

# Renal profile and urinary fractional excretion of calcium, phosphorus, and magnesium of lambs fed with different contents of phosphorus

## *Perfil renal e excreção fracionada urinária de cálcio, fósforo e magnésio de cordeiros alimentados com teores diferentes de fósforo*

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**ABSTRACT:** Obstructive urolithiasis is common in confined sheep, and a predominant factor predisposing animals to this condition is nutritional management. The objective of this study was to evaluate the effect of different concentrations of phosphorus (P) on urinalysis, serum urea and creatinine, and serum and urine concentrations of calcium (Ca), P, and magnesium (Mg), as well as to establish the fractional excretion (FE) of these elements in confined lambs. Thirty male Santa Inês x Ile de France sheep at four months of age were divided into two groups according to the diet: Group 1 (G1, n = 15), with 0.43% P in their feed; and Group 2 (G2, n = 15) with 0.65% P. The diets consisting of 10% Coast-cross hay and 90% of a concentrate of soybean meal, wheat, and crushed corn were provided for 90 consecutive days, with water ad libitum. The time points (M) of blood and urine collection were determined as M0, immediately before the beginning of the diet, and at 15-day intervals: M1, M2, M3, M4, M5, and M6. Phosphorous-rich diets favored the appearance of triple phosphate crystals in both groups, causing calculogenesis in more than one third of the animals (11/30), emphasizing the relevance of conducting laboratory tests to aid in urolithiasis diagnosis. In addition, FE revealed an increased renal excretion of excess minerals in the diet, and therefore a greater predisposition for stone formation in relation to nutritional management.

**KEYWORDS:** Feedlot; Kidney; Sheep; Urinary obstruction; Urolithiasis

**RESUMO:** A urolitíase obstrutiva é frequente em ovinos confinados, sendo o manejo nutricional um dos fatores predisponentes mais importantes à formação dos cálculos. O objetivo desse estudo foi avaliar o efeito da alimentação, com diferentes concentrações de fósforo (P), sobre a urinálise, ureia e creatinina séricas, concentração de cálcio (Ca), P e magnésio (Mg) séricos e urinários, bem como estabelecer a excreção fracionada (EF) desses elementos em cordeiros confinados. Utilizaram-se 30 ovinos machos, não castrados, Santa Inês x Ile de France, com quatro meses de idade, que foram divididos em dois grupos de acordo com a dieta: Grupo 1 (G1, n = 15), com 0,43% de P; e Grupo 2 (G2, n = 15) com 0,65% de P. As dietas foram fornecidas por 90 dias consecutivos, com 10% de feno de Coast-cross; 90% de concentrado composto por farelo de soja, trigo e milho triturado; água ad libitum. Os momentos (M) de colheita de sangue e urina foram determinados como M0, imediatamente antes do início da dieta, e em intervalos de 15 dias nos momentos M1, M2, M3, M4, M5, M6. As dietas ricas em fósforo favoreceram o aparecimento de cristal fosfato triplo em ambos os grupos, o que provocou a calculogênese em um terço dos animais (11/30). Ressaltou-se a relevância da execução de exames laboratoriais para auxílio diagnóstico de urolitíase. Além disso, a EF comprovou a maior excreção renal dos minerais em excesso na dieta, que mostrou a maior predisposição para formação dos cálculos conforme o manejo nutricional.

**PALAVRAS-CHAVE:** confinamento; obstrução urinária; ovinos; rim; urolitíase.

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## INTRODUCTION

Urolithiasis is a multifactorial pathological process characterized by the presence of stones in the urinary system, which has a high incidence in confined sheep (RIET-CORREA; SIMÕES; VASCONCELOS, 2008; GUIMARÃES et al., 2012). The disease particularly affects males, causing reproductive inability in rams and carcass conviction of lambs (ANTONELLI et al., 2012; FERREIRA et al., 2015).

Intensive breeding systems with a Ca: P imbalance or plants containing a large amount of silica or oxalate can trigger the disease (RIET-CORREA; SIMÕES; VASCONCELOS, 2008). Grain-dominated diets with high levels of phosphorus (P) and magnesium (Mg), low concentrations of calcium (Ca), and a Ca: P ratio ranging from 1:4 to 1:6 can influence the occurrence of urolithiasis (FREEMAN et al., 2010). The chemical imbalance results in increased excretion of P in the urine, which in ruminants is normally alkaline, therefore rendering the P insoluble, causing precipitation and consequent formation of crystals (FERREIRA et al., 2014; MACIEL et al., 2017).

Excretion of excess P in ruminants occurs through saliva from the rumination process. In intensive farming systems using diets containing a high concentration of grains and low roughage, saliva production is reduced, and a higher concentration of P is eliminated by the kidneys, contributing to the formation of uroliths (EMERICK; EMBRY, 1963; LOUVADINI; VITTI, 1994).

The optimal chemical composition of the feed must be determined to correct the factors that are involved in stone formation, and thus avoid the appearance of obstructive urolithiasis and the consequent economic losses (FERREIRA et al., 2015). In addition, mineral levels should be measured in the serum and urine in order to verify the homeostasis of these elements. Furthermore, it is necessary to ascertain the renal function of animals by determining the serum urea and creatinine, as they allow for the estimation of the glomerular filtration rate (SACCO; LOPES, 2011). Factors such as water intake and renal blood flow can interfere with this rate, thereby influencing the excretion of minerals. In addition, calculating the fractional excretion (FE) of the elements can verify their resorption and excretion, which also considers the urinary creatinine level (KING, 1994; FERREIRA et al., 2018). Thus, the aim of this work was to study the renal profile of lambs subjected to two diets with different levels of phosphorus, through urinalysis findings and serum urea and creatinine readings, as well as to determine the urinary FE of Ca, P, and Mg.

## MATERIAL AND METHODS

This work was submitted and approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science (FMVZ), São Paulo State University (UNESP), Botucatu Campus, SP, Brazil, under protocol 156/2012. The average temperature was 21.03 °C and relative humidity was 71.38% in the period from September to December 2013.

Thirty male lambs, non-castrated, crossbred from the Ile de France and Santa Inês breeds from commercial flocks, recently weaned with an average age of 120 days and average live weight (LW) of  $18.7 \pm 1.2$  kg were identified, separated, and randomly distributed into two groups of 15 animals (G1 and G2). Initially, the lambs were dewormed with 1% injectable Moxidectin (200 µg/kg, Cidectin® Zoetis Indústria de Produtos Veterinários Ltda, Campinas-SP, Brazil) and vaccinated against Clostridiosis (Sintoxan polyvalente T®, Boehringer Ingelheim, Campinas-SP, Brazil). After a 15-day adaptation to the environment and diets, the animals were confined for 75 days for five collection samples, one every 15 days, totaling 90 days of confinement. The sheep were allocated to four collective 12 m<sup>2</sup> masonry stalls, with seven or eight animals each (average of 1.5 m<sup>2</sup>/head), all in the same location inside the Veterinary Hospital of FMVZ/UNESP, Botucatu. Two stalls were assigned to G1, with seven and eight animals, respectively, and the other two were allocated to G2, in which the animals were under the same nutritional conditions. The animals received water ad libitum in automatic drinkers.

There was a visual assessment of the leftovers, but it was not quantified in order to guarantee the daily adjustment of 10% of leftovers. The hay was crushed and mixed with the concentrate, which provided a complete homogeneous diet, and was offered in feeders with sufficient area available for all sheep to feed without dispute. The experiment covered the usual growth and finishing time of lambs for slaughter, weaning at 60 to 70 days of age at an average weight of 18 kg, confining for 90 days, and reaching a final average weight of 40 kg at 150 to 160 days old (PIRES et al. 2000). During this period, the sheep presented an average daily consumption of 3% of the dry matter (DM) PV, following the recommendations of the NRC (2007). Thus, the animals had an average daily intake of DM of 1 to 1.3 kg and an average daily weight gain of 0.244 g (CIRNE et al., 2013).

The groups received diets differing in Ca and P ratios, but within the recommendations indicated by the NRC (2007) for raising sheep, using ratios of 1.9:1 for G1 and 1.5:1 for G2, as follows: G1–Ca 0.8% (8 g/kg DM), P 0.43% (4.3 g/kg DM); and G2–Ca 0.97% (9.7 g/kg DM), P 0.65% (6.5 g/kg DM). Based on the average consumption of 1 kg/day of DM, lambs from G1 ingested 8 g of Ca and 4.3 g of P per day, and animals in G2 consumed 9.7 g of Ca and 6.5 g of P per day. These values were above the absolute requirement for this animal category of 183 mg of Ca and 103 mg of P per kg of BW/day (GERASEEV et al., 2000; NRC, 2007). Based on this, at the end of the experiment lambs with 40 kg BW would require 7.32 g of Ca and 4.12 g of P daily.

The chemical-bromatological composition and percentage of ingredients in the diets are listed in Table 1. In order to ensure experimental safety, the diet of 10% crushed Coast-cross hay and 90% concentrate (soy bran, wheat, and crushed corn), was subjected to chemical-bromatological analysis by the

Department of Animal Improvement and Nutrition of FMVZ/UNESP, Botucatu. The DM, Crude Protein (CP), Etheric Extract (EE), and Mineral Matter (MM) were analyzed by the Weende method, and the neutral detergent fiber (NDF) and acid detergent fiber (ADF) by the Van Soest procedure (VAN SOEST; WINE, 1967). Mineral analysis was performed at the Campineiro Institute for Soil and Fertilizer Analysis (ICASA) to determine the percentage of phosphorus, calcium, magnesium, potassium, sodium, and sulfur (BRANCHINI, 2014).

Urine and whole blood samples were collected for the following analyses: urinalysis, serum and urinary Ca, P and Mg levels, serum urea, and serum and urinary creatinine, at seven points, M0 (immediately before the supply of the diets), and at 15-day intervals: M1 (15 days); M2 (30 days); M3 (45 days); M4 (60 days); M5 (75 days); and M6 (90 days).

Blood and urine samples were collected in the morning (06:00) before feeding, by haltering the sheep in a quadrupedal position. Urine was collected by natural, spontaneous urination or by induction after asphyxia for approximately 15 seconds (GARCIA-NAVARRO, 2005). After collecting at least 10 mL of urine in a sterile 70 mL flask (J. Prolab. Indústria e Comércio de Produtos para Laboratório Ltda., São José dos Pinhais-PR, Brazil), the samples were sent to the Commercial Pathology Laboratory Veterinary Clinic (Vida Vet®, Botucatu-SP), for urinalysis. On physical examination, volume, appearance, and density were evaluated by

refractometry (Atago® T2 refractometer, NE Clinical, Atago Brasil Ltda. Ribeirão Preto-SP, Brazil); for the chemical examination dry biochemical reagent strips were used (Combur10 Test®, Roche Diagnóstica Brasil Ltda. São Paulo-SP, Brazil), to evaluate pH, proteins (mg/dL), glucose (mg/dL), acetone, urobilinogen, bilirubin, occult blood, and bile salts.

For the sedimentoscopy exam, 5 mL of urine was centrifuged (Excelsa II, Fanen, São Paulo-SP, Brazil) in conical tubes at 400 g for 5 min. Subsequently, after discarding the supernatant, 0.5 ml of urine was used to identify possible cells for desquamation of the urinary tract (kidney, pelvis, bladder, and urethral cells), along with prostate cells and other structures such as red blood cells, leukocytes, cylinders, bacteria, sperm, mucus, and crystals. Large-field magnification counting (cga) was performed with ordinary optical microscopy, using 400× magnification. Urine aliquots were then stored in 2 mL tubes in a -20 °C freezer (Eppendorf do Brasil Ltda. São Paulo-SP, Brazil), for subsequent mineral dosing.

Samples of 10 mL of blood from the jugular vein of each animal were collected in vacuum tubes without anticoagulant (BD Vacutainer®, BD Medical, Curitiba-PR, Brazil) at each sampling point (M0, M1, M2, M3, M4, M5 M6, and M7). After the clot retraction, the samples were centrifuged (Combate Celm® - Cia. Equipadora de Laboratórios Modernos, Barueri-SP, Brazil) at 2000 g for 5 min to obtain serum, and subsequently frozen at -20 °C in 2 mL aliquots in tubes.

Biochemical analyses were performed at the Service of Clinical Pathology, Department of Veterinary Clinic, FMVZ / UNESP, Botucatu, using commercial reagents (Katal® Biotecnológica Ind. Com. Ltda. Belo Horizonte-MG, Brazil) by reading on a spectrophotometer (SB- 190 Celm® - Company of Modern Laboratories, Barueri-SP, Brazil). Serum measurements were performed according to the following methods: colorimetric enzyme to determine the urea concentration (modified Berthelot); colorimetric kinetic for creatinine (Jaffe); and colorimetric for Ca (cresolphthalein complexone), P (ammonium molybdate), and Mg (sulfonated Magon). The dosage of calcium and phosphorus were obtained after acidification of the samples, in accordance with the technique described by Fleming et al. (1991).

Serum and urine electrolytes and creatinine were measured, and the fractional excretion calculations were performed. Thus, it was possible to compare the electrolyte clearance with that of endogenous creatinine to determine the renal excretion of this element, using the concentrations of urinary electrolytes (EU), urinary creatinine (CRU), serum electrolytes (ES), and serum creatinine (CRS). Fractional excretion was calculated:  $FE (\%) = [(EU / ES) \times (CRS / CRU)] \times 100$  according to LEFEBVRE et al. (2008).

The distribution of the studied variables was considered for the choice of statistical methods, descriptive statistics, and non-parametric analyses. Due to the non-normal distribution of the results, the medians for the Wilcoxon non-parametric test were used to assess the difference between the groups (G1 and G2), within each of the seven sampling

**Table 1.** Chemical-bromatological composition and percentage of ingredients in the diets of feedlot lambs subjected to diets with different levels of phosphorus, 0,43% (G1) and 0,65% (G2).

Variables	G1	G2
DM (%)	86.82	87.59
CP (%)	19.22	18.39
EE (%)	2.33	2.91
MM (%)	4.97	5.02
NDF (%)	17.40	17.62
ADF (%)	6.43	6.50
Crude Energy (%)	38.13	38.76
Phosphorus (P -%)	0.43	0.65
Calcium (Ca - %)	0.8	0.97
Magnesium (%)	0.19	0.22
Potassium (%)	0.78	0.83
Sodium (%)	0.4	0.3
Sulfur (%)	0.14	0.14
Ca:P ratio	19:1	1.5:1
crushed Coast-cross hay (%)	10	10
Soy bran (%)	26	21.40
Wheat bran (%)	8	15
Crushed corn (%)	54	51.3
Calcitic limestone (%)	2	2.3

points (M0 to M6). The correlation between the variables was estimated by Spearman's correlation coefficient. The analyses were performed with the SAS software (SAS Institute, 2011), adopting a significance level of 5% ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

During the experiment, three lambs presented obstructive urolithiasis, two lambs from G1 (2/15) and one lamb from G2 (1/15). At the end of the experiment, eight more animals showed phosphate calculations in the urinary system at necropsy, four from each group for a total of 36.67% (11/30) of lambs that developed the condition.

### Urinalysis

The urinary density averages (Table 2) of the samples remained between 1010 and 1020, which is within the standard of normality of 1015–1045 (GARCIA-NAVARRO, 2005; ARAÚJO et al., 2009). Comparing the experimental groups, only M2 and M5 showed a difference in urinary density ( $p < 0.05$ ), with G1

showing higher medians than G2, indicating that the urine was more concentrated at these times. Similar to this study, Ferreira et al. (2020) reported that the urine density of confined lambs remained within normal values. Urinary density varies according to the degree of hydration, as well as the presence of other sediments such as flaking cells, crystals, and cylinders (ARAÚJO et al., 2009). Although some samples points revealed greater density in G1, the factors related to the high concentration of solutes, which caused urine supersaturation with calculogenic crystalloids (ARAÚJO et al., 2009), occurred in both groups, favoring the formation of uroliths in 11 lambs.

The average pH values ranged from 6.5 to 8.0, within the normal range for sheep of 6.0–8.5, according to Garcia-Navarro (2005). There was a difference between groups in M1 only (15 days of confinement), when G1 had a higher median than G2, but subsequently there was no significant variation between groups, although the median of G2 was higher in M5 and M6. Physiologically, the urine pH of ruminants is alkaline, which favors the precipitation of solutes (GARCIA-NAVARRO, 2005; ARAÚJO et al., 2009).

In a contrasting study with sheep, Ferreira et al. (2014) observed that even before treatment with ammonium chloride and vitamin C, a lower pH in relation to the normal pH of the species was observed in the three groups studied when they did not receive urinary acidifier, which can be explained by the richness of the concentrate diet. Ferreira et al. (2020) observed the effectiveness of urinary acidification in sheep treated with 400 mg/kg/PV of ammonium chloride/day and the failure of 4 mg/kg of vitamin C, both administered orally.

Although they were receiving different treatments, the two experimental groups in the present study showed similar results in urinalysis. The variables (results): color (light to dark yellow), appearance (clear or slightly cloudy), protein (absent or +), glucose (absent), urobilinogen (normal), bilirubin (absent), occult blood (absent), red blood cells (absent or rare), urinary tract cells (renal, pelvic, bladder, and urethral absent or rare), cylinders (absent) and mucus (absent) were not statistically different between the groups and remained within the normal range for the species (GARCIA-NAVARRO, 2005; ARAÚJO et al., 2009).

The median urinary leukocyte count showed a statistical difference ( $p < 0.05$ ) between groups only at M0, with G2 showing higher values compared to G1 (Table 3). G1 presented normal values of up to five/cga (GARCIA-NAVARRO, 2005) in six of the seven collection points studied, however, in M5 there was an inversion with G2, revealing counts above five/cga in M0, M1, M2, M3, M4 and M6. There is a need for an organic matrix for deposition of inorganic crystals for calculogenesis, which develop into the urolith, a mucopolysaccharide or mucoprotein where leukocytes, fibrin, cellular debris, and/or bacteria are deposited (ARAÚJO et al., 2009). Therefore, this leukocyturia may have contributed to the matrix for the calculations formed, particularly in G2.

**Table 2.** Urinary density and pH of feedlot lambs subjected to diets with different levels of phosphorus, 0,43% (G1) and 0,65%(G2), at different sampling times (M).

Times	Variables	G1 (n=15)		G2 (n=15)	
		$\bar{X} \pm s$	md	$\bar{X} \pm s$	Md
M0	Density	1011.8 ± 8.2	1010.0	1016.9 ± 7.7	1017.5
	pH	8.3 ± 1.0	9.0	7.9 ± 1.1	8.0
M1	Density	1013.0 ± 7.7	1010.0	1017.7 ± 7.5	1020.0
	pH	8.1 ± 1.0	8.0 <sup>A</sup>	7.4 ± 0.9	7.5 <sup>B</sup>
M2	Density	1017.0 ± 5.6	1020.0 <sup>A</sup>	1018.3 ± 8.8	1015.0 <sup>B</sup>
	pH	7.6 ± 0.9	8.0	7.2 ± 1.2	7.0
M3	Density	1017.9 ± 5.8	1015.0	1020.0 ± 6.8	1020.0
	pH	7.4 ± 0.9	7.0	7.0 ± 0.9	7.0
M4	Density	1021.8 ± 9.1	1020.0	1013.2 ± 8.0	1012.5
	pH	7.5 ± 1.3	8.0	7.8 ± 1.1	8.0
M5	Density	1020.4 ± 5.2	1020.0 <sup>A</sup>	1014.3 ± 8.1	1015.0 <sup>B</sup>
	pH	6.8 ± 1.2	6.5	7.4 ± 1.0	8.0
M6	Density	1014.6 ± 9.7	1015.0	1013.9 ± 9.4	1010.0
	pH	7.6 ± 1.2	7.0	7.4 ± 1.0	8.0

Mean ( $\bar{X}$ ), standard deviation (s) e median (md).

<sup>A,B</sup>Medians followed by different capital letters on the line differ statistically between groups within the time point by the Wilcoxon Test ( $p < 0.05$ ).

Urinary density: 1015-1045; pH: 6.0-8.5 (GARCIA-NAVARRO, 2005).



The bacteria count in the urine (Table 3) was within the normal parameters between the groups and the sample times of up to five/cga (GARCIA-NAVARRO, 2005). G2 showed higher values when compared to G1, with a statistical difference ( $p < 0.05$ ) at M2 and M4. Urinary tract infection is an important factor in the development of urolithiasis, since bacterial colonization favors the appearance of organic nuclei and modifies the secretion of inhibitory elements by the inflammatory process. More importantly, urease-producing bacteria change the pH level of urine (SACCO; LOPES, 2011). Thus, bacteriuria and leukocyturia in G2 associated with a more alkaline pH, especially at the end of the experiment, favored the appearance of uroliths in these animals.

The presence of triple phosphate crystals (Figure 1) was constant from the first urine collection to the end of the experiment. There was no significant difference between the groups, although it was observed that G1 always returned higher values than G2, with the exception of M4 and M6 (Figure 2). Triple phosphate crystals can be formed in alkaline, neutral, or slightly acidic pH, but they are more prominent in a basic pH (SUN et al., 2010; JONES et al., 2017). In the present study, triple phosphate crystals occurred because of the high levels of phosphorus in the diet, and they were more evident in G1 where an alkaline pH was observed in five sample points, M0, M1, M2, M4, and M6.

**Table 3.** Leukocyte and bacteria count in urine of feedlot lambs subjected to diets with different levels of phosphorus, 0,43% (G1) and 0,65%(G2), at different sampling times (M).

Times	Variables	G1 (n= 15)		G2 (n= 15)	
		$\bar{X} \pm s$	md	$\bar{X} \pm s$	md
M0	Leukocyte	74 ± 8.7	3.3 <sup>A</sup>	124 ± 10.3	9.0 <sup>B</sup>
	Bacteria	0.6 ± 0.8	0.0	1.1 ± 1.1	1.0
M1	Leukocyte	6.4 ± 5.2	3.5	9.0 ± 5.4	9.0
	Bacteria	0.7 ± 1.0	0.0	1.1 ± 0.9	1.0
M2	Leukocyte	4.9 ± 3.0	4.0	14.3 ± 12.7	8.0
	Bacteria	0.3 ± 0.6	0.0 <sup>A</sup>	1.3 ± 1.2	1.0 <sup>B</sup>
M3	Leukocyte	26.9 ± 41.4	3.3	46.6 ± 52.5	6.0
	Bacteria	0.9 ± 1.1	0.5	1.3 ± 0.9	1.0
M4	Leukocyte	14.4 ± 28.1	5.0	23.6 ± 35.5	7.0
	Bacteria	0.5 ± 0.7	0.0 <sup>A</sup>	1.9 ± 1.4	2.0 <sup>B</sup>
M5	Leukocyte	11.7 ± 10.0	11.0	9.5 ± 9.4	5.0
	Bacteria	1.1 ± 1.5	0.0	1.1 ± 1.4	1.0
M6	Leukocyte	12.5 ± 24.2	2.5	24.3 ± 38.5	7.5
	Bacteria	0.6 ± 1.0	0.0	0.4 ± 0.8	0.0

Mean ( $\bar{X}$ ), standard deviation (s) e median (md).

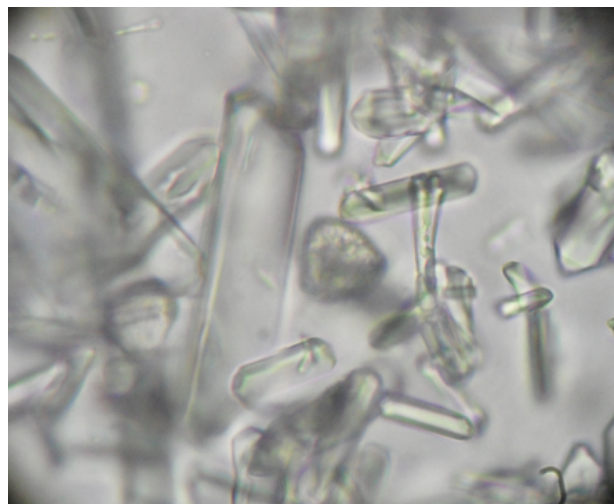
<sup>A,B</sup>Medians followed by different capital letters on the line differ statistically between groups within the time point by the Wilcoxon Test ( $p < 0.05$ ).

Leukocytes: up to five/cga; Bacterias: up to five/cga (GARCIA-NAVARRO, 2005).

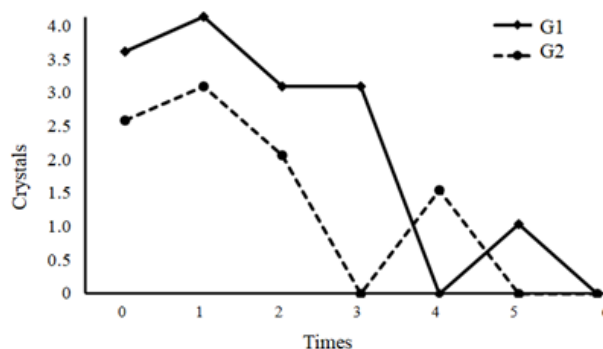
The difference between animals in terms of the presence of crystals was evaluated (Table 4) with two subgroups (Gc - calculation and Gw - without calculation). In this analysis, it was possible to observe that the animals that produced urinary stones presented a greater quantity of crystals in the urine, with statistical differences in M2, M3, M4, and M6. This was first noted at 30 days of confinement (M2) due to the elapsed time of depositing solutes in the lambs' urine, which was sufficient to show crystalluria, due to the excessive intake of minerals present in the diet. There was a decrease in the crystal count in the last collection points (M5 and M6), likely a result of the cementation of the calculations, since the formation occurs from the precipitation of solutes in the form of crystals around the organic matrix nucleus (FERREIRA et al., 2015).

### Serum dosage of urea and creatinine

During the experiment, the urea concentration (Table 5) of the confined lambs subjected to diets with different levels of P ranged from 42.70 mg/dL to 90.40 mg/dL, which were above the reference level of 17.12–42.80 mg/dL (KANEKO et al., 2008). There was a difference between the groups at M5 and



**Figure 1.** Triple phosphate crystals observed under the microscope, at 400× magnification.



**Figure 2.** Variation in the presence of crystals (crosses by large field of increase) between the groups with a diet composed of 0.43% (G1) and 0.65% (G2) of P, at different times.

M6 ( $p < 0.05$ ), with G1 revealing higher medians than G2, since two animals obstructed in G1 causing post-renal azotemia. The values obtained for urea were similar to those obtained by VinodhKumar et al. (2010), who reported serum urea values above the reference range, even in healthy animals. These authors identified levels of 45.28 mg/dL in lambs considered healthy, 67.38 mg/dL in suspect animals, and 146.34 mg/dL in those with confirmed urolithiasis. In the present study, the mean serum urea and creatinine levels of sheep with urolithiasis were 143.73 mg/dL and 1.98 mg/dL, respectively.

According to Rennó et al. (2000), the serum dosage of urea is influenced by the high concentration of CP in the diet, which in the present study was 19.22% and 18.39% in the respective groups G1 and G2 (Table 1), therefore the high urea levels may be a consequence of the concentrate-rich diet.

Serum creatinine values (Table 5) ranged from 0.68 to 1.71 mg/dL during the experiment, within the reference level of 1.2 to 1.9 mg/dL (KANEKO et al., 2008). The two groups returned similar results, apart from in M4, where G2 showed a higher median than G1. Creatinine proved to be a more efficient marker of kidney damage than urea because it was not influenced by the diet, and therefore faithfully represented the glomerular filtration rate. The serum creatinine showed no values above the reference, which indicated that the diets did not compromise the functioning of the nephrons (KOZLOSKI et al., 2005; KIRSZTAJN, 2007).

### Serum, urine, and FE measurement of Ca, P, and Mg

The serum Ca values (Table 6) were below the reference values (MACIEL et al., 2016; FERREIRA et al., 2020) for the sheep species, which are 4.82 to 9.37 mg/dL. There was a statistical difference between the groups only at two times, M1 (15 days of the diet) and M2 (30 days), when G2 showed higher medians than G1 since the Ca concentration in the G2 diet

**Table 4.** Count of crystals (represented by crosses) in the urine of feedlot lambs subjected to diets with different levels of phosphorus, with urinary calculations (Gc) and without (Gw), at different sampling times (M).

Times	Gc (n=11)		Gw (n=19)	
	$\bar{X} \pm s$	md	$\bar{X} \pm s$	md
M0	3.27 ± 1.42	4.00	2.25 ± 1.65	2.50
M1	3.45 ± 1.29	4.00	2.42 ± 1.84	3.00
M2	3.36 ± 0.67	3.00 <sup>A</sup>	1.79 ± 1.72	2.00 <sup>B</sup>
M3	3.30 ± 1.25	4.00 <sup>A</sup>	0.89 ± 1.32	0.00 <sup>B</sup>
M4	2.11 ± 1.17	3.00 <sup>A</sup>	1.06 ± 1.47	0.00 <sup>B</sup>
M5	1.89 ± 1.83	1.00	0.72 ± 1.13	0.00
M6	1.89 ± 1.76	2.00 <sup>A</sup>	0.39 ± 0.98	0.00 <sup>B</sup>

Mean ( $\bar{X}$ ), standard deviation (s) e median (md).

<sup>A,B</sup>Medians followed by different capital letters on the line differ statistically between groups within the time point by the Wilcoxon Test ( $p < 0.05$ ).

(0.97%) was greater than G1 (0.8%). According to Ferreira et al. (2018; 2020), urolithiasis is more frequent in confined animals, where the feed generally consists of grains, since this type of food normally has a high content of P and Mg, but a low level of Ca. However, in this study, the Ca concentration was higher, and an increase in serum Ca values of M0 was observed in relation to M6 for both groups.

The urinary Ca dosage (Table 6) presented levels between 0.69 mg/dL and 3.39 mg/dL, differing in G2 having greater medians than G1 in M0 and M1 only. Ferreira et al. (2020) reported a broad value for urinary calcium in their control group but with a higher average. Tiruneh (2006) studied biochemical changes in the urine of cattle from farms with a history of urolithiasis, and observed that animals affected by urolithiasis had low urinary Ca.

**Table 5.** Serum urea (mg/dL) and creatinine (mg/dL) dosages of feedlot lambs subjected to diets with different levels of phosphorus, 0,43% (G1) and 0,65% (G2), at different sampling times (M).

Times	Variables	G1 (n=15)		G2 (n=15)	
		$\bar{X} \pm s$	md	$\bar{X} \pm s$	md
M0	Urea	47,56 ± 12,49	42,70	51,28 ± 20,81	45,30
	Creatinine	0,75 ± 0,13	0,73	0,69 ± 0,18	0,69
M1	Urea	46,56 ± 11,52	48,90	55,33 ± 35,54	47,10
	Creatinine	0,78 ± 0,29	0,68	0,89 ± 0,32	0,84
M2	Urea	63,40 ± 23,93	62,10	69,37 ± 14,63	70,00
	Creatinine	0,79 ± 0,17	0,81	0,82 ± 0,16	0,79
M3	Urea	86,66 ± 44,04	74,40	75,06 ± 17,17	74,00
	Creatinine	0,91 ± 0,23	0,91	0,95 ± 0,24	0,94
M4	Urea	68,42 ± 24,90	69,60	73,52 ± 15,96	75,20
	Creatinine	1,28 ± 0,37	1,25 <sup>B</sup>	1,54 ± 0,31	1,53 <sup>A</sup>
M5	Urea	82,75 ± 22,47	82,20 <sup>A</sup>	61,99 ± 10,91	64,65 <sup>B</sup>
	Creatinine	1,51 ± 0,46	1,37	1,34 ± 0,30	1,32
M6	Urea	96,61 ± 32,63	90,40 <sup>A</sup>	64,10 ± 14,97	65,75 <sup>B</sup>
	Creatinine	1,63 ± 0,59	1,53	1,72 ± 0,31	1,71

Mean ( $\bar{X}$ ), standard deviation (s) e median (md).

<sup>A,B</sup>Medians followed by different capital letters on the line differ statistically between groups within the time point by the Wilcoxon Test ( $p < 0.05$ ).

Urea: 17,12-42,8mg/dL; Creatinine: 1,2-1,9mg/dL (KANEKO et al., 2008).

**Table 6.** Serum (mg/dL), urinary (mg/dL), and FE (%) of Ca of feedlot lambs subjected to diets with different levels of phosphorus, 0,43% (G1) and 0,65%(G2), at different sampling times (M).

Times	Variables	G1 (n=15)		G2 (n=15)	
		$\bar{X} \pm s$	md	$\bar{X} \pm s$	md
M0	Serum	6.22 ± 1.95	5.89	7.26 ± 2.96	7.41
	Urinary	1.43 ± 1.44	0.94 <sup>B</sup>	4.40 ± 3.42	2.63 <sup>A</sup>
	FE	0.20 ± 0.17	0.18 <sup>B</sup>	1.09 ± 1.88	0.52 <sup>A</sup>
M1	Serum	6.44 ± 2.79	5.57 <sup>B</sup>	7.82 ± 1.74	7.76 <sup>A</sup>
	Urinary	1.09 ± 0.83	0.69 <sup>B</sup>	3.15 ± 2.19	2.43 <sup>A</sup>
	FE	0.25 ± 0.34	0.13 <sup>B</sup>	0.66 ± 0.63	0.46 <sup>A</sup>
M2	Serum	5.91 ± 1.84	5.42 <sup>B</sup>	7.93 ± 0.88	8.06 <sup>A</sup>
	Urinary	1.42 ± 1.12	1.08	1.96 ± 2.05	1.37
	FE	0.20 ± 0.15	0.14	0.23 ± 0.19	0.14
M3	Serum	5.20 ± 1.94	4.82	6.16 ± 3.09	6.08
	Urinary	2.44 ± 1.60	1.72	4.32 ± 4.32	2.44
	FE	0.25 ± 0.08	0.24 <sup>B</sup>	0.51 ± 0.41	0.35 <sup>A</sup>
M4	Serum	8.60 ± 3.37	7.78	8.76 ± 3.17	9.37
	Urinary	2.86 ± 1.78	2.44	2.42 ± 1.74	2.23
	FE	0.68 ± 0.54	0.50	1.21 ± 0.73	1.12
M5	Serum	7.91 ± 3.12	7.07	8.89 ± 3.02	8.09
	Urinary	2.75 ± 1.06	2.56	2.36 ± 1.00	2.31
	FE	0.68 ± 0.28	0.69	0.63 ± 0.42	0.55
M6	Serum	5.70 ± 2.19	4.91	4.99 ± 1.34	5.14
	Urinary	3.77 ± 1.62	3.39	4.03 ± 4.24	2.86
	FE	1.41 ± 1.13	1.18	1.95 ± 1.39	1.47

Mean ( $\bar{X}$ ), standard deviation (s) e median (md).

<sup>A,B</sup>Medians followed by different capital letters on the line differ statistically between groups within the time point by the Wilcoxon Test ( $p < 0.05$ ).

Serum Ca: 11.5-12.8mg/dL (KANEKO et al., 2008).

As there are wide variations of minerals and electrolytes in the urine, oscillating according to the absorption and excretion of water (LEFEBVRE et al., 2008), the fractional excretion of the analyzed minerals was calculated.

The FE values of Ca (Table 6) varied during the experiment from 0.13% to 1.47% of the median, being lower than the medians reported by Ferreira et al. (2020) for confined lambs (1.74% to 23.03%). The results of FE Ca in animals in G1 remained stable from M0 to M3, whereas there was an increase in M4 to M5, but they were similar to each other, and in M6, G1 showed higher Ca levels. In G2, M1, M2, M3, and M5 showed mean FE of Ca between 0.231% and 0.644%, however M0, M4, and M6 returned an increase of 1.097% to 1.95%. G2 had higher medians than G1 in M0, M1, M3, M4, and M6. During the study, FE revealed an increase when comparing the initial sampling times with the end of the experimental period (M6), due to the greater excretion of Ca, likely related to the longer period of intake of the diet with 0.8% (G1) and 0.97% (G2) of Ca.

Serum P measurements (Table 7) ranged from 7.83 to 12.29 mg/dL, and showed no statistical difference between the groups studied, with hyperphosphatemia in both groups throughout the experiment, due to the high concentrations of P in the diet. Crookshank et al. (1967) and Hoar et al. (1970) confirmed the importance of phosphorus concentration in the feeding of small ruminants and proved that hyperphosphatemia contributes to the formation of stones (EMERICK; EMBRY, 1963).

The urinary P measurements (Table 7) presented levels between 17.85 mg/dL and 85.25 mg/dL with a difference only in M1, revealing higher medians in G2 compared to G1. The measurements of FE of P varied during the experiment from 2.73% to 22.10%. FE P values in G1 animals at M0, M1, M2, and M3 showed medians between 3.18% and 5.68%, while at M4 and M6 the medians were similar to each other, and in M5, G1 higher levels were observed. In the G2, at M0, M2 and M3, the medians of FE were between 3.77% and 8.64%, while at M1, M5, and M6 the medians were from 16.16% to 19.85%. In M4, G2 showed a higher level, reaching 31.81%. G2 returned higher medians than G1 at four times, M0, M1, M3 and M4, suggesting increased urinary P excretion, which was more evident in the G2 group fed with a higher concentration of P (0.65%) in the diet. This result corroborated that of Hoar et al. (1970), who described those diets rich in P increased serum phosphate, and consequently increased urinary phosphorus excretion, favoring calculogenesis. In contrast, other authors reported hypophosphatemia ( $4.0 \pm 3.0$  mg/dL) in 107 goats with obstructive urolithiasis (GEORGE; HIRD; GEORGE, 2007), which was not observed in the present study, even in animals that did not present calculus, but were subjected to diets rich in P.

The serum dosages of Mg (Table 8) ranged from 2.50 mg/dL to 4.98 mg/dL. A statistical difference was observed only in M4, when G2 returned a higher median than G1.

**Table 7.** Serum (mg/dL), urinary (mg/dL), and FE (%) of P of feedlot lambs subjected to diets with different levels of phosphorus, 0,43% (G1) and 0,65%(G2), at different sampling times (M).

Times	Variables	G1 (n= 15)		G2 (n= 15)	
		$\bar{X} \pm s$	md	$\bar{X} \pm s$	md
M0	Serum	9.06 ± 2.85	7.62	9.37 ± 3.87	7.83
	Urinary	45.63 ± 56.91	35.05	34.28 ± 38.02	17.85
	FE	5.62 ± 4.73	4.23	3.77 ± 3.83	1.991
M1	Serum	15.30 ± 10.31	11.65	9.43 ± 4.01	8.62
	Urinary	47.67 ± 53.45	28.60 <sup>B</sup>	104.05 ± 83.57	75.00 <sup>A</sup>
	FE	3.18 ± 2.70	2.73 <sup>B</sup>	16.16 ± 12.81	15.38 <sup>A</sup>
M2	Serum	10.97 ± 2.71	11.14	11.25 ± 2.24	10.90
	Urinary	61.50 ± 38.34	50.20	82.97 ± 86.82	62.00
	FE	5.68 ± 3.40	5.23	8.64 ± 9.49	4.10
M3	Serum	9.63 ± 2.76	9.54	10.50 ± 2.2	10.09
	Urinary	77.96 ± 72.65	67.00	119.40 ± 129.88	85.25
	FE	5.59 ± 5.13	4.10	8.28 ± 6.87	5.90
M4	Serum	10.50 ± 4.67	9.22	11.10 ± 4.39	10.43
	Urinary	59.28 ± 71.76	23.80	100.81 ± 121.26	50.05
	FE	11.51 ± 10.45	10.11 <sup>B</sup>	31.81 ± 33.27	22.10 <sup>A</sup>
M5	Serum	12.08 ± 4.92	11.12	11.21 ± 4.01	10.49
	Urinary	125.33 ± 110.46	82.90	100.04 ± 117.70	64.85
	FE	22.67 ± 20.53	20.51	19.85 ± 16.52	18.94
M6	Serum	12.05 ± 3.55	12.29	11.36 ± 2.79	11.54
	Urinary	63.02 ± 67.42	40.20	70.91 ± 108.72	18.52
	FE	12.48 ± 12.51	10.93	16.70 ± 21.89	4.78

Mean ( $\bar{X}$ ), standard deviation (s) e median (md).

<sup>A,B</sup>Medians followed by different capital letters on the line differ statistically between groups within the time point by the Wilcoxon Test (p<0.05).

Serum P: 5-7.3mg/dL (KANEKO et al., 2008).

**Table 8.** Serum (mg/dL), urinary (mg/dL), and FE (%) of Mg of feedlot lambs subjected to diets with different levels of phosphorus, 0,43% (G1) and 0,65%(G2), at different sampling times (M).

Times	Variables	G1 (n= 15)		G2 (n= 15)	
		$\bar{X} \pm s$	md	$\bar{X} \pm s$	md
M0	Serum	3.75 ± 1.10	3.52	3.66 ± 0.85	3.94
	Urinary	46.14 ± 19.51	39.05	38.11 ± 14.41	36.35
	FE	12.18 ± 7.44	10.87	10.32 ± 5.10	11.25
M1	Serum	2.66 ± 0.90	2.50	2.98 ± 0.81	2.96
	Urinary	32.15 ± 21.80	20.70 <sup>B</sup>	48.43 ± 19.86	54.80 <sup>A</sup>
	FE	12.26 ± 8.51	9.32	21.13 ± 10.58	18.48
M2	Serum	4.33 ± 1.17	4.42	4.80 ± 0.95	4.79
	Urinary	41.76 ± 14.24	43.10	38.66 ± 15.28	38.55
	FE	8.95 ± 4.10	7.55	9.03 ± 3.88	9.20
M3	Serum	4.73 ± 0.90	4.98	4.91 ± 1.01	4.80
	Urinary	33.18 ± 13.31	31.15 <sup>B</sup>	47.01 ± 16.04	49.85 <sup>A</sup>
	FE	4.45 ± 2.39	4.15	6.12 ± 2.246	6.03
M4	Serum	2.84 ± 0.93 <sup>B</sup>	2.69 <sup>B</sup>	3.97 ± 2.33	3.57 <sup>A</sup>
	Urinary	57.30 ± 21.35	62.50	50.26 ± 18.04	48.00
	FE	43.45 ± 31.89	33.20	62.26 ± 43.38	58.44
M5	Serum	3.99 ± 1.09	3.67	3.78 ± 1.10	3.94
	Urinary	57.52 ± 20.19	65.20 <sup>A</sup>	41.71 ± 17.23	40.05 <sup>B</sup>
	FE	26.24 ± 7.68	23.57 <sup>A</sup>	25.15 ± 13.85	21.37 <sup>B</sup>
M6	Serum	3.44 ± 1.24	3.03	3.63 ± 0.90	3.73
	Urinary	52.37 ± 28.15	62.70	49.49 ± 23.14	53.25
	FE	27.36 ± 16.60	29.12	32.30 ± 17.19	30.39

Mean ( $\bar{X}$ ), standard deviation (s) e median (md).

<sup>A,B</sup>Medians followed by different capital letters on the line differ statistically between groups within the time point by the Wilcoxon Test (p<0.05).

Serum Mg: 2.2-2.8mg/dL (KANEKO et al., 2008).



The urinary Mg dosage had levels between 20.70 mg/dL and 65.20 mg/dL, with statistical differences between the groups in M1, M3, and M5. At M1 and M3, G2 showed higher medians compared to G1. The FE of Mg varied during the experiment from 4.15% to 58.44%. The FE values of Mg showed different medians in G1 at M0, M1, M2, and M3, with variation between 4.15% and 10.32%, whereas in M5 and M6 there were medians of 23.57% and 29.12%, respectively. M4, however, revealed the highest median FE (33.20%). In G2, M0, M1, M2, and M3 presented medians between 6.03% and 18.48%. M5 and M6 obtained similar medians, 21.37% and 30.39%, respectively. M4 returned the highest median (58.44%) of all sample points. G2 showed higher medians than G1 in M0, M1, M2, M3, M4, and M6. Throughout the experimental period, the FE of Mg increased significantly from M3 to M4, remaining high until the end, possibly related to the chronic ingestion of the concentrate-rich diet.

Changes in Mg metabolism are determining factors in the development of urolithiasis, although abnormal P metabolism is also a requirement (KUNKEL et al., 1953). In a study by Haag and Palmer (1928), it was noticed that when rats were supplemented with diets containing a high concentration of Mg and P, there was a greater occurrence of phosphate calculations. In the present study, similar to the

findings with FE of P, Mg showed a higher urinary concentration at the end of the experiment, which showed a synergism between these elements.

## CONCLUSIONS

Under the conditions of this experiment, following the recommendations of Ca and P proportionality (1:1 to 2:1) in the literature, it was concluded that both diets promoted calcu-logenesis in lambs. In addition, the concentration of these elements should be considered in the nutritional planning of diets, as the levels of minerals are often higher than expected. From the results obtained, it is not possible to suggest precise levels for the elaboration of a diet; however, caution is necessary regarding the concentrations of these minerals. The relevance of conducting chemical-bromatological analysis of food is emphasized, and in a complementary way, laboratory tests for the diagnosis of urolithiasis.

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