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### Αξιολόγηση καρδιακών βιοδεικτών ορού σε πρόβατα με οξεία γαλακτική οξέωση

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## Evaluation of serum cardiac biomarkers in sheep with acute lactic acidosis

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**ABSTRACT.** In this study we investigated the changes of cardiac injury biomarkers in serum samples from 200 sheep with acute ruminal lactic acidosis (ARLA) and 50 healthy controls belonging to the Ghezel breed. After clinical examination and recording of vital signs (heart rate, respiratory rate, rectal temperature and hydration status), rumen fluid and venous blood samples were collected. The pH of rumen fluid was determined using a paper tape pH-meter and lactic acid concentration of serum was measured using a commercially available method. Similarly, activities of AST, LDH, CK-MB and serum concentration of cTnI were measured using special commercial kits. According to the findings serum activities of AST ( $p = 0.007$ ) and CK-MB ( $p = 0.002$ ) in sheep with ARLA were significantly higher than in healthy animals. Serum LDH activity in the disease group was higher than in the control group, however this difference was statistically non-significant. cTnI concentration were  $0.684 \pm 0.03$  ng/ml in sheep with ARLA, which was significantly higher than in healthy sheep ( $p = 0.000$ ). There were significant negative correlations between ruminal pH and serum lactate levels, heart rate, respiratory rate and dehydration degree. The sheep with the lowest ruminal pH (3 cases with  $< 4.5$ ) had the highest levels of cTnI (2.28 to 3.06 ng/mL), and all died. It can be concluded that lactic acidosis may cause some degree of heart damage, although further studies are needed to support this speculation.

**Keywords:** Acute Lactic Acidosis, Cardiac Biomarkers, Cardiac Troponin I, CK-MB

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## INTRODUCTION

Acute ruminal lactic acidosis (ARLA), also called grain overload, grain poisoning and acute indigestion, develops in sheep and cattle that have ingested large amounts of unaccustomed feeds rich in ruminally fermentable carbohydrates (Radostits et al., 2007). Rapid fermentation of carbohydrates lead to production of lactic acid and a decrease in ruminal pH to physiologically inappropriate levels and finally metabolic acidosis occurs (Patra et al., 1996; Jafari-Dehkordi et al., 2011).

The disease is characterized by anorexia, depression, dehydration, ruminal stasis, profuse diarrhea, severe toxemia, weakness and ataxia leading to recumbency and high mortality rate (Radostits et al., 2007). Several biomarkers have been used for the detection of myocardial injury including cardiac troponins, lactate dehydrogenase (LDH), creatine kinase (CK), MB isoenzyme of CK (CK-MB), along with electrocardiogram (ECG), but measurement of serum troponins has been shown to be more specific and sensitive than others (Coudrey et al., 1998; Babuin and Jaffe, 2005; Collinson and Gaze, 2007). Cardiac troponin I (cTnI) is a more sensitive and persistent indicator of cardiac injury, with high tissue specificity, than other markers in the presence of marked skeletal muscle injury, liver disease, and chronic renal failure (O'Brien et al., 1997; Adin et al., 2005). The molecular structure of troponin proteins is highly conserved across species, and some current assays developed for their detection in humans have been used and validated in several other species (Oyama and Solter, 2004; Varga et al., 2009). The central hypothesis of this study is that acid-base and electrolyte disturbances associated with ARLA and secondary complications, lead to some degree of cardiovascular injury. Accordingly, the goals of this study were to (1) quantify the vital signs abnormalities, (2) investigate changes in serum levels of cTnI, aspartate aminotransferase (AST), LDH and CK-MB; and (3) evaluate the association of biochemical abnormalities and vital signs in sheep with clinical signs of ARLA.

## MATERIALS AND METHODS

### Animals

The study involved 200 adult Ghezel sheep of both sexes with acute ruminal lactic acidosis (ARLA), and 50 healthy sheep with the same age, randomly selected from the same flock as a control group. Sheep with ARLA symptoms that were mentioned previously and ruminal fluid pH below 5.5 were included in disease group. Animals having clinical signs of a disease other than ruminal lactic acidosis were excluded. Control sheep were excluded if any abnormality was found. All of the animals used in this study were aged between 1 - 4 years old. This study was approved by the Ethics Committee of Faculty of Veterinary Medicine, Islamic Azad University.

### Clinical examination

After a routine clinical examination of all animals, vital signs including heart rate, respiratory rate, rectal temperature and dehydration degree were recorded. Rumen fluid samples were obtained via rumenocentesis using an 18-gauge 1½ inch (about 4 cm) disposable needle attached to a 20 mL eccentric syringe. A 2 cm by 2 cm area was shaved on the left paralumbar fossa, and the sampling needle was inserted firmly into the rumen using aseptic protocol. Physical properties of the ruminal fluid samples were examined and then their pH was measured with commercial test strips (Merck®, Germany). Ruminal fluid pH less than 5.5 indicated ARLA and animals were included in the disease group.

For biochemical analysis, approximately 6 mL blood samples were collected from the jugular vein into serum tubes.

### Biochemical assay

Serum samples were analyzed for cardiac troponin I (cTnI) by chemiluminescent method (CLIA) using commercial human system (LIAISON® Troponin I, DiaSorin, Italy), and AST, CK-MB and LDH activities were determined by an automatic analyzer (WS-ROCHE912, Roche Hitachi, Japan), using commercial kits (Pars Azmoon Co. INC., Karadj, Iran). This CLIA assay is able to measure troponin in range

**Table 1.** Vital signs parameters, ruminal pH and serum lactate levels in sheep with ARLA (mean  $\pm$  SD)

	Heart Rate (BPM)	Respiratory Rate (/min)	Temperature (°C)	Ruminal Fluid pH	Serum Lactate (mg/dL)
Disease	106.5 $\pm$ 9	38.3 $\pm$ 4	39.7 $\pm$ 0.5	5.28 $\pm$ 0.2	95.2 $\pm$ 9.6
Control	75.0 $\pm$ 6	28.4 $\pm$ 3	38.9 $\pm$ 0.4	6.93 $\pm$ 0.3	31.2 $\pm$ 4.8
<i>p</i> value	0.000	0.004	0.002	0.001	0.000

0.005-100 ng/mL. Levels of serum lactate were measured with commercial kits (Randox®, UK).

### Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences for Windows, version 19.0 (SPSS Inc.). Data normality was tested by Kolmogorov-Smirnov test. The independent samples *t* test was used for comparison of measured factors between two groups (controls and clinical cases). 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

All vital signs parameters, ruminal fluid pH and blood lactate levels showed significant differences between clinically healthy and sick animals (Table 1). There were significant negative correlations between ruminal pH and serum lactate levels ( $r = 0.56$ ;  $P = 0.002$ ), heart rate ( $r = 0.71$ ;  $P = 0.000$ ), respiratory rate ( $r = 0.52$ ;  $P = 0.003$ ) and dehydration degree ( $r = 0.82$ ;  $P = 0.000$ ). The mean ( $\pm$  SD) cardiac troponin I concentration in healthy sheep and ARLA cases was  $0.005 \pm 0.00$  ng/mL and  $0.684 \pm 0.03$  ng/mL respectively, which showed significant increase in the disease group ( $P = 0.000$ ). cTnI levels ranged from

0.391 – 3.06 ng/mL in sheep with ARLA and  $< 0.005 - 0.021$  ng/mL in healthy ones.

There was significant negative correlation between ruminal fluid pH and serum lactate levels ( $r = 0.85$ ;  $P = 0.004$ ); so that three sheep with ruminal pH  $< 4.5$  had the highest levels of cardiac troponin I (2.28, 2.51 and 3.06 ng/mL), and all died within 4-6 hours. Mean serum activities of CK-MB, LDH and AST were  $117 \pm 15$  U/L,  $1332 \pm 62$  U/L and  $352 \pm 32$  U/L in the disease group respectively, which showed increase in sheep with ARLA (Table 2).

### DISCUSSION

Although cardiac troponins are routinely assessed in

**Table 2.** Serum cTnI concentration and AST, LDH, CK-MB activities in sheep with ARLA (mean  $\pm$  SD)

	cTnI (ng/mL)	AST (U/L)	LDH (U/L)	CK-MB (U/L)
Disease	0.684 $\pm$ 0.03	352 $\pm$ 32	1332 $\pm$ 62	117 $\pm$ 15
Control	0.005 $\pm$ 0.00	137 $\pm$ 16	1296 $\pm$ 85	58 $\pm$ 10
<i>p</i> value	0.000	0.002	0.063	0.007

people with myocardial infarction, such lesions are not common in animals. Cardiac troponins were evaluated in some pathologic conditions in ruminants including white muscle disease in lambs, (Gunes et al., 2010), experimental coronary ligation in sheep (Leonardi et al., 2008), foot and mouth disease in calves (Gunes et al., 2005; Tunca et al., 2008), bovine theileriosis (Fartashvand et al., 2013), experimental endotoxemia in calves (Peek et al., 2008). There are some reports about myocardial injury in sheep with acute lactic acidosis (Kirbas et al., 2014) which ranged from changes in ECG (Jafari-Dehkordi et al., 2011; Onmaz et al., 2011) to severe focal myocarditis (Dshurov, 1976). Although history taking, clinical findings and determination of ruminal fluid pH are sufficient for the diagnosis of ARLA, the severity of myocardial damage is not known and prognosis of the disease cannot be fully clarified.

In this study, the activities of AST and CK-MB in the disease group were higher as compared to that of the healthy group. Similar results had been reported previously (Patra et al., 1996; Kirbas et al., 2014). Binding of endotoxins to cellular membrane receptors initiate inflammation and cytokines production, which lead to hepatic dysfunction and increase in AST activity. The MB isoenzyme of creatine kinase is normally present in the skeletal muscle in low amounts and substantial injury to skeletal muscle can increase CK-MB activity and significantly elevate values to abnormal levels (Coudrey, 1998).

This is an important consideration in ARLA, in which liver impairment and secondary rhabdomyolysis due to recumbency can occur. Thus in ruminal lactic acidosis, serum enzyme activities such as AST and CK-MB could also be high because of both muscle and liver disease (Radostits et al., 2007). Significant elevation of serum LDH activity in ewes with experimental ARLA has been reported, which is inconsistent with the results of this study (Brown et al., 1999). Muscle and liver tissue, as well as red blood cells are the main sources of LDH; thus increasing of the LDH alone can not confirm heart damage (Radostits et al., 2007). Possible reasons for cardiac damage in lactic acidosis include: direct inhibitory effect of low blood pH on cardiac myocytes contraction

and conductive system (Orchard and Cingolani, 1994; Abera et al., 2001), endotoxemia and its complications (Constable, 1999; Pelander et al., 2008), dehydration and reduced blood supply to the heart muscle, and hypocalcemia (Orchard and Kentish, 1990; Orchard et al., 1993). Cardiac troponin I is released from myocytes in both reversible and irreversible myocardial injury (Fartashvand et al., 2013; Varga et al., 2013).

Our results mainly demonstrate that sheep diagnosed with ARLA had significantly higher cTnI concentrations in comparison with the healthy ones. It may be speculated that increased oxygen consumption by the myocardium during a prolonged period of tachycardia is combined with a reduced oxygen supply to the myocardium due to the shortened diastole during tachycardia (Fartashvand et al., 2013). Although one of the presumptive reasons of increased serum cTnI concentration in sheep with ARLA may be attributed to the occurrence of tachycardia secondary due to dehydration, its significance is unclear. A possible cause for the increased cTnI concentrations could be the presence of endotoxins into the circulation. Depending on the extent of endotoxin release, the result is varying manifestations of inflammation, from local to systemic and cellular damage, which could potentially affect myocardial cells and thereby result in elevated serum concentrations of cTnI (Pelander et al., 2008). Cardiac dysfunction due to experimental endotoxemia has been reported previously (Constable, 1999). Increase of the heart rate following metabolic acidosis is due to adrenergic system.

Release of catecholamines lead to stimulation of the carotid body reflexes and secondarily increase the respiratory rate, or directly stimulate the cardiac center in the brain because of the elevation of blood CO<sub>2</sub> levels (Cunningham and Klein, 2007). Significant elevation of the heart rate was reported in some experimental studies (Coa et al., 1987; Pourjafar et al., 2004; Jafari-Dehkordi et al. 2011).

The prognostic value of cTnI concentrations in sheep with ARLA deserves further investigations. Studies on the prognostic significance of cTnI concentrations in human patients with non-primary

cardiac disorders have also found that cTnI can predict disease outcome (Wang et al., 2009; Ilva et al., 2010). Long-term follow-up of serum cTnI concentrations in sheep with lactic acidosis would also be valuable in assessing the relationship between acidosis and myocyte damage.

## CONCLUSION

In conclusion, this study has demonstrated that sheep with acute ruminal lactic acidosis have higher serum cTnI concentration compared to clinically healthy sheep,

which confirms the occurrence of some degree of cardiac damage. Serum cTnI concentrations may compose an important indicator to determine the prognosis of sheep with clinical signs of ARLA, although further study is needed to support this hypothesis.

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