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Assessment of Biochemical and Antioxidant Enzymes and Stress Hormone in Crossbred-Cows during Peripartum Period

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A B S T R A C T

The whole study was conducted on six adult pregnant Crossbred cows during Peripartum period. Nine ml of whole blood was collected and plasma was separated and stored in deep freeze at -20˚C until analyzed. Serum concentration of ALT (U/L) and AST (U/L) were significantly ($P < 0.05$) decreased on the day parturition. While ALP (IU/L) and ACP (IU/L) levels were significantly ($P < 0.05$) increased on the day of parturition. Level of cortisol was significantly ($P<0.05$) higher on the day parturition than any other days during the peri-partum period. Antioxidant enzymes like SOD (U/ml), LPO (nmol) and GPx (nmol/min/ml) were significantly ($P<0.05$) elevated on the day of parturition. The biochemical parameters and enzymes were influenced significantly ($P<0.05$) by the physiological changes occurring during the peri-partum period in the crossbred cows.

Introduction

The transition (or periparturient) period is the most critical period in dairy cows regarding health status and production. Lactation is the period of increased physiological stress, which is observed especially in early lactation. Rapid differentiation of secretory parenchyma, intense mammary gland growth, and the onset of copious milk synthesis and secretion in early lactation give rise to the high energy and oxygen requirements (Gitto *et al.*, 2002), thus resulting in the generation of reactive oxygen species (ROS). Under normal physiological condition, the generated ROS are neutralized by the antioxidant system and the redox

homeostasis is maintained in the body. In contrast, oxidative stress occurs when there is an imbalance between increased production of ROS and reduced capacity of the antioxidant system. Several studies showed that ROS production in the blood of dairy cows during the periparturient period can overwhelm certain antioxidant defence and result in increased oxidative stress (Sordillo *et al.,* 2007). Immediately after calving, animals undergo severe negative energy balance, indicated by alterations in blood metabolites and hormone profiles (Wathes *et al.,* 2009).

The hormonal pattern established during the latter portion of gestation triggers parturition. During the last three weeks of gestation, the fetus begins to release hormones from the adrenal cortex (predominantly cortisol) due to increased fetal stress and this stress hormone cortisol released from the fetus is responsible for production of other important hormones like, estrogen and PGF2α from mother. A combination of hormones from the fetus, placenta and dam induce a series of events leading to parturition (Rhodes, 2003). Also this stress hormone influences the metabolic functions and feed intake by the animal after parturition (Ingvartsen and Andersen, 2000).

After parturition cow experiences a period of high energy demand for milk production and insufficient feed intake. However, just after calving the high yielding cows cannot increase Dry Matter Intake (DMI) as fast as the increased nutrient demands required for lactation (Roche *et al.,* 2009; Hayirli *et al.,* 2003; NRC, 2001). Therefore to deal with this nutrient shortage, the cow mobilises body reserve fat and protein. This situation causes the Negative Energy Balance (NEB) and increases fat mobilization (Rukkwamsuk *et al.,* 2000) and resulted into cell structure damage and alteration in various important enzyme activity such as AST, ALT, GGT and ALP in the liver.

To know more about the stress experienced by the animals during the peripartum period particularly in crossbred cattle an investigation was planned at Department of Physiology and Biochemistry, College of Veterinary Science and A.H., Anand on Crossbred cows during their peripartum period.

Materials and Methods

The whole study was conducted on six adult pregnant, apparently healthy, Crossbred cows during Peripartum period i.e. from 45 days before parturition to 45 days after parturition. The research was approved by the Institutional animal Ethics Committee (IAEC.No.235/VBC/2016). The experimental animals were reared in semi-open housing system which is made up of concrete floor under asbestos roofed housing system constructed east west direction and well covered with trees. These experimental cows were not separated from other cows. The experimental animals were maintained on ICAR feeding standard (1998).

Nine ml of whole blood from each experimental animal was collected from jugular vein in heparinized vaccutainer. The blood was collected from 45 days before parturition at weekly interval i.e. -45day,-38day,-31day, -24day, -17day, -10day,-3 day, on the day of parturition and upto 45 days after parturion i.e. on +3day, +7day, +10day, +15day,+30day and +45day in accordance with the probable date of parturition. Plasma from the blood samples was separated by centrifugation at 3000rpm for 15 minutes and stored in deep freeze at -20◦C until analyzed for enzymes like acid phosphatase, alkaline phosphatase, aspartate transaminase and alanine transaminase, hormone like cortisol along with antioxidant enzymes like glutathione peroxidase, superoxide dismutase and melondialdehyde (Lipid Hydroperoxidase).

These plasma biochemical estimation were carried out in the Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand using Diagnostic kits manufactured by Crest Biosystems, Coral Clinical Systems, Goa, by Spectrophotometer (model Visiscan 167). Blood Plasma cortisol was estimated by ELISA method using Bovine Cortisol (CORT) ELISA kit (Cat.No: MBS028594, manufactured in Sandiego, USA). Estimation of Glutathione peroxidase (GPx), Superoxide Dismutase and Lipid Hydroperoxide (LPO) were performed by assay Kits manufactured by Cayman Chemicals, USA. The data obtained in the present study was statistically analyzed by the Completely Randomised Design (CRD). Statistical analysis performed at Department of Agriculture Statistics, B. A. College of Agriculture, Anand Agricultural University, Anand.

The statistical model for CRD with one observation per unit

$Yij = \mu + ti + eij$
μ = overall mean effect
ti = true effect of the i
th treatment
eij = error term of the jth unit receiving ith treatment

Results and Discussion

The observed overall mean value of Aspartate Aminotransferase (AST) (IU/L) in crossbred cows was 40.03 ± 1.24 which ranged from 33.11±0.47 to 49.49 ±1.60 during the peripartum period. Lowest value of AST was observed on the of day parturition as compared to any other peri-partum days. There were no any significant differences observed in AST levels during pre-partum period in the study. On the day of parturition the level of AST decreased significantly ($P<0.05$) and after parturition from day 3 onwards the value started increasing significantly ($P<0.05$) reaching the highest value on day 45 of the peri-partum period. Results of present experiment were in consonance with observations of Nessim (2010) in Baladi cows, Liu *et al.,* (2012) in Holstein cows and Yehia and Salem (2015) in dairy cows. However, Abdulkareem (2013) observed non- significant differences in AST levels in Iraqi riverine buffaloes around calving and postpartum period. While, Alameen and Abdelatif (2012) reported that the AST level was significantly higher during late pregnancy in crossbred cows. AST activity was numerically higher during early post parturient periods as compared to activity observed at calving indicating that hepatic metabolism might be more stressed and tissue catabolism was more pronounced during this period. However, the observed enzyme activities were within the normal range, integrity and functionality of liver tissue was obviously maintained during these periods. A similar trend was observed by Reist *et al.,* (2003) for Holstein dairy cows, where AST activity was lowest pre - partum and reached its peak during the first week post-partum.

The observed overall mean value of Alanine aminotransferase (ALT) (IU/L) in crossbred cows was 14.50 ± 0.49 which ranged from 12.39 ±0.27 to 17.87 ±0.45 during the peripartum period. Lowest value of ALT was observed on the day parturition as compared to any other peri-partum days. There were no any significant differences observed in the values of ALT levels during pre-partum period. After parturition the value of ALT started increasing significantly ($P<0.05$) from day 3 onwards reaching the highest value on day 45 of the peri-partum period. Results of present experiment were in consonance with observations of Liu *et al.,* (2012) in Holstein cows and Yehia and Salem (2015) in dairy cows. However, Mohamed *et al.,* (2015)

observed non- significant difference in ALT levels in buffalo heifers during pre and postpartum period. While, Nessim (2010) in Baladi cows reported that the ALT level tended to decrease significantly during postpartum period. Determination of Alanine aminotransferase (ALT) activity plays an important role in the diagnosis of cows manifest controlled to excess fat mobilization in early lactation which causes liver function injury and lead to variation of activity (Kaneko, 2008).The observed peripartum increase in serum enzymes indicated increased hepatic effort during this period.

The observed overall mean value of Alkaline phosphatase (ALP) (IU/L) in crossbred cows was 22.03 ± 1.59 which ranged from 13.15±0.51 to 39.47±3.87 during the peripartum period. Highest value of ALP enzyme was observed on the day parturition as compared to any other peri-partum day. There was no any significant difference observed in ALP levels during pre-partum period. On the day of parturition the level of ALP was increased significantly (P<0.05) and after parturition the value started decreasing significantly (P<0.05) from day 3 onwards reaching the lowest value on day 45. Results of present experiment were in consonance with observations of Liu *et al.,* (2012) in Holstein cows. However, Mostafa *et al.,* (2014) observed non- significant differences in ALP levels in crossbred cows during pre and postpartum period. While, Abdulkareem (2013) reported that the ALP level dramatically declined at calving in Iraqi riverine buffaloes. Serum ALP activity increases in cases of hepatitis, biliary disorders or during growth due to active bone metabolism. Sato *et al.,* (2005) noted that the serum ALP activity was found to be higher in lactation periods in comparison with the dry period because the bone metabolism and liver functions are more active during lactation period.

The observed overall mean value of Acid phosphatase (ACP) (IU/L) in crossbred cows was 1.37 ± 0.02 which ranged from 1.26±0.01 to 1.53±0.02 during the peripartum period. Highest value of ACP was observed on day 45 as compared to any other peri-partum day. There was no any significant difference found in ACP levels during pre-partum period. The value of ACP started increasing significantly (P<0.0.5) from 3 days before to the day of calving. After parturition the ACP level started decreasing significantly (P<0.05) from 3 to 7 days after calving. The ACP values started increasing significantly (P<0.05) from 10 to 45 days after calving. Information on Acid phosphatase related to our study were scantly. However, a report of Chaurasia *et al.,* (2016) represented that the value of ACP was significantly (P<0.01) highest in repeat breeder followed by reduced in normal cyclic and lowest in anestrous condition in Murrah buffaloes. The result was also similar to Ganguly (2013) who reported that increase in Acid phosphate concentration decreases with increase in follicular size. It can be inferred that higher concentration of acid phosphatase increases follicular activity resulting in repeat breeding. On the contrary, Sharma *et al.,* (1986) reported that mean values of ACP were significantly (P<0.01) higher in normal cyclic Kankrej heifers. Increased activity of ACP might be helpful in hydrolysing the organic phosphomonoesters and thus provide energy in the form of phosphates in normal cyclic animals. The observed overall mean value of cortisol (µg/dl) in crossbred cows was 25.63 ± 0.75 which ranged from 20.98 ±0.72 to 31.36 ±1.87 during the peripartum period. Highest level of cortisol was observed on the day of calving as compared to all other peri-partum days. The concentration of cortisol differed non-significantly upto 10 days before calving, than cortisol level started increasing significantly (P<0.05) reaching the highest level on the day of parturition (Table 1).

Table.1 Mean (±SEM) values of enzymes in crossbred cows (n=6) during peripartum period

Days	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ACP (IU/L)	Cortisol (µg/dl)
-45	13.90^{cde}±0.54	37.75^{de}±1.68	23.07^{bcde}±4.20	1.26^{d}±0.01	20.98^{g} ±0.72
-38	13.05^{e}±0.44	35.36^{ef}±0.35	18.24^{e}±1.76	1.29^{cd}±0.02	22.39^{fg} ±0.37
-31	13.02^{e}±0.33	37.95^{de}±1.70	18.61^{de}±1.41	1.34^{c}±0.02	23.04^{efg} ±0.56
-24	13.28^{de}±0.17	38.85^{cde}±1.94	19.60^{cde}±1.66	1.28^{cd}±0.02	23.24^{efg} ±1.2
-17	13.51^{de}±0.47	36.71^{def}±1.07	18.79^{de}±1.45	1.34^{c}±0.02	24.46^{def} ±0.85
-10	12.93^{e}±0.45	38.04^{de}±1.58	22.16^{bcde}±1.49	1.30^{cd}±0.04	25.16^{de} ±0.85
-3	12.97^{e}±0.36	38.24^{de}±0.94	23.60^{bcd}±1.53	1.44^{b}±0.06	26.18^{cd} ±1.09
0	12.39^{e}±0.27	33.11^{f}±0.47	39.47^{a}±3.87	1.48^{ab}±0.05	31.36^{a} ±1.87
+3	14.80^{cd}±0.59	38.50^{de}±0.67	25.34^{b}±2.21	1.32^{cd}±0.02	29.82^{ab} ±2.10
+7	15.25^{bc}±0.52	40.52^{cd}±0.81	21.60^{bcde}±1.43	1.28^{cd}±0.03	27.85^{bc} ±2.07
+10	15.18^{c}±0.47	42.83^{bc}±1.59	24.63^{bc}±3.52	1.35^{c}±0.04	26.65^{cd} ±1.57
+15	16.84^{ab}±0.61	45.61^{ab}±1.29	21.95^{bcde}±2.38	1.45^{ab}±0.02	26.09^{cd} ±1.65
+30	17.45^{a}±0.56	47.35^{a}±1.59	18.14^{e}±2.00	1.48^{ab}±0.02	26.18^{cd} ±1.55
+45	17.87^{a}±0.45	49.49^{a}±1.60	13.15^{f}±0.51	1.53^{a}±0.02	26.23^{cd} ±1.54
GM	14.50 ± 0.49	40.02 ± 1.24	22.03 ± 1.59	1.37 ± 0.02	25.63 ± 0.75
CD	1.31	3.76	6.59	0.094	3.94
CV %	7.87	8.16	23.02	5.92	13.32

Values having different superscripts differed significantly (P < 0.05) within column

Table.2 Mean (±SEM) values of antioxidant enzymes in crossbred cows (n=6) during peripartum period

Days	GPX (nmol/min/ml)	LPO (nmol/min/ml)	SOD (U/ml)
-45	2.15^{e} ±0.04	2.82^{gh} ±0.30	51.84^{e} ±0.47
-38	2.25^{de} ±0.02	3.19^{fg} ±0.43	51.61^{e} ±0.46
-31	2.44^{bcde} ±0.02	3.28^{efg} ±0.16	53.67^{de} ±0.52
-24	2.56^{bcd} ±0.01	4.00^{bcde} ±0.76	55.24^{cd} ±0.28
-17	2.60^{bcd} ±0.02	4.04^{bcd} ±0.65	55.42^{cd} ±0.25
-10	2.66^{bc} ±0.01	3.75^{cdef} ±0.34	56.55^{cd} ±0.19
-3	2.66^{bc} ±0.03	4.24^{bc} ±0.30	57.50^{c} ±0.25
0	3.57^{a} ±0.02	5.93^{a} ±0.24	62.33^{a} ±0.20
+3	3.43^{a} ±0.01	4.50^{b} ±0.40	62.73^{a} ±0.15
+7	3.24^{a} ±0.02	4.06^{bcd} ±0.28	63.40^{a} ±0.12
+10	3.35^{a} ±0.01	2.96^{gh}±0.34	61.75^{ab}±0.45
+15	3.24^{a} ±0.02	2.50^{h} ±0.30	61.40^{ab} ±0.22
+30	3.17^{a} ±0.01	3.40^{defg} ±0.34	58.41^{bc} ±0.34
+45	3.29^{a} ±0.01	3.98^{bcde} ±0.27	56.90^{cd} ±0.10
GM	2.90 ± 0.47	3.76 ±0.22	57.77 ± 4.02
CD	0.059	1.12	0.90
CV %	1.77	25.97	1.36

Values having different superscripts differed significantly (P < 0.05) within column

After parturition from day 3 to day 10 the level of cortisol started decreasing significantly (P< 0.05). The decrease in the cortisol level was non-significant from day 10 to day 45. Results of present experiment were in consonance with observations of Ghanem *et al.,* (2012) in Friesian cows, Teama and Gad (2014) in crossbred cows and Ashmawy (2015) in buffaloes and cows. Cortisol also, induced gluconeogenesis that provide adequate glucose supplement necessary for the fetal nutrition (Bell, 1995). The hormone pattern established during the latter portion of gestation triggers parturition. During the last three weeks of gestation, the fetus begins to release hormones from the adrenal cortex (predominantly cortisol). Cortisol from the fetus stimulates estrogen production by the placenta (Rhodes, 2003). Disturbed rest and uncomfortable resting areas during transition may result in a physiological stress response indicated by altered levels of plasma cortisol and increased heart rate in dairy cattle (Ladewig and Smidt, 1989).

The observed overall mean value of Glutathione peroxidase (GPX) (nmol/min/ml) in crossbred cows was 2.91 ± 0.48 which ranged from 2.15 ±0.04 to 3.57 ±0.02 during the peripartum period. Highest value of GPX was observed on the day of parturition as compared to any other peri-partum day. The GPX level started increasing non-significantly from 45 day before calving. But on the day of parturition the GPX level was significantly (P<0.05) higher than any other day of the peri-partum period. After parturition the variations in concentration of GPX were observed to be non-significant from day 3 onwards to day 45. The results of the present experiment were in consonance with the observation of Pilarczyk *et al.,* (2012) in HF cows and Gong and Xiao (2015) in HF cows. Whereas, Sharma *et al.,* (2011) reported that the GPX value was significantly lower in early lactating cows as compared to advanced pregnant cows. While, Konvicna *et al.,* (2015) reported that a significant (P<0.05) decrease in GPX activity was recorded one week after calving as compared to the weeks 6 and 9 after calving. Glutathione peroxidase is selenium dependent enzyme and it has also antioxidant property. Plasma glutathione peroxidase is considered as an indicator of oxidative stress (Tüzün *et al.,* 2002; Sharma *et al.,* 2011). In present study GPX activity was found to be increased in cows towards parturition indicating more oxidative stress at the time of parturition (Table 2).

The observed overall mean value of Lipid peroxidase (LPO) (nmol/min/ml) in crossbred cows was 3.76 ±0.22 which ranged from 2.15 ±0.04 to 3.57 ±0.02 during the peripartum period. Highest value of LPO was observed on the day of parturition as compared to any other peri-partum day. The LPO level started increasing non-significantly from 45 days before calving. But on the day of parturition the LPO level was significantly (P<0.05) higher than any other days of the peri-partum period. The value of LPO started decreasing significantly (P<0.05) from day 3 onwards to day 15. The value of LPO was increased significantly (P<0.05) from day 30 to day 45. The results of the present experiment were in consonance with the observation of Sharma *et al.,* (2011) in Holstein X Sahiwal crossbred dairy cows. Singh *et al.,* (2014) reported high lipid peroxidation in fresh cows (3-30 days in milk). Castillo *et al.,* (2005) and Castillo *et al.,* (2006) showed a increased level of LPO around parturition and early lactation in dairy cows. Gong and Xiao (2015) also reported significantly (P<0.05) increased level of melondialdehyde in early lactation as compared to dry period and peak lactation. Whereas, Adela *et al.,* (2008) reported that in the second week after parturition the level of melondialdehyde decreased significantly, and remained approximately at same levels during the experiment. Lipid peroxidation is one of

the important consequences of oxidative stress (Kumaraguruparan *et al.,* 2002). MDA is generated as a consequence of lipid peroxidation and, as such, is assayed as a biomarker of oxidative stress. Metabolic demand associated with the initiation of lactation would be expected to increase the production of ROS. Lipid peroxidation occurs when ROS react with polyunsaturated fatty acids. Peroxidation of lipids within cellular membranes can lead to changes in fluidity. This may explain why the highest serum MDA or LPO level was observed in early-lactation cows. In addition, lower antioxidant potential as a consequence of lactation stage can result in excess accumulation of ROS, which can induce lipid peroxidation.

The observed overall mean value of superoxide dismutase (SOD) (U/ml) in crossbred cows was 57.77 ± 4.03 which ranged from 51.61 ±0.46 to 63.40 ±0.12 during the peripartum period. Highest value of SOD was observed on the day 7 as compared to any other peri-partum days. The SOD level started increasing non-significantly from -30 day of the peri-partum period. But on the day of parturition the SOD level was significantly ($P<0.05$) higher as compared to any other pre-partum days. The value of SOD was higher on day 7 as compared to the day of parturition. The value of SOD was decreased non-significantly from day 10 to day 45. The result of the present study was similar with the results obtained by Chandra and Agrawal (2009) in crossbred cows where they found that SOD activity increased upto calving and then decreased. Maurya *et al.,* (2014) also found that the SOD value remained low at 60 days before calving and it is found to be increased on the day of parturition and after that the level start decreasing, Sheikh *et al.,* (2015) also reported similar type of result in Sahiwal and Karan Fries cows. While Singh *et al.,* (2014) reported that Superoxide dismutase levels were higher in pregnant cows than heifers. Whereas, Konvicna *et al.,* (2015) reported in his study that the SOD activities were found to increase during whole monitored time i.e. from 3 weeks before parturition to 9 weeks after parturition in dairy cows. Adela *et al.,* (2008) found that the SOD activity increases with passing days after parturition upto 6 weeks. The higher erythrocyte SOD activity on the day of parturition indicates higher oxidative stress and lower antioxidant status (Bernabucci *et al.,* 2005). During our experiment, the SOD activities were gradually elevated, which was probably caused by a response of the organism to higher superoxide generation, especially after calving. Although the SOD activity increases after calving in dairy cows (Gaál *et al.,* 2006), several recent studies have shown that the antioxidant capacity in periparturient dairy cows is insufficient to counteract the increase in ROS supply (Bernabucci *et al.,* 2005; Castillo *et al.,* 2005).

Serum concentration of ALT (U/L) and AST (U/L) were significantly ($P < 0.05$) lower on the day parturition than any other days of the peri-partum period. While ALP (IU/L) and ACP (IU/L) levels were significantly ($P < 0.05$) higher on the day of parturition as compared to any other days of the peri-partum period. Concentration of hormone cortisol was significantly ($P<0.05$) higher on the day parturition than any other days during the peri-partum period. Antioxidant enzymes like SOD (U/ml), LPO (nmol) and GPx (nmol/min/ml) were significantly ($P<0.05$) elevated on the day of parturition than any other days of the peri-partum period. The values of biochemical parameters including enzymes (AST, ALT, AKP and ACP) were influenced significantly ($P<0.05$) by the physiological changes occurring during the peri-partum period in the crossbred cows. Activity of antioxidant enzymes (LPO, SOD, GPx) were significantly increased during

calving and also after parturition to encounter the parturition stress and post-parturient stress in animals.

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