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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, α 1-antitrypsin, haptoglobin, α 1-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.

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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 known 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 2500g 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 Committee 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 (IgG_L) and heavy (IgG_H) chains), ceruloplasmin (Cp), transferrin (Tf), albumin (Ab), α 1-antitrypsin (α 1-AT), haptoglobin (Hp), α 1-acid glycoprotein (α 1-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, IgG_L, Tf, α 1-AT, α 1-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); IgG_L (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 highest 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

Table 1

Mean \pm standard deviation and coefficient of variation (CV) of serum protein concentrations (mg/dL) by SDS – PAGE, fibrinogen - Fib (mg/dL) obtained by heat precipitation and total serum protein - TP (g/dL) obtained by biuret method of sheep with ARA. Harvest moments: control^{*}, 4 h, 8 h, 12 h, 16 h, 20 h, 24 h, 28 h, 32 h, 36 h, 48 h, 72 h, 96 h, 120 h, 144 h, 2nd, 3rd and 4th weeks after oral sucrose administration.

	TP (g/dL)	IgA (mg/dL)	Ceruloplasmin (mg/dL)	Transferrin (mg/dL)	Albumin (mg/dL)	Haptoglobin (mg/dL)	Fib (mg/dL)
Control [*]	7,06 \pm 0,13	0,016 \pm 0,007	0,056 \pm 0,010	0,387 \pm 0,021	4,414 \pm 0,102	0,012 \pm 0,002	0,15 \pm 0,02
04 h	6,60 \pm 0,19	0,008 \pm 0,002	0,054 \pm 0,007	0,356 \pm 0,032	4,168 \pm 0,112	0,014 \pm 0,002	–
08 h	7,19 \pm 0,18	0,016 \pm 0,005	0,060 \pm 0,009	0,368 \pm 0,041	4,621 \pm 0,141	0,022 \pm 0,005	–
12 h	7,85 \pm 0,18	0,016 \pm 0,005	0,080 \pm 0,013	0,418 \pm 0,026	4,808 \pm 0,151	0,039 \pm 0,020	–
16 h	7,69 \pm 0,15	0,011 \pm 0,003	0,057 \pm 0,011	0,375 \pm 0,043	4,987 \pm 0,135	0,020 \pm 0,004	–
20 h	7,70 \pm 0,15	0,019 \pm 0,008	0,059 \pm 0,008	0,344 \pm 0,060	5,152 \pm 0,128	0,023 \pm 0,005	–
24 h	7,66 \pm 0,16	0,011 \pm 0,003	0,050 \pm 0,007	0,384 \pm 0,047	5,084 \pm 0,108	0,033 \pm 0,007	0,22 \pm 0,04
28 h	7,24 \pm 0,16	0,013 \pm 0,005	0,059 \pm 0,006	0,376 \pm 0,042	4,750 \pm 0,105	0,034 \pm 0,007	–
32 h	7,12 \pm 0,11	0,010 \pm 0,004	0,064 \pm 0,008	0,345 \pm 0,049	4,681 \pm 0,096	0,039 \pm 0,007	–
36 h	7,15 \pm 0,15	0,027 \pm 0,013	0,079 \pm 0,009	0,391 \pm 0,038	4,646 \pm 0,110	0,028 \pm 0,005	–
48 h	6,99 \pm 0,14	0,008 \pm 0,004	0,077 \pm 0,011	0,433 \pm 0,049	4,567 \pm 0,075	0,065 \pm 0,017	0,54 \pm 0,06
72 h	6,76 \pm 0,12	0,016 \pm 0,006	0,100 \pm 0,010	0,403 \pm 0,028	4,353 \pm 0,118	0,093 \pm 0,030	0,48 \pm 0,07
96 h	6,61 \pm 0,11	0,028 \pm 0,008	0,097 \pm 0,009	0,416 \pm 0,024	4,327 \pm 0,172	0,063 \pm 0,028	0,44 \pm 0,04
120 h	6,59 \pm 0,13	0,009 \pm 0,003	0,103 \pm 0,011	0,429 \pm 0,025	4,100 \pm 0,096	0,051 \pm 0,020	0,44 \pm 0,05
144 h	6,77 \pm 0,09	0,019 \pm 0,010	0,083 \pm 0,012	0,433 \pm 0,033	4,303 \pm 0,083	0,033 \pm 0,015	0,32 \pm 0,04
2nd week	6,72 \pm 0,20	0,012 \pm 0,005	0,063 \pm 0,008	0,410 \pm 0,034	4,193 \pm 0,133	0,053 \pm 0,041	0,22 \pm 0,05
3rd week	7,10 \pm 0,12	0,008 \pm 0,003	0,062 \pm 0,010	0,379 \pm 0,025	4,519 \pm 0,115	0,014 \pm 0,002	0,10 \pm 0,04
4th week	7,06 \pm 0,14	0,017 \pm 0,007	0,077 \pm 0,014	0,392 \pm 0,019	4,478 \pm 0,072	0,015 \pm 0,001	0,04 \pm 0,02
CV	4,68	117,24	34,18	21,17	6,44	115,77	45,62

^{*} Control: mean of the harvests -15, -7 days and immediately before the sucrose administration.

Table 2

Mean \pm standard deviation and coefficient of variation (CV) of serum protein concentrations (mg/dL) by SDS – PAGE of sheep with ARA. Harvest moments: control^{*}, 4 h, 8 h, 12 h, 16 h, 20 h, 24 h, 28 h, 32 h, 36 h, 48 h, 72 h, 96 h, 120 h, 144 h, 2nd, 3rd and 4th weeks after oral sucrose administration.

	α 1AT (mg/dL)	α 1 GA (mg/dL)	IgG1 (mg/dL)	IgGh (mg/mL)	MWP95 (mg/dL)	MWP46 (mg/dL)	MWP36 (mg/dL)	MWP31 (mg/dL)
Control [*]	0,165 \pm 0,011	0,031 \pm 0,003	0,461 \pm 0,055	1,100 \pm 0,250	0,012 \pm 0,002	0,003 \pm 0,008	0,005 \pm 0,009	0,007 \pm 0,001
04 h	0,175 \pm 0,012	0,034 \pm 0,004	0,474 \pm 0,064	1,045 \pm 0,100	0,009 \pm 0,001	0,005 \pm 0,001	0,006 \pm 0,001	0,013 \pm 0,002
08 h	0,187 \pm 0,008	0,034 \pm 0,005	0,454 \pm 0,056	1,105 \pm 0,082	0,010 \pm 0,002	0,004 \pm 0,001	0,005 \pm 0,009	0,012 \pm 0,002
12 h	0,199 \pm 0,011	0,044 \pm 0,005	0,541 \pm 0,076	1,243 \pm 0,075	0,012 \pm 0,002	0,004 \pm 0,001	0,006 \pm 0,009	0,014 \pm 0,003
16 h	0,209 \pm 0,128	0,036 \pm 0,004	0,553 \pm 0,076	1,104 \pm 0,073	0,012 \pm 0,002	0,003 \pm 0,007	0,005 \pm 0,001	0,019 \pm 0,004
20 h	0,217 \pm 0,018	0,045 \pm 0,005	0,499 \pm 0,079	1,028 \pm 0,087	0,013 \pm 0,004	0,005 \pm 0,001	0,005 \pm 0,007	0,015 \pm 0,003
24 h	0,223 \pm 0,012	0,041 \pm 0,005	0,546 \pm 0,067	0,893 \pm 0,123	0,011 \pm 0,002	0,002 \pm 0,005	0,005 \pm 0,008	0,013 \pm 0,002
28 h	0,211 \pm 0,009	0,062 \pm 0,027	0,489 \pm 0,057	0,997 \pm 0,053	0,011 \pm 0,002	0,003 \pm 0,007	0,004 \pm 0,005	0,013 \pm 0,003
32 h	0,207 \pm 0,016	0,038 \pm 0,004	0,482 \pm 0,040	0,982 \pm 0,065	0,012 \pm 0,003	0,006 \pm 0,003	0,005 \pm 0,002	0,011 \pm 0,002
36 h	0,192 \pm 0,011	0,028 \pm 0,005	0,520 \pm 0,060	0,996 \pm 0,061	0,034 \pm 0,018	0,003 \pm 0,009	0,002 \pm 0,003	0,025 \pm 0,018
48 h	0,196 \pm 0,014	0,036 \pm 0,006	0,480 \pm 0,045	0,941 \pm 0,064	0,012 \pm 0,002	0,004 \pm 0,001	0,003 \pm 0,006	0,010 \pm 0,001
72 h	0,188 \pm 0,013	0,040 \pm 0,005	0,462 \pm 0,043	0,872 \pm 0,057	0,012 \pm 0,003	0,007 \pm 0,002	0,005 \pm 0,001	0,014 \pm 0,003
96 h	0,196 \pm 0,011	0,026 \pm 0,003	0,425 \pm 0,055	0,885 \pm 0,076	0,018 \pm 0,004	0,005 \pm 0,001	0,002 \pm 0,004	0,014 \pm 0,003
120 h	0,187 \pm 0,011	0,032 \pm 0,006	0,420 \pm 0,054	0,936 \pm 0,059	0,025 \pm 0,005	0,004 \pm 0,001	0,002 \pm 0,006	0,014 \pm 0,001
144 h	0,195 \pm 0,016	0,032 \pm 0,006	0,440 \pm 0,044	0,959 \pm 0,050	0,024 \pm 0,005	0,003 \pm 0,009	0,004 \pm 0,005	0,012 \pm 0,002
2nd week	0,186 \pm 0,013	0,031 \pm 0,005	0,495 \pm 0,053	0,926 \pm 0,108	0,024 \pm 0,005	0,004 \pm 0,005	0,004 \pm 0,007	0,012 \pm 0,002
3rd week	0,203 \pm 0,018	0,039 \pm 0,005	0,496 \pm 0,056	1,068 \pm 0,078	0,021 \pm 0,004	0,004 \pm 0,001	0,007 \pm 0,001	0,011 \pm 0,002
4th week	0,194 \pm 0,027	0,034 \pm 0,005	0,486 \pm 0,047	1,082 \pm 0,082	0,020 \pm 0,005	0,007 \pm 0,002	0,006 \pm 0,009	0,019 \pm 0,002
CV	14,77	59,94	21,41	18,59	102,19	95,60	63,54	110,21

^{*} Control: mean of the harvests -15, -7 days and immediately before the sucrose administration.

prognosis of diseases such as mastitis, enteritis, peritonitis, pneumonia, endocarditis and endometritis [37]. On the present study, the Hp values increased from 08 h, remaining changed until the second week and then returning to values similar to the control moment. The increase in Hp values was associated with the ruminal acid ambient [38], which increased the lysis of Gram-negative bacteria, LPS release and its translocation into the systemic circulation, resulting in inflammatory response [17,39]. However Huo et al. [40] showed that only LPS cannot be the sole inducer of inflammatory response and subsequently increasing the concentration of Hp. Skinner and Roberts [41] confirmed that Hp was a better indicator of bacterial infection than the white blood cell count. Danscher et al. [23] compared the APR in heifers with ARA induced with silage, indicating that the inflammatory response also depends on pre-existing conditions in the rumen, which can affect the degree of ruminitis, changing the bacterial population and the amount of lactic acid produced.

The α 1-AT showed an increase from 08 h, with the highest mean obtained at 24 h. This protein has anti-proteinase activity, and the values increase can be explained by their characteristic to inhibit proteases released by phagocytes or pathogens, limiting the tissue damage at the site of inflammation [42].

The use of Cp as a marker of inflammation is not as common as the use of other APP as Hp [36]. In the present study, this APP presented a different dynamic of other proteins, and its mean showed an increase from 32 h, with the higher mean founded at 120 h. This increase is considered late. On the compiled literature there was no study describing the Cp alterations in animals with ARA. Pfeffer and Rogers [43] observed an increase on concentration of Cp in sheep with pneumonia and bronchial obstruction, and this increase was detected on early days. Pereira et al. [44] and Costa et al. [9] studied lambs with copper poisoning and induced acute mastitis in sheep, they also reported an increase in this protein. The study of Cp is little explored, however Murata et al. [36]

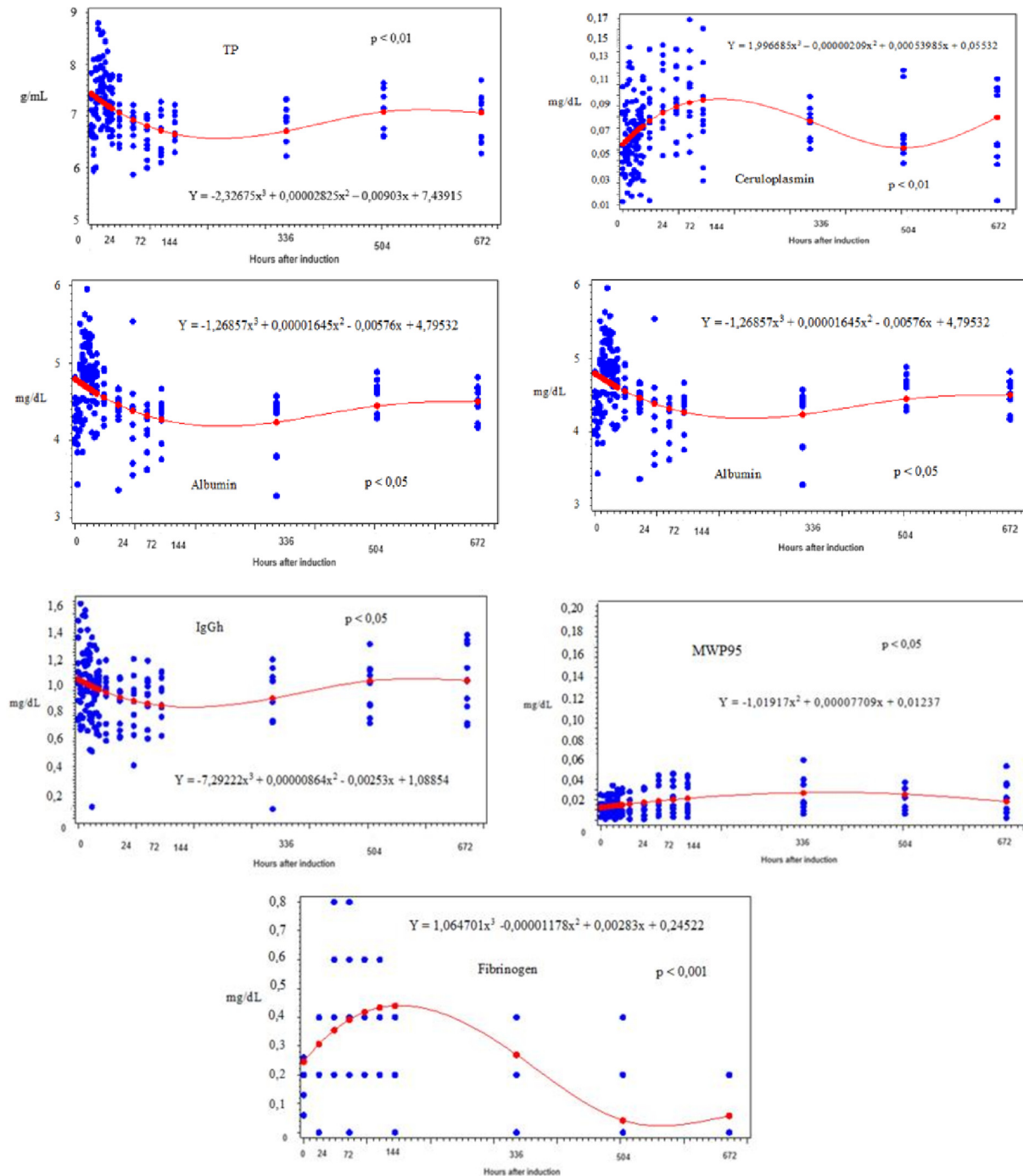


Fig. 1. Graphical representations of the regression analysis of the proteins with statistical significance in sheep with acute ruminal lactic acidosis.

believed that this ferroxidase can be used as an indicator of infection in cattle.

Albumin is classified as a negative APP because the need of amino acids for the APR synthesis of positive proteins is greatly increased, making it necessary to readjust the synthesis of hepatic proteins [42]. The highest Ab mean was obtained at 20 h, same period that the animals showed clinical signs of dehydration, returning to values similar to the control with 144 h. Costa et al. [9] induced acute mastitis in sheep and described a slight increase in Ab at 96 h after infection and then values returned to normal, coinciding with the clinical improvement of animals. Another protein that is also classified as negative is Tf, which has the characteristic

of bind iron molecules, inhibiting the multiplication of some virus, bacteria and fungi and limiting their access to iron [42]. The increase in serum concentration during the inflammatory process can be used as a non-specific immune response against pathogenic microorganisms [36].

The IgA values did not change significantly, but it is believed that this immunoglobulin can protect surfaces against infections [9]. IgG also showed no significant difference, but in a study conducted by Costa et al. [9], this immunoglobulin is considered one of the responsible for the control of various infections. In cattle, the α 1-GA is known to be an important anti-inflammatory protein and capable of modulating monocytes and polymorphonuclear

cells in the inflammatory response, including chemotaxins, free reactive oxygen radicals and degranulation of other cells [45]. Eckersall [21] suggested that this protein might be used as a marker for chronic diseases in sheep, and also described an increase in its concentration in cases of caseous lymphadenitis. In cattle this APP shows an increase at 24 to 72 h following inflammatory conditions [46], but in dairy cattle with traumatic reticuloperitonitis this APP showed high values in animals with acute inflammation compared to chronic [47]. In this study, the highest value was found with 28 h, returning to normal with 120 h. This protein may modulate the recruitment and platelet aggregation, reducing local inflammation and preventing changes on the injured tissue [48].

Fibrinogen is classified as a minor/moderate APP in cattle, and in response to inflammation and infection can increase their values in two to three times [37]. The plasma concentration rises in the stimulatory action of interleukins (IL-1 and 6) of the necrotic factor released during the inflammatory process [49]. According to Schalm et al. [50] during the acute inflammatory process, the plasma concentration of this substance increases, reaching the highest values between the fifth and seventh day. The stimulation of Fb synthesis according to Jain [51] occurs in six to eight hours after injury, and in this study the maximum values were obtained after 48 h of induction; returning to normal after 144 h. The obtained data showed that Fb can be used as a marker of acute inflammatory response in sheep as described by Pfeffer and Rogers [43]; Pfeffer et al. [52], Costa et al [9], Gonzalez et al. [22], Danscher et al. [23]. But authors like Vieira et al. [28] stated that Fb values do not change after induction of acidosis through sucrose or diets with high concentrate values [20,22,38]. Kirbas et al. [12] stated that the Fb concentration is preferable as a marker to APR as compared to the leukogram in evaluating traumatic inflammatory processes in cattle, such as traumatic reticuloperitonitis.

On the compiled literature there is no mention about MWP95, MWP46, MWP36 and MWP31. Therefore their functions are unknown, however, several of them show potential for use as APPs. A variety of new APPs and their mediators can be introduced to veterinary science due to a continuous request for those substances and intensive research about their applications, thus accelerating the understanding of innate immune mechanisms and pathophysiology of infection and inflammation [36].

5. Conclusions

Changes in serum levels of total protein, haptoglobin, ceruloplasmin, fibrinogen and acute negative phase proteins can assist the clinical evaluation and diagnosis of acute ruminal lactic acidosis in sheep. The SDS-PAGE electrophoresis technique allows the identification of a variety of serum proteins, some of them with unknown functions.

Competing interests

The authors declare that they have no competing interests.

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