Mundim *et al*., 2007) [27]. However, no evidence was found in the literature to explain the relationship between the observed variations in the concentrations of these enzymes and the different stages of lactation.

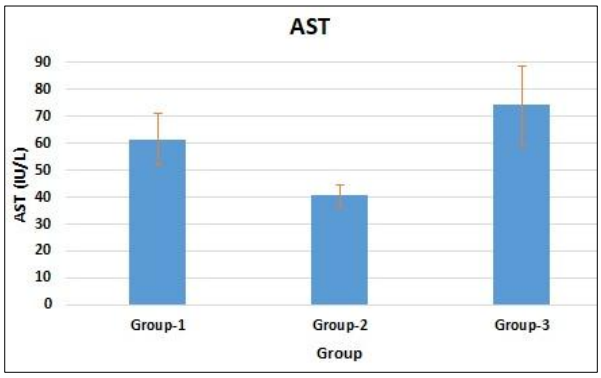

Fig 4: Graphical representation of AST in three lactation groups of Murrah buffaloes

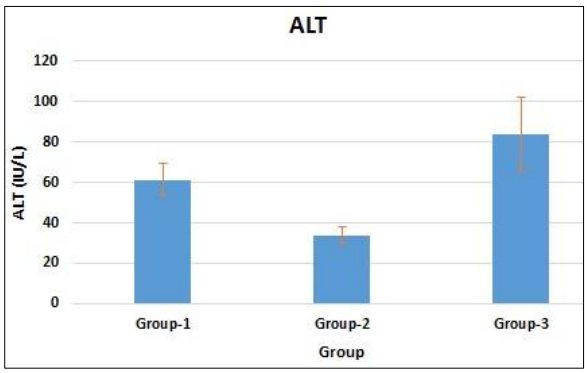

Fig 5: Graphical representation of ALT in three lactation groups of Murrah buffaloes

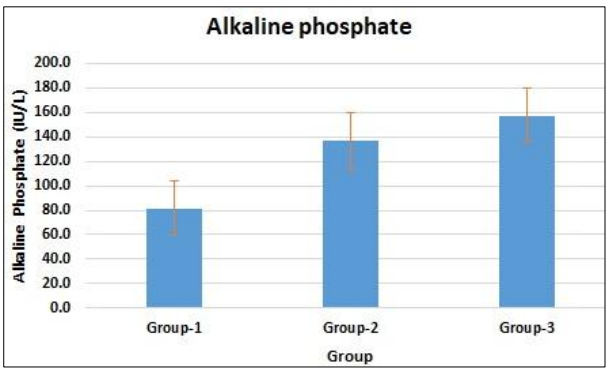

Fig 6: Graphical representation of Alkaline phosphatase in three lactation groups

The total protein level 7.457±0.198 g/dL was found to be slightly higher in group II as compared to group I and group III animals. This observation corroborates to the finding of Yaylak *et al*. (2009) [37], who recorded lower protein values in late and early stages of lactation in case of Holstein cows. This observation is in contrast to the findings of Krajnicakova and his co-workers. (2003) [20], who observed an increasing trend in serum total protein content as lactation progressed in lactating goats that this was due to protein depletion for milk synthesis. Variations can be attributed to differences in species, diet, husbandry, environment, and research methods

(Beaunoyer, 1992; Osman and Albusadah, 2003) [5, 28]. However, it was reported that the highest protein levels in the early phase of lactation, which is comparable to the current results (Das *et al*., 2017, Hagawane *et al*., 2012) [11, 16]. Total protein content is usually used to assess the nutritional status of an animal and reflects food intake and metabolism.

It was found that the albumin concentration in the blood is highest in the early phase of lactation and lowest in the late phase of lactation. Globulin concentration, on the other hand, showed the opposite trend (Hemen Das *et al*., 2016) [10]. A previous study reported that albumin concentration decreased significantly on the first day after birth ($P < 0.05$), and a nonsignificant decrease in content was observed from the second day after birth (Lone *et al*., 2003) [23]. These results justify the high blood albumin concentration during the early phase of lactation observed in the present study. Our results regarding albumin, globulin, and A/G ratio are in agreement with the findings of earlier reports (Maria *et al*., 1990 and Singh *et al*., 1982) [24, 35], observed that most milk proteins stabilize about a week after calving, with significant changes in Ig content occurring during this period, which contributes significantly to total proteins at this time. It was observed that there was a rapid decrease in total plasma protein and Ig concentrations within 24 hours of birth in buffalo and this could be due to decreased transfer of Ig from maternal blood, decreased synthesis by mammary glands, and/or depletion of stored Ig with subsequent increased number of milking's (Abraham, 1988) [1].

The concentration of blood cholesterol was lowest in early stage and increased subsequently as the lactation advances. The trend in the change in total cholesterol can be attributed to the increased energy requirements of buffalo during lactation. The higher cholesterol content as lactation progressed was a physiological adaptation to the demands of lactation (Lone *et al*., 2003) [23]. It was also found that triglycerides are used by the mammary glands to form milk lipids and their demand increases until the peak of lactation (Karapehlivan *et al*., 2007) [19].

Blood glucose level is considered to be one of the indicators of buffalo energy status. The present study also showed that the mean concentration of blood glucose was lowest in the early phase (41.142±4.177 mg/dL) and subsequently increased as lactation progressed. In the present study blood glucose values for middle and late phases of lactation were 46.142±1.944 mg/dL and 58.571±4.433 mg/dL, respectively (Fig. 7). The lower mean values of blood glucose concentration in the early lactation period were due to the large amount of blood glucose taken from the mammary gland for the synthesis of lactose (Schultz, 1968) [34]. The current trend of changes is consistent with the earlier reports of lactating ewes (Roubies *et al*., 2006) [32] and lactating mares (Heidler *et al*., 2002) [17]. It is described that lactose synthesis and milk yield had shown a linear positive correlation with glucose intake and thus lactose synthesis potential is associated with greater glucose uptake by the lactating mammary gland (Afshar and Fathi, 2012) [2].

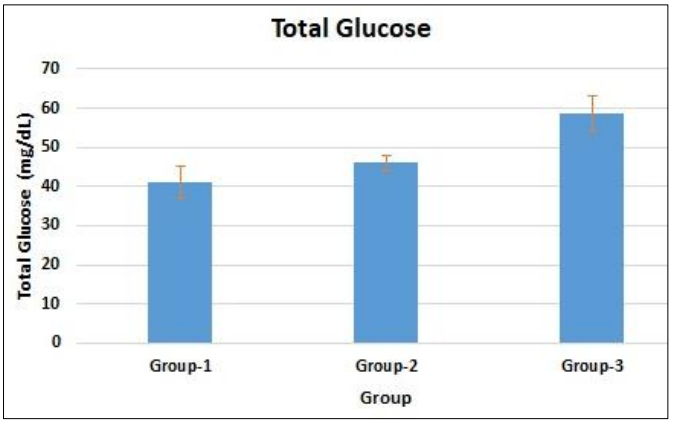

Fig 7: Graphical representation of Total Glucose in three lactation groups

4. Conclusions

The study results indicate that the stage of lactation did not affect most of the parameters. The unaffected parameters include haemoglobin, RBC count, WBC count, platelet count, packed cell volume, MCH, MCHC, serum glucose, total protein, albumin, globulin, A:G ratio and total cholesterol. Few parameters differed significantly with the stage of lactation. Those parameters include, MCV, BUN, Creatinine, Alkaline phosphate, ALT and AST. However, the significant difference did not show any pattern, and also has high intra-group range. Hence, these differences cannot be attributed to the stage of lactation alone and the factors other than stage of lactation, like individual variation and environmental impact could have influenced these differences. In order to understand such factors, more studies are required to understand the effect of lactation stage on blood profile under diversified conditions and different seasons. Since, the buffalo is a less studied milk animals, compared to cattle, this present study was carried out as an attempt to provide data for further research.

5. Acknowledgements

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6. Conflict of Interest

The authors declare that there is no conflict of interest.

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