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Comparative study on hemato-biochemical profiling in primiparous and multiparous Surti buffaloes during early lactation

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Abstract
The present study was conducted to compare the hemato-biochemical profile in primiparous and multiparous Surti buffalo during early lactation. 14 Surti buffaloes in advance gestation were selected and divided into 2 groups (primiparous and multiparous) of 7 each. Whole blood was collected at calving, 1 month and 2 months and used for hematological as well as biochemical analysis. Significantly (P<0.05) higher levels of lymphocytes in primiparous at calving and day 60 postpartum and granulocytes in multiparous buffaloes at calving was observed. Within multiparous buffaloes at day 30 significantly (P<0.05) higher platelets, lymphocytes and monocytes and lower granulocytes were observed. Significant (P<0.05) lower values of blood glucose was seen in primiparous as compared to multiparous buffaloes. At calving significant (P<0.05) lowering of protein levels within primiparous and of albumin within both groups was observed. At calving significantly (P<0.05) higher lactate and significantly (P<0.05) lower bicarbonate levels were observed within primiparous buffaloes. Thus it was concluded that lymphocytic migration to other tissues and contribution of neutrophil component in granulocytes led to lowered lymphocyte levels and higher granulocyte levels in multiparous buffaloes. At calving negative balance for energy was seen in terms of low glucose in primiparous both between and within the group and for nitrogen in terms of total protein within primiparous and of serum albumin within both groups. At calving lactate levels were higher within primiparous group and bicarbonate levels were lower within both the groups.

Keywords: Hemato-biochemical, primiparous, multiparous, Surti buffalo, postpartum, early lactation

Introduction
Buffalo population in India as per 2012 Census is 108.7 million that contributes around 21.23% of the total livestock population. The state of Gujarat is ranked 4th by possessing 9.55% of total population out of which Surti breed is integral primarily serving the purpose of milk production. Parturition and lactation are the stressful phases in the life of dairy buffaloes. Immediately after parturition the milk production starts and the animal suffers from the stress of parturition as well as milk production. Following parturition milk production increases and reaches a peak level within 50-60 days of parturition and thereafter it starts declining. Hematological and blood biochemical metabolites present in blood reflect the metabolic status and health in general. The metabolic demand of the primiparous buffaloes may be slightly different from that of the multiparous buffaloes. Immediately after parturition there is increased demand of metabolites for milk synthesis and therefore alterations in their blood levels takes place. Changes in hematobiochemical profile has been reported to occur during peripartum Joksimović and Davidović (2012) [1] and different stages of lactation Das *et al*., (2016) [2] .Therefore the present study was planned with the objective to compare the hemato-biochemical profile of primiparous and multiparous Surti buffaloes at parturition and during early lactation.

Materials and methods
The study was conducted at Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NAU, Navsari (Gujarat). The study was done following ethical guidelines and was approved by IAEC vide NAU/NVC/IAEC/12/2017, 11/11/2017. Study was conducted on 14 post parturient buffaloes that were divided equally into two groups of 7 each as primiparous (first parity) and multiparous (number of parity more

than one). The selected animals were maintained under standard feeding and management system at Livestock Research Station, Navsari Agricultural University, Navsari. They were housed in pucca shed with concrete floor. Feeding and watering was done as per Indian Council of Agricultural Research (ICAR) feeding standards, 1998. Blood sample collection was done at calving and thereafter at monthly interval up to peak lactation. Whole blood from jugular vein was collected in vacutainers for hematological analysis (containing anticoagulant K_3EDTA) and for serum separation (without anticoagulant). Serum was separated and stored at -20ºC in deep freeze until analyzed for biochemical parameters. Whole blood was used for the analysis of hematological parameters such as haemoglobin (Hb), packed cell volume/hematocrit (HCT), total erythrocyte count/total red blood cell count (TEC), total leukocyte count, differential leukocyte count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets by using fully automated haematology cell counter (Exigo EOS, Boule Diagnostics AB, Sweden). Serum samples were used for analysis of biochemical parameters such as glucose, albumin, total protein, urea, bicarbonate, pyruvate and lactate. All the biochemical parameters were analyzed using kits (DIATEK healthcare Pvt. Ltd). On Biospectrometer (Eppendorf AG, 22331 Hamburg, Germany). Results obtained were analysed statistically by Two-way ANOVA using DMRT for interpreting effect of different groups for the parameters under study. DMRT was used for mean separation at 5% level of significance (Snedecor and Cochran 1994) [3].

Results and Discussion

Hematological Parameters

Changes in the haematological parameters (Mean±SE) from parturition (at calving) to peak lactation (day 60 postpartum) in primiparous and multiparous Surti buffaloes are given in Table 1.

Lymphocytes at calving and day 60 postpartum were significantly (P<0.05) lower in multiparous as compared to primiparous buffaloes whereas granulocytes were significantly (P<0.05) higher in multiparous buffaloes at calving. Other parameters did not show any significant variation between the groups however at calving and day 60 postpartum concentrations of TEC, Hb, HCT and total leukocytes were slightly higher.

Within the group of multiparous buffaloes the platelets, lymphocytes and monocytes were significantly (P<0.05) higher at day 30 postpartum than at calving and day 60 postpartum whereas granulocytes were significantly (P<0.05) lower at day 30 postpartum than other two postpartum stages. Rest of parameters did not show any significant changes within both the groups however there was slight decrease in the concentration of RBC and Hb from calving to day 60 postpartum within primiparous buffalo group.

Significantly (P≤0.05) high granulocyte was observed at calving in multiparous buffaloes. Highest neutrophil and low lymphocyte at calving has also been reported in 3rd lactation in HF cows by Joksimović *et al*., 2012 [1] and in heifers by Piccinini *et al*., 2004 [4]. Neutrophils are part of granulocyte and in present study high granulocyte% was observed. High leukocyte count on day of parturition was observed on calving in present study. However, values were non-significant. Decrease in number of lymphocyte at calving may be due to their migration into different tissue (Alon *et al.* 1995) [5]. Increase in neutrophils is usually associated with bacterial infection. Stress of calving and milking at early stage of lactation may also be factors associated with increase in granulocytes%. Neuroendocrine changes also change the neutrophils and lymphocytes % during peripartum period (Kehrli *et al.* 1989) [6].

Non-significant variation in all the haematological parameter has been reported in early, mid and late lactation in Mehshani buffaloes (Das *et al.* 2016) [2] , in Hb and PCV at 60 days post-partum in riverine buffaloes (Abdulkareem 2013) [7] and in Murrah, Jafarabadi, Nagpuri and non-descript buffaloes (Hagawane *et al.* 2012) [8]

Blood Biochemical Parameters

Changes in the glucose, total protein, urea, lactate, pyruvate and bicarbonate (Mean±SE) of primiparous and multiparous Surti buffaloes from parturition to peak lactation are given in Table 2.

Significant (P<0.05) lower values of blood glucose between group was observed at calving in primiparous as compared to multiparous Surti buffaloes.

Within primiparous group of buffaloes the serum protein level was significantly (P<0.05) lower at calving and day 30 of lactation as compared to day 90 postpartum. Albumin levels were also significantly (P<0.05) lower at calving within both groups. At calving significantly (P<0.05) higher lactate within primiparous group and significantly (P<0.05) lower bicarbonate levels within both groups were observed as compared to later stages of lactation of buffaloes. Other parameters did not vary significantly.

Metabolic profile of animals varies with the different lactation stages. Significant (P≤0.05) low value of glucose was observed at calving as compared to 30 days and 60 days of lactation in primiparous as compared to multiparous Surti buffaloes. The decrease in blood glucose in primiparous may be due to negative energy balance at calving and its use in synthesis of milk lactose. Blood glucose level may decrease or may not change. If there is no change in blood glucose it indicates that body spares glucose for the synthesis of lactose as observed in present study. Low glucose may be attributed to decreased gluconeogenesis at beginning of lactation (Joritsma, 2003; Doepel *et al*., 2002) [9, 10]. Low blood glucose at parturition may be result of decline in insulin level of cows during initiation of lactation (Accorsi *et al.* 2005) [11]. A drop of 17.2% blood glucose in first week post-partum has been reported by Teama and Gad (2014) [12]. Significant increase in blood glucose during early and mid-lactation in dairy cows has been reported (Djokowik *et al*., 2014) [13]. Significant difference (P≤0.05) in blood glucose during different lactation phase comprising of more than 60 days has been reported by Monterio (2012) [14] and at 15 day in primiparous by Joksimovik *et al*., (2012) [1]. Non-significant difference in glucose in buffaloes has also been reported during different lactation stages (Jambh *et al*., 2016) [15].

Significantly (P≤0.05) low total protein and albumin was observed at calving in the present study. The decrease in protein near parturition and during early lactation may be due to synthesis of milk protein as the physiological changes takes place from gestation, parturition and then lactation. The findings of significant low total protein and albumin in early lactation as compared to mid and late lactation in Murrah buffaloes has been reported (Hagawane *et al.*, 2009) [8] which corroborates with our findings. Non-significant increase in total protein and albumin occurs in riverine buffaloes from 15 to 60 days post-partum Abdulkareem, 2013 [7] and at different stages of lactation in dairy cattle (Djokowik *et al*., 2014) [13].

Abd-El Naser *et al*., (2014) [16] also reported decrease in total protein with increase in lactation, which is contrary to our study.

Table 1: Changes in haematological parameters (Mean±SE) from parturition to peak lactation in primiparous and multiparous Surti buffaloes

Parameter	Primiparous			Multiparous		
	At calving	30 days	60 days	At calving	30 days	60 days
Lymphocytes (%)	45.90^{B}±4.07	46.27±3.81	47.43^{B}±3.99	29.44^{Aa}±2.93	46.1^{b}±3.19	35.87^{Aa}±1.54
Monocytes (%)	7.94±0.58	7.49^{A}±0.37	9.30±1.09	8.57^{ab}±0.66	10.07^{Bb}±0.94	7.83^{a}±0.28
Granulocytes (%)	46.16^{A}±3.90	46.24±3.81	43.27±3.70	62.09^{Bb}± 3.47	43.81^{a}±3.48	56.30^{b}±1.44
Total leukocyte count(10^{3}×µl)	9.80±1.58	9.09±0.99	8.34±0.73	7.77±0.74	7.34±0.37	7.81±0.48
Haemoglobin (g/dl)	12.13±0.46	11.06±0.44	11.47±0.22	11.74±0.63	11.30±0.27	11.34±0.61
HCT (%)	33.59±1.43	30.41±1.16	30.94±0.93	32.57±1.65	30.74±0.69	30.56±1.52
TEC (10^{6}×µl)	6.17±0.26	5.73±0.26	6.10±0.10	5.89±0.34	6.14±0.29	5.90±0.38
MCV (fl)	54.66±1.33	53.30±1.25	50.73±1.23	55.44±1.00	50.69±2.46	52.17±1.20
MCH (pg)	19.76±0.33	19.41±0.44	18.89±0.29	19.94±0.23	18.59±0.72	19.33±0.34
MCHC (g/dl)	36.23±0.31	38.49±2.15	37.23±0.45	36.00±0.28	36.81±0.74	37.11±0.32
Platelets (10^{3}×µl)	374.57±36.30	329.29±60.71	274.00±23.90	305.00^{a}±27.18	661.29^{b}±141.68	305.00^{a}±50.16

Upper case shows significant difference between the groups at $P<0.05$
Lower case shows significant difference within the groups at $P<0.05$

Table 2: Changes in glucose, total protein, albumin, urea, pyruvate, lactate and bicarbonate (Mean±SE) from parturition to peak lactation in primiparous and multiparous Surti buffaloes

Parameter	Primiparous			Multiparous		
	At calving	30 days	60 days	At calving	30 days	60 days
Glucose (mg/dl)	39.087^{Aa}±1.41	44.917^{ab}±2.63	51.294^{b}±2.93	47.411±3.412^{B}	37.383±3.385	45.033±3.929
Total protein (g/dl)	6.144^{a}±0.33	6.391^{a}±0.37	7.453^{b}±0.27	6.157±0.231	6.771±0.265	6.907±0.249
Albumin (g/dl)	3.220^{a}±0.09	3.326^{ab}±0.16	3.670^{b}±0.09	3.286^{a}±0.09	3.391^{ab}±0.06	3.556^{b}±0.09
Urea (mg/dl)	39.184±1.78	34.506±1.20	38.303±2.88	36.687±1.96	35.141±1.26	39.083±2.32
Pyruvate (mg/dl)	1.751±0.14	1.728±0.14	2.013±0.12	1.830±0.31	1.570±0.13	1.863±0.17
Lactate (mg/dl)	10.780^{b}±0.46	8.205^{a}±0.70	8.336^{a}±0.39	9.550±0.79	8.961±0.24	8.420±0.23
Bicarbonate (mmol/l)	20.536^{a}±1.57	31.789^{b}±2.59	28.566^{b}±3.43	19.137^{a}±2.23	29.296^{b}±1.92	27.560^{b}±2.28

Upper case shows significant difference between the groups at $P<0.05$
Lower case shows significant difference within the groups at $P<0.05$

In present study, non-significant increase in urea concentration was observed between and within primiparous and multiparous animals at different days of lactation. The concentration of serum urea is also dependent on the dietary intake of protein. Significant increase in urea has been reported during late lactation as compared to the late gestation and thebeginning of lactation (Piccione *et al*., 2012) [17]. Increase in serum urea levels during thelactation period as compared to the late gestation may be due to more dietary intake of proteins, because of the increased requirements (Roubies *et al*., 2006) [18]. Significantly ($P\leq0.05$) low concentration of blood urea occurs in early lactating stage as compared to the normal healthy buffaloes (Hagawane *et al.*, 2009) [8]. Non-significant difference in urea corroborates with the findings of Dias *et al.* (2017) [19] and Milani *et al.*, (2011) [20] in dairy cows and Jambh *et al.*, (2016) [15] in buffaloes.

In present study, significant high level of lactate was observed at calving in primiparous Surti buffaloes. Significant high level of lactate at calving may be due to more conversion of pyruvate to lactate. The production of lactate in enterocytes has been reported to be quite high during early stage of lactation (Okiane 1995) [21]. Lactate through Cori cycle may enter into liver and get converted in to glucose through the process of gluconeogenesis. More amount of lactate may be due to more energy required for calving. Accumulation of lactate in tissue may increase the level of blood lactate. The bicarbonate level of 20.53±1.57 mmol/L at calving in the present study in primiparous was nearer to the values of 21.55 mmol/L as reported by (Sarwan *et al.*, 2007) [22]. In the present study, concentration of bicarbonate increased and the concentration of lactate decreased. An increase in bicarbonate with increase in lactation may be due to change in the diet. The concentration of bicarbonate also depends upon the amount of Na^{+} entering the renal tubular cells. Entry of more Na^{+} will lead more bicarbonate in blood. High bicarbonate level occurs in prepartum than 2nd week post-partum in cows (Utim *et al.*, 2009) [22].

Conclusion

It was concluded from the present study that in multiparous lowered lymphocyte levels and higher granulocyte levels as compared to primiparous buffalo indicated possible lymphocyte migration to other tissues and contribution of neutrophil component in granulocytes. Significant lowering of serum glucose in primiparous buffaloes indicated presence of negative energy balance as compared to multiparous. At calving lower serum protein levels within primiparous and lowered serum albumin levels within both groups indicated negative nitrogen balance. Levels of lactate were higher within both groups at parturition that probably served as energy substrate during high demand.

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