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# Haemato-biochemical Profiling in Buffaloes in Relation to Metabolic Changes during Transition Period

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## ABSTRACT

**Background:** Transition period is a critical period in which most dairy animals undergo a period of negative energy balance (NEB), hypocalcemia, insulin resistance, hypoglycemia and susceptibility to infectious diseases. The critical changes in biochemical indicators are well reported in dairy cows whereas; in dairy buffaloes limited data related to alterations in hemato-biochemical changes have been reported so far. The current study aimed to evaluate the hemato-biochemical changes in buffaloes during transition period

**Methods:** A total number of 210 buffaloes aged between 3 to 14 years with parity ranged from 1st to 4th, divided in three groups (n=70; each) during transition period were used for the study. Three groups *viz* Group I (-30 days), Group II (0 days) and Group III (+30 days) were subjected for evaluation of hemato-biochemical parameters.

**Result:** Our study showed significant increase ($p<0.05$) in WBC ($10^3/\mu l$), lymphocytes and granulocytes count at the time of calving. Significant decrease ($p<0.05$) in the level of RBC, Hb and HCT were seen in Group-I and II. In biochemical analysis, parameters like albumin, total protein, triglyceride and A:G ratio showed significant ($p<0.05$) decrease from Group-I to II. Calcium and Ca:P ratio decreased significantly ($p<0.05$) along with suppressed levels of sodium and potassium levels in Group-III. Thus, the present study showed significance of hemato-biochemical analysis during transition period for possibility of occurrence of production diseases.

**Key words:** Lipid profile, Metabolic changes, Protein profile, Transition period.

## INTRODUCTION

Transition period is described as period from 3 weeks pre-calving to 3 weeks post-calving (Grummer, 1995). During the critical prepartum period, feed intake is at the lowest point of the lactation-gestation cycle and nutrient demand of the foetus is met by lipolytic agents (Kour *et al*., 2022). This period is characterized by negative energy balance (NEB), fat mobilization, elevation of circulating non-esterified fatty acids, ketone bodies hypocalcemia condition (milk fever) (Goff and Horst, 1997) and oxidative stress leading low level of immunity (Maurya *et al*., 2014). Various alterations in biochemical and hematological parameters are used to identify and evaluate metabolic disturbances in buffaloes along the progression of this period viz; serum protein, lipid profile, electrolyte concentration, haemoglobulin, packed cell volume, total erythrocyte count *etc* (El-Deeb and El-Bahr, 2017). In view of such considerations, the aim of the study was to evaluate the changes in hematological and metabolic parameter during the transition period in buffaloes.

## MATERIALS AND METHODS

The experiment was conducted during period of 2019-2021 at Division of Veterinary Medicine, F.V.Sc and AH, Sher-e-Kashmir University of Agricultural sciences and Technology, Jammu. Buffaloes (N=210) aged between 3 to 14 years with parity ranged from 1st to 4th during their transition period were selected randomly from organised and unorganised farms of Jammu region, UT J and K, India. They were categorised into three groups, each having seventy animals; group-I (3 weeks pre-partum period), group-II (near gestation) and group-III (3 weeks post-partum). In organised farms, animals were maintained under semi-intensive housing system with standard feeding management. The buffaloes were given were given stall feeding (containing 25-30 kgs of dry fodder (bhoosa, prali) (no availability of green fodder during winters and concentrate mixture were not given, salt lick and adlib water provide . Blood samples

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from mentioned group of study were collected in EDTA (Ethylene diamine tetra acetic acid) containing vacutainers VACUETTE (Cat. 455036, Greiner bio-one, Austria) for estimation of haematological parameters *viz*; total WBC ($\times 10^3/\mu L$), lymphocytes (%), monocytes (%), granulocytes (%), RBC ($\times 10^6/\mu L$), Hb (g/dl), HCT(%), MCV (fl), MCH (pg), MCHC (pg) and platelets ($\times 10^3/\mu L$) using MYTHIC 18 VET Haematology Analyser, (Compact diagnostics India Pvt Ltd.) and in serum clot activator vacutainers for estimation of biochemical parameters.

All the biochemical parameters viz; total protein (g/dl), albumin (g/dl), globulin (g/dl), A:G ratio, SGPT (serum glutamic pyruvic transaminase; U/L), SGOT (Serum glutamate oxaloacetic transaminase; U/L), GGT (Gamma glutamyl transferase; U/L), cholesterol (mg/dl), triglyceride (mg/dl), calcium (mg/dl) were estimated by UV spectrophotometer using Erba diagnostic kits (Transasia Biomedical Ltd. Mumbai, India) except for blood electrolytes; sodium, potassium using Liquizyme kit (Beacon Diagnostics Pvt. Ltd. Navsari, India) and magnesium, inorganic phosphorus using Agappe diagnostic kits (Ernakulam, Kerala).

ANOVA was used to analyze the significance of differences. The data is represented in the form of Mean±SE.

## RESULTS AND DISCUSSION

### Serum protein profile

The mean concentration of total protein and albumin decreased significantly ($p<0.05$) from group-I to group-III (pre-partum to post-partum period) as shown in Table 1. Similar findings were seen in study of Ashmawy (2015), with significant decrease ($p<0.05$) in total protein (g/dl) levels from pregnant to early lactation group. However, non-significant difference ($p>0.05$) among the mean concentrations of total protein, albumin and globulin were seen in study of Abdulkareem (2013), conducted on riverine buffaloes of Iraq during transition period. Serum protein profile in dairy cows has been reported earlier with similar findings of Mohri *et al*. (2007) with decrease in protein profile from late pregnancy to early lactation reflecting the requirement of protein source for lactation and providing immunoglobulins. In last trimester of pregnancy with prevailing endocrine situations, there may be insulin resistance and decreased level in insulin growth factor-1, which can cause net mobilisation of amino acid reserves and decreases the plasma level of albumin and protein (Block *et al*., 2001). Albumin is considered as negative acute phase protein and it may get decreased during transition period due to encounter of high inflammatory responses (Bionaz *et al*., 2007).

### Serum liver enzymes

A significant increase ($p<0.05$) in the mean± SE values of serum glutamic-oxaloacetic transaminase (SGOT) has been observed in buffaloes of Group-I to Group-III (Table 1). The levels of Gamma-glutamyl transferase (GGT) (U/L) showed significant ($p<0.05$) difference with highest value in group II (24.33±0.14) as compared to group I (15.38±0.18) and group III (21.29±0.20). Whereas, highest level of Serum glutamic pyruvic transaminase (SGPT) (U/L) was observed in group III (Table 1). Mills *et al*. (1986 ) and EA Mohammed *et al*. (2019)bserved high level of SGOT during calving and postpartum period indicating of stressed hepatic metabolism and pronounced catabolism of body reserves. Similar findings related to levels of SGOT and SGPT were found in the study of Grasso *et al*. (2004), Abdulkareem . (2013), and Fiore *et al*. (2017) with no significant changes in the concentration of GGT (U/L). The changes in the concentrations of liver enzymes indicate metabolic disorders with involvement of liver even at sub-clinical levels (Fiore *et al*., 2015).

**Table 1:** Biochemical parameter changes during transition period in buffaloes.

| Parameters | -30 days (Gp-I) (n=70) | 0 day (Gp-II) (n=70) | +30 days (Gp-III) (n=70) |
|---|---|---|---|
| Albumin (g/dl) | $3.12^{c}$±0.02 | $2.93^{b}$±0.02 | $2.81^{a}$±0.02 |
| Total protein (g/dl) | $7.13^{c}$±0.03 | $6.96^{b}$±0.02 | $6.83^{a}$±0.02 |
| Globulin (g/dl) | 4.01±0.01 | 4.03±0.01 | 4.02±0.01 |
| A:G ratio | $0.78^{c}$±0.01 | $0.73^{b}$±0.01 | $0.70^{a}$±0.01 |
| SGOT (U/L) | $98.39^{a}$±0.51 | $102.27^{b}$±0.28 | $109.69^{c}$±0.54 |
| SGPT (U/L) | $24.60^{b}$±0.26 | $23.76^{a}$±0.23 | $25.79^{c}$±0.21 |
| GGT (U/L) | $15.38^{a}$±0.18 | $24.33^{c}$±0.14 | $21.29^{b}$±0.20 |
| Cholesterol (mg/dl) | $79.16^{b}$±0.67 | $70.76^{a}$±0.54 | $81.62^{c}$±0.47 |
| BUN (mg/dl) | $11.05^{a}$±0.14 | $11.61^{a}$±0.19 | $12.56^{b}$±0.29 |
| Creatinine (mg/dl) | $1.22^{a}$±0.01 | $1.24^{b}$±0.01 | $1.20^{a}$±0.01 |
| Triglyceride (mg/dl) | $24.24^{c}$±0.20 | $21.49^{b}$±0.32 | $19.04^{a}$±0.23 |
| Calcium (mg/dl) | $8.80^{c}$±0.03 | $8.5^{b}$±0.02 | $8.35^{b}$±0.02 |
| Sodium (mEq/L) | $138.05^{b}$±0.36 | $137.73^{b}$±0.30 | $135.70^{a}$±0.26 |
| Potassium (mEq/L) | $3.88^{b}$±0.03 | $3.86^{b}$±0.02 | $3.72^{a}$±0.01 |
| Magnesium (mg/dl) | $1.18^{b}$±0.01 | $1.16^{a}$±0.01 | $1.16^{a}$±0.01 |
| Phosphorus (mg/dl) | $4.90^{b}$±0.01 | $4.88^{b}$±0.01 | $4.74^{a}$±0.01 |
| Ca:P ratio | $1.71^{c}$±0.01 | $1.66^{a}$±0.01 | $1.69^{b}$±0.00 |

**Serum lipid profile, Urinary nitrogen and creatinine**

The mean values showed significant decrease ($p<0.05$) in the level of cholesterol and triglyceride as transition period progresses (Table 1 ). Similar findings were found in study of Karapehlivan *et al*. (2007) and Rambachan *et al*. (2019) with low level of triglyceride as progression of lactation occurs, as they are believed to be used by the mammary glands for production of milk fat. Kweon *et al*. (1986) observed that higher rate of disease occurrence in cattle with low cholesterol level at the time of calving. However, the observation of Singh *et al*. (2012) are not in accordance to our findings with non-significant difference ($p>0.05$) in cholesterol level (mg/dl) among buffaloes in different transition stage. Total cholesterol reflects the indirect availability of exogenous source along with hepatic functionality (Grum *et al*. 1996). The level of serum cholesterol (mg/dl) may get decreased during calving and got build with progression of lactation in response to physiological adaptation. In ruminants, cholesterol and triglyceride are transported from liver *via* lipoproteins and these lipoproteins get decreased during periparturient period (Katoh, 2002) which eventually leads to decrease level of cholesterol and triglyceride around calving (Akamatsu *et al*., 2007). There was significant ($p<0.05$) increase in the level of urinary nitrogen (mg/dl) from 0 day to +30 days. Roubies *et al*. (2006) observed that level of BUN are influenced by many actors *viz* dietary intake, protein quantity in feed, rumen degradability and catabolism of body reserved protein. The significant increase ($p<0.05$) in the concentration of creatinine during calving is suggestive of dehydration causing decreased glomerular filteration whereas, decrease in their level during post-partum period points toward skeletal muscle wasting (Megahed *et al*., 2019).

**Serum electrolyte concentrations**

The calcium level (mg/dl) showed significant decrease ($p<0.05$) from Group-I to Group-II buffaloes (Table 1), where as non-significant difference ($p>0.05$) was observed between Group-II and Group-III. For phosphorus level, non-significant ($p<0.05$) decrease in the Group-I and Group-II was seen. But there was a significant decrease ($p<0.05$) in the mean±SE values of Group-III from other two's. Mean±SE values of Ca:P ratio showed significant decrease ($p<0.05$) from Group-I to III, with lowest value seen in Group-II. Similar findings were found in Kronqvist *et al*. (2011) study in which calcium level decreased from 0 day, 2, 4 and 7 days after calving till peak lactation attains. Wu *et al*. (2008) also observed that calcium level of plasma on the day of calving was significant lower than the prepartum period (8.33 vs 9.30 mg/dl). Parathyroid hormone regulates the level of calcium via stimulation of osteocytic osteolysis mechanisms that releases calcium from lacunae to blood stream (Hernández-Castellano *et al*., 2020). During the lactation, calcium homeostasis becomes less effective in high yielding cattle (Kovacs, 2011). The Mean±SE values of sodium and potassium (mEq/L) showed non-significant ($p<0.05$) decrease from Group-I to Group-II, whereas significant decrease ($p<0.05$) in the Group-III was observed. Serum magnesium level (mg/dl) showed a significant decrease ($p<0.05$) from Group-I to Group-II, but there was non-significant ($p>0.05$) decrease amongst Group-II and III (Table 1). The decrease in the potassium level during postpartum period was seen in study of Jacob *et al*. (2011) suggesting of transfer of the cation in the milk during lactation. Skrzypczak *et al*. (2014) reported that lower Na+ concentration in the first week of lactation could be due to Na loss associated with decreased plasma rennin activity. Additionally, Asif *et al*. (1996) indicated that as a consequence of high prostaglandin concentrations, which increase excretion of Na+ by the kidneys, significant decrease in blood Na+ concentration can be seen.

**Haematological parameters**

In our study, the blood concentration of WBC(x10³/µl) in all the groups increased significantly ($p<0.05$) with highest values in Group-III (7.57±0.09). The levels of lymphocytes and granulocytes as depicted in Table 2 differ significantly ($p<0.05$) in all three groups, with highest value in Group-II particularly. Similar findings were found in the study of Preisler *et al*. (2000) with increased amount of WBC, lymphocytes and neutrophil due to rise in concentration of

**Table 2:** Haematological parameters during transition period in buffaloes.

| Parameters | -30 days (Gp-I) (n=70) | 0 day (Gp-II) (n=70) | +30 days (Gp-III) (n=70) |
|---|---|---|---|
| WBC (×10³/µL) | $6.45^{a}$±0.05 | $7.27^{b}$±0.05 | $7.57^{c}$±0.09 |
| Lymphocytes (%) | $45.78^{a}$±0.19 | $48.12^{b}$±0.19 | $46.17^{a}$±0.19 |
| Monocytes (%) | $4.51^{c}$±0.04 | $4.09^{a}$±0.02 | $4.41^{b}$±0.02 |
| Granulocyte (%) | $43.66^{a}$±0.33 | $46.65^{b}$±0.67 | $43.62^{a}$±0.1 |
| RBC (×10⁶/µL) | $5.19^{b}$±0.06 | $4.98^{a}$±0.03 | $5.11^{b}$±0.04 |
| Hb (g/dl) | $10.65^{c}$±0.13 | $9.67^{b}$±0.11 | $9.25^{a}$±0.08 |
| HCT (%) | $33.34^{b}$±0.31 | $30.89^{a}$±0.45 | $31.22^{a}$±0.23 |
| MCV(fl) | $57.20^{a}$±0.33 | $56.80^{b}$±0.14 | $56.64^{b}$±0.09 |
| MCH (pg) | $17.90^{b}$±0.10 | $16.07^{a}$±0.20 | $17.83^{b}$±0.09 |
| MCHC (pg) | $32.41^{a}$±0.18 | $31.86^{a}$±0.59 | $32.67^{a}$±0.11 |
| PLT (×10³/µL) | $338.81^{a}$±6.10 | $380.40^{b}$±5.67 | $397.97^{c}$±5.90 |

a,b,c means with different superscripts between rows differs significantly ($p<0.05$).

cortisol at the time of calving. Cortisol is suppose to cause demargination of neutrophil from endovascular linings eventually leads to increase concentration of neutrophil in peripheral blood circulation (Shoenfeld *et al*., 1981). The mean±SE values of RBC's ($10^6$/µl) and Hematocrit (HCT %) in all the groups as depicted in Table 2 showed significant ($P<0.05$) decrease in Group-II from other groups. Similarly, haemoglobin (Hb, g/dl) decreased significantly ($p<0.05$) along the progression of transition period from -30 days to +30 days (Table 2). Our results showed agreement to the findings of Gavan *et al*. (2010) who observed a marked reduction in the values of RBC, Hb and HCT in periparturient period. Kumar and Pachauri (2000) observed that Hb concentration in blood decreases during the calving time that might be due to decreased erythropoiesis in periparturient buffaloes. Chikazawa and Dunning (2016) suggested that during time of parturition, there is increased in level of pro-inflammatory cytokines (TBF-alpha and interferon's) which are inhibitors of erythropoiesis through the action on erythroid precursors in bone marrow. Also significant increase ($p<0.05$) in the levels of platelet count has been observed along the transition period, suggestive of activated COX-2 pathway (cyclooxygenases) which ultimately increases the activating platelet factors and thromboxane A2 (Sordillo *et al*., 2009).

## CONCLUSION

The present study highlights significant changes in hematological and biochemical parameters during transition period in buffaloes, which may be served as a guide to monitor health status, disease diagnosis, routine herd investigations and monitoring any future studies related to buffaloes.

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