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Association studies on biochemical parameters and uterine health in crossbred cows of central dry zone of Karnataka

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Abstract

It is essential to understand underlying metabolic stress influencing uterine inflammatory conditions. Therefore, the present study was undertaken to assess the metabolic stress influencing endometritis in crossbred dairy cows reared in the central dry zone of Karnataka. The cows considered for the present study were divided into four groups viz., Group- I (Control N=8): crossbred cows at 3 weeks to 11 weeks of postpartum estrus with negative White side test (WST), Group- II (N=8): crossbred cows at 3 weeks to 5 weeks of postpartum estrus, Group- III (N=8): crossbred cows at 6 weeks to 8 weeks of postpartum estrus, Group- IV (N=8): crossbred cows at 8 weeks to 11 weeks of postpartum estrus. Group II to Group IV with positive WST and designated as clinical endometritis cases (CE). Metabolic profile included estimation of Non-esterified free fatty acids (NEFA), beta hydroxy butyric acid (BHBA), blood glucose, total protein, albumin, total cholesterol, total bilirubin, blood urea nitrogen (BUN), creatinine, aspartate amino transferase (AST), Alanine amino transferase (ALT) and mineral profile included estimation of calcium and phosphorous. Statistical analysis of data revealed a significant difference ($p<0.05$) between concentrations of BHBA and NEFA in groups of II, III, IV when compared to group-I. There is no significant difference ($p<0.05$) found in blood glucose and plasma total proteins levels in all groups compared to control group. Concentrations of BUN and the cholesterol in groups II, III, IV significantly higher compared to group-I. However, the mean values of creatinine were within the normal threshold range in all groups. There was a significant ($p<0.05$) difference between total bilirubin concentrations in group-II compared to control group. However, group-III and group-IV were comparable with control group. There were no significant findings with respect to ALT and AST enzymes. There is a significant ($p<0.05$) difference in the concentrations of calcium and phosphorous of all the groups compared with control group. The present revealed that the associations of elevated NEFA, BHBA, lower levels of calcium and phosphorous may affect the uterine health and estimation of such biochemical parameters are helpful in monitoring udder health in dairy cows.

Keywords: endometritis, crossbred cows, white side test, metabolic profile

Introduction

The stress can stimulate changes in biochemical pathways involved in the maintenance of the immune functions, which in turn might increase the susceptibility for uterine diseases. Animals with metabolic stress are more prone to get a uterine infection than healthy animals during exposure to the same bacterial load. Cross bred cows are presented to the Veterinary Dispensaries with a history of repeat breeding, decreased conception rates and decreased milk production. Repeat breeding of crossbred cows due to subclinical endometritis is a major problem in the region of the central dry zone of Karnataka. A systematic biochemical investigation to find out the biochemical basis for uterine health status is essential and there is a research gap in region-wise case studies. In view of the above, the present study is undertaken to study the association of some of the biochemical parameters indicating metabolic profile and udder health in postpartum crossbred cows with cytological endometritis.

Materials and Methods

Selection of Animals

Apparently healthy crossbred cows at 3-11th week postpartum were taken for the experiment. Age, previous breeding history, breed, milk yield, feeding status, and behaviour of the cows were noted from the owner. The cows considered for the present study were divided into four

groups viz., Group- I (Control N=8): crossbred cows at 3 weeks to 11 weeks of postpartum estrus with negative White side test (WST), Group- II (N=8): crossbred cows at 3 weeks to 5 weeks of postpartum estrus, Group- III (N=8): crossbred cows at 6 weeks to 8 weeks of postpartum estrus, Group- IV (N=8): crossbred cows at 8 weeks to 11 weeks of postpartum estrus. Group II to Group IV with positive WST.

Estimation of Biochemical parameters

Estimation of Non-esterified free fatty acids (NEFA), beta hydroxy butyric acid (BHBA), blood glucose, total protein, albumin, total cholesterol, total bilirubin, blood urea nitrogen (BUN), creatinine, aspartate amino transferase (AST), Alanine amino transferase (ALT), calcium and phosphorous were estimated using commercially available kits viz., NEFA - colorimetric assay kit (Elabscience, USA., supplied by Clementia biotech, New Delhi, India). ALT, AST (Alpha technologies, Chennai, India) BUN, creatinine, calcium, phosphorous, total protein, albumin, total cholesterol, total bilirubin- Kits from Erbamaneim company, Manheim, Germany. BHBA - electronic hand-held meters (Abbott Healthcare pvt. Ltd, India), Blood glucose - electronic hand-held meters (One touch plus Glucometer, India). All estimations were done in biochemical analyzer (HY-SAC Vet Version: A/6 Semi-auto Chemistry Analyzer, Hycel Handelsges Austria).

Statistical Analysis

Data was statistically analyzed using SAS (2003) computer package. Data obtained were subjected to generalized linear model one-way analysis of variance (ANOVA) procedure followed by Duncan's multiple range tests. The values obtained from the experiments were expressed as Mean \pm SE. Correlations were made for Pearson correlation coefficients

using Graph Pad Prism software programme (Graph Pad[®] software Inc., trial version 5.0; San Diego, CA, USA).

Results and Discussion

NEFA, BHBA and blood glucose were considered as metabolic biomarkers of energy status. NEFA and BHBA are important energy metabolites that are traditionally used as indicators of negative energy balance (NEB) during transition period and are responsible for a suppression of immune responsiveness that consequently leads to the occurrence of metabolic and infectious diseases (Hammon *et al.*, 2006) [12]. The mean (\pm SE) blood NEFA concentrations in control and CE affected cows were found to be 0.24 ± 0.13 and 0.59 ± 0.36 , 0.65 ± 0.26 , 0.6 ± 0.37 ($p < 0.05$) mmol/L respectively. The NEFA concentrations were significantly higher in the CE affected groups when compared to control group. Previously Pascottini and LeBlanc, (2020) found similar higher levels of NEFA in postpartum CE infections. Conversely, Sensoy *et al.* (2011) reported that there was not any impact of NEFA concentrations on the occurrence of subclinical endometritis diagnosed at 5th, 6th and 7th WPP in his study. In the study the mean (\pm SE) BHBA concentrations in control and CE affected cows are 0.24 ± 0.13 and 0.59 ± 0.36 , 0.65 ± 0.26 , 0.6 ± 0.37 ($p < 0.05$) mmol/L respectively. The BHBA concentrations were significantly higher in CE affected groups when compared to control group. The results were in accordance with the LeBlanc *et al.* (2005) [14] and Yasui *et al.* (2014) where they concluded that a lower energy status due to BHBA in the first few weeks of postpartum was associated with CE. There was no significant ($p < 0.05$) difference found in glucose levels in cows affected with CE when compared to control group. The results were in accordance with Pascottini and Le Blanc, (2020).

Table 1: Mean (\pm SE) of association between body condition score with metabolic markers of energy status in blood of CE affected cows.

Particulars	Group-A (BCS-2.5-3.0)	Group-B (BCS 3-3.50)	Group-C (BCS >3.5)
BHBA (mmol/L)	0.73 ± 0.35	0.56 ± 0.23	0.46 ± 0.25
NEFA (mmol/L)	0.24 ± 0.14	0.3 ± 0.22	0.26 ± 0.09
GLUCOSE (mg/dl)	42.75 ± 11.11	63.12 ± 25.83	56.98 ± 16.25

*Values are expressed as Mean \pm SE, Significant at $P < 0.05$. The pair of Mean \pm SE values with different superscript are significantly different and with common superscripts are non-significantly different.

BUN, creatinine, total protein and albumin are metabolic biomarkers of protein status. Evaluating protein status in dairy animals is not as easy as that of energy balance because there are number of factors which influence protein status of the animal. These factors may be dietary protein intake, rumen degradability, dietary amino acid composition and protein intake relative to requirement (Van Saun, 1997) [12]. The mean (\pm SE) BUN concentrations in control and CE affected cows are 8.58 ± 1.33 , 22.66 ± 9.99 , 23.1 ± 11.56 and 25.1 ± 14.66 ($p < 0.05$) mg/dl respectively. In the study mean values of BUN were significantly higher in cows with CE groups when compared to control group. Similar results were reported by (Ahmed *et al.*, 2004) where he opined that BUN concentrations increased around calving and it could be due to feeding of urea treated fodder and high concentrate diet after parturition. The mean (\pm SE) albumin concentrations in control and CE affected cows are 6.48 ± 0.68 and 6.38 ± 2.07 , 6.86 ± 1.62 , 7.27 ± 1.34 ($p < 0.05$) g/dl respectively. In the present study there was no significant differences found in mean values of total protein concentrations in all the groups. However, Prodanovic *et al.* (2012) reported that differences in

the TP concentrations in cows influenced by dietary protein intake, dietary amino acid composition. The mean (\pm SE) creatinine concentrations in control and CE affected cows are 1.09 ± 0.15 , 1.32 ± 0.29 , 1.29 ± 0.33 , 1.4 ± 0.36 ($p < 0.05$) mg/dl respectively. There was no significant differences found in creatinine concentrations in CE affected cows when compared to control group. The liver functionality in cows can be evaluated using the concentrations of albumin, cholesterol, bilirubin, enzymes AST and ALT (Bertoni and Trevisi, 2013). The mean (\pm SE) cholesterol concentrations in control and CE affected cows are 190.26 ± 53.21 and 250.43 ± 56.91 , 415.11 ± 138.75 , 238.35 ± 109.0 ($p < 0.05$) mg/dl respectively. The mean values of total cholesterol were increased only in 6-8th week of CE affected cows compared to control group. The results were in accordance with Sensoy *et al.* (2011) where he had reported higher total cholesterol concentrations in endometritic cows. Ruegg *et al.* (1995) [10] reported that the concentration of cholesterol during the postpartum period was inversely related to the loss of body condition. The mean (\pm SE) total bilirubin concentrations in control and CE affected cows are 0.23 ± 0.21 and 0.79 ± 0.48 ,

0.37 ± 0.18 , 0.59 ± 0.48 ($p < 0.05$) mg/dl respectively. There was a significant raise in total bilirubin levels observed in Group-II CE affected cows than control group. Group-III and IV cows were comparable with control group and it indicates cows were recovering from metabolic stress and immunosuppression. Previously, Reid *et al.* (1977)^[9] reported that serum bilirubin is elevated in early lactation owing to its diminished hepatic uptake in favour of NEFA uptake. In the present study the mean (\pm SE) ALT concentrations in control and CE affected cows were 17.23 ± 4.25 and 42.24 ± 30.16 , 30.83 ± 15.74 , 29.39 ± 8.12 ($p < 0.05$) μ /L respectively. There

was no significant differences found in CE affected cows compared to control group. However, ALT values gradually decreased from group-II to group-IV indicating recovery of hepatic tissue damage from metabolic stress and immunosuppression as days passes from calving. Similar findings observed by Andela *et al.* (2019), Pascottini and LeBlanc, (2020) where there was no significant difference of ALT levels observed at any time point in primiparous and multiparous CE affected cows. ALT has much diagnostic value especially as liver specific enzyme in dogs and cats but not in cattle (Kaneko *et al.*, 2008)^[3].

Table 2: Mean (\pm SE) of association between body condition score with biochemical profiles in serum of cows with cytological endometritis

Particulars	Group-A (BCS-2.5-3.0)	Group-B (BCS 3-3.50)	Group-C (BCS >3.5)
BUN (mg/dl)	23.3 \pm 10.2	20.81 \pm 10.31	19.23 \pm 7.33
Creatinine (mg/dl)	1.33 \pm 0.36	1.23 \pm 0.29	1.33 \pm 0.43
Total protein (g/dl)	5.54 \pm 1.15	6.92 \pm 1.78	6.93 \pm 1.93
Albumin (g/dl)	4.1 \pm 0.61	4.25 \pm 0.89	3.39 \pm 0.54
Cholesterol (mg/dl)	293.03 \pm 86.06	349.56 \pm 127.87	228.3 \pm 61.39
Total bilirubin (mg/dl)	0.79 \pm 0.42	0.37 \pm 0.26	0.5 \pm 0.34
AST(μ /L)	119.47 \pm 43.13	86.28 \pm 21.17	86.85 \pm 22.35
ALT(μ /L)	43.33 \pm 18.4	25.56 \pm 11.47	34.48 \pm 23.95

*Values are expressed as Mean \pm SE, Significant at $P < 0.05$. The pair of Mean \pm SE values with different superscript are significantly different and with common superscripts are non-significantly different.

The mean values of calcium concentrations were significantly decreased in group-II and group-III cows with CE compared to control group. Subclinical hypocalcemia has been linked to an increased risk of development of several health disorders at first few weeks after parturition in CE affected cows were reported in many studies (Mateus *et al.* 2002; Martinez *et al.*, 2012; Pascottini and LeBlanc, 2020)^[6, 5]. A low profile of

plasma calcium in the cows with CE could be due to some metabolic disturbance after parturition. The mean (\pm SE) phosphorous concentrations in control and CE affected cows were 5.84 ± 0.55 and 4.35 ± 0.42 , 4.65 ± 0.57 , 5.74 ± 1.25 ($p < 0.05$) mg/dl respectively. The mean values of phosphorous were significantly ($p < 0.05$) reduced in group-II and III cows with CE, comparable to control group.

Table 3: Mean (\pm SE) of association between body condition score with minerals profiles in serum of CE affected cows.

Particulars	Group-A (BCS-2.5-3.0)	Group-B (BCS 3-3.50)	Group-C (BCS >3.5)
Calcium (mg/dl)	7.85 \pm 2.26	7.45 \pm 0.94	8.83 \pm 1.71
Phosphorous (mg/dl)	4.57 \pm 0.56 ^b	4.38 \pm 0.52 ^b	5.63 \pm 1.27 ^a

*Values are expressed as Mean \pm SE, Significant at $P < 0.05$. The pair of Mean \pm SE values with different superscript are significantly different and values with common superscripts are non-significantly different.

Conclusion

The present revealed that the associations of elevated NEFA, BHBA, lower levels of calcium and phosphorous may affect the uterine health and estimation of such biochemical parameters are helpful in monitoring udder health in dairy cows.

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