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Predictive Elements of Obstructive Urolithiasis in Sheep*

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ABSTRACT

***Background*:** Urolithiasis is an economically important disease that has considerable significance for sheep farming. With the tissue and biochemical changes resulting from the development of this disease, metabolic disorders and immune response are established. Hemogasometric evaluation allows the identification of systemic acid-base imbalances quickly. Acute phase proteins (APP) have in the last two decades had become the biomarkers of choice in human and veterinary medicine. To date, no biomarker studies have been published for sheep with obstructive urolithiasis. Thus, this study aimed to analyze the hemogasometric kinetics in obstructive urolithiasis in sheep and the APP that can be used as early biomarkers in this disease.

***Materials, Methods & Results*:** In this study, 14 healthy male Santa Inês sheep, aged approximately 90 days, were fed on calculogenic diet for 120 days. The sheep were examined weekly to observe the clinical signs. Blood and urine analysis were also performed. For comparative analysis purposes, at the end of the experiment, sheep that developed obstructive urolithiasis were extracted from the initial experimental group D1 (without urolithiasis) and moved to the second experimental group D2 (with urolithiasis). In the pre-experimental period and on the day of slaughter, venous blood was sampled for hemogasometric tests, with a maximum time of 15 minutes between collection and analysis to ensure the reliability of the results obtained. The pH, pCO_2, pO_2, EB, tCO_2, HCO_{3-}, $stHCO_{3-}$, tHb, sO_2 and Hct, Na^+, K^+ and Ca^{2+} ions were quantified. To identify and measure immunoglobulins (A and G) and APP, samples from sheep that developed obstructive urolithiasis (D2) were analyzed. Blood samples were harvested weekly until the clinical manifestation of the disease, totaling 16 samples, of with IgA, IgG, ceruloplasmin, transferrin, albumin, α1-antitrypsin, haptoglobin and α1-acid glycoprotein concentrations were measured. Elevation of pCO_2 was observed between D1 and D2, but there was a significant difference ($P < 0.05$) only in the final moments (FMs). Although EB, tCO_2, HCO_{3-}, $stHCO_{3-}$ increased between moments in the same group and between groups at the same time, significant differences were recorded only in the FMs. Higher values were observed for Na^+, K^+ in the FMs. The APP of sheep that developed the disease oscillated between moments, however, significant difference ($P < 0.05$) over time was observed only in haptoglobin and transferrin.

***Discussion*:** The disease occurred in five of the 14 studied sheep, demonstrating the effectiveness of the formulated diet in inducing the disease. Through the analysis of blood gases, plasma bicarbonate concentration and excess base or deficit it was possible to diagnose disturbances in acid base balance, characterizing a picture of metabolic alkalosis in sheep with urolithiasis. Mean pH was not significantly different between groups, but sheep that developed urolithiasis had alkalosis. Final values of tCO_2 and HCO_{3-} indicate the compensatory organic response, that which, together with the analysis of the averages of HCO_{3-} and EB, reflect the metabolic alkalosis picture. The APP have different responsiveness among them. Haptoglobin and transferrin were the most reliable biomarkers among the studied APP to predict obstructive urolithiasis, with transferrin showing atypical behavior, characteristic of positive APP..

Keywords: urinary tract, acid-base balance, transferrin, haptoglobin, small ruminants.

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INTRODUCTION

Monitoring the inflammatory response is a challenge because the signs of inflammation are not always clear [27]. Hemogasometry is an important technique for assessing acid-base balance in various diseases, capable of clarifying severity and predicting clinical evolution [15,26,28]. However, in the acute phase of disease, it may not detect changes suggestive of inflammatory process [1].

Acute phase proteins are early markers of inflammation, capable of providing an alternative means of monitoring animal health, in addition to those commonly used in clinical routine, such as blood count and biochemistry [12]. There is a difference in responsiveness of different APP due to varied inflammatory stimuli. During the acute phase response, the serum concentration of these proteins changes dramatically [3].

Although the feasibility of venous blood samples for hemogasometric examination is short [15] compared to APP dosing [27], both analyzis may provide diagnostic and prognostic information if adequate sampling time is warranted [19,28].

Due to the clinical, surgical and economic importance of urolithiasis in sheep, besides the scarcity of studies on hemogasometric kinetics [8] and on acute phase proteins in urinary disorders [5,7], added to the inexistence of studies on APP in this disease, this study aims to analyze the behavior of these variables in obstructive urolithiasis disease in sheep and APP that can be used as early biomarkers to predict the development of this disease.

MATERIALS AND METHODS

Animals used

Fourteen healthy male (non-castrated) Santa Inês sheep, approximately 90 days old, were used. To maintain animal health and prevent interference from other diseases, parasitological examinations, control of endoparasites with oral sulfaquinoxaline (Sulfaquinoxaline)[1] and sodium closantel (Diantel 10%)[2] and vaccination against clostridiosis (Poly-Star)[3] were performed in the pre-experimental period (15 days).

Facilities, diets and experimental groups

Sheep were confined in the small ruminant experimentation pen of Garanhuns Academic Unit (UAG), of the Federal Rural University of Pernambuco (UFRPE), in individual masonry pens, 2.0 m x 1.0 m, throughout the experimental period. Natural source water was supplied in plastic containers to measure daily consumption. In the pre-experimental phase, the animals received a balanced diet (Table 1), and subsequently, there was a gradual decrease in the roughage: concentrate ratio for 15 days to adapt the rumen microbiota.

Starting the experiment, the sheep received, for 90 days, experimental diet with calculogenic potential, Ca/P ratio 1:2 (Table 1). After this period, the diet was modified to obtain a 1:3 Ca/P ratio, provided for 30 days. Diets were fed fractionally at two times, at 8 and 14 hours, in plastic troughs with dimensions of 50x20x30 cm, and the leftovers were weighed daily to determine consumption.

Table 1. Percent composition of experimental diets.

Ingredient	Pre-experimental diet Roughage/conc. (70/30) Ca/P ratio (1.9:1)	Experimental diet Roughage/conc. (70/30) Ca/P ratio (1:2)
$Tifton_{85}$ Hay	70	30
Ground corn	18.05	56.07
Soybean meal	10	9.6
Anhydrous Dibasic Sodium Phosphate	-	2.2
Mineral supplement *	1,5	1.5

*Ovinofós® Mineral Salt: Zinc (3,800.00 mg), Sodium (147,00 g), Manganese (1,300,00 mg), Cobalt (40,00 mg), Iron (1,800,00 mg), Copper (590,00 mg), Sulfur (18.00 g), Selenium (15.00 mg), Iodine (80.00 mg), Chromium (20.00 mg), Molybdenum (300.00 mg), Calcium (120.00 g), Fluorine (max – 870.00 mg), Phosphorus (87.00 g).

The total confinement period of 119 days was established to ensure urolith formation. For the purpose of comparative analysis, at the end of the experiment those that developed obstructive urolithiasis were extracted from the initial experimental group D1 (without urolithiasis), and moved to started a second group D2 (with urolithiasis).

For experimental safety, the feed ingredients were submitted to bromatological analysis, and the water, to its chemical composition analysis.

Clinical evaluation and sample collection

The sheep were examined weekly, which allowed the immediate identification of urolithiasis [6].

Samples of 2 mL of jugular vein blood were harvested from all sheep in the pre-experimental period and on the day of slaughter for the performance of hemogasometric tests [28] following the care of sample conservation [16]. The maximum time elapsed between collection and blood gas analysis was 15 minutes.

To identify and measure APP with predictive value for urolithiasis, we analyzed the samples of sheep that developed the disease (D2). Blood samples were harvested from all individuals weekly until clinical manifestation of the disease, totaling 16 samples. All blood collections were performed prior to feeding between 6 h and 8 h. To this end, blood was collected by jugular venipuncture after local antisepsis, using vacuum tubes[4] without anticoagulant. After blood coagulation, the samples were centrifuged for ten minutes. The obtained serum was separated by aspiration, and 1 mL aliquots were stored in 2 mL plastic tubes[5] and frozen at -20°C until processing.

Hemogasometric analysis

The pH, pCO_2, pO_2, EB, tCO_2, HCO_{3-}, $stHCO_{3-}$, tHb, sO_2 and Hct were measured in a blood gas analyzer with electrolyte measurement (OPTI CCA-TS)[6], added to the determination of Na^+, K^+ and Ca^{2+} ion values using specific cassette according to the manufacturer's recommendations [4,24]. After the introduction of the blood aliquot in the hemogasometer, the hemoglobin and temperature values were corrected, since the standard value of the device is related to the human species.

Determination of serum concentrations of acute phase proteins

APP analyzis were performed at the Research Support Laboratory of the Department of Veterinary Clinic and Surgery (UNESP-Jaboticabal). Serum total protein (TP) concentrations were obtained by the biuret method, with the aid of reagent kits[7] in a semi-automatic biochemical analyzer. Protein fractionation was performed by polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE). After fractionation, the gel was stained for 10 min in Coomassie Blue solution, followed by washing in 7% acetic acid solution to remove excess dye until the protein fractions became clear. The concentration of these proteins was determined by means of a computerized densitometer[8]. As a reference, a marker solution with molecular weights 29,000, 45,000, 66,000, 97,400, 116,000 and 205,000 daltons (Da) was used, as well as purified proteins[9] - IgA, IgG, ceruloplasmin, transferrin, albumin, α1-antitrypsin, haptoglobin and α1-acid glycoprotein.

Statistical analysis

For hemogasometric analysis, a comparison was made between two groups. Data from each group were subjected to the Shapiro-Wilk normality test. With normality ($P > 0.05$), the two groups were compared by parametric tests. Initially, the variances were compared using the F test. If the variances were equal ($P > 0.05$), both groups were compared using the t test; if not, the groups were compared by the Welch test. When data were not normal, the comparison was performed by means of the Wilcoxon rank sum test. Significant difference was considered when $P < 0.05$. Analyzes were performed using the R software.

For the analysis of APP an evaluation of the effect of time was made. To this end, it was used the methodology of longitudinal data analysis (repeated measures over time) by nonparametric ANOVA [2]. The analysis was performed using the nparLD package, software.

RESULTS

During the 119 days of the experimental period, five of the 14 sheep manifested total obstructive urolithiasis. At the onset of clinical signs, appetite was not affected but was reduced or absent after 24 hours. The disease was clinically manifested by: apathy or restlessness, strangury, contraction and abdominal kicking with exposure and penis licking, postural changes (back arching, pelvic limbs abduction and raised tail), congested glans and urethral process and areas of necrosis and rigid to palpation, as well

as discomfort and sensitivity to the urogenital tract examination. Different degrees of ruminal repletion were observed, ranging from moderately empty to full, and decreased motility. Tympanism was observed in only one of the sheep. Emphasis should be given to the depraved appetite (gnawing cuttings and stall walls) observed in some sheep.

There was dehydration in all sheep, but in varying degrees (from mild to severe), enophthalmia and slightly congested conjunctivae, slight elevation of heart and respiratory rates (124 ± 19 beats/min. and 60 ± 35 movements/min., respectively). Penile urethral rupture occurred in only one animal, which presented anuria, edema and tenderness to palpation of the ventroabdominal region (from xiphoid cartilage to scrotal region) and swollen foreskin preventing exposure of the penis, as well as vocalization, bruxism and digging movements.

The hemogasometric findings confirmed the pCO2 elevation observed between the initial moment (IM) (39.6 ± 3.65) and the final moment (FM) (41.6 ± 3.78) in the group of sheep with urolithiasis, however, significant difference ($P < 0.05$) was found only when there was a comparison between groups in the FMs (healthy 36.33 ± 4.18, and affected 41.6 ± 3.78). The EB analysis showed elevation between moments in the same group and between groups at the same moment, with significant difference ($P < 0.05$) for FM (healthy 1.34 ± 3.22, and affected 6.9 ± 3.67) between groups. This behavioral pattern was also observed for tCO_2 (healthy 26.2 ± 3.47, and affected 32.24 ± 3.58), HCO_{3-} (healthy 25.06 ± 3.37, and affected 30.98 ± 3.55) and $stHCO_{3-}$ (healthy 25.44 ± 2.61, and affected 30.16 ± 3.20), with significant differences only in the FMs. Although there was no significant difference between Na^+, K^+ and Ca^{2+} ions at the moments or groups, higher values were observed at the final moments for sodium and potassium, with an increase in sodium values and a decrease in potassium and calcium concentrations between IM and FM (Table 2).

The IgA and IgG and APP analysis of sheep that developed obstructive urolithiasis showed a temporal oscillation behavior of the means (Tables 3 and 4). IgA showed peak concentration at M5 (39.86 ± 8.45) and lowest value at M14 (10.99 ± 5.92). IgG increased from M0 (1286.80 ± 459.01) to M8 (1698.25 ± 315.03), starting to oscillate and decreasing at M15 (1586.67 ± 674.06). Ceruloplasmin had lower means at M0 (30.76 ± 10.74) and M9 (30.50 ± 12.03), with similar values, but with substantial elevation and peak at M12 (68.90 ± 18.32), decreasing until M15 (67.70 ± 73.00), when it presented new elevation. Albumin maintained discrete linear growth, but with two moments of decreasing concentrations recorded in M2 (3283.40 ± 710.04) and M15 (3786.33 ± 596.88). Already α1-antitrypsin and α1-acid glycoprotein maintained close average values between M0 (244.60 ± 94.44 and 64.60 ± 35.41) and M15 (313.00 ± 129.98 and 59.63 ± 41.09) respectively.

Significant difference ($P < 0.05$) could be noted in haptoglobin, which showed large oscillations over time, with peaks at three moments, M2 (123.78 ± 92.10), M6 (131.48 ± 52.39) and M13 (77.56 ± 55.33), and lowest value recorded in M8 (5.39 ± 3.88). Similarly, transferrin showed a significant increase from M3 (400.40 ± 57.99) to M7 (510.80 ± 70.94), and occasional decreases in values, as recorded in M13 (490.00 ± 157.77), but with minimum and maximum values at M0 (351.00 ± 52.36) and M15 (599.66 ± 98.05), respectively (Figure 1).

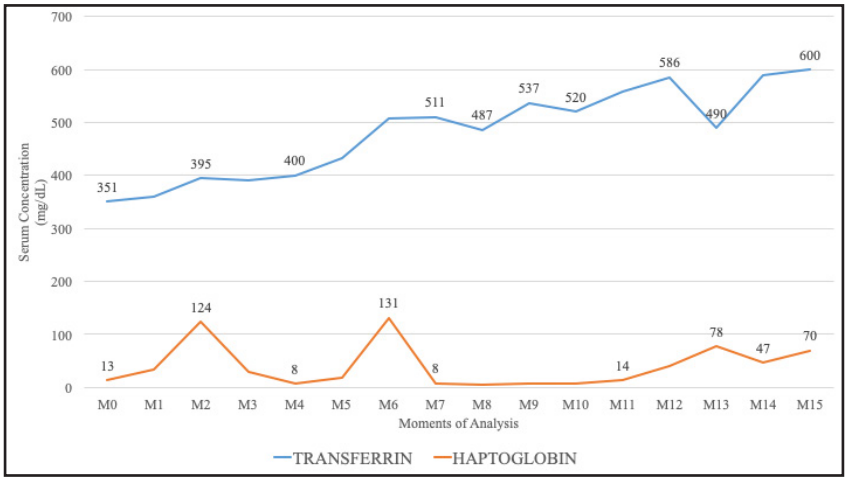

Figure 1. Temporal evolution of haptoglobin and transferrin in healthy sheep until clinical manifestation of obstructive urolithiasis.

Table 2. Hemogasometric profile (mean ± standard deviation) of venous blood of young male sheep, submitted to calculogenic diets (n = 14).

Parameter	D_1 (without urolithiasis) (n = 9)	D_2 (with urolithiasis) (n = 5)	*P* (*t*/Welch test)	*P* (nonparametric test)
IM pH	7.39 ± 0.04	7.42 ± 0.05	0.21	-
FM pH	7.45 ± 0.03	7.49 ± 0.05	0.14	-
IM pCO_2	40.44 ± 3.43	39.6 ± 3.65	0.67	-
FM pCO_2	36.33 ± 4.18	41.6 ± 3.78	0.04*	-
IM pO_2	36.11 ± 3.76	32.2 ± 7.01	0.20	-
FM pO_2	37.22 ± 4.47	40 ± 9.11	0.46	-
IM EB	-1.04 ± 1.36	0.42 ± 2.24	0.15	-
FM EB	1.34 ± 3.22	6.9 ± 3.67	0.01*	-
IM tCO_2	25.06 ± 1.15	26.08 ± 2.04	0.25	-
FM tCO_2	26.2 ± 3.47	32.24 ± 3.58	0.01	0.02*
IM HCO_3^-	23.82 ± 1.13	24.88 ± 2.03	0.23	-
FM HCO_3^-	25.06 ± 3.37	30.98 ± 3.55	0.01	0.02*
IM st HCO_3^-	23.23 ± 1.03	24.64 ± 1.88	0.09	-
FM st HCO_3^-	25.44 ± 2.61	30.16 ± 3.20	0.01*	-
IM tHb	13.79 ± 0.59	13.46 ± 1.20	0.50	-
FM tHb	12.68 ± 1.05	10.18 ± 3.97	0.23	0.06
IM sO_2	-	-	-	0.67
FM sO_2	70.13 ± 4.05	75.75 ± 7.27	0.11	-
IM Ht	41.33 ± 1.80	40.4 ± 3.78	0.54	-
FM Ht	38.11 ± 3.06	36.6 ± 3.85	0.43	0.42
IM Na^+	138.78 ± 32.94	149.4 ± 2.07	0.36	1.0
FM Na^+	148.56 ± 1.13	151 ± 4.85	0.33	0.63
IM K^+	4.49 ± 0.57	4.74 ± 0.60	0.45	-
FM K^+	3.68 ± 0.26	4 ± 1.06	0.54	-
IM Ca^+	1.29 ± 0.10	1.17 ± 0.14	0.12	-
FM Ca^+	0.56 ± 0.07	0.68 ± 0.19	0.24	-

IM: Initial Moment; FM:Final Moment; D_1: without urolithiasis; D_2: with urolithiasis; *Significant difference between groups ($P < 0.05$). pCO_2 (mmHg), pO_2 (mmHg), EB (mmol/L), tCO_2 (mmol/L), HCO_3^- (mmol/L), tHb (g/dL), sO_2 (%), Ht (%), Na^+ (mmol/L), K^+ (mmol/L), Ca^+ (mmol/L).

Table 3. Acute phase protein kinetics in healthy young male sheep until clinical manifestation of obstructive urolithiasis (D2 - n = 5).

	Ceruloplasmin (mg/dL)	α_1-antitripsin (mg/dL)	α_1-acid glicoprotein (mg/dL)	Haptoglobin* (mg/dL)	Transferrin* (mg/dL)
M0	30.76 ± 10.74	244.60 ± 94.44	64.60 ± 35.41	13.34 ± 2.45	351.00 ± 52.36
M1	39.74 ± 19.24	159.00 ± 115.44	66.04 ± 44.48	32.94 ± 25.36	359.50 ± 40.07
M2	58.38 ± 34.26	150.40 ± 148.22	74.04 ± 29.11	123.78 ± 92.10	395.20 ± 84.71
M3	39.52 ± 4.69	237.80 ± 155.38	56.60 ± 27.23	30.28 ± 32.70	391.40 ± 61.66
M4	39.78 ± 13.05	203.60 ± 124.18	50.24 ± 18.36	7.62 ± 2.11	400.40 ± 57.99
M5	41.22 ± 13.25	208.00 ± 121.74	50.60 ± 24.71	18.66 ± 27.40	432.00 ± 59.55
M6	56.82 ± 20.90	199.20 ± 120.22	48.40 ± 31.64	131.48 ± 52.39	508.00 ± 61.48
M7	43.06 ± 15.99	201.60 ± 121.20	50.38 ± 26.90	8.07 ± 6.91	510.80 ± 70.94
M8	35.52 ± 19.11	260.50 ± 38.17	57.10 ± 31.74	5.39 ± 3.88	486.50 ± 89.89
M9	30.50 ± 12.03	254.50 ± 114.73	41.15 ± 23.72	7.26 ± 3.12	536.75 ± 90.48
M10	39.06 ± 15.65	305.00 ± 112.60	72.70 ± 44.45	6.80 ± 4.62	520.33 ± 125.43
M11	58.70 ± 26.55	255.66 ± 40.50	65.76 ± 42.16	13.88 ± 7.86	559.00 ± 43.55
M12	68.90 ± 18.32	267.33 ± 72.52	61.46 ± 45.08	40.84 ± 42.06	585.67 ± 85.29
M13	54.76 ± 30.32	259.00 ± 57.58	66.70 ± 37.24	77.56 ± 55.33	490.00 ± 157.77
M14	45.53 ± 14.18	262.00 ± 72.18	63.96 ± 38.33	46.61 ± 57.45	588.00 ± 87.50
M15	67.70 ± 73.00	313.00 ± 129.98	59.63 ± 41.09	69.50 ± 96.08	599.66 ± 98.05

D_2 - with urolithiasis. Data presented as mean ± standard deviation. *Variables with significant time effect: Transferrin ($P = 0.0061$) and Haptoglobin ($P = 0.0276$). Other variables without significant effect of time ($P > 0.05$).

Table 4. Serum IgA and IgG concentrations (mg/dL) in healthy young male sheep until clinical manifestation of obstructive urolithiasis (n = 5).

	IgA	IgG
M0	37.48 ± 14.65	1286.80 ± 459.01
M1	30.62 ± 21.77	1371.80 ± 278.97
M2	32.20 ± 28.70	1780.20 ± 861.19
M3	32.08 ± 29.62	1676.60 ± 367.69
M4	31.60 ± 12.25	1571.00 ± 179.14
M5	39.86 ± 8.45	1596.00 ± 174.05
M6	25.82 ± 14.13	1602.40 ± 139.05
M7	32.16 ± 20.97	1615.80 ± 155.81
M8	24.65 ± 11.19	1698.25 ± 315.03
M9	15.60 ± 6.42	1541.75 ± 182.47
M10	14.99 ± 6.49	1649.67 ± 432.49
M11	26.93 ± 7.36	1522.67 ± 238.04
M12	39.16 ± 20.04	5674.33 ± 7176.02
M13	19.43 ± 0.70	1561.67 ± 298.87
M14	10.99 ± 5.92	1661.33 ± 389.42
M15	29.90 ± 15.69	1586.67 ± 674.06

Data presented as mean ± standard deviation. Variables without significant effect of time ($P > 0.05$).

DISCUSSION

The symptoms observed in the present study for sheep that manifested the clinical picture of obstructive urolithiasis is in agreement with the reports of other authors [18,23,25]. Symptoms are due to the release of prostaglandins, adrenaline and noradrenaline after urinary stasis and pain [22,30]. Thus, to evaluate the systemic effects of this disease on blood gas variables, healthy sheep (D1) were compared with those that developed the disease (D2).

The harvesting of jugular blood proved to be effective in reflecting the metabolic status and acid-base balance when compared to the arterial, presenting even easier sampling in ruminants [28]. The methodology of this experiment used the maximum time of 15 minutes for the hemogasometric analysis, deadline already established [16]. Researchers demonstrated the viability of bovine venous blood stored in a cold water bath for up to six hours after harvesting for this examination, and may extend to sheep for up to 24 hours [15].

Hemogasometry provides information that reflects acid-base balance quickly and practically through blood gas analysis, plasma bicarbonate concentration, and base excess or deficit [9]. The mean recorded pH (7.4 ± 0.05) showed no significant difference between groups, but based on the reference values described [11] the sheep that developed urolithiasis had alkalosis.

The averages observed for HCO_{3-} and EB were higher than those described (19 to 25 mmol/L and -4.0 to 2.0 mmol/L) respectively [11], and reflect the installed metabolic alkalosis in sheep with urolithiasis, also observed in goats [10,14].

Metabolic alkalosis can settle in different situations. A consequent digestive disturbance, anorexia and cessation of rumination, as well as diseases that result in tubular injury that cause accumulation of blood bicarbonate [21], conditions that could justify such elevations in the group with urolithiasis.

The average recorded for pCO_2 in FMs (41.6 ± 3.78) in sheep with urolithiasis showed a significant difference in the comparison between groups, but with values close to the described upper limit of normality (37 to 42 mmHg) [11]. Unfeasibility of generalizing blood gas analysis between ruminant species can be verified when compared with the goat species. Goat normality data for tCO_2 [20] were higher than those proposed for sheep in Brazil (19 to 26 mmol/L) [21].

In this study, the final tCO_2 values observed in the urolithiasis group (32.24 ± 3.58) were higher than the normal range recorded for the species. And it was found that the higher the bicarbonate contents IM (24.88 ± 2.03) and FM (30.98 ± 3.55), the higher the tCO_2, IM (26.08 ± 2.04) and FM (32.24 ± 3.58) values, a fact also observed for bovine species [28]. These findings, together with the pCO_2 values reflect the compensatory organic response due to the installed metabolic alkalosis, in which the organism tends to retain CO_2. The difference between bicarbonate and tCO_2 concentrations in this study was between 1 and 2 mmol/L, indicating correct sample conditioning and anaerobic maintenance [13].

Mean sodium values for the urolithiasis (149.4 ± 2.07 and 151 ± 4.85) and potassium (4.74 ± 0.60 and 4 ± 1.06) groups in IM and FM respectively were within normal range for sheep (139 to 152 mmol/L and 3.9 to 5.4 mmol/L) [8,13].

The APP have different species responsiveness based on the inflammatory stimulus that has settled, becoming in the last two decades the biomarkers of choice in human medicine and, by extension, in veterinary medicine. Acid α1-glycoprotein was considered effective as a biomarker for early diagnosis in monitoring the prognosis of sepsis [31], such potential has been proved in cattle with mastitis and photosensitization [27]. Moreover, they demonstrated the sensitivity of ceruloplasmin and haptoglobin in cattle with mastitis, photosensitization and onfalophlebitis.

Some biomarkers have already been used for the early evaluation of urinary injuries such as Neutrophil Gelatinase-Associated Lipocalin (NGAL) and fetuin-A in humans [17]. Since haptoglobin, ceruloplasmin and albumin, among other acute phase proteins, were increased in camels with urinary tract infection, the authors recommend the investigation of these biomarkers in other species [7].

To date, no early biomarker studies for sheep with obstructive urolithiasis have been reported. Thus, we investigated ceruloplasmin, albumin, α1-antitrypsin, α1-acid glycoprotein, haptoglobin and transferrin, and immunoglobulins (A and G) in an attempt to identify other biomarkers besides those already studied [7] for urinary tract infections. Haptoglobin and transferrin were the most reliable biomarkers among APP studied in this species to predict obstructive urolithiasis.

Although transferrin is classified as negative APP, it was elevated until obstructive urolithiasis manifested.

Similar behavior has been observed in chickens [29,32], and although this mechanism remains unexplained so far, it is believed to be involved in passive immunity, which aids host nonspecific defenses against microbial challenges.

CONCLUSIONS

The formulated diet was effective in inducing the disease and can be used as an experimental model in future research.

Hemogasometric evaluation of bases and venous blood gases showed alterations in obstructive urolithiasis, which lead to acid-base imbalance, characterized by metabolic alkalosis with an attempt to compensate by retaining CO_2.

Haptoglobin and transferrin were sensitive biomarkers for obstructive urolithiasis in sheep, with transferrin presenting atypical behavior, characteristic of positive AFP.

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***Ethical approval*.** All methodology adopted for the development of the present research was submitted and approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal Rural University of Pernambuco (UFRPE), Recife-PE, under protocol number 13726/2012-97.

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