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Serum proteinogram in sheep with acute ruminal lactic acidosis

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ABSTRACT

The electrophoretic fractionation represents one of the most reliable methods for the identification of blood proteins in ruminants. The aim of this study was to evaluate the serum proteinogram of sheep with acute ruminal lactic acidosis (ARA) using the SDS-PAGE electrophoresis technique. Ten Santa Inês ewes were used and blood was collected to establish the basal values for induction of ARA. Sucrose was administered orally in a single dose of 15 g/kg body mass. After the administration, blood samples were obtained at the following moments: 4, 8, 12, 16, 20, 24, 28, 32, 36, 48, 72, 96, 120 and 144 h. Subsequently, samples were obtained every seven days for three further weeks, until complete one month. The total of 13 proteins were identified: immunoglobulins A and G, ceruloplasmin, transferrin, albumin, α1-antitrypsin, haptoglobin, α1-acid glycoprotein, proteins of molecular weight 95, 46, 36 and 31 kDa. The increase of haptoglobin from 08 h coincides with the ruminal pH decrease, possibly due to the death of Gram negative bacteria and also the inflammatory process on the rumen. Fibrinogen was presented on highest mean at 48 h and returned to normal with 144 h. We can conclude that changes in serum levels of acute phase proteins can assist the clinical evaluation and diagnosis of ARA in sheep.

1. Introduction

According to metabolic function, the serum proteins can be divided into categories such as the acute phase (APP), immunoproteins, complement, coagulation and transport proteins. The APP alter the concentration in animals subject to challenges such as infection, inflammation, surgical trauma and stress. Its activities contribute to host defense by neutralizing inflammatory agents, reducing the extent of tissue damage and acting on tissue repair, and these proteins may change their serum concentrations up to 25% in response to stimulation of pro-inflammatory cytokines [1]. In cattle, the major knows APP are serum amyloid A (SAA), haptoglobin (Hp) and fibrinogen (Fb) [2–4].

Tissue injury, infection or exposure to pro-inflammatory molecules such as lipopolysaccharide (LPS) results in an immediate defense mechanism that is not specific, named acute phase response (APR). The APR involves both local and systemic components and a complex mechanism of many cell types and organs as well as other mediators and cytokines [5].

The electrophoretic fractionation is one of the most reliable blood protein identification method. The technique of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) has been widely used to evaluate the APP of inflammatory response, contributing to the diagnosis and prognosis of several diseases, such as equine laminitis [6], equine acute abdomen [7], pneumonia in calves [8] and mastitis in ewes [9].

Acute ruminal lactic acidosis (ARA), is a ruminal fermentative disorder with acute indigestion resulting from the sudden digestion of foods containing easily fermentable carbohydrates, such as barley, wheat, sugar beet and sugar in high quantities in cattle and sheep [10–12]. The systemic acidosis influences on cellular and humoral immunity [13,14] and it is known that ruminal acidosis is particularly present in dairy cows during the transition period, a period in which other metabolic disorders such as ketosis and hypocalcemia are prevalent [15,16]. Dairy cows are susceptible to the occurrence of subacute ruminal acidosis (SARA) or ARA, besides the occurrence of metabolic acidosis due to the high concentrations of carbohydrate diet used in these animals. Several studies have shown that SARA leads to increases in serum concentrations of SAA and Hp in this species [17–20].

The serum concentration of APP can provide useful quantitative information for study and diagnosis of metabolic diseases, and monitoring the therapy response [21]. Studies relating the elec-

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trophoretic profile of serum proteins in ruminants and the occurrence of ARA have been described on literature [22,23] however, we found no similar study in sheep. The number of sheep in Brazil is constantly growing, and intensive production models are used in order to obtain short-term weight gain goals due to the increase of animal confined and susceptible to various diseases. This study aimed to evaluate the serum proteinogram including the acute phase response in sheep with experimentally induced ARA.

2. Materials and methods

2.1. Study design

For induction of ARA, 10 adult, healthy, non-pregnant Santa Inês ewes, fed exclusively with grass, were used. All animals were dewormed and fed in a basal diet with coast cross hay (Cynodon dactylon), they also had free access to drinking water and mineral salt mixture. The adaptation period lasted 30 days. At the beginning of the experiment (Day [D] -15, -7 and 0), blood was collected from all animals to establish the basal values for serum proteinogram analysis, and the mean values were calculated. The ARA induction was preconized conformed as described by Sabes [24]. After the sucrose administration, blood samples were obtained at the following intervals: 4, 8, 12, 16, 20, 24, 28, 32, 36, 48, 72, 96, 120 and 144 h. Subsequently, samples were obtained every seven days for three further weeks until complete one month, and frozen at −20 °C.

2.2. SDS-PAGE electrophoresis analysis

Blood samples were obtained by puncture of the jugular vein after using vacuum system (BD Vacutainer™) with multiple collection needle 25 × 8 mm and sterile plastic tube without anticoagulant and 10 mL capacity. The samples were centrifuged at 2500*g* for 10 min to obtain the serum fraction. Serum proteins were fractionated by means of SDS-PAGE gel electrophoresis [25]. Concentration of protein fractions was determined by use of computerized densitometry (Shimadzu CS 9301, Shimadzu Corporation, Tokyo, Japan). Proteins were identified by means of reference markers (Sigma Chemical Company, St Louis, MO, USA) with molecular weights of 24,000 Da, 29,000 Da, 36,000 Da, 45,000 Da, 55,000 Da, 66,000 Da, 97,000 Da, 116,000 Da and 205,000 Da. Serum protein concentrations (mg/dL) were determined by multiplying the percentage of each fraction by the total serum protein (TP) concentration obtained by semi-automatic spectrophotometer (Labquest, Labtest, Brazil) using the biuret method.

Fibrinogen was determined by heat precipitation [26] and measured using a refractometer (Atago T2-NE Clinical, Atago Co. Ltd., Japan). These analyses were performed at the Research Support Laboratory, Department of Veterinary Clinic and Surgery, School of Agrarian and Veterinary Sciences, São Paulo State University, Jaboticabal, Brazil. This study received approval from the Ethics Comittee of FCAV/Unesp, Jaboticabal, São Paulo, Brazil (Protocol n°. 03888/14).

2.3. Data analysis

The data were submitted to variance analysis with repeated measures in which the factor tested was the harvest (18 levels for serum protein fractions values and ten levels for fibrinogen values), with the animals constituting the 10 blocks. A regression analysis (linear, quadratic and cubic) was performed using the computer program General Linear Models Procedure (GLM). The means were subjected to analysis by Tukey test at 5% significance.

3. Results

Thirteen proteins were identified using the SDS-PAGE method: Immunoglobulin A (IgA) and G (IgG – light (IgGl) and heavy (IgGh) chains), ceruloplasmin (Cp), transferrin (Tf), albumin (Ab), α1-antitrypsin (α1-AT), haptoglobin (Hp), α1-acid glycoprotein (α1-GA), and proteins of molecular weight 95 (MWP95), 46 (MWP46), 36 (MWP36) and 31 (MWP31) kDa.

The following proteins were not associated with time ($p > 0.05$): IgA, IgGl, Tf, α1-AT, α1-GA, MWP46, MWP36 and MWP31. Time showed cubic regression with the values of Cp (on the Tukey test the means showed a significant difference at 72, 96 and 120 h), Al (significant difference was observed with 12, 16, 20 and 24 h on Tukey Test); Hp (only with 72 h there was significant difference by Tukey test); IgGl (no significant difference was observed at Tukey Test), MWP95 (Tukey Test showed no significant difference), TP (difference was observed at 12, 16, 20 and 24 h) and Fb (significant difference observed at 48, 72, 96 and 120 h). The mean, standard deviation and coefficient of variation (CV) of the serum proteinogram are shown on Tables 1 and 2. The graphical representations of the regression analysis of the proteins with statistical significance were presented at Fig. 1.

4. Discussion

All animals showed clinical signs of ARA and also metabolic acidosis as previously described by Sabes [24] such as apathy, appetite loss, bilateral abdominal distension, ruminal atony, dehydration, tachycardia, tachypnea and five animals developed acute laminitis clinically noticed. Other analyses were performed such as arterial blood hemogasometry, evaluation of urinary pH and lactate blood levels during the experimental period to confirm the ruminal and metabolic acidosis. Although the literature says that mortality cases are extremely common in animals with ARA and metabolic acidosis [27], no animal was treated during the period and no animal died in this experiment.

The inflammation resulting from ARA and some clinical signs can be noticed through alterations beyond serum protein concentrations. The TP indicates changes from 08 h, with the hightest value at 12 h, and at the same moment the animals showed signs of dehydration and hemoconcentration, resulting from the fluid influx from the blood stream into the rumen. At 36 h, the TP values returned to normal, remaining until the end of the observation period. These data do not corroborate with the study conducted by Vieira et al. [28] in which the TP variations were not observed until 48 h after induction of ARA in small ruminants.

Some studies have shown that lactic acid presented in high levels on the rumen and on systemic circulation is the key factor on triggering the inflammatory response in cases of ARA [29–32]. The ruminitis, presence of LPS and other pro inflammatory substances on systemic circulation can lead to the increase on the expression of APP. Jacobsen et al. [31] reported that LPS is a potent inducer of APP and it is known that LPS concentrations on the ruminal fluid increase during lactic acidosis, and also may occur the migration from the rumen to the systemic circulation, stimulating the hepatic synthesis of APP [33–35]. Furthermore, the acid pH and certain organic acids can activate components of humoral and cellular response, improving the inflammatory process [29,30,32] as observed after ARA induction in heifers using oligofructose.

It is known that the APR in ruminants is different in relation to other species, and the Hp is one of the most important APP. In healthy bovine serum, the Hp concentration is less than 20 mg/L, but it may increase to values higher than 2 g/L in two days in cases of infections [36]. In cattle, the Hp is effective on the diagnosis and