



Blood metabolite concentrations and body condition score in relation to postpartum resumption of ovarian cyclicity in crossbred cows

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Received: 6 November 2018; Accepted: 11 December 2018

Key words: BCS, Blood metabolite, Cattle, Postpartum cyclicity

BCS is a subjective estimate of the energy reserves in adipose tissues of a dairy cow. It is an acceptable, non-invasive, quick and inexpensive method to estimate degree of fatness. The changes of BCS had been associated with the resumption of oestrous cycle during postpartum period (Borpujari *et al.* 2018). The lactation phases affect significantly the metabolic profile and so the variation recorded during different physiological phases is expected.

The transition from gestation to lactation is a period of great metabolic stress for dairy cows (Rollin *et al.* 2010). The milk production and its composition are influenced by the metabolical status of dairy cows (Heck *et al.* 2009). The body condition scoring may be used as an efficient tool in this regard. Changing BCS have implications on milk yield, health, reproduction, longevity and overall profitability of an animal (Mishra *et al.* 2016, Borpujari *et al.* 2018). All animals require calcium (Ca), magnesium (Mg) and phosphorus (P) for growth, reproduction and lactation, which often affect specific requirements, and serve as catalytic components of enzymes or regulate several mechanisms involved just in pregnancy and lactation (Talukdar *et al.* 2016). At the beginning of lactation, Ca homeostatic mechanisms have to react to a tremendous increase in demand for Ca, and its mobilization from bone and increased absorption from the gastrointestinal tract are required to re-establish homeostasis (Talukdar *et al.* 2016). Hence, minerals have one of the important roles in reproduction. The present study was undertaken to study the body condition score and certain blood metabolite for

establishment of cyclicity during postpartum periods.

Apparently healthy crossbred cows (20) of first to fourth parity with normal calving history and free from any immediate post-parturient complications were divided equally into Gr A—cows exhibited oestrus within 60 days postpartum (cyclic) and in Gr B—cows that did not exhibit oestrus within 60 days postpartum (non-cyclic). The experimental cows were maintained under standard feeding and managerial conditions. Their body condition score was evaluated on every fortnightly interval from the day of parturition upto 8 weeks, i.e. day 0, 15, 30, 45, 60 on the basis of body condition scoring system (5 point scale) provided by Elanco Animal Health (Lilly Corporate Center, Indianapolis).

Blood samples (100) were collected from 20 cows for estimation of blood biochemical profile. Blood sample (10 ml) was collected into a vacuum clot activator vial containing no additives by jugular puncture with a sterile 18 gauge needle fitted with a plastic syringe, from the day of calving at fortnightly interval up to 8 weeks postpartum. Following standing at room temperature for 20 min, the clot activator vials were centrifuged at 3,000 rpm for 10 min. The obtained sera were kept at –20°C until further analysis.

The serum levels of different biochemical parameters such as glucose, cholesterol, total protein, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), aspartate amino transferase (AST), calcium (Ca), phosphorus (P) and magnesium (Mg) were analysed by using commercial diagnostic kit in Fujifilm (Dri Chem 4000i) autoanalyzer.

The data collected from the study were subjected to statistical analysis using suitable formula for meaningful and accurate comparison, and interpretation as per Snedecor and Cochran (1994).

BCS is a subjective visual and tactile measure of body condition and temporary changes in BCS were used to monitor nutritional and health status of cows during their productive cycle (Berry *et al.* 2007). Changes in BCS are associated with the resumption of estrous cycle during

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Table 1. BCS and level of blood biochemical and mineral profile in postpartum cyclic and non-cyclic crossbred cows (Mean $\pm$ SE, N=10)

Parameter		Days					Overall mean	F value
		0	15	30	45	60		
BCS	Cyclic	3.22 ^{aA} $\pm$ 0.05	3.12 ^{aA} $\pm$ 0.04	2.82 ^{bA} $\pm$ 0.06	2.42 ^{cA} $\pm$ 0.06	2.17 ^{dA} $\pm$ 0.05	2.75 $\pm$ 0.06	61.367**
	Noncyclic	2.60 ^{aB} $\pm$ 0.04	2.57 ^{aB} $\pm$ 0.03	2.32 ^{bB} $\pm$ 0.03	2.10 ^{cdB} $\pm$ 0.04	2.00 ^{dB} $\pm$ 0.00	2.32 $\pm$ 0.03	58.850**
	t-value	8.778**	9.731**	6.626**	4.230*	3.280**		
Glucose (mg/dL)	Cyclic	64.50 ^A $\pm$ 2.16	60.60 ^A $\pm$ 2.11	58.30 $\pm$ 2.29	58.40 ^A $\pm$ 3.07	58.40 $\pm$ 3.70	59.8 $\pm$ 1.05	1.563 ^{NS}
	Noncyclic	49.40 ^B $\pm$ 4.51	45.60 ^B $\pm$ 2.27	50.50 $\pm$ 3.32	50.60 ^B $\pm$ 2.22	49.70 $\pm$ 3.58	49.16 $\pm$ 1.42	0.388 ^{NS}
	t-value	3.017**	4.833**	1.931 ^{NS}	2.330*	1.843		
Protein (g/dL)	Cyclic	7.74 ^{bA} $\pm$ 0.20	8.04 ^{abA} $\pm$ 0.18	8.20 ^{abA} $\pm$ 0.19	9.48 ^{aA} $\pm$ 0.33	9.48 ^{aA} $\pm$ 0.33	8.44 $\pm$ 0.12	9.194**
	Noncyclic	5.12 ^{aB} $\pm$ 0.21	5.48 ^{aB} $\pm$ 0.19	6.05 ^{abB} $\pm$ 0.31	6.49 ^{abB} $\pm$ 0.36	7.31 ^{bB} $\pm$ 0.44	6.09 $\pm$ 0.17	7.197**
	t-value	8.723**	9.347**	5.798**	5.671*	3.904**		
Cholesterol (mg/dL)	Cyclic	90.40 ^{cA} $\pm$ 3.86	108.70 ^c $\pm$ 4.46	150.40 ^{bA} $\pm$ 6.46	204.70 ^{aA} $\pm$ 7.40	204.70 ^{aA} $\pm$ 7.40	148.28 $\pm$ 6.71	66.007**
	Noncyclic	66.70 ^{aB} $\pm$ 4.45	98.40 ^b $\pm$ 5.43	129.30 ^{bB} $\pm$ 6.71	153.90 ^{cB} $\pm$ 8.25	180.10 ^{dB} $\pm$ 6.45	140.42 $\pm$ 5.15	48.885**
	t-value	4.020**	1.463 ^{NS}	2.264*	3.055**	2.504*		
BUN (mg/dL)	Cyclic	9.75 ^{bA} $\pm$ 0.57	10.10 ^{bA} $\pm$ 0.70	12.56 ^{aA} $\pm$ 1.26	12.54 ^a $\pm$ 0.90	12.54 ^{aA} $\pm$ 0.90	11.94 $\pm$ 0.49	5.283**
	Noncyclic	13.33 ^{aB} $\pm$ 0.66	14.48 ^{abB} $\pm$ 0.95	16.20 ^{bcdB} $\pm$ 1.11	17.60 ^{cd} $\pm$ 0.81	20.04 ^{dB} $\pm$ 0.58	16.33 $\pm$ 0.49	9.636**
	t-value	4.081**	3.686**	2.158*	1.927 ^{NS}	6.934**		
Creatinine (mg/dL)	Cyclic	1.06 $\pm$ 0.08	1.10 $\pm$ 0.07	1.17 $\pm$ 0.10	1.32 $\pm$ 0.15	1.32 $\pm$ 0.15	1.19 $\pm$ 0.04	1.214 ^{NS}
	Noncyclic	1.15 $\pm$ 0.06	1.03 $\pm$ 0.08	1.07 $\pm$ 0.12	1.08 $\pm$ 0.10	1.17 $\pm$ 0.12	1.1 $\pm$ 0.04	0.324 ^{NS}
	t-value	0.886 ^{NS}	0.606 ^{NS}	0.612 ^{NS}	1.632 ^{NS}	0.750 ^{NS}		
AST (U/L)	Cyclic	69.00 ^c $\pm$ 3.45	78.10 ^{cA} $\pm$ 3.27	98.50 ^{bA} $\pm$ 4.27	90.40 ^{abA} $\pm$ 3.19	95.00 ^{aA} $\pm$ 3.58	86.2 $\pm$ 2.17	11.864**
	Noncyclic	60.90 ^a $\pm$ 4.26	66.00 ^{abB} $\pm$ 4.43	71.90 ^{abcB} $\pm$ 2.65	76.90 ^{bcB} $\pm$ 3.43	81.90 ^{cB} $\pm$ 4.07	71.52 $\pm$ 1.93	4.782**
	t-value	1.475 ^{NS}	2.196*	5.288**	2.877*	2.415*		
ALT (U/L)	Cyclic	26.00 $\pm$ 3.82	18.30 ^A $\pm$ 1.35	17.80 ^A $\pm$ 2.24	22.60 ^A $\pm$ 2.71	21.50 ^A $\pm$ 2.62	21.24 $\pm$ 1.21	1.575 ^{NS}
	Noncyclic	32.60 $\pm$ 3.86	27.00 ^B $\pm$ 2.66	31.30 ^B $\pm$ 4.23	34.00 ^B $\pm$ 2.45	38.40 ^B $\pm$ 1.82	32.66 $\pm$ 1.43	1.739 ^{NS}
	t-value	1.213 ^{NS}	2.911**	2.818*	3.114**	5.289**		
Calcium (mg/dL)	Cyclic	6.40 ^a $\pm$ 0.29	7.97 ^{abA} $\pm$ 0.61	10.35 ^{abA} $\pm$ 0.70	10.53 ^{cA} $\pm$ 0.60	10.63 ^{cA} $\pm$ 0.54	8.92 $\pm$ 0.32	9.308**
	Noncyclic	6.18 ^a $\pm$ 0.44	6.09 ^{abB} $\pm$ 0.22	7.16 ^{abB} $\pm$ 0.18	7.80 ^{bB} $\pm$ 0.32	6.71 ^{bB} $\pm$ 0.55	6.78 $\pm$ 0.18	3.661*
	t-value	0.413 ^{NS}	2.874*	4.388**	3.959**	3.424**		
Phosphorus (mg/dL)	Cyclic	5.11 ^a $\pm$ 0.36	5.86 ^a $\pm$ 0.28	6.66 ^{ab} $\pm$ 0.49	7.01 ^{ab} $\pm$ 0.48	6.98 ^c $\pm$ 0.52	6.32 $\pm$ 0.21	3.473*
	Noncyclic	4.58 ^a $\pm$ 0.27	5.68 ^{ab} $\pm$ 0.20	6.13 ^{ab} $\pm$ 0.28	6.55 ^{bc} $\pm$ 0.42	7.07 ^c $\pm$ 0.27	6.00 $\pm$ 0.17	10.018**
	t-value	1.152 ^{NS}	0.521 ^{NS}	0.938 ^{NS}	0.714 ^{NS}	0.151 ^{NS}		
Magnesium (mg/dL)	Cyclic	3.01 ^{bA} $\pm$ 0.09	2.98 ^{abA} $\pm$ 0.19	3.55 ^a $\pm$ 0.27	3.53 ^{abA} $\pm$ 0.19	3.27 ^{ab} $\pm$ 0.27	3.26 $\pm$ 0.09	3.626*
	Noncyclic	1.81 ^B $\pm$ 0.29	2.07 ^B $\pm$ 0.16	2.79 $\pm$ 0.33	2.76 ^B $\pm$ 0.18	2.72 $\pm$ 0.17	2.43 $\pm$ 0.11	1.589 ^{NS}
	t-value	3.878**	3.573*	1.772 ^{NS}	2.905*	1.676 ^{NS}		

Means bearing different superscript in a row and column differ significantly (**P<0.01, *P<0.05), ^{NS}Nonsignificant.

postpartum period (Borpujari *et al.* 2018). BCS in postpartum cyclic cows decreased significantly (P<0.01) from the day of parturition (day 0) to day 60 of postpartum and it ranged from 2.17–3.22. In the postpartum non-cyclic cow, the corresponding value ranged from 2.0 to 2.60, and it significantly decreased (P<0.05) at day 0 to day 60 of postpartum (Table 1). These changes of BCS might be due to negative energy balance (NEB) in which there may be increased lipolysis in adipose tissue, increased gluconeogenesis and glycolysis in liver, protein mobilization in muscle tissue, mineral mobilization in bone and increased capacity and activity of GI tract (Dampney *et al.* 2014). Veena *et al.* (2015) observed that a loss of 1 unit of BCS following calving is equivalent to about of 400 mcal cumulative energy. Domecq (1997) observed that changes of BCS during first month of lactation have a stronger influence on milk yield than those at parturition. Thus loss of body condition was found associated with increased milk yield as the animals under this group were producing 15–

20 litre of milk/day (Mishra *et al.* 2016).

Cows with a BCS of 2.75–3.0 (on a scale of 5) at calving and which managed to lose BCS of not more than 0.5 units between calving and first service were found associated with a good reproductive performance (Mishra *et al.* 2016). Cows in low body condition at calving, or cows with excess BCS loss early postpartum, had higher calving to conception intervals (Roche *et al.* 2009) due to impaired oocyte competence (Snijders *et al.* 2000). The cows that calved in poor BCS (<2.5 on a five-point score) had a prolonged anoestrous period due to lower LH pulse frequency (Dampney *et al.* 2014). The BCS in cyclic cow was significantly higher (P<0.01) compared to noncyclic postpartum crossbred cows, which might be due to lactational stress and restricted feed intake resulting in low BCS, which consequently led to prolonged postpartum estrus in non-cyclic cows.

The serum glucose level in postpartum cyclic cows from day of parturition (day 0) to day 60 of postpartum ranged

from 58.30 to 64.50 mg/dL and in postpartum non-cyclic the corresponding value ranged from 45.60 to 50.60 mg/dL (Table 1). The level of glucose nonsignificantly decreased from day 0 to day 60 of postpartum in both the groups which might be due to production stress as massive energy demand to support milk production is largely made through gluconeogenesis (Piccione *et al.* 2012).

Energy is the first limiting dietary factor for cows in early lactation. Energy demands in the form of milk output and body maintenance exceeds energy inputs in early postpartum. Cows mobilize energy stores from their body reserve to make up the difference between energy intake and energy output. This puts cows into a negative energy state, which is one of the most important factors affecting days to first ovulation. Cows with poor body conditions (negative energy) may not show cycle until 60 days postpartum, increasing open days (Veena *et al.* 2015). Piccione *et al.* (2012) opined that there exists direct relationship between positive energy status at early postpartum and diameter of largest follicle on day 10 postpartum. The serum glucose level in cyclic cows were significantly higher ($P<0.01$) than the postpartum non-cyclic cows, which might be due to positive effect of energy on reproduction. The resumption of cyclicity within 2 months of postpartum was due to positive energy balance as it increases luteinizing hormone (LH) / follicle stimulating hormone (FSH).

The lactation phases affect significantly the metabolic profile and so the variation recorded during different physiological phases is expected. The transition from gestation to lactation is a period of great metabolic stress for dairy cows (Rollin *et al.* 2010). In fact, the milk production and its composition profoundly influence the metabolic status of dairy cows (Heck *et al.* 2009). The serum total protein level in postpartum cyclic cows from day of parturition to day 60 of postpartum ranged from 7.74 to 9.48 g/dL and in postpartum non-cyclic cows from 5.12 to 7.31 g/dL (Table 1). The serum level of total protein significantly increased ($P<0.01$) in both the groups at different days of postpartum, i.e. day 0 to day 60. The variations reflect the maternal requirements of proteins need for milking and providing immunoglobulins (Mohri *et al.* 2007). The higher concentrate-to-forage ratio provided during the lactation is generally associated with lower levels of fibre and higher levels of starch in the diet, which gives rise to an increased production of propionic acid in the rumen and an increased microbial protein supply (Heck *et al.* 2009). This is reflected by an increase of total serum protein during the period.

The role of proteins in reproduction of ruminants is equivocal. Diets deficient in protein resulted in weak expression of estrus, cessation of estrus, repeat breeding, etc. However, total proteins in circulation represent a balance between the biosynthesis and catabolism or mechanical loss. In this study, level of protein was significantly higher ($P<0.01$) in cyclic cows compared to the cows which did not come to heat within 2 months of

postpartum. Piccione *et al.* (2012) reported higher protein levels during fertile cycles. The lower level of serum proteins may cause deficiency of certain amino acids required for the synthesis of proteins in the body resulting in late expression of estrus after calving.

As the parity and postpartum intervals have a significant effect on serum cholesterol concentration, the serum cholesterol concentration was comparatively higher in multiparous cows. The serum cholesterol concentration steadily increased after parturition. The serum cholesterol level significantly increased ($P<0.01$) in both the groups from day 0 to day 60 of postpartum (ranged from 90.40 to 204.70 mg/dL in cyclic and 66.70–180.10 mg/dL in non-cyclic postpartum cows) which might be due to fat mobilization that occurs during the period (Saqib *et al.* 2018).

Significantly higher ($P<0.01$) level of serum cholesterol was present in animals which came to estrus within 2 months compared to those which did not come to estrus before 2 months postpartum. Plasma cholesterol concentrations were consistently important in predicting nutritional status of dairy cows and plasma insulin, and IGF-I concentrations increase concomitantly with plasma cholesterol during early lactation, which resulted in resumption of ovarian activity in the cyclic postpartum cows (Veena *et al.* 2015).

The serum BUN level in postpartum cyclic cows from day of parturition (day 0) to day 60 of postpartum ranged from 9.75 to 12.54 and in non-cyclic cow from 13.33 to 20.04 mg/dL, which significantly increased ($P<0.01$) from the day of parturition upto 60 days postpartum (Table 1). It might be due to lactational stress and protein metabolism (Piccione *et al.* 2012).

Significantly higher ($P<0.01$) level of serum BUN was found in animals which did not come to estrus before 2 months postpartum in comparison with the cows that came to estrus within 2 months. The late and non-cyclic postpartum cows were in poorer nutritional status, which could have elicited a situation of energy deficiency, limiting microbial protein synthesis and increasing urea concentrations in the plasma (Piccione *et al.* 2012). During periods of energy restrictions, the shortfall in energy may be met by catabolism of body proteins, which results in increased urea concentrations in the blood (Jeong *et al.* 2015) of non-cyclic cows.

The serum creatinine level in postpartum cyclic cows from day of parturition (day 0) to day 60 of postpartum ranged from 1.06 to 1.19 and in non-cyclic cows from 1.03–1.17 mg/dL (Table 1) which nonsignificantly increased from the day of parturition upto 60 days postpartum which might be due to lactational stress and protein metabolism (Piccione *et al.* 2012). The differences in level of serum creatinine during postpartum period might be due to prolonged active tissue protein catabolism which suppressed the resumption of ovarian activity (Dampney *et al.* 2014).

Significantly higher ($P<0.01$) level of serum creatinine was present in cows which came to estrus within 2 months compared to those which did not come to estrus before 2

months postpartum. Similar findings were reported by Dampsey *et al.* (2014). The concentration of creatinine in the blood is directly related to muscle mass, as it is a product of muscle metabolism and as a result, is significantly correlated to live weight (Kalem *et al.* 2017). As creatinine excretion is influenced by muscle mass, the early cycling cows with higher BCS excreted more creatinine than the late cycling cows with lower body weight.

ALT and AST reveal liver function during postpartum (Kahn and Scott 2010). The serum ALT and AST levels ranged from 17.8 to 26; 69–98.5 U/L and 27–38.4; 60.9–81.9 U/L (Table 1) in postpartum cyclic and non-cyclic cows, respectively, which was within the normal range (Kahn and Scott 2010). Significantly higher ($P<0.01$) level of serum AST was present in cows which came to estrus within 2 months compared to those which did not come to estrus before 2 months and significantly higher ($P<0.01$) level of ALT was present in non-cyclic cows than the cyclic cows which is similar to the findings of Kalem *et al.* (2017).

The calcium level significantly increased ($P<0.01$) from day 0 to day 60 of postpartum in cyclic cows which might be due to lactational stress as the milk production increased day by day (Piccione *et al.* 2012). Reduced blood calcium concentration increases the incidence of postpartum reproductive disorders and fertility (Talukdar *et al.* 2015) as calcium stimulates the secretion of gonadal hormones and also sensitizes the tubular genital tract. Significantly higher ($P<0.01$) level of calcium was present in cyclic cows than that in non-cyclic cows, which is similar as reported by Kalem *et al.* (2017).

The serum phosphorus level ranged from 5.11 to 7.01 mg/dL in cyclic and 4.58–7.07 mg/dL in non-cyclic cows (Table 1), which was within the normal range (Kahn and Scott 2010). The phosphorus level significantly increased ($P<0.05$) in both the groups from day 0 to day 60 of postpartum which might be due to lactational stress as the milk production increased day by day (Piccione *et al.* 2012, Talukdar *et al.* 2016).

Reduced blood phosphorus concentration may influence ovarian activity (Jeong *et al.* 2015, Talukdar *et al.* 2015) thus affecting the fertility of the cow. Higher level of serum phosphorus was present in cyclic cows than in non-cyclic cows without any significant difference which is similar to the findings of Kalem *et al.* (2017).

The serum magnesium level ranged from 2.98 to 3.55 mg/dL in cyclic and 1.81–2.79 mg/dL in non-cyclic cows (Table 1), which was within the normal range (Kahn and Scott 2010). The magnesium level significantly increased in postpartum cyclic cows ($P<0.05$) from day 0 to day 60 of postpartum, which might be due to lactational stress as the milk production increased day by day (Piccione *et al.* 2012). In non-cyclic cows, the level increased with the advancement of postpartum days, but there was no significant difference between days postpartum which might be due to reduced dry matter intake, which leads to NEB during this period (Piccione *et al.* 2012). The magnesium concentration was significantly lower ($P<0.01$) in non-cyclic group than that

in cyclic group, as also reported by Jeong *et al.* (2015). As magnesium is not stored in the body, lower levels of magnesium in cows with delayed resumption of postpartum cyclicity might be due to reduced dry matter intake.

It can be inferred from this study that body condition score (BCS) ≥ 3.25 (on a scale of 5) at calving had a normal postpartum oestrus within 2 months, and $BCS \leq 2.25$ at calving had a prolonged postpartum anoestrus. There was positive relation between the level of serum glucose, total protein, cholesterol, BUN, calcium, phosphorus and magnesium with postpartum cyclicity of the crossbred cows.

SUMMARY

Apparently healthy crossbred cows (20) of first to fourth parity with normal calving history and free from any immediate post-parturient complications were selected to study the body condition score (BCS) and certain blood metabolite for establishment of cyclicity during postpartum periods. The cows were divided equally into 2 groups—Gr A (cows exhibited oestrus within 60 days postpartum (cyclic)) and Gr B (cows did not exhibit oestrus within 60 days postpartum (non-cyclic)). The study revealed that the BCS, level of serum total protein, cholesterol, BUN, calcium, phosphorus and magnesium differed significantly at different days of postpartum, i.e. day 0, 15, 30, 45, 60 and between the groups. The BCS, level of serum glucose, total protein, cholesterol, BUN, calcium, phosphorus and magnesium had a positive relation with respect to the postpartum cyclicity of the crossbred cows.

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