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Metabolic Predictors of Subclinical Mastitis during Early Lactation in Crossbred Cows

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ABSTRACT

Mastitis is a complex disease with critical outcomes. Subclinical mastitis (SCM) although lacks clinical signs but it has a profound effect on milk production if occurs during early lactation. The purpose of the study, was to investigate the changes that occur in plasma metabolite levels before development of mastitis during early lactation. To evaluate the association, blood samples were collected from 50 pregnant Karan Fries (KF) cows at weekly interval from the day of partum till +90th days of calving or till the occurrence of SCM, whichever was earlier. Positive diagnosis for SCM was confirmed in 15 cows by both modified Californian mastitis test (mCMT) and increased Somatic cell count (SCC) between 4 10 lakh cells/ml within 68 week (56 days) of lactation. Thus 15 SCM cows were compared with 15 healthy ones for plasma metabolic profile till 56th day of lactation. The significantly ($P<0.05$) higher plasma nonesterified fatty acid (NEFA) and plasma (β Hydroxy Butyric Acid) βHBA levels of SCM cows over healthy ones before disease occurrence signifies severe metabolic load along with unsuccessful metabolic adaptation from transition, might be a potential factor for causing SCM during early lactation.

Keywords: SCM, Early lactation, Nonesterified fatty acid (NEFA) and β Hydroxy butyric acid (βHBA)

Selective breeding of dairy cattle has led to a dramatic increase in milk yield over recent decades giving India an honour to become the top milk producer of world. But at the same time this increase has been accompanied with high incidence of health problems like mastitis in modern dairy cows (Oltenacu and Algers, 2005). Mastitis is a multifactorial and a costly problem affecting dairy animals in India. Most experts agree that the economic impact of subclinical forms of mastitis is larger than clinical mastitis (Singh *et al*., 2016). The overall prevalence of sub-clinical mastitis was found to be 59.43 per cent (%) and quarter level prevalence was at 34.78% (Bhat *et al*., 2016). During transition period, reduced feed intake along with initiation of lactation leads to energy deficit. This energy deficit can be compensated by the mobilization of fat from adipose tissues. Energy status particularly in terms of fat mobilization and efficiency of use of mobilized fat can be evaluated from plasma metabolites like glucose, NEFA, and βHBA. The immunosuppression normally observed during the transition is partly resulted from altered metabolic profile that may increase the incidence of mastitis during early lactation (Mehrzad *et al*., 2001). Compared to other stages of the lactation cycle, early lactation is relatively more critical period as it follows the transition period and is also directly associated with health, production, and profitability of dairy cows. Therefore, early identification of SCM during early lactation may be useful (Huzzey *et al.,* 2006) to overcome future production losses.

Generally, plasma NEFA and βHBA levels are used as a diagnostic tool to assess the metabolic outcomes in transition cows. But the present study, was designed to determine the important metabolic parameters in plasma during peripartum period as potential markers for development of infectious outcomes like SCM in early lactation.

MATERIALS AND METHODS

The experimental protocol was duly cleared by the Institute Animal Ethic Committee.

For the study, 50 Karan Fries cows approaching parturition were selected from the experimental herd of National Dairy Research Institute (NDRI), Karnal, Haryana (latitude 29.43° N & longitude 77.2° E), India. All the experimental animals were healthy and free from any anatomical, physiological and infectious disorders. All these cows were offered *ad lib* green fodder and calculated amount of concentrate mixture. Fresh tap water was also made available *ad lib* at all times of the day. Blood samples were collected from them at weekly interval from the day of calving till 90th day post calving or till the development of SCM whichever was earlier. Milk samples were collected from them at weekly interval from the day of partum till 90th day post calving for cow side mCMT and SCC to screen the SCM cows.

The SCM cows, were screened using modified of California Mastitis Test (mCMT) and SCC. Group I cows (n=15) were healthy during the entire period of study without sign of postpartum complications; Group II cows (n=15) were those who developed SCM within 6th to 8th week of calving.

The copper soap solvent extraction method modified by Shipe *et al*., (1980) was adopted for the estimation of plasma NEFA. βHBA were estimated by commercially available ELISA kit (Mybiosource). Plasma glucose was estimated by GODPOD kits of Span Diagnostics Ltd. All analysis was done using SYSTAT software package. Data from different experiments are presented as mean ± SE. Significance was tested by employing one way ANOVA and two way ANOVA.

RESULTS AND DISCUSSION

Table 1 shows mean plasma NEFA values at different postpartum days of healthy and mastitic cows. NEFA registered a massive rise around calving in both the groups. Plasma NEFA levels were maximum on the day of calving. A nonsignificant reduction was recorded at 7th day postcalving. Thereafter on 15th day a significant (P<0.001) reduction was seen in group I but not in group II. The percent reduction in NEFA values on 15th day postcalving was only 1516% in group II in relation to 26% of group I.

In group II, the plasma NEFA levels in postpartum period followed almost the similar pattern like that of group I but the values recorded were higher.

Table 1: Plasma NEFA concentrations (Mean ±SEM) in healthy and mastitic crossbred cows at different days with respect to calving

Days	Group I (n=15)	Group II (n=15)
0	480.80^{ax}±11.19	512.00^{ax}±15.41
7	442.20^{abx}±11.65	467.71^{abx}±15.65
15	355.60^{cx} ±13.86	432.43^{bcy}±17.50
21	288.90^{dex}±15.57	347.14^{dy}±14.59
28	279.00^{ex}±8.82	312.71^{dey} ±10.32
35	271.40^{ex}±10.74	290.57^{ex}±10.82
42	280.30^{dex}±13.68	294.00^{ex} ±15.94
49	282.10^{dex}±10.61	289.57^{ex} ±14.76
56	250.70^{ex}±12.39	274.43^{ex}±14.80

Values within a row having different superscripts (x,y) are significantly (P<0.05) different from each other. Values within a column having different superscripts (a,b,c,d,e) are significantly (P<0.05) different from each other.

Significantly (P<0.05) higher levels were recorded from 15th to 28th day postpartum in group II when compared with group I. That is the NEFA levels did not decline to a basal level in group II as it was there in group I. Highest levels of plasma NEFA levels at partum in both the groups of crossbred cows clearly reflect that the transition period is an energy demanding period and excessive body fat mobilization takes place during this period. The data is in agreement with earlier reports (Park *et al*., 2010) in cattle. In group I cows after 21st day, NEFA levels were reduced may be because of homeostatic mechanism, slow increase in DM intake, reduction in calving stress as well as a decline in severity of lactation stress. Results showed the NEFA levels continues to be higher even after 21st day in group II i.e. the mastitis prone group. The normal physiological increased plasma NEFA levels during parturition normalized in mastitis prone cows at a later part of postpartum life compared to healthy ones. This indicates that metabolic strain during transition was more severe in cows that suffered from mastitis in subsequent period. As increased NEFA concentrations is indication of increased lipolysis, cows that developed SCM during

postpartum mobilized more body energy reserves than cows that did not develop SCM.

Table 2: Plasma βHBA concentrations (Mean ±SEM) in healthy and mastitic crossbred cows at different days with respect to calving

Days	Group I	Group II
	(n=15)	(n=15)
0	0.43^{bcx}±0.02	0.51^{cy}±0.01
7	0.52^{bx}±0.03	0.61^{by}±0.02
15	0.62^{ax}±0.03	0.70^{ay}±0.02
21	0.52^{bx}±0.03	0.59^{bx}±0.02
28	0.45^{bcx}±0.04	0.49^{cx}±0.02
35	0.46^{bcx}±0.02	0.46^{cdx}±0.02
42	0.45^{bcx}±0.03	0.43^{cdx}±0.03
49	0.43^{bcx}±0.03	0.45^{cdx}±0.03
56	0.36^{dx}±0.02	0.39^{dex}±0.03

Values within a row having different superscripts (x,y) are significantly (P<0.05) different from each other. Values within a column having different superscripts (a,b,c,d,e) are significantly (P<0.05) different from each other.

Table 2 shows mean plasma βHBA values at different postpartum days. βHBA registered massive rise in both the groups immediately after calving. Peak levels were obtained in both the groups on 15th day postcalving. βHBA levels were significantly (P<0.05) higher from day of partum to 15th day postcalving in group II when compared with healthy group. After 15th day the values declined gradually and prepartum levels were attained after 49th day in both the groups. Higher concentrations of ketone bodies (βHBA) indicate that adaptability for altered metabolism was exceeded, which affects the whole body homeostasis (Aeberhard *et al*., 2001). The result we obtained indicates that higher βHBA at partum and in early postpartum period might be associated with the development of SCM after calving. This was in agreement with finding of Mohammed and Mohsin, (2013).

Table 3 shows mean plasma glucose values at different postpartum days. There was nonsignificant variations in glucose levels of both the groups at different early lactation days. Glucose is the primary metabolic fuel, required for many vital functions, including milk production. It is the main precursor of lactose in the milk of lactating ruminants. In the present study, plasma glucose were found to be minimal on the day of calving and then increase gradually.

Table 3: Plasma glucose concentrations (Mean ±SEM) in healthy and mastitic crossbred cows at different days with respect to calving

Days	Group I	GroupII
	(n=15)	(n=15)
0	42.20^{cx}±1.40	45.00^{cx}±2.53
7	45.40^{bcx}±1.62	47.20^{bcx}±1.83
15	49.70^{abx}±1.26	49.20^{abx}±1.53
21	50.50^{abx}±2.11	49.80^{abx}±1.16
28	50.00^{abx}±1.51	51.20^{abx}±1.36
35	49.10^{abx}±2.47	52.20^{ax}±1.16
42	48.00^{abx}±1.81	50.40^{abx}±1.03
49	48.30^{abx}±1.63	51.60^{abx}±2.00
56	49.40^{abx}±1.54	50.60^{abx}±1.63

Values within a column having different superscripts (a,b,c) are significantly (P<0.05) for glucose different from each other.

Similar changes in peripartum glucose levels were reported by many workers (Chandra *et al*., 2012). During the early postpartum period, due to the low dry matter intake (DMI) propionate production is insufficient to synthesize the total amount of glucose needed. Therefore, glucose concentration decreases and as an alternative energy source NEFA concentration increases (as a result of massive lipolysis) (ShehabElDeen *et al*., 2010). The lowest glucose levels at calving could be a result of high demand of glucose for lactose synthesis (Doepel *et al*., 2002). The results obtained signifies that the prepartum glucose metabolism may have an effect on identification of energy status during transition but plasma glucose is a poor analyte for monitoring mastitis because plasma glucose concentration is under strict homeostatic control. Leblanc, (2010) suggested that blood glucose just does not vary a lot, which make it difficult to relate back to problems.

CONCLUSION

Transition period is the physiologically most disturbed period. But the metabolic alterations that are seen during this period get normalized in the early postpartum period because of body's own homeostatic mechanism, which

can be called as successful metabolic adaptation. But those having poor metabolic adaptation take longer to adjust and this is reflected in circulating concentration of NEFA and βHBA which negatively affects the immune status of dairy cow, which may increase the risk of mastitis. From our study, it can be concluded that by monitoring these changes during postpartum period disease incidence can be reduced and animal performance can be improved.

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