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Research Article
Biochemical Markers of Ketosis in Dairy Cows at Post-paturient Period: Oxidative Stress Biomarkers and Lipid Profile

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Abstract

Background: Oxidative stress biomarkers and lipid profiles were used successfully as prognostic and diagnostic biomarkers of many animal diseases. However, their use in the diagnosis of ketosis in dairy cows at post-paturient period is not completely elucidated. **Materials and Methods:** Therefore, 25 cows suffered from ketosis at post-paturient period were used in the current study together with 20 healthy cows who served as a control. Blood samples were collected from diseased and healthy animals and the harvested serum were used for determination of oxidative stress biomarkers and the profiles of lipids, protein and enzymes. **Results:** The obtained results declared that, there was a significant ($p \leq 0.05$) increase in the levels of aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), non-esterified free fatty acids (NEFA), β-hydroxylbutyric acids (BHBA), malonaldehyde (MDA) and nitric oxide (NO) in dairy cows affected with ketosis compared to control. Conversely, a significant ($p \leq 0.05$) decrease in the levels of glucose, total cholesterol, cholesterol ester, free cholesterol, triacylglycerol (TAG), superoxide dismutase (SOD) and reduced glutathione (GSH) were detected in diseased cows compared to control. Serum BHBA, NEFA, MDA and NO levels were positively correlated with each other's and inversely correlated with activity of SOD and GSH concentration in cows affected with ketosis. **Conclusion:** Oxidative stress biomarkers and lipid profiles could be used as promising biomarkers for ketosis in dairy cows at post-paturient period. The antioxidant therapy may useful in the treatment of ketosis in cows at post-paturient period.

Key words: Cows, ketosis, antioxidant, oxidative stress, lipid profile, liver enzymes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The post-paturient (transition) period is the most critical period in dairy cows[1] due to severe economic losses for dairy farmers as results of drop in milk production and high culling rates[2,3]. This period constitutes 3 weeks before and after parturition[4]. In this period cows are affected by different metabolic and infectious disease for examples ketosis and mastitis, respectively[5]. Ketosis is mostly occurred after calving because the stimulus for milk production is at its maximum and the demand of the mammary gland for glucose is often greater than the glucose available in blood creating negative energy balance. Negative energy balance stimulate the secretion of hormone sensitive lipase, triggering lipolysis with subsequent release of non-esterified fatty acids from adipose tissues (NEFA) into blood in a bioprocess named lipid mobilization[6]. Incomplete oxidation of NEFA in the liver resulted in ketone bodies formation (acetoacetate, β-hydroxybutyrate and acetone) and subsequent ketosis[7]. The most common ketone body in dairy cows is β-hydroxybutyric acid (BHBA). Therefore, NEFA and BHBA are the most common biomarkers for evaluation of ketosis and lipid mobilization[8]. Lipid peroxidation also may be induced as a result of intensified oxidation of NEFA in liver[9]. The MDA levels in blood and tissues reflect the status of lipid peroxidation[10]. The SOD is an antioxidant enzyme catalyzes the conversion of superoxide anion into hydrogen peroxide which is transformed into water by a series of reaction catalyzed by catalase and glutathione peroxidase[11]. Oxidative stress biomarkers and lipid profiles have been presented as prognostic and diagnostic biomarkers for many animal diseases[12-14]. However, the investigation of these golden biomarkers in dairy cows at post-paturient period is not completely elucidated so far. Therefore, the current study aimed to evaluate potentials of oxidative stress biomarkers and lipid profile as diagnostic markers of ketosis in dairy cows at post-paturient period.

MATERIALS AND METHODS

Animals: This study was carried out on a total number of 45 cows (3-9 weeks post-parturient), aging from 4-7 years old with average body weight of 650±15 kg from a private farm. The selected cows were assigned to two groups, first group represented control cows (n = 20) whereas second group (n = 25) consisted of ketotic cows. All cows were clinically examined every day until 4 weeks after parturition[15]. All applicable international, national and/or institutional guidelines for the care and use of animals were described.

Samples collection: Blood samples were collected from the jugular vein into plain tubes and were allowed to clot at room temperature. The harvested serum stored frozen at -20°C until the time of analysis of AST, GGT, glucose, total protein, albumin, NEFA, BHBA, total cholesterol, cholesterol ester, free cholesterol, TAG, MDA, NO, SOD and GSH. Urine samples have been collected from all animals for detection of ketonuria.

Biochemical analysis: The presence of ketone bodies in the urine was detected by commercial kits (Fujisawa pharmaceutical Co., Osaka, Japan). The levels of serum glucose, triglyceride, cholesterol as well as AST and GGT activities were determined in serum samples on a Beckman CX-7 auto-analyzer using the corresponding kits (Sigma Chemical Co., Ltd., Poole, Dorset, UK). Serum BHBA was determined by a kinetic enzymatic method using a commercially available kit (Ranbut D-3-hydroxybutyrate, Randox, Crumlin Co., Antrim, UK). Serum concentration of NEFA was carried out using commercially available test kits supplied by Randox laboratories Ltd.

Statistical analysis: All data was presented as mean±standard error of mean by using one way analysis of variance (ANOVA). All tests were performed using computer package of the statistical analysis system[16].

RESULTS

Clinical examination: The diseased cows showed anorexia, ruminal stasis, constipation and significant reduction of milk production.

Profiles of proteins, lipids and enzymes: The profiles of proteins, lipids and enzymes showed in Table 1. The presented data indicated a significant increase ($p \leq 0.05$) in AST and GGT activities as well as concentrations of NEFA and BHBA in the serum of ketotic animals compare to the control. The data shown in the same Table 1 revealed a significant reduction ($p \leq 0.05$) in the values of glucose, total cholesterol, cholesterol ester, free cholesterol, TAG, total proteins, albumin and globulins in the serum of ketotic animals compare to the control.

Oxidative stress biomarkers: The values of oxidative stress biomarkers are illustrated in Table 2. The data summarized in this Table 2 revealed significant elevation of MDA and nitric

Table 1: Lipids, enzymes and protein profiles in serum of control and cows affected with ketosis

Variables	Control	Ketosis
AST (U L^{-1})	70.20±1.23	140.60±3.60*
GGT (U L^{-1})	7.32±0.53	14.23±0.63*
Glucose (mmol L^{-1})	3.23±0.23	1.60±0.12*
Total cholesterol (mg dL^{-1})	53.23±1.36	23.20±1.23*
Cholesterol ester (mg dL^{-1})	25.32±1.20	9.80±0.52*
Free cholesterol (mg dL^{-1})	27.23±1.23	13.90±0.93*
TAG (mg dL^{-1})	30.23±1.23	19.80±1.62*
NEFA (mg dL^{-1})	365.32±11.2	623.32±9.32*
BHBA (mmol L^{-1})	0.60±0.01	1.90±0.023*
Total protein (mg dL^{-1})	6.20±0.35	4.40±0.23*
Albumin (mg dL^{-1})	2.60±0.23	1.60±0.05*
Globulin (mg dL^{-1})	5.30±0.23	2.80±0.12*

*Means are significantly different at the level ($p \leq 0.05$), AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase, TAG: Triacylglycerol, NEFA: Non-esterified free fatty acids, BHBA: β-hydroxylbutyric acid

Table 2: Oxidative stress biomarkers in serum of control and cows affected with ketosis

Variables	Control	Ketosis
MDA (mg dL^{-1})	5.80±0.56	14.23±0.89*
NO (mg dL^{-1})	11.30±4.80	22.40±8.30*
SOD (mmol L^{-1})	7.43±0.31	4.55±0.45*
GSH (mmol L^{-1})	9.84±0.21	6.47±0.53*

*Means are significantly different at the level ($p \leq 0.05$), MDA: Malonaldehyde, NO: Nitric oxide, SOD: Superoxide dismutase, GSH: Reduced glutathione

Table 3: Spearmen's correlation coefficient among traditional (BHBA and NEFA) and suggested (MDA, SOD, NO and GSH) biomarkers of ketosis in cows affected with ketosis

Variables	MDA (μmol L^{-1})	SOD (U mL^{-1})	NO (μmol L^{-1})	GSH (μmol L^{-1})	BHBA
MDA (μmol L^{-1})					
SOD (U mL^{-1})	-0.899				
NO (μmol L^{-1})	0.952	-0.875			
GSH (mmol L^{-1})	-0.886	0.853	-0.853		
BHBA (mmol L^{-1})	0.955	-0.933	0.933	-0.894	
NEFA (mg dL^{-1})	0.963	-0.908	0.949	-0.910	0.974

MDA: Malondialdehyde, SOD: Superoxide dismutase, NO: Nitric oxide, GSH: Reduced glutathione, BHBA: β-hydroxylbutyric acids, NEFA: Non-esterified free fatty acids

oxide values and significant reduction in GSH concentration as well as SOD activity in the serum of ketotic animals compare to the control.

Correlation between traditional (BHBA and NEFA) and suggested (MDA, SOD, NO and GSH) biomarkers of ketosis in cows affected with ketosis: The data summarized in Table 3 indicated that, BHBA was positively correlated with NEFA ($r = 0.974$, $p = 0.000$). Furtherly, both BHBA and NEFA were inversely correlated with SOD ($r = -0.933$ and -0.908) and GSH ($r = -0.894$ and -0.910), respectively. In addition, MDA was inversely correlated with SOD ($r = -0.899$) and GSH ($r = -0.886$) and positively correlated with NO ($r = 0.952$), BHBA ($r = 0.955$) and NEFA ($r = 0.963$).

DISCUSSION

The occurrence of ketosis in cows at post-parturient phase perhaps owed to lack of dry matter intake around parturition, increase demands for glucose and insufficient propionate production[17,18]. Ketotic animals have been diagnosed by positive findings of clinical examination and confirmed by positive ketone bodies test in the urine. The clinical signs observed in the ketotic cows were the same observed in previous study on cows[19] and buffaloes[20,21]. Beside the clinical signs that observed in ketotic cows of the current study, ketosis has been approved by positive test of ketone bodies in urine and ketonuria. In addition, the significant increase of NEFA and BHBA in the serum of ketotic cows at post-parturient period confirmed the observed clinical findings. The significant increase of AST and GGT activities in the serum of ketotic cows compared to control as observed in the current study indicated liver dysfunction[22,23]. The release of liver enzymes (AST and GGT) may attribute to increased hepatic cell membrane permeability as a result of infiltration of hepatic cells with fat[24]. The hepatic dysfunction in ketotic cows has been confirmed also by observed low TAG level along with high AST and GGT activities compare to control animals. In addition, the significant decrease in total protein and albumin level in ketotic animals of the current study compare to control indicated liver dysfunction[25]. The significant reduction of cholesterol levels in ketotic cows compared with normal ones as reported in the current study may attribute to liver dysfunction which reduces cholesterol biosynthesis[26]. Similar findings have been observed in cattle[27] and buffaloes[20]. In the contrast other study reported significant increase in cholesterol levels in ketotic animals[28]. The significant decrease in cholesterol ester in ketotic cows may attribute to the negative effect of postpaturient ketosis on synthesis of LCAT, an enzyme responsible for formation of cholesterol ester from peripheral cholesterol[20,29]. Similar results have been obtained in ketotic cows and bufflaoes[20,25]. The significant reduction in glucose level and higher BHBA in ketotic cows has been reported earlier in cows[30-32] and buffaloes[20,33]. Hypoglycemia may occur due to imbalance between glucose intake[34] and glucose utilization in the mammary gland during lactation period postpartum[31]. As a response to low blood glucose level, fat mobilization is initiated[7,35] and subsequent elevation of NEFA oxidation and production of BHBA has been occurred to compensate the energy loss as a result of absence of glucose[36]. However, as a result of elevated rate of fatty acid oxidation, free radicals have been produced causing lipid peroxidation and oxidative stress. In addition the formed ketone bodies (BHBA) considered as important source of free radicals

and initiation of oxidative stress. Therefore, in the current study, MDA level has been increased significantly in cows affected with ketosis compared to control. Similar findings have been reported in cows[37], buffaloes[20,21], human[38] and rabbits[39]. In the current study NO level has been elevated in in cows affected with ketosis compared to control. It has been postulated that ketosis enhanced the NO production[40]. The significant positive correlation between MDA and BHBA and the negative correlation between MDA as well as BHBA and NO have been observed earlier in buffaloes[21]. The negative correlation among MDA, NEFA as well as BHBA and SOD and GSH was reported for first time in cows affected with ketosis in the current study.

CONCLUSION

At post-paturient period in cows, blood glucose level decreased, lipid mobilization and fatty acid oxidation increased with subsequent increase in ketone bodies (BHBA) which creating a state of lipid peroxidation (MDA) and oxidative stress. The enzymatic (SOD) and non-enzymatic (GSH) antioxidants have been depleted as a trial to counteract the stressful situation.

SIGNIFICANCE STATEMENTS

Oxidative stress biomarkers and lipid profiles could be used as promising biomarkers for ketosis in dairy cows at post-paturient period. The antioxidant therapy may useful in the treatment of ketosis in cows at post-paturient period.

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