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# Alterations in the Hemato-biochemical, Endocrine, Somatic Cell Count and Milk Composition in Lactating Tarai Buffaloes during Different Lactation Stages

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## ABSTRACT

**Background:** Tarai buffalo is indigenous buffalo breed of Uttarakhand state, dual purpose, well adapt to hot-humid climatic condition of Tarai area. This study aimed to evaluate the alterations in the hemato-biochemical, endocrine, milk somatic cell count and milk composition in lactating Tarai buffaloes.

**Methods:** Thirty-six healthy Tarai buffaloes were selected from four different Gujjar farms and divided into four groups consisting nine buffaloes in each group as early (60±30 days), mid (120±30 days) and late (180±30 days) lactation stages and dry buffaloes. Both blood and milk samples were collected once from each lactation stage of selected animals. Hematological parameters and milk composition were evaluated by standard procedures and methods. Biochemical and endocrine parameters were evaluated using commercially available kits as per manufacturer's protocol. Milk somatic cell count (SCC) was done by direct microscopic counts using Newman Lampert stain.

**Result:** It observed significantly ($p<0.05$) higher TLC value but lower plasma glucose, cholesterol, calcium, phosphorus and urea levels during early lactation stage. Significantly ($p<0.05$) declined in plasma total protein during late lactation stage and triglyceride in dry cows. Plasma hormone significantly ($p<0.05$) higher in cortisol during early lactation while higher $T_4$ and insulin in late lactation stages. Milk fat, urea and total solids were significantly ($p<0.05$) decreased during early lactation and again increased during late lactation while milk SCC significantly ($p<0.05$) higher during early and late lactation stages.

**Key words:** Blood, Endocrine, Lactation, Milk, Tarai buffalo.

## INTRODUCTION

Tarai buffalo is a buffalo breed native to tropical tarai region located at the foothill of Himalaya and Siwalik hills of northern India in Uttarakhand state, reared by the marginal and small land holding farmers "Van Gujjar" living in the tarai region. These farmers socio-economically poor and their livelihood solely depend on their livestock's growth and products. Tarai buffalo has a unique physical appearance and physiology to adapt adverse climatic conditions of tarai region (Manjari *et al*. 2016). They require less external inputs and survive mostly on natural forest products that are available round the year (Anonymous, 2014). This breed is known to have excellent draught power and resist to many tropical diseases. Recently, the milk production capabilities of this buffalo breed have been explored. The average milk yield of this buffalo breed was reported 1054.08±1.95 kg during the lactation cycle of about 291 days by Singh and Barwal, (2014).

There is much scope for improvement and utilization of Tarai buffalo as a dual purpose buffalo breed. Milk production of the animal is highly related to the physiological status and metabolic profiles interaction with hormones. Hemato-biochemical and endocrine profiles were used as an indicator of the health status in animals (Antunovic *et al*. 2011). Further, the alterations in milk composition and blood profiles during different lactation stages in tarai buffalo still needs to be standardized. Therefore, the present study was designed to evaluate the hemato-biochemical, endocrine, milk SCC and milk composition in Tarai buffalo during different lactation stages.

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## MATERIALS AND METHODS

The study was conducted at Gujjar farms in and around Lalkaun, Udham Singh Nagar District which lying in northern upper Gangetic plains of tarai region in Uttarakhand, India. It is located at 344 m above the mean sea level (79°E longitude

and 29°N latitude). Tarai region have average air temperature of max. 29°C and min. 17°C with relative humidity of max. >80% and min.55% throughout the year. It is hot-humid during summer and rainy seasons with extreme cold during winter and short spring season. Animal ethical permission was taken (IAEC/VPB/CVASc/119) and the study was done for one complete lactation cycle.

Thirty-six healthy multiparous Tarai buffaloes weighing 280 to 350 kg B.W. with average lactation length of about 290 days were selected from four Gujjar farms. The animals were divided into four groups of nine buffaloes in each group as; early (60±30 days), mid (120±30 days), late (180±30 days) lactation stages and another group of dry buffaloes. The buffaloes were provided with minimal concentrate but ad lib access to water and natural forest products (grasses and fodders) available year round. Open housing system for the animals and roof made of locally available materials to provide shelter and comfort during summer and rainy seasons.

The milk production data was collected from the farmers. Both milk and blood samples collected once from each lactation stage. All the analytical works were performed within the Department of Veterinary Physiology and Biochemistry laboratory, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology.

Morning milk sample collected in a sterile vial after discarding initial few streaks of milk by manual full hand milking technique. Before which udder was washed with lukewarm water and mammary teat dip into the dipping solution (0.5% iodine or 4% hypochlorite) for at least 20-30s before milking. Then teats were wiped with a muslin cotton cloth moistened with 70-80% ethyl alcohol. Milk SCC was estimated by direct microscopic counts using Newman Lampert stain (Schalm *et al.,* 1971) and milk composition analysis of Ph (Microprocessor, HPG System, Chandigarh, INDIA), fat (IS:1224, 1977), lactose (Oser, 1979), protein (Kjeldahl's method of AOAC, 2000), total solids and solids-non-fat (IS:1479, 1961), milk urea (Dhali *et al.,* 2006) were done.

Blood samples (7 ml) collected from jugular vein aseptically into a vial containing disodium EDTA @ 1 mg/ml (Wittgenstein, 1953) as anticoagulant, from which 2 ml blood was utilized for haematological analysis of Hb (Drabkin and Austin, 1932), PCV (Dacie and Lewis, 1975), TEC and TLC (Schalm *et al.,* 1975) and 5 ml blood for biochemical analysis of plasma total protein, albumin, globulin, urea, cholesterol, triglycerides, electrolytes (calcium, phosphorus) and glucose using commercially available kits (Autospan Liquid Gold, Erba Mannhein). Plasma hormones estimation for $T_3$, $T_4$, Insulin and cortisol were done by radioimmunoassay kit (Beckman Coulter RIA kit) where determined in Stratec 12 well Gamma Counter (Gewerbaactr, Germany).

Effect of lactation stages on blood and milk parameters were analyzed by one-way ANOVA between different lactation stages using statistical analysis software IBM® SPSS® Version 20.

## RESULTS AND DISCUSSION

The alterations in the milk yield and milk SCC were depicted in Fig 1. The total milk yield per lactation was reported around 812.55±23.42 kg with highest around mid-lactation (415±23.21 kg) and peak milk yield (3.12±0.04 kg/day) was seen around 212 days. The average milk SCC was $1.05\pm0.06 \times 10^5$/ml throughout the lactation period. Milk SCC showed significant ($p$<0.01) difference during different lactation stages and lowest SCC during mid-lactation stage. Milk yield recorded in our present observations was lower compared to the earlier report of Singh and Barwal, (2014). The total lactation yield of Tarai buffaloes was found comparable to Nagpuri buffaloes, another dual purpose breed of India with less peak lactation yield (Panicker *et al*. 2016). The milk SCC of Tarai buffaloes reported in present investigation was lower than that reported by Singh and Ludri, (2001) but within the range as reported by Dang *et al*. (2010). The pattern of alterations in milk SCC during different lactation stages was also corroborated with the earlier reports of Singh and Ludri, (2001) in Murrah buffaloes.

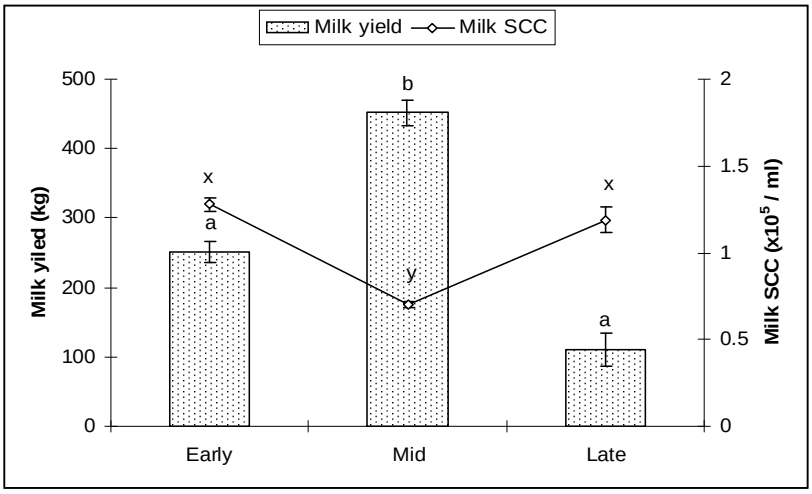


**Fig 1:** Alterations in the milk yield and milk SCC during different stages of lactation cycle in Tarai buffaloes.
Values were expressed as mean±SE. Values with different superscripts are significantly ($p$<0.01) different (a, b milk yield; x, y milk SCC).

Hematological parameters during different lactation stages have presented in Table 1. No alterations were observed in the hematological parameters except TLC, which was significantly ($p$<0.05) higher during early lactation compared to other stages. The hematological parameters of Tarai buffaloes were within the normal range as reported earlier (Manjari *et al*. 2016). The results revealed stability in the PCV, Hb and TEC values during different lactation stages. Our results corroborated with the earlier reports in Indian buffaloes (Hagawane *et al*. 2012; Das *et al*. 2016). Higher TLC during early stage of lactation observed in our investigation was similar with the earlier reports of Meglia *et al*. (2001) which could be due to higher levels of glucocorticoids around parturition that facilitates increased in neutrophil outputs from bone marrow (Lee and Kehrli, 1998).

Biochemical parameters during different lactation stages in Tarai buffaloes have presented in Table 1. Blood glucose, cholesterol, calcium, phosphorus and urea levels were significantly ($p$<0.05) lower during early lactation whereas total protein during late lactation stages while triglyceride level was significantly ($p$<0.05) lower in dry cows. These values were within the range as reported earlier (Hagawane *et al*. 2012; Das *et al*. 2016). The lower blood glucose levels during early lactation was in accordance with the earlier reports of Wu *et al*. (2019) in cattle but contrary to the reports of Das *et al*. (2016) in buffaloes. Lower blood glucose during early lactation may be due to partitioning of nutrients (Sakowski *et al*. 2012). Decreased levels of blood triglycerides during late lactation were reported earlier in cows (Wu *et al*. 2019). Das *et al*. (2016) reported no alteration in triglyceride levels during different lactation stages in buffaloes. The decreasing pattern of plasma proteins with the advancement of lactation was corroborated with the earlier reports in cows (Cavestany *et al.* 2005) but contrary to the reports in buffaloes (Das *et al*. 2016). These pattern may be correlated with maternal requirements of proteins providing immunoglobulins (Mohri *et al*. 2007). In our current investigation, we observed progressive increasing in urea, calcium and phosphorus levels as lactation advanced. A similar finding was reported earlier in cows (Coroian *et al.* 2017). The reason might be due to decreased efficiency of protein utilization with increase in

**Table 1:** Hemato-Biochemcal parameters, endocrine profiles and milk constituents in lactating Tarai buffaloes during different stages of lactation cycle.

| Parameters | Stages of lactation | | | |
|---|---|---|---|---|
| | Early | Mid | Late | Dry cows |
| **Hematological parameters** | | | | |
| PCV (%) | 39.47±0.46 | 39.12±0.23 | 38.92±0.62 | 39.04±0.31 |
| Hb (g %) | 11.90±0.17 | 12.01±0.23 | 11.67±0.16 | 11.97±0.16 |
| TEC ($\times10^6$/µl) | 6.02±0.37 | 6.17±0.23 | 6.21±0.62 | 6.31±0.31 |
| TLC ($\times10^3$/µl) | $10.80^{b}$±0.48 | $9.54^{a}$±0.41 | $9.82^{a}$±0.41 | $9.39^{a}$±0.68 |
| **Biochemical parameters** | | | | |
| Total protein (g/dl) | $8.84^{b}$±0.04 | $8.42^{b}$±0.06 | $7.27^{a}$±0.08 | $8.25^{b}$±0.03 |
| Albumin (g/dl) | 3.54±0.05 | 4.30±0.04 | 3.35±0.05 | 3.25± 0.08 |
| Globulin (g/dl) | 4.81±0.03 | 3.81±0.04 | 4.48±0.05 | 4.80±0.07 |
| Urea (mg/dl) | $33.68^{a}$±0.61 | $41.49^{b}$ ±0.66 | $45.90^{c}$±0.60 | $46.69^{c}$±0.59 |
| Triglyceride (mg/dl) | $31.25^{a}$±0.55 | $35.21^{c\pm}$0.63 | $33.41^{b}$±0.66 | $30.67^{a}$±0.43 |
| Cholesterol (mg/dl) | $60.88^{a}$±0.91 | $75.82^{c}$±0.83 | $70.09^{b}$±0.74 | $71.23^{b}$±0.88 |
| Calcium (mg/dl) | $8.26^{a\pm}$0.04 | $8.52^{a}$±0.04 | $9.34^{b}$±0.10 | $10.48^{c}$±0.15 |
| Phosphorous (mg/dl) | $4.13^{a\pm}$0.02 | $4.26^{a}$±0.02 | $4.57^{a}$±0.05 | $5.24^{b}$±0.07 |
| Glucose (mg/dl) | $39.84^{a\pm}$0.67 | $42.82^{b}$±0.75 | $46.87^{c}$±0.81 | $49.53^{d}$±0.61 |
| **Endocrine parameters** | | | | |
| $T_3$ (ng/ml) | 1.02±0.08 | 1.077±0.09 | 1.13±0.08 | 0.978±0.09 |
| $T_4$ (ng/ml) | $35.33^{a}$±1.10 | $37.55^{b}$±0.98 | $38.48^{b}$±1.11 | $35.40^{a}$±0.95 |
| Cortisol (ng/ml) | $7.82^{b}$±0.03 | $4.32^{a}$±0.06 | $5.38^{a}$±0.05 | $6.42^{b}$±0.04 |
| Insulin (µIU/ml) | $16.31^{a}$±0.21 | $24.31^{b}$±0.32 | $25.03^{b}$±0.33 | $17.32^{a}$±0.11 |
| **Milk constituents** | | | | |
| Fat (%) | $6.13^{a}$±0.06 | $6.65^{a}$±0.05 | $7.13^{bc}$±0.05 | - |
| Protein (%) | 4.70±0.04 | 4.76±0.04 | 4.79±0.04 | - |
| Lactose (%) | 4.46±0.01 | 4.49±0.04 | 4.50±0.04 | - |
| Urea (mg/dl) | $26.52^{a}$±0.54 | $33.83^{b}$±0.61 | $42.82^{c}$±0.41 | - |
| SNF (%) | 8.33±0.02 | 8.61±0.03 | 8.85±0.03 | - |
| TS (%) | $12.44^{a}$±0.10 | $13.38^{ab}$±0.10 | $14.70^{b}$±0.17 | - |
| pH | 6.58±0.17 | 6.70±0.17 | 6.78±0.17 | - |

$^{abcd}$Means±SE values in the same row without common letter are statistically different at p<0.05.

milk production (Roy *et al*. 2003) and increased demand of calcium for lactogenesis (Das *et al*. 2016). The alterations in the blood cholesterol levels observed during different lactation stages was in accordance with the reports of Rowlands *et al*. (1980) in cows.

Alterations in the metabolic hormones during different lactation stages have been presented in Table 1. A significant ($p$<0.05) increased in insulin and T4 concentrations from early to late lactation stages where plasma cortisol level showed opposite trend. Plasma T3 did not show any significant variation between different lactation stages. Hormonal profiles of Tarai buffaloes obtained in our investigation were in accordance with the study of Fiore *et al*. (2018). The increasing trend of plasma insulin level with advancement in lactation stage may be due to increase feeding rather than nutrient partitioning as glucose uptake by mammary gland is independent of insulin (Tsuda *et al*. 1991). Thyroid hormones played important role during the lactation period (Nikolic *et al*. 1997), lower level of T4 with advancement in lactation stage might be due to increasing number of hormone receptors within the mammary gland (Wilson and Gorewit, 1980). Cortisol level during different lactation stages found similar to the reports of Fukasawa *et al*. (2008) in cattle. Higher level of cortisol during early lactation might be due to parturition stress as there was a very low correlation between milk yield and cortisol level (Fukasawa *et al*. 2008).

Variation in milk composition during different lactation stages of Tarai buffaloes has represented in Table 1. A significant ($p$<0.05) gradual increased in milk fat, urea and total solids from early to late lactation stages were observed in our investigation. The other parameters remained stable in respect to lactation stages.

Milk fat percentage of Tarai buffalo was lower than Murrah (Yadav *et al.* 2013), but found similar to the reports of Patbandha *et al*. (2015) in Jaffrabadi buffalo. The lower milk fat content during early lactation and its subsequent increments with advancement of lactation may be associated with milk production as they are negatively correlated (Friggens *et al.* 2007). We did not find any significant alterations in the milk protein content of Tarai buffaloes during different lactation stages as reported earlier in Jaffrabadi buffaloes (Garaniya *et al.* 2013). The milk lactose percentage was found stable in respect to different lactation stages in contrary to the reports of Patbandha *et al*. (2015) in Jaffrabadi buffaloes. The pattern of milk urea level in Tarai buffalo during different lactation stages was in accordance with the earlier report in cows (Henao-Velásquez *et al.* 2014). Milk pH of Tarai buffalo was comparable with the milk of other buffalo breeds and observed no definite pattern of alterations during different lactation stages (Johanson *et al.* 2019).

## CONCLUSION

Milk production potential of Tarai buffaloes was poor, but milk composition found comparable with other milch buffalo breeds. Milk SCC of Tarai buffaloes indicate a good udder immunity and less prone to mastitis. Alterations in tarai buffalo blood and milk entities during different lactation stages as part of physiological homeostasis and performed well in hot-humid climatic condition of tarai region. Improvement on socio-economic and livelihood for landless and small land holding farmers by encouraging farmers to take up tarai buffalo rearing due to low external inputs and easy management. It is suggested that improvement in their feeding regime and shelter during unfavourable seasons might improve their performance further.

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