

EVALUATION OF HEPATIC FUNCTION MARKERS OF SERUM IN DAIRY CATTLE WITH LACTIC ACIDOSIS

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ABSTRACT

Rumen lactic acidosis is a metabolic disorder which develops in ruminants that have ingested large amounts of unaccustomed feeds rich in ruminally fermentable carbohydrates. In this study the relationship between serum lactate levels and rumen pH with liver profile was investigated in 100 dairy cattle with ruminal lactic acidosis. After complete clinical examination and recording of vital signs including heart rate, respiratory rate, rectal temperature and dehydration, rumen fluid and venous blood samples were collected. pH of rumen fluid was determined using pH strips and serum biochemical parameters were measured via biochemical method. Based on ruminal fluid pH, sick animals were divided in 3 group: acute form (20 cattle), sub-acute (60 cattle) and chronic form (20 cattle). According to the findings, heart rate, respiratory rate and percentage of dehydration among the three study groups showed a significant difference. There were significant differences between serum activities of AST ($p=0.018$), ALT ($p=0.048$) and GGT ($p=0.043$) but there was no any statistically significant changes in serum LDH activity ($p=0.503$). Total bilirubin level in acute form of disease was 0.64 ± 0.2 mg/dl that was significantly higher than other forms of disease and healthy cattle ($p=0.036$). Significant changes were not found in total protein, albumin and direct bilirubin of serum. Serum lactate levels and ruminal acidity (pH) revealed significant differences between all groups. There were significant negative correlations between pH of rumen fluid with serum lactate ($p=0.001$; $r=-0.495$), heart rate ($p=0.000$; $r=-0.615$), respiratory rate ($p=0.002$; $r=-0.455$) and dehydration ($p=0.000$; $r=-0.656$). In this study, significant negative correlations were found between ruminal fluid pH with AST activity of serum ($r=-0.041$; $p=0.310$) and serum total bilirubin levels ($r=-0.046$; $p=0.283$). Also there were correlation between serum lactate levels with AST ($r=0.583$; $p=0.000$), ALT ($r=0.439$; $p=0.003$) and GGT ($r=0.432$; $p=0.005$) activities of serum. Liver abscesses secondary to the lactic acidosis and ruminitis in grain fed cattle, is one of major reason of alteration of liver function markers in serum. In conclusion, this study has demonstrated that cattle with ruminal lactic acidosis have some degree of liver involvement. The reliability and prognostic value liver function markers in cattle with ruminal lactic acidosis is deserving of further investigations.

Keywords: *Lactic Acidosis, Liver Function Test, Dairy Cattle*

INTRODUCTION

Acidosis is a pathological condition associated with the accumulation of acid or depletion of alkaline reserves in blood and body tissues, and characterized by increased hydrogen ion concentrations (Bramley *et al.*, 2008). Ruminal acidosis refers to a series of conditions that reflect a decrease in pH in the rumen of cattle. Rumen lactic acidosis (grain overload, grain poisoning, acute indigestion) develops in sheep and cattle that have ingested large amounts of unaccustomed feeds rich in ruminally fermentable carbohydrates (Crichlow and Chaplin, 1985; Nocek, 1997). Ruminants, regardless of age, breed, and sex are susceptible to overeating with grains and carbohydrates (Radostits *et al.*, 2007).

The resulting production of large quantities of volatile fatty acids (VFA) and lactic acid decreases rumen pH to non-physiological levels, simultaneously weakening the buffering capacity of the rumen, and reduces the efficiency of rumen flora and fermentation. Lactic acidosis can cause ruminitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death (Lean *et al.*, 2007). Accumulation of lactic acid in the rumen result in damage to the ruminal epithelium ultimately leads to breakdown of the barrier between the ruminal fluid and the blood, which may result in translocation of bacteria or potentially

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harmful molecules, such as lipopolysaccharide/endotoxin, to the portal and systemic circulation (Danscher, 2011).

The aim of this study was to investigate the effect of ruminal lactic acidosis in Holstein dairy cattle on the biochemical profile in the livers.

MATERIALS AND METHODS

Animals

Blood samples were collected randomized from 100 adult Holstein dairy cattle (>1year) presented to the Veterinary Teaching Hospital, Veterinary Faculty, Islamic Azad University- Tabriz Branch during the September 2013 to July 2014. These were clinically sick animals. A total of 30 clinically healthy Holstein adult cattle from same farms were used as a control group. Control cattle were excluded if any abnormality was found. All animal used in this study were aged between 2.5 - 6 years old. This study was approved by the Ethics Committee of Faculty of Veterinary Medicine, Islamic Azad University.

Clinical Examination and Sampling

The ruminal pH, dehydration degree, rectal temperature, respiratory rate and cardiovascular system of the cattle deemed to be undergoing ruminal acidosis were examined and clinical observations were recorded. Ruminal fluid samples collected using a 16-gauge 1½ inch (about 4 cm) disposable needle attached to a 30 mL syringe. A 2 cm by 2 cm area was shaved on the left paralumbar fossa approximately one hand length ventral to the lumbar spinal transverse processes and one hand width caudal to the last rib, avoiding the major muscle masses. This area was surgically prepared and the sampling needle was inserted firmly through into the rumen. A minimum of 4 mL normally was obtained from most animals using this procedure. Ruminal fluids pH were determined using pH test strips. Based on ruminal fluid pH, animals classified as three groups including acute (pH<5.5), sub-acute (pH 5.5 to 6) and chronic (pH 6 to 6.5) groups. After confirmation of ruminal lactic acidosis, approximately 10 mL blood samples were collected from jugular vein into serum tubes for biochemical analysis.

Biochemical Assay

Serum samples were analyzed for lactate using commercial kits (Randox[®], UK) and aspartate amino transferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-Glutamyl transferase (GGT) activities and total protein, albumin, total and direct bilirubin levels were determined by an automatic analyzer (WS-ROCHE 912, Roche Hitachi, Tokyo, Japan) using commercial kits (Pars Azmoon Co. INC., Karadj, Iran).

Statistical Analysis

All statistical analyzed by Statistical Package for Social Sciences for Windows, version 17.0 (SPSS Inc.). Data normality was tested by Kolmogorov-Smirnov test. Analysis of variance (ANOVA) test was used for comparison of measured factors in all groups. Pearson's correlation coefficient was used for determination of the relationship between parameters. All values were expressed as mean and standard deviation (SD), and $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Result

Based on ruminal fluid pH, sick animals were divided in 3 group: acute form (20 cattle), sub-acute (60 cattle) and chronic form (20 cattle). The results of the clinical and physical examination of animals are summarized in Table 1. Heart rate, respiratory rate, and degree of dehydration among the three disease subgroups showed significant difference. Serum lactate levels and ruminal fluid acidity (pH) revealed significant differences between all subgroups (Table 1).

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Table 1: Vital signs, ruminal fluid pH and serum lactate levels in cattle with lactic acidosis

	Heart rate (b.p.m)	Respiratory rate (per min.)	Rectal temperature (°C)	Dehydration (%)	Ruminal pH	Serum lactate (mg/dl)
Acute	105.5 ± 2 ^a	38.3 ± 0.8 ^a	39.0 ± 0.5 ^a	7.0 ± 1.7 ^a	4.8 ± 0.2 ^a	95.2 ± 9.6 ^a
Sub-acute	99.0 ± 6 ^b	36.2 ± 1.8 ^b	38.8 ± 0.4 ^a	4.7 ± 1.4 ^b	5.5 ± 0.0 ^b	31.1 ± 4.8 ^b
Chronic	91.1 ± 6 ^c	35.5 ± 2.0 ^c	38.7 ± 0.3 ^c	2.8 ± 1.6 ^c	6.0 ± 0.0 ^c	20.0 ± 5.3 ^c
p value	0.000	0.004	0.031	0.000	0.000	0.001

Means (±SD) within a row with different superscript letters (a,b,c,d,e) denote significant differences (p<0.05)

In sick cattle, significant negative correlations were observed between ruminal fluid pH with serum lactate levels (r=-0.495; p=0.001), heart rate (r=-0.615; p=0.000), respiratory rate (r=-0.455; p=0.002), dehydration degree (r=-0.656; p=0.000). There were positive correlation between serum lactate levels with heart rate (r=0.408; p=0.006) and dehydration degree (r=0.393; p=0.008).

Table 2: Liver function biomarkers levels in serum of cattle with lactic acidosis

	AST (U/L)	ALT (U/L)	LDH (U/L)	GGT (U/L)	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Control	62.0 ± 11 ^a	17.3 ± 3 ^a	1325 ± 94 ^a	16.9 ± 8 ^a	6.8 ± 0.9 ^a	3.4 ± 0.4 ^a	0.25 ± 0.3 ^a	0.04 ± 0.01 ^a
Acute	107.8 ± 17 ^b	26.9 ± 7 ^b	1361 ± 60 ^a	25.0 ± 7 ^b	6.3 ± 0.5 ^a	2.8 ± 0.3 ^a	0.64 ± 0.2 ^b	0.07 ± 0.02 ^a
Sub-acute	96.3 ± 14 ^c	21.9 ± 1 ^a	1296 ± 160 ^a	25.5 ± 11 ^b	6.6 ± 0.1 ^a	3.1 ± 0.1 ^a	0.30 ± 0.0 ^a	0.04 ± 0.00 ^a
Chronic	85.7 ± 16 ^b	19.4 ± 2 ^a	1373 ± 19 ^a	20.1 ± 11 ^a	6.3 ± 0.6 ^a	2.6 ± 0.1 ^a	0.53 ± 0.1 ^b	0.04 ± 0.02 ^a
p value	0.018	0.048	0.503	0.043	0.816	0.082	0.036	0.391

Means (±SD) within a row with different superscript letters (a,b,c,d,e) denote significant differences (p<0.05)

In this study, significant negative correlations were found between ruminal fluid pH with AST activity of serum (r=-0.041; p=0.310) and serum total bilirubin levels (r=-0.046; p=0.283). Also there were correlation between serum lactate levels with AST (r=0.583; p=0.000), ALT (r=0.439; p=0.003) and GGT (r=0.432; p=0.005) activities of serum.

Discussion

Ruminal acidosis refers to a series of conditions that reflect a decrease in pH in the rumen which is commonly develops in cattle that have ingested large amount of highly fermentable carbohydrates (Bramley *et al.*, 2008; Nocek, 1997). This condition leads to production of large quantities of the volatile fatty acids (VFA) in the rumen (Lean *et al.*, 2007).

Rumen microbial population of cattle and sheep is affected by ration. Naturally, most of the rumen bacteria are anaerobic and gram negative (Cunningham and Klein, 2007; Ortolani, 1995). Several hours after eating of the large amount of fermentable rations, important changes was occur in the microbial flora and production of lactic acids in the rumen is significantly increased that causes to reduction of ruminal pH (Radostits *et al.*, 2007). Ruminal lactic acidosis results in pathological changes in the ruminal wall, such as chronic inflammation, ulceration and/or hyperkeratosis (Gabel, 1990; Lechoski, 1996). A damaged ruminal wall facilitates the absorption of substances such as endotoxins, which then pass to the

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liver via the portal route, so causing damage (Ainmalamali *et al.*, 1992). This has been confirmed by Adamski (1992), Bieniek (1981) and Das and Misra (1992), who observed an increased activity of the liver enzymes in the serum and necrotic lesions in the liver in the course of acidosis in sheep, cows and goats.

West (1990) demonstrated that during the acidosis, AST activity increased in the liver of cows, so that these changes were correlated with the liver damages. Evaluation of AST activity of serum is more useful than ALT activity measurement for diagnosis of liver diseases in large animals; because changes of ALT levels mostly are insignificant. However, AST is not specific for liver and increase of AST activity is seen in the cellular necrosis of most tissues such as cardiac and skeletal muscles and also liver parenchyma. So, interpretation of this enzyme should be made after assure the health of other organs (Stockham and Scott, 2002; Huxtable, 1988). It is believed that bilirubin and AST activity levels are sensitive indicators of liver function during the periparturient period in cattle (Lee *et al.*, 2007). Clearance of LPS in the liver kupffer cells can cause to increasing of AST levels (Marchesini *et al.*, 2013). Also, severe congestion and advanced hepatocellular degeneration has been seen in the Nubian goats suffering from experimental lactic acidosis (Mohamed-Nour *et al.*, 1998). Similar mechanism may be contributed for elevation of serum AST activity in cattle with lactic acidosis. Significant increases of serum activities of liver enzymes including AST, ALT and GGT had been reported in the pregnant dairy cattle suffering from experimental acidosis. But this increase was last only 30 days after administration of sucrose, which may be attributed to the adaptive ability of hepatocytes (Lechowski, 1996). In contrary to the results of Lechowski (1996) study, the highest enzyme changes were seen in the acute form of disease rather than chronic form. So that there is no any pathognomonic clinical signs of liver involvement in animals. The increase in the liver enzyme activities, without clinical signs, will have been the result of compensatory functions in the course of metabolic acidosis. These compensatory mechanisms lead to intracellular functional changes and cellular damage, especially in the liver (Lachman *et al.*, 1986). However, similar results have been reported, previously. For example elevation of liver enzymes activities in the serum of cattle after feeding with barley for several months has been reported (Stockham and Scott, 2002). This depicts the hepatocellular damage during the initial days of feeding with grains, which recovery has been occurred after one month (Radostits *et al.*, 2007).

In this study the changes in serum ALT activity had significant difference in the cattle with acute form of ruminal lactic acidosis compared to other groups ($p=0.048$). Since, the change of ALT (GPT) of the serum is minor, increase of AST (GOT) activity compared with ALT is important and sensitive indicator for liver damage (Brown *et al.*, 1999).

LDH changes of the serum were statistically insignificant among three groups of the patients and control group. The increase of serum LDH in the ewes with experimental acidosis was observed as a result of injection of glucose in the rumen (Brown *et al.*, 1999). LDH is a non-specific enzyme that muscles, liver tissue and red blood cells are considered its main resources (Stockham and Scott, 2002). For this reason, measuring of this enzyme activity is not recommended for diagnosis of the liver diseases (Brown *et al.*, 1999). The results of this study showed impracticality of LDH.

GGT is an appropriate enzyme for evaluation of biliary ducts rather than liver tissue. In this study the serum GGT was increased in the cattle with lactic acidosis that it was significant in the acute and sub-acute form of disease. Significant elevation of serum GGT activity had been reported in the pregnant dairy cows suffering from acidosis as consequent of feeding with sucrose (Lechowski, 1996). The increase of GGT level in serum was seen in 27 percent of cattle with liver abscess secondary to the ruminal acidosis. But other enzymes including AST, ALT and ALP changes were in normal ranges (Dore *et al.*, 2007).

In a study conducted on the eighteen dairy Holstein cattle affected with liver abscesses as a result of lactic acidosis, increase of globulin and decrease albumin of serum were seen in 11 and 6 cases, respectively (Dore *et al.*, 2007). Although, increase of globulin, albumen and total protein has been reported in the experimental lactic acidosis in ewes (Brown *et al.*, 1999), in the cases of liver involvement, decrease of total protein and serum albumen is expected. Because of serum protein alteration does not occur in the

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early stages of liver disease, measurement of serum protein it is not useful in rapid and primary diagnosis of liver diseases. It is believed that liver disease can be diagnosed when serum total protein is decreased lower than 5 g/dl; this situation occurs in the late stage of the liver disease (Stockham and Scott, 2002).

Although, in this study total bilirubin of serum in cattle with lactic acidosis was higher than healthy ones; this change was not enough large (1mg/dl) to be considered as hyperbilirubinemia. Therefore the jaundice was not seen in any animal in spite of increase of serum bilirubin. Significant increase of serum total bilirubin was observed in the pregnant dairy cows suffering from experimental acidosis (Lechowski, 1996). Also elevation of serum total bilirubin was found only in 3 of 18 cases of liver abscess secondary to ruminal lactic acidosis (Dore *et al.*, 2007). Increase of bilirubin occurs in liver disease; but serum bilirubin change in the liver disease of cattle is trivial and therefore Von Den Berg test is not always useful (Stockham and Scott, 2002).

Liver abscesses secondary to the lactic acidosis and ruminitis in grain fed cattle, is one of major reason of alteration of liver function markers in serum. *Fusobacterium necrophorum* then gains entry into the blood and portal circulation via damaged ruminal wall and localized in liver parenchyma as liver abscess (Tadepalli *et al.*, 2009). Efforts have been made for estimation of rumen pH based on clinical symptoms or blood and metabolic parameters. However none of them could predict the rumen condition (Marchesini *et al.*, 2013). In conclusion, this study has demonstrated that cattle with ruminal lactic acidosis have some degree of liver involvement. The reliability and prognostic value liver function markers in cattle with ruminal lactic acidosis is deserving of further investigations.

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