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Studies on metabolic parameters during late gestation and early post-partum period in crossbred cows

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Abstract

Periparturient period is critically important for dairy cows for subsequent health and reproductive performances as marked alterations occur in various metabolic parameters during this period. Thus, establishing the base line values for various metabolic parameters (haemoglobin (Hb), packed cell volume(PCV), total erythrocyte count (TEC), total leucocyte count (TLC), total plasma protein (TPP), albumin, plasma urea nitrogen(PUN), creatinine, glucose, beta-hydroxyl butyric acid (BHBA),non-esterified fatty acid (NEFA), calcium (Ca), magnesium (Mg),plasma inorganic phosphorus (Pi), sodium (Na), potassium (K),copper (Cu), iron (Fe) and zinc (Zn) during this period will help in the future for differentiating between the healthy and diseased animals and also for the advanced prediction of various metabolic diseases. A total of 51 crossbred cows were sampled thrice during the transition period. A decrease was noticed in the Hb, PCV,TEC, TPP, albumin, glucose, Ca, Na, K, Fe and Zn, whereas an increased was observed in TLC, PUN, BHBA, NEFA, Mg and Cu levels from the pre-parturient to the post-parturient levels.

Keywords: Metabolic profiling Crossbred cows, Minerals, BHBA, NEFA.

The transition period (from 21 days before to 21 days after calving) is an important period in the production cycle of dairy cows (Drackley, 1999). The success of the next lactation and reproduction depends upon this period, as during this period a lot of changes in physiological conditions, nutritional status, and metabolic responses of cows occur during this period as during this period, most of the animals are in negative energy balance due to the increase demand of the body for glucose required for the synthesis and secretion of milk after calving (Esposito *et al.,* 2014). The development of a negative energy balance will increase lipid mobilization and production of fatty acids that further exacerbate metabolic stress and inflammation response (Bell, 1995; Overton *et al.,* 2017) leading to increased risk of metritis, fatty liver, ketosis, and mastitis in postpartum cows (Contreras and Sordillo, 2011). Thus, in order to monitor, detect and predict such diseases, Compton Metabolic Profile Test (CMPT) was first developed by Payne *et al.* (1970). However the values established by different scientists cannot be used exactly in our country as the laboratory procedures, target population, environmental conditions, types of feeding, management systems are not similar, so the study was planned to establish the base line values for the metabolic parameters in crossbreed cows during the periparturient period so that these values can be used in future for the early detection, treatment and prevention of subclinical and clinical forms of various diseases.

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Materials and Methods

A total of 51 crossbred cows (thirty seven from Farm no.1, seven from Farm no.2 and seven cows from Farm No. 3) from various farms of Amritsar district of Punjab were sampled during their last trimester of pregnancy. Blood samples were taken thrice from each cow, (i) Far off dry (FOD)- >10 days following dry off and not < 30 days prior to calving, (ii) close up dry (CUD)- Between 3 and 21 days prior to calving, (iii) Fresh - 3 to 30 days in milk) to evaluate the changes in the values of various parameters of CMPT, viz. hemoglobin (Hb), Packed cell volume (PCV), total erythrocyte count (TEC),total leucocyte count (TLC), total plasma protein (TPP), albumin, plasma urea nitrogen (PUN), creatinine, glucose, beta- hydroxyl butyric acid (BHBA), non-esterified fatty acid (NEFA), calcium (Ca), magnesium (Mg), plasma inorganic phosphorus (Pi),sodium (Na), potassium (K), copper (Cu), iron (Fe) and zinc (Zn).

All the haematological parameters (Hb, PCV, TEC, TLC) were estimated on the Haematology Analyzer (ADVIA 2120, SIEMENS, USA). Various biochemical parameters (TPP, albumin, PUN, creatinine and glucose) were accessed on semiautomatic Biochemistry Analyzer using kits. Both plasma BHBA and NEFA levels were estimated in the ELISA plates with the help of kits. The plasma samples were digested as per the procedure described by Kolmer *et al*. (1951) and the digested samples were estimated for copper, iron and zinc by Atomic Absorption Spectrophotometer (AAS). The Ca, Mg, Na and K concentrations in plasma were estimated on the AAS directly from the plasma samples as described in Perkin Elmer, (2000). Plasma Pi was determined by using method given by Tausky and Shorr (1953) and the readings were taken using spectrophotometer. Mean, standard errors of means of various parameters were estimated and test of significance (one way analysis of variance), were performed using SPSS for windows (version 16.0; Microsoft).

Results and Discussion

All the cows had normal parturition and they were not found suffering from any clinical disease during the periparturient period, however marked alterations were observed in the various metabolic parameters during the periparturient period.

The overall mean Hb, PCV, TEC and TLC levels observed in crossbred cows from various farms was within the normal range and is presented in Table 1. However marked alterations were recorded in various parameters during the different phases of parturient period. Between farms, significantly lower ($p<0.05$) values were recorded for Hb on Farm no. 1 as compared to the Farm no. 2 and 3 from FOD to CUD to fresh period, where as within a farm, a significant decrease was noted in the mean Hb and PCV in Farm no. 1and 3 between the FOD and fresh period (Table 1).

For TLC values, within a farm, a significant increase ($p<0.05$) was observed in the mean TLC values between the FOD and fresh period in Farm no. 2 (Table 1). Similarly, Khamas *et al.* (2013) observed an increase in the leucocyte count with the advancement of lactation.

The overall mean Hb was within the normal range on all the farms, as described by Feldman *et al*. (2000) while the mean Hb was within the normal range, but a constant decrease was observed in all the farms from FOD up to fresh period. On the contrary, Ate *et al.*(2009) did not find any significant difference in the mean Hb, PCV, RBC and WBC values during pregnancy and early lactation period.

Biochemical Profile

Within a farm, significant decrease was observed in the mean TPP levels from the FOD to CUD to the fresh period in Farm no. 1 (Table 2). Similarly, a significant decrease was also recorded in total protein and albumin values in crossbred cows from different districts (Singh *et al.,* 2016). Paquay *et al.* (1972) also reported a decrease in plasma proteins, as the cow loses 37 pound of body proteins during the first two weeks of lactation in order to provide amino acid and glucose required for milk

Table 1: Haematological indices in crossbred cows from Amritsar district (Mean ± S.E.)

Parameters	Period	Farm No.1 (n=37)	Farm No.2 (n=7)	Farm No.3 (n=7)
Hb (g/dl)	FOD	10.04 ± 0.14^{axz}	12.73 ± 0.85^{bdx}	12.10 ± 0.16^{cdxw}
	CUD	9.69 ± 0.14^{axy}	11.42 ± 0.68^{bdx}	11.51 ± 0.25^{cdxy}
	Fresh	8.97 ± 0.13^{adw}	10.34 ± 0.48^{bx}	9.31 ± 0.32^{cdz}
PCV (%)	FOD	32.50 ± 0.69^{axz}	40.71 ± 2.51^{bdx}	37.05 ± 0.74^{cdxw}
	CUD	31.61 ± 0.50^{axy}	37.58 ± 2.31^{bx}	34.72 ± 0.66^{abxy}
	Fresh	28.46 ± 0.44^{adw}	33.79 ± 2.04^{bx}	28.98 ± 1.05^{cdz}
TEC ($x10^6/\mu l$)	FOD	6.77 ± 0.35^{adwx}	8.10 ± 1.08^{abx}	5.10 ± 0.25^{cdx}
	CUD	6.67 ± 0.25^{awy}	6.52 ± 0.81^{ax}	5.63 ± 0.30^{ax}
	Fresh	5.98 ± 0.14^{ayz}	6.29 ± 0.60^{ax}	5.72 ± 0.17^{ax}
TLC ($x10^3/\mu l$)	FOD	8.19 ± 0.26^{ax}	7.34 ± 0.48^{awx}	8.34 ± 0.50^{ax}
	CUD	8.11 ± 0.22^{ax}	8.26 ± 0.37^{ayx}	9.15 ± 0.47^{ax}
	Fresh	8.83 ± 0.26^{ax}	10.43 ± 0.68^{az}	9.76 ± 0.77^{ax}

Values bearing different superscript (a, b, c, d) in a row differ significantly
Values bearing different superscript (w, x, y, z) in a column differ significantly

Table 2: Biochemical profile in crossbred cows from Amritsar district (Mean± S.E.)

Parameters	Period	Farm No.1 (n=37)	Farm No.2 (n=7)	Farm No.3 (n=7)
TPP (g/dl)	FOD	7.20 ± 0.12^{ax}	7.38 ± 0.29^{ax}	7.15 ± 0.29^{ax}
	CUD	6.82 ± 0.95^{ay}	7.12 ± 0.23^{ax}	7.28 ± 0.40^{ax}
	Fresh	6.42 ± 0.11^{az}	6.55 ± 0.27^{bx}	7.20 ± 0.26^{bx}
Albumin (g/dl)	FOD	2.75 ± 0.07^{azw}	2.27 ± 0.15^{bcx}	2.44 ± 0.86^{acx}
	CUD	2.64 ± 0.07^{azx}	2.29 ± 0.15^{ax}	2.30 ± 0.65^{ax}
	Fresh	2.53 ± 0.07^{axy}	2.20 ± 0.20^{ax}	2.41 ± 0.09^{ax}
PUN (mg/dl)	FOD	9.27 ± 0.69^{ax}	9.71 ± 0.74^{axz}	7.14 ± 0.76^{ax}
	CUD	11.35 ± 0.51^{ay}	9.14 ± 0.73^{abxy}	6.00 ± 0.89^{cx}
	Fresh	14.32 ± 0.46^{az}	12.14 ± 1.14^{abz}	6.57 ± 0.86^{cx}
Creatinine (mg/dl)	FOD	1.03 ± 0.04^{ax}	1.10 ± 0.05^{ax}	1.08 ± 0.06^{ax}
	CUD	0.99 ± 0.04^{ax}	1.21 ± 0.59^{ax}	1.01 ± 0.22^{ax}
	Fresh	0.98 ± 0.05^{ax}	1.22 ± 0.05^{ax}	0.95 ± 0.07^{ax}
Glucose (mg/dl)	FOD	65.51 ± 2.75^{adzw}	62.00 ± 5.74^{bdxw}	93.14 ± 5.03^{cx}
	CUD	60.56 ± 2.18^{azx}	54.28 ± 2.98^{abxz}	89 ± 4.64^{cx}
	Fresh	55.83 ± 2.03^{axy}	46.14 ± 3.99^{abyz}	80.85 ± 3.75^{cx}
BHBA (mmol/L)	FOD	0.47 ± 0.02^{aw}	0.37 ± 0.09^{axw}	0.50 ± 0.03^{ax}
	CUD	0.63 ± 0.02^{axy}	0.46 ± 0.06^{bxz}	0.56 ± 0.03^{abx}
	Fresh	0.69 ± 0.02^{axz}	0.59 ± 0.04^{azy}	0.62 ± 0.04^{ax}
NEFA (mmol/L)	FOD	0.10 ± 0.01^{ax}	0.14 ± 0.01^{axw}	0.17 ± 0.01^{axw}
	CUD	0.19 ± 0.02^{ay}	0.18 ± 0.01^{axy}	0.19 ± 0.02^{axz}
	Fresh	0.25 ± 0.02^{az}	0.24 ± 0.02^{az}	0.27 ± 0.02^{azy}

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production. Similarly a significant decrease was observed in the mean plasma albumin levels on Farm 1 between the FOD and fresh period (Table 2).

The fall in serum albumin towards the end of pregnancy might be due to the increasing nutrient requirement of the growing fetus (Lone *et al.,* 2003) and/or due to inadequate nourishment during advance stages of pregnancy (Sivaraman *et al.,* 2003).

Within a farm, a significant ($p<0.05$) increase was observed in the PUN values on the Farm no. 1 from the FOD to CUD to the fresh period and on Farm no. 2, from CUD to the fresh period.

The mean creatinine levels in our study showed non-significant patterns during pregnancy in our study. It is well recognized that during the late gestation, the mother, assumes the load of organic waste of the newborn for the foetal maternal circulation (Ferrell, 1991). So the increase in serum creatinine levels observed in some farms could be attributed to the development of the foetal musculature which is well documented in sheep and ewes too (Roubies *et al.,* 2006).

A significant decrease was observed in the mean glucose values, on the Farm 1 and 2, between FOD and fresh period (Table 2). In the present study, the plasma glucose concentration showed a transient decrease starting from the FOD period up to the early lactation which could be as during the last weeks of fetal development, the fetus uses around 46 per cent of maternal glucose taken up by the uterus. Bulent *et al.* (2006) too reported a significant decrease in blood glucose levels as the cow approached parturition.

Significantly ($p<0.05$) higher values were observed for BHBA on the Farm no. 1 during CUD period as compared to the Farm no. 2. Within a farm, a significant increase was observed in BHBA values on Farm no. 1 and 2 in between the FOD and fresh period (Table 2).

The mean plasma NEFA levels observed from FOD to fresh period was within the normal range, though a increase was observed from FOD to fresh period in all the farms. Within farms, a significant ($p<0.05$) increase was observed in the all the farms from the FOD period up to the fresh period.

The elevated serum NEFA level is one of the indicators of negative energy balance (NEB) in postpartum dairy cattle (Bell, 1995). Other indicators of NEB are an increased plasma concentration of beta-hydroxybutyrate (BHB) (Bell, 1995), decreased plasma glucose concentration (Grum *et al.,* 1996), decreased amount of insulin and insulin-like growth factor-1 (IGF-1) (Butler *et al.,*2003) and a decreased body condition score (BCS) (Oldick, 1999).

Concentrations of Plasma Minerals

Significantly ($p<0.05$) lower mean plasma Ca concentrations were observed in cows from the Farm no. 3 during the FOD, CUD and fresh period, as compared to the cows from the other two farms (Farm no. 1 and 2), and the values were lower than the normal range during the fresh period (Table 3).

Horst *et al.* (2005) also observed a decrease in the plasma Ca concentration in the older cows and cited that one of the reason was decreased number of receptors for 1, 25-dihydroxy Vitamin-D in the intestine resulting in decreased absorption of mineral.

Significantly ($p<0.05$) lower mean plasma Mg concentration was observed for the Farm 3 in comparison to the Farm no. 1 and 2 during the FOD period, and

Table 3: Plasma mineral concentrations in crossbred cows from Amritsar district (Mean± S.E.)

Parameters	Period	Farm No.1 (n=37)	Farm No.2 (n=7)	Farm No.3 (n=7)
Ca (mg/dl)	FOD	11.92 ± 0.32^{ax}	11.23 ± 0.27^{acx}	9.76 ± 0.45^{bzw}
	CUD	10.82 ± 0.30^{ay}	11.05 ± 0.32^{abx}	8.87 ± 0.53^{cxz}
	Fresh	9.65 ± 0.33^{az}	10.26 ± 0.48^{abx}	7.99 ± 0.55^{cxy}
Mg (mg/dl)	FOD	3.38 ± 0.16^{adx}	3.29 ± 0.24^{cdx}	2.41 ± 0.29^{bx}
	CUD	3.60 ± 0.14^{adx}	3.36 ± 0.29^{dx}	2.75 ± 0.27^{bax}
	Fresh	3.62 ± 0.16^{ax}	3.39 ± 0.29^{ax}	2.97 ± 0.15^{ax}
Pi (mg/dl)	FOD	4.81 ± 0.17^{ax}	4.58 ± 0.41^{ax}	4.47 ± 0.23^{ax}
	CUD	4.91 ± 0.13^{bdx}	4.86 ± 0.18^{dx}	4.14 ± 0.28^{cx}
	Fresh	4.61 ± 0.11^{ax}	4.89 ± 0.27^{ax}	4.37 ± 0.36^{ax}
Na (mmol/l)	FOD	114.74 ± 1.27^{axz}	111.82 ± 3.80^{ax}	112.67 ± 3.12^{ax}
	CUD	117.71 ± 1.69^{awz}	116.67 ± 3.00^{ax}	110.33 ± 5.10^{ax}
	Fresh	105.99 ± 1.28^{ay}	107.77 ± 1.78^{ax}	108.87 ± 0.05^{ax}
K (mmol/l)	FOD	4.76 ± 0.11^{abzw}	5.52 ± 0.48^{bdx}	5.85 ± 0.42^{cdx}
	CUD	4.50 ± 0.11^{axz}	5.67 ± 0.38^{bdx}	5.90 ± 0.24^{cdx}
	Fresh	4.34 ± 0.08^{axy}	5.25 ± 0.42^{abx}	5.09 ± 0.22^{bx}
Cu (ppm)	FOD	1.05 ± 0.07^{adx}	1.50 ± 0.14^{bdx}	1.87 ± 0.23^{cx}
	CUD	1.01 ± 0.08^{acx}	1.29 ± 0.16^{acx}	1.64 ± 0.10^{bx}
	Fresh	0.89 ± 0.06^{ax}	1.37 ± 0.20^{bdx}	1.42 ± 0.15^{cdx}
Fe (ppm)	FOD	2.52 ± 0.18^{ax}	3.44 ± 1.06^{ax}	2.44 ± 0.30^{ax}
	CUD	2.42 ± 0.17^{ax}	3.37 ± 1.09^{ax}	2.54 ± 0.34^{ax}
	Fresh	2.62 ± 0.20^{ax}	3.52 ± 1.11^{ax}	2.54 ± 0.28^{ax}
Zn (ppm)	FOD	1.38 ± 0.08^{adx}	1.20 ± 0.11^{abx}	1.90 ± 0.46^{cdx}
	CUD	1.12 ± 0.07^{ady}	0.95 ± 0.11^{bax}	1.47 ± 0.18^{cdx}
	Fresh	0.92 ± 0.05^{az}	0.86 ± 0.10^{ax}	0.99 ± 0.19^{ax}

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in comparison to Farm no. 2 during the CUD period (Table 3). A transient increase in mean plasma Mg level was found after calving. This has also been recorded in other studies (Goff and Horst, 1997). Results of Thilsing-Hansen *et al.* (2002) indicated that the increase in plasma Mg was related to the decrease in plasma Ca level occurring at calving.

Significantly lower values were recorded for the mean plasma Pi from the Farm no. 3 as compared to the Farm no. 1 and 2 during the CUD period (Table 3).This postpartum decrease in plasma Pi levels observed in the present study might be due to the fact that a large amount of Pi was drained from the blood for milk production and higher PTH levels, induced by low plasma Ca, increased urinary loss of phosphorus (House and Bell, 1993).

Within a farm, the mean plasma Na and K concentration showed a significant decrease from late pregnancy up to the early lactation period on Farm no. 1 respectively (Table 3). During the early lactation period, there is continuous drainage of sodium and potassium in milk, due to which a decrease could occur during the early lactation period (Deshpande *et al.,* 1998).

Significantly ($p<0.05$) higher values were observed for plasma copper levels on the Farm no. 3 during all the three periods as compared to the Farm 1. Within a farm, a non significant decrease was observed in the plasma copper levels from the FOD period up to the fresh period on all the farms (Table 3). The variations in plasma Cu concentrations under the present experimental conditions are a reflection of the net result of foetal demand and/or lactation. Thus the plasma Cu concentrations in pregnant cattle observed in the present study are in agreement with those reported by Gooneratne *et al.* (2013).

Non significant differences were observed for the plasma iron levels between the farms during various periods (Table 3). Insignificant patterns i.e decrease during the late pregnancy and increase during early lactation might be due to the increased transfer of this nutrient across the placenta and hemodilution during late pregnancy and at calving, together with the initiation of ovarian follicular activity postpartum, leading to high circulatory estrogens which stimulated the binding of iron with the proteins in liver and, thereby, increased concentration in plasma (Jacob *et al.,* 2003). Significant decrease was observed in plasma zinc levels on Farm no. 1 from FOD up to fresh period (Table 3). As during the early lactation period, zinc is required for colostrum synthesis (Kincaid and Cronrath, 1992), and also during late gestation, fetuses start accumulating Zn at a rate of about 12mg/day (House and Bell, 1993) due to which continuous decrease is recorded in Zn levels from late pregnancy to early lactation.

Base line values were established for various metabolic parameters in crossbred cows during periparturient period which will help in future for early prediction and diagnosis of various metabolic diseases in future.

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