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Evaluation of oxidant-antioxidant status, serum cytokine levels and some cardiac injury biomarkers in acute ruminal lactic acidosis in goats

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ABSTRACT

Fifteen goats clinically diagnosed with acute ruminal lactic acidosis (ARLA group) and fifteen healthy goats (control group) were included in the study. The samples of whole blood and rumen liquor were collected for the analysis of oxidative stress indices, serum cytokines, cardiac biomarkers and intraruminal changes. The acidotic goats showed moderate to severe dehydration, elevation of pulse and respiration rates, ruminal atony, reduced population of ruminal protozoa and a marked increase in ruminal glucose concentration. Compared to the control group, ARLA group had a significantly higher ($p < 0.0001$) level of malondialdehyde (MDA), a marker of lipid peroxidation (LPO), whereas the activities of catalase (CAT) and superoxide dismutase (SOD), total antioxidant capacity (TAC) and levels of reduced glutathione (GSH) were significantly lower ($p < 0.0001$) in ARLA goats. The secretion of tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β (pro-inflammatory cytokines) and an anti-inflammatory cytokine, interleukin (IL)-10, was significantly increased ($p < 0.0001$) in goats with ARLA, with the highest increase in TGF-β. A significant elevation ($p < 0.001$) of creatine kinase isoenzyme MB (CK-MB) and lactate dehydrogenase (LDH) was detected in goats with ARLA as compared to healthy goats. These results highlight the occurrence of oxidative stress with a notable decrease in antioxidant defenses, increased expression of several inflammatory cytokines and the myocardial injury induced during ARLA in goats.

1. Introduction

Acute ruminal lactic acidosis (ARLA) is a commonly encountered gastrointestinal disease of goats characterised by high mortality rates (Oliveira et al., 2009). The clinical form of disease is characterized by abnormal ruminal distension, rumen stasis, anorexia, diarrhoea, lethargy, and in severe cases, death of the animal. The morbidity rate varies from 10 to 50 percent, however, case fatality rate may be as high as 90 percent in untreated goats while it is only 30–40 percent after treatment (Rahman et al., 2014). ARLA is a management problem and occurs primarily due to the rapid ingestion of great amounts of readily fermentable carbohydrates, like barley, wheat, potatoes, and sugar beet and excessive accumulation of lactic acid in the rumen. Consequently, it leads to decrease in rumen pH below 5.5 and abnormal proliferation of Gram-positive bacteria, such as *Streptococcus bovis* and *Lactobacillus* spp. (Xu and Ding, 2011; Gozho et al., 2007; Garry, 2002). Lactic acid isomers along with lipopolysaccharide (LPS) released after the death of Gram-negative bacteria in goats with ARLA, are absorbed into the blood circulation and lead to endotoxic shock, cardiovascular collapse, renal failure, liver abscess, muscular weakness, thiamine deficiency, laminitis and finally, death (Kirbas et al., 2014).

The excessive production of highly toxic reactive oxygen species (ROS) including superoxide anion, hydrogen peroxide and hydroxyl radicals, during physiological and metabolic processes, causes oxidative stress (Celi et al., 2011). ROS causes peroxidation of several biomolecules such as DNA, proteins, and carbohydrates, however, polyunsaturated fatty acids (PUFAs) of cell membranes are the main targets (Bogdan et al., 2000). The lipid peroxida-

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tion (LPO) of PUFAs results in the production of LPO end-products, mainly malondialdehyde (MDA). MDA is the most frequently used indicator of an increased oxidative stress in the body (Gawel et al., 2004). The most important enzymatic antioxidants which combat cellular oxidative damage include superoxide dismutase (SOD) and catalase (CAT) while non-enzymatic antioxidant is reduced glutathione (GSH) (Kirbas et al., 2014). There have been no studies on oxidant-antioxidant status in goats clinically infected with ARLA.

The circulating LPS can elicit local and systemic inflammatory responses (Khafipour et al., 2009). Moreover, it leads to the synthesis of excessive pro-inflammatory cytokines in the liver and a significant increase in their blood concentrations, such as interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α (Vels et al., 2009). Endotoxaemia, which is recorded in ARLA, can interfere with cardiovascular functions and thus, cardiac biomarkers and isoenzymes show significant alterations during ARLA associated endotoxaemia. The levels of circulating enzymes, such as creatine kinase isoenzyme MB (CK-MB), cardiac troponin I (cTnI), lactate dehydrogenase (LDH) and homocysteine (Hcy) are elevated (Chalmeh et al., 2013). The enzymes CK-MB and LDH are present in cytoplasm with their major activities in myocardium, skeletal muscle, liver, kidney, and erythrocytes. Their increased blood concentrations are indicative of cellular injury and inflammatory changes in tissues, especially heart (Chang et al., 2013). There are only few reports regarding the analysis of serum cardiac biomarkers during ARLA in goats. Therefore, the objective of the current study was to evaluate the blood levels of LPO and antioxidant defenses along with the serum concentrations of cytokines and cardiac injury biomarkers in goats naturally suffering from ARLA.

2. Materials and methods

2.1. Area of study

The present study was conducted at Referral Veterinary Polyclinic, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh and in adjoining goat farms of Bareilly.

2.2. Animals and clinical examination

Fifteen goats, of either sex and breed, with clinical signs of ARLA (ARLA group) were used for this study and an equal number of healthy goats, free from any clinical abnormality, served as the control group. The goats of both the groups were subjected to a routine clinical examination including recording of rectal temperatures, respiratory and pulse rates, strength of ruminal contractions, examination of mucous membranes and degree of dehydration.

2.3. Sampling

Ten ml of ruminal fluid, for examination of odor, color, protozoal motility, pH, and glucose, was collected from all the goats with ARLA as well as healthy goats through ruminocentesis. A 16G needle with a disposable syringe was used to withdraw rumen liquor from the ventral part of left flank region. The collected sample was immediately sent to the laboratory and pH analysis was done within two minutes. After straining, it was used for the estimation of ruminal protozoal motility and glucose concentration. The strained ruminal samples were deproteinised using 10 percent zinc sulphate and 0.5 N sodium hydroxide solutions (1:10), centrifuged for 15 min at 3000 rpm and after separation of supernatant, were stored at −20 °C till further analysis.

Jugular blood samples were taken in sodium fluoride and disodium EDTA containing vacutainers for the estimation of blood glucose and other hemato-biochemical parameters, respectively. After centrifugation, plasma was separated and kept at −20 °C till analysed. EDTA blood was used for complete blood count (CBC) and the preparation of 10 percent haemolysate and subsequent determination of oxidative stress indices.

2.4. Ruminal fluid odor, color, pH, protozoal motility and glucose

The physical examination was undertaken to classify odor as normal (aromatic) or abnormal (acidic) and color as normal (olive/brownish green) or abnormal (milky grey). Rumen pH was measured using commercially available pH indicator paper strips and categorised as very low (4–4.9), moderately low (5–5.9) and normal (6–7). Three to four drops of rumen liquor were kept on a clean glass slide and examined under low power objective. The protozoal motility was classified as normal, reduced, or absent (Kirbas et al., 2014). Rumen glucose level was measured by O'toludine method (Hultmann, 1959), using a commercially available kit and values were expressed as mg/dl.

2.5. Blood pH

A wide range pH indicator paper was used to measure the blood pH. The pH paper was dipped into serum and the color of the strip was matched with the standard colors.

2.6. Blood biochemistry

2.6.1. MDA estimation

LPO level in 10 percent haemolysate was determined by thiobarbituric acid (TBA) reaction according to the method of Placer et al. (1966) and the values were expressed in terms of nmol MDA per ml.

2.6.2. SOD estimation

SOD activity in 10 percent haemolysate was measured using nitroblue tetrazolium (NBT) as a substrate according to the method of Marklund and Marklund (1974) and the values were expressed as units/mg of haemoglobin.

2.6.3. CAT estimation

Catalase activity was estimated in 10 percent haemolysate after appropriate dilution by the method of Cohen et al. (1970) and the values were expressed as units/mg of haemoglobin.

2.6.4. Reduced GSH estimation

The activity of reduced GSH was estimated in the RBC suspension by dithio-bis-2-nitro benzoic acid (DTNB) method as per the procedure of Prins and Loos (1969) and the values were expressed as mmol per litre.

2.6.5. Total antioxidant activity (TAC) estimation

TAC was measured by the method of Miller and Rice-Evans (1997) by using a commercial test kit (Sigma-aldrich, USA) and the values were expressed as mmol per litre.

2.6.6. Serum pro-inflammatory and anti-inflammatory cytokines

The pro-inflammatory cytokines quantified in ARLA goats included TNF-α and transforming growth factor (TGF)-β. Serum TNF-α concentration was determined by using a commercially available goat-specific ELISA kit (CSB-E09811G, Cusabio Life Sciences, China) and the results were expressed as pg/ml. Serum TGF-β concentration was determined by using a commercially available goat-specific ELISA kit (CSB-E09811G, Cusabio Life Sciences, China) and the results were expressed as pg/ml.

Likewise, serum IL-10, an anti-inflammatory cytokine, concentration was determined by using a commercially available