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Biochemical analytes during different stages of lactation in Surti buffaloes

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Abstract
The present study was conducted to investigate biochemical analytes during different stages of lactation in Surti buffaloes. 10 healthy Surti buffaloes were selected at postpartum (n=10). Blood samples from all the animals were collected at early (30±5 days), mid (100±5 days) and late lactation (200±5 days) and serum was obtained and analysed for various biochemical metabolites. Serum biochemical metabolites such as total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, total protein, albumin and globulin increased significantly ($p \leq 0.05$) whereas urea and creatinine significantly ($p \leq 0.05$) decreased from early to late stages of lactation. It is thus concluded that biochemical metabolites such as total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, total protein, albumin and globulin increased whereas urea and creatinine decreased from early to late lactation. These changes suggest close monitoring of these metabolites in Surti buffaloes during different stages of lactation for nutritional intervention if required.

Keywords: Biochemical analytes, Stages of lactation, Surti buffaloes

Introduction
Lactation, the period of milk production following parturition, is a critical phase in the reproductive cycle of dairy animals such as buffaloes. Surti buffaloes are mainly reared for milk production and have adaptability to various environmental conditions. It undergoes distinct biochemical changes throughout lactation, influencing both milk quality and quantity. Understanding these alterations is essential for optimizing dairy management practices and ensuring the well-being of the animals (Singh *et al.*, 2015) [17].

During lactation, Surti buffaloes undergo dynamic metabolic shifts to meet the demands of milk synthesis and secretion. Extensive lipolysis and adipose mobilization that overwhelm hepatic metabolism are hallmark processes that occur during this time. These changes are reflected in various biochemical analytes such as those associated with lipid profile and protein metabolism. For instance, studies have indicated fluctuations in these metabolites during different lactation phases, which play pivotal roles in regulating mammary gland development and milk synthesis (Bhutto *et al.*, 2017) [2]. Additionally, alterations in urea and creatinine have been observed, reflecting the metabolic activity and health status of the mammary gland (Singh *et al.*, 2015) [17]. Serum levels of urea and creatinine are also indicators of renal health. Furthermore, the composition of milk constituents, including protein, fat, lactose and minerals, undergoes variations across different lactation stages in Surti buffaloes (Patel *et al.*, 2019) [15]. These variations not only influence milk yield but also impact its nutritional quality and commercial value.

Understanding the biochemical changes occurring during different lactation stages in Surti buffaloes is crucial for implementing effective management strategies, including nutritional interventions, to maximize milk production efficiency while ensuring animal welfare. Moreover, insights into these changes can aid in the development of biomarkers for assessing mammary gland health and diagnosing metabolic disorders in dairy animals.

Materials and Methods

Animal selection

Total 10 healthy lactating postpartum Surti buffaloes (n=10) were selected for the study that were maintained at Livestock Research Station, Navsari Agricultural University campus, Kamdhenu University, Navsari.

Sample collection and laboratory analysis

Blood samples were collected at early (30±5 days), mid (100±5 days) and late lactation (200±5 days) from selected Surti buffaloes (n=10). Approximately 10 ml of whole blood from animals were collected aseptically from jugular vein in clot activator vacutainer. Vacutainer then were placed in slanting position undisturbed at room temperature and serum was obtained after clot formation without disturbing the clot. Separated serum was stored at -20°C in deep freeze until used for analysis of biochemical parameters.

Laboratory analysis

Standard commercially available analytical kits were used for the analysis of cholesterol (Randox), triglyceride (Diatek), HDL-Direct (Diatek), LDL-Direct (Diatek), total protein (Randox), albumin (Randox), urea (Diatek) and creatinine (Randox). Semi-automated clinical chemistry analyzer (Merck) and spectrophotometer were used for analysing these parameters. Serum globulin was calculated by subtracting albumin levels of serum from total protein concentration in serum.

Statistical analysis

Descriptive statistics was used to determine Mean±SE values for biochemical analytes at different stages of lactation in Surti buffaloes. Means obtained were compared statistically using one way ANOVA along with DMRT to interpret effect of different stages of lactation on the studied biochemical parameters. DMRT was used for determining significant difference of mean at 5% level (Snedecor and Cochran, 1997) [19].

Results

The result of changes in serum biochemical analytes (Mean±SE) during different stages of lactation in Surti buffaloes are presented in table 1.

During early, mid and late stages of lactation in Surti buffaloes total cholesterol levels (mg/dl) were 116.96±0.59, 122.89±0.50 and 130.32±0.63; triglyceride levels (mg/dl) were 19.72±0.85, 24.43±0.36 and 26.09±0.29; high density lipoprotein (HDL) (mg/dl) were 59.86±0.29, 61.67±0.18 and 65.15±0.20 and low density lipoprotein (LDL) (mg/dl) were 82.29±0.85, 101.72±0.73and 122.82±0.91, respectively.

Lipid profile parameters representing fat metabolism differed significantly ($p \leq 0.05$) between different stages of lactation. Significantly ($p \leq 0.05$) low values of total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein were observed at early stage of lactation. Significantly ($p \leq 0.05$) high values of total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein were observed at late stage of lactation. Significantly ($p \leq 0.05$) different to early and late stages, the intermediate levels of these parameters were observed at mid stage of lactation.

During early, mid and late stage of lactation in Surti buffaloes total protein (g/dl) concentration were 8.12±0.28, 9.17±0.22 and 10.11±0.29; albumin (g/dl) concentration were 4.87±0.17, 5.50±0.13 and 6.07±0.17; globulin (g/dl) concentration were 3.25±0.11, 3.67±0.09 and 4.05±0.12; urea (mg/dl) concentration were 32.50±2.04, 24.63±0.27 and 20.88±0.68 and creatinine (mg/dl) concentration 1.93±0.02, 1.45±0.07 and 0.92±0.07 respectively.

All the protein metabolites differed significantly ($p \leq 0.05$) between different stages of lactation. Significantly ($p \leq 0.05$) low values of total protein, albumin and globulin were observed at early stage of lactation. Significantly ($p \leq 0.05$) high values of total protein, albumin and globulin were observed at late stage of lactation followed by intermediate but significant ($p \leq 0.05$) lower values at mid stage of lactation. Urea and creatinine were significantly ($p \leq 0.05$) high at early stage of lactation followed by ($p \leq 0.05$) low values at mid stage and ($p \leq 0.05$) lowest values at late stage of lactation.

Table 1: Changes in serum biochemical analytes (Mean±SE) during different stages of lactation in Surti buffaloes

Parameters	Early lactation (30±5 days) (n=10)	Mid lactation (100±5 days) (n=10)	Late lactation (200±5 days) (n=10)
TC (mg/dl)	116.96^{c}±0.59	122.89^{b}±0.50	130.32^{a}±0.63
TG (mg/dl)	19.72^{c}±0.85	24.43^{b}±0.36	26.09^{a}±0.29
HDL (mg/dl)	59.86^{c}±0.29	61.67^{b}±0.18	65.15^{a}±0.20
LDL (mg/dl)	82.29^{c}±0.85	101.72^{b}±0.73	122.82^{a}±0.91
TP (g/dl)	8.12^{c}±0.28	9.17^{b}±0.22	10.11^{a}±0.29
Albumin (g/dl)	4.87^{c}±0.17	5.50^{b}±0.13	6.07^{a}±0.17
Globulin (g/dl)	3.25^{c}±0.11	3.67^{b}±0.09	4.05^{a}±0.12
Urea (mg/dl)	32.50^{a}±2.04	24.63^{b}±0.27	20.88^{c}±0.68
Creatinine (mg/dl)	1.93^{a}±0.02	1.45^{b}±0.07	0.92^{c}±0.07

Means bearing different superscripts across rows differ significantly ($p \leq 0.05$) between different stages of lactation in Surti buffaloes

Discussion

During early stage of lactation, there is increased demand of energy to support milk production. As a result of energy demand animal body utilizes stored energy (lipid) to meet the energy demand which ultimately leads to increased lipolysis at an early stage of lactation. Increased demand of energy by lactating mammary gland is met by physiological phenomena of insulin resistance that begins during late gestation and is continued during early lactation gradually declining as the lactation advances. As per Brockman and Laarveld (1986) [3], increased insulin responsiveness decreases gluconeogenesis from other sources and favour use of propionate for it. Thus, vice versa insulin resistance stimulates gluconeogenesis from non-carbohydrate sources and therefore utilization of such lipid metabolites will alter their circulating levels in blood and cause them to decline and is thus beneficial. In the present study, insulin resistance during early lactation might be the reason for decline of triglycerides, total cholesterol, high density lipoprotein and low density lipoprotein. Increased NEFA during insulin resistance in early lactation is a characteristic finding in the study. As NEFA (indicator of negative energy balance) has inverse relation with blood cholesterol levels (Djokovic *et al.*, 2011) [7], lowering of cholesterol in serum of early lactating buffaloes was imminent observation. Findings of Kessler *et al.* (2014) [12] showed that despite slightly increased processes of cholesterol biosynthesis at onset of lactation, the levels of cholesterol fractions associated with lipoproteins (LDL-C, VLDL-C, HDL-C) were decreased. The low blood LDL-C levels at the onset of lactation seems

to be a consequence of the low rate of extrahepatic export of VLDL that in turn gives rise to LDL by receiving cholesteryl esters. Limited extrahepatic VLDL export during initial stages of lactation has also been suggested by Gross *et al.* (2013) [10] that also is responsible for triglyceride accumulation in liver leading to fatty liver. This finding of Gross *et al.* (2013) [10] further substantiates lowering of triglyceride during early lactation in the present study. As the lactation progressed peripheral cells of the body became more insulin sensitive and responsive, these lipid metabolites got spared from gluconeogenesis and increased in circulation during mid as well as late stage of lactation. Further, NEFA also decreases as the lactation advances and lipid profile returns to normal.

Triglycerides have shown a similar trend to that of total cholesterol and HDL- cholesterol with lowest level during early lactation, followed by an increase during mid-lactation, possibly due to increased demand of the udder for fatty acid synthesis for milk fat, as well as fluctuations in estrogen and thyroxine levels influencing lipid metabolism (Tripathi *et al.*, 2010) [20].

In accordance to the present study, similar increasing trend from early to late stage of lactation were reported for cholesterol by Hagawane *et al.* (2009) [11], for triglycerides by Monteiro *et al.* (2012) [14], for both cholesterol and triglycerides by Saqib *et al.* (2022) [16] and Djoković *et al.* (2014) [7], for cholesterol, triglycerides, HDL and LDL by Tripathi *et al.* (2010) [20].

During early lactation in buffaloes, insulin resistance diverts glucose towards mammary gland and leads to negative energy balance in the body. Diminished insulin sensitivity and responsiveness lowers anabolic processes and increases protein catabolism (Bell *et al.*, 2000; Drackley *et al.*, 2001) [1, 8]. Increase in protein catabolism provides amino acids like alanine and glutamine as substrate for hepatic gluconeogenesis (Kuhla *et al.*, 2011) [7] in order to suffice energy demand during negative energy balance. Therefore, the levels of total protein declined during early lactation in the present study. As blood levels of total protein is comprised of albumin and globulin fractions, lowering of total protein is a reflection of basically lowered albumin and globulin levels in blood. As urea and creatinine are catabolic products of nitrogen metabolites like protein, they have inverse relation with levels of total protein. Higher catabolism of total protein will lower total protein levels but increase urea and creatinine as was seen during early lactation and higher anabolism of total protein will result in lower urea and creatinine as was seen during late lactation in the present study. As lactation advances insulin sensitivity as well as responsiveness increases in peripheral tissues that stimulates protein synthesis and inhibit its degradation (Brockman and Laarveld, 1986; Sjaastad *et al.*, 2010) [3].

Similar to present study, increasing trend for total protein and albumin were reported by Castillo *et al.* (2006) [4] and Gianesella *et al.* (2019) [9] from early to late lactation. The trend for decreasing levels from early to late lactation as observed for present study was also reported by Das *et al.* (2016) [6] for urea and creatinine and by Chacha *et al.* (2018) [5] for urea.

Conclusions

It is thus concluded that biochemical metabolites such as total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, total protein, albumin and globulin increased whereas urea and creatinine decreased from early to late lactation. These changes suggest close monitoring of these metabolites in Surti buffaloes during different stages of lactation for nutritional intervention as well as metabolic disorders.

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