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Nutraceuticals for Dissolving Nephrolites in Cats

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ABSTRACT

***Background*:** Nephrolithiasis is a condition frequently observed in felines, with or without concomitant chronic kidney disease, constituting the major cause of ureteral obstruction and causing progressive damage to the renal parenchyma. About 90% of nephrolites are composed of calcium oxalate, which cannot dissolve, and its incidence has increased substantially in recent years, along with its recurrence. There are functional foods known as nutraceuticals provides health benefits such as renoprotection. Due to these benefits and the high prevalence of nephrolithiasis in feline species, the present study aimed to evaluate the efficacy of the association of some nutraceuticals in the dissolution of nephrolites.

***Materials, Methods & Results*:** The included cats had an ultrasound diagnosis of nephrolithiasis that, on clinical examination, showed: (i) the absence of previous or concomitant treatment for urinary infections and/or vesical and renal lithiasis; (ii) absence of genitourinary clinical manifestations; (iii) absence of obstructive processes; and (iv) absence of concomitant ureterolithiasis. We separated 51 cats with nephrolithiasis into 2 groups: control (n = 12) and study (n = 39). The control group received a placebo and the study group, nutraceuticals (magnesium chelate, resveratrol, vitamin K2, docosahexaenoic acid, and eicosapentaenoic acid). Laboratory tests (blood and urine) and abdominal imaging (ultrasound) were performed at day 0 and 30 days after enrollment. Monitoring the therapeutic efficacy in both groups was performed on day 30 for the CG and on days 30, 60, and 90 for the EG. The CG cats, after this step, were referred to surgery or to the EG, with prior authorization from their tutors. The diameter of nephrolites was significantly different before and after treatment, indicating a reduction in nephroliths over time. During the use of the nutraceutical formulation by the EG and use of placebo by the CG, there were no clinical and/or laboratory manifestations of side effects.

***Discussion*:** The dissolution of nephrolites was correlated with the synergism promoted by the combination of nutraceuticals and not with the individual beneficial action of each nutraceutical since nutraceuticals have individually been used in isolation for a long time in veterinary medicine without having yielded the same benefit. Those undissolved were attributed to a different type of mineral composition than the one associated with the nutraceutical compound in this study, such as struvite or ammonium urate, of mixed composition or comprising solidified dried blood, which has shown an increasing increase in use in recent years. The dissolution of nephrolites was correlated with the synergism promoted by the combination of nutraceuticals and not with the individual beneficial action of each nutraceutical since nutraceuticals have individually been used in isolation for a long time in veterinary medicine without having yielded the same benefit. The undissolved nephrolites were attributed to the nephrolites that obtained partial dissolution at the end of 90 days, in that they possibly needed a longer time to obtain complete dissolution or that they present another mineral composition in their nucleus; that is, it is a compound-type urolith. The nutraceutical compound proved to be effective in the dissolution of nephrolites in the cats included in this study, and the time of use (up to 90 days) showed no negative influence on the clinical or laboratory tests during the entire treatment period.

Keywords: cats, food supplement, functional foods, natural products, nephrolithiasis, nutraceuticals.

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INTRODUCTION

Nephrolithiasis occurs frequently in cats and can lead to urinary tract obstruction. As a manifestation of this condition, nephrolites can form from the precipitation of lithogenic ions in supersaturated urine in the presence of pre-existing kidney damage at a favorable urinary pH or by other predisposing factors [2,20]. Clinical manifestations, when present, are variable and are related to evolution speed [1]. Ultrasonography and abdominal radiography are the most commonly used diagnostic methods [2,20]. However, computed tomography has a greater sensitivity and specificity [4].

Treatment is aimed at the resolution of the underlying cause, prevention of growth, and prevention and/or reduction of the damage the condition causes to the renal parenchyma. Surgery is appointed as the treatment of choice, as even if the obstruction is only partial, the kidney may suffer irreversible damage. However, one must take into account the high rate of mortality (20~30%) and post-surgical complications (20~40%) of this treatment modality [16,21].

The initiative to develop a nutraceutical compound as an alternative to the surgical management of nephrolithiasis was due to their health benefits and the high prevalence of cats affected by calcium oxalate nephrolithiasis (CaC_2O_4) [1,4,7,16,21]. This study aimed to evaluate the therapeutic efficacy of a nutraceutical compound in dissolving nephrolites in cats.

MATERIALS AND METHODS

Local

This study was conducted at the Small Animal Veterinary Hospital of the Federal Rural University of Rio de Janeiro (UFRRJ).

Animals

The included cats had an ultrasound diagnosis of nephrolithiasis that, on clinical examination, showed: (i) the absence of previous or concomitant treatment for urinary infections and/or vesical and renal lithiasis; (ii) absence of genitourinary clinical manifestations; (iii) absence of obstructive processes; and (iv) absence of concomitant ureterolithiasis.

Treatment safety assessment

Blood samples were collected after the cats were made to fast (8-12 h) to detect the blood count and serum urea, creatinine, and magnesium levels. Urine samples were collected via cystocentesis for the analysis of abnormal sediment elements. Samples were collected every 30 days.

Distribution of groups

The animals were divided into 2 groups in a simple random way using a free number draw (https://sorteador.com.br/sorteador/numeros). One group comprised animals that received the nutraceutical formulation (EG) and the other group, animals that received a placebo (CG).

Treatment

The treatment of both groups was initiated (day 0) with the EG receiving the nutraceutical formulation (manipulated capsules) containing the following compounds: eicosapentaenoic acid (EPA) 40 mg/kg; docosahexaenoic acid (DHA) 25 mg/kg; vitamin K2 (MK-7) 2 µg/kg; resveratrol 2 mg/kg; and magnesium chelate 600 µg/kg. The CG received a placebo containing microcrystalline cellulose excipient (Celulomax E®)[1]. Both formulations were orally administered to the animals by their respective tutors every 24 h for a period of 90 days for the EG and 30 days for the CG. The tutors were also instructed to always administer the capsules after feeding, to increase the water supply by adding more pots of water to the environment, and to include a moist diet.

Effectiveness assessment

Monitoring the therapeutic efficacy in both groups was performed on day 30 for the CG and on days 30, 60, and 90 for the EG. The CG cats, after this step, were referred to surgery or to the EG, with prior authorization from their tutors.

Statistical analysis

To test the effectiveness of the treatment over time, the analysis of variance was used with data from both groups. To test the relationship of the decrease in nephrolites with serum concentrations of magnesium, creatinine, and urea in both the CG and EG, Pearson correlation tests were performed. All of the analysis were conducted in R for Windows2 v.4.0.3 with the help of the R studio[2] v1.3.1093 graphical interface and use of FSA[2] v0.8.31 packages; car[2] v3.0-10; agricolae[2] v1.3-3c and the graphics were built with the R package ggplot[2] v3.3.2d.

RESULTS

Animals

A total of 51 cats were included, divided into the CG (n = 12) and EG (n = 39). Of these, 70.6% (36/51) were female and 29.4%, male (15/51). Regarding the distribution of animals by age group, 25.5% (13/51) were aged between 7 months and 5 years, 31.4% (16/51) aged between 6 and 10 years, 39.2% (20/ 51) aged between 11 and 15 years, and 3.9% (2/51) aged over 16 years. The majority of the cats were mixed breed, with 96% (49/51) and the remaining 4%, represented by the breeds Persian 2% (1/51) and Siamese 2% (1/51), respectively. There was no difference between the animals in different age groups regarding the presence of nephrolites (Figure 1).

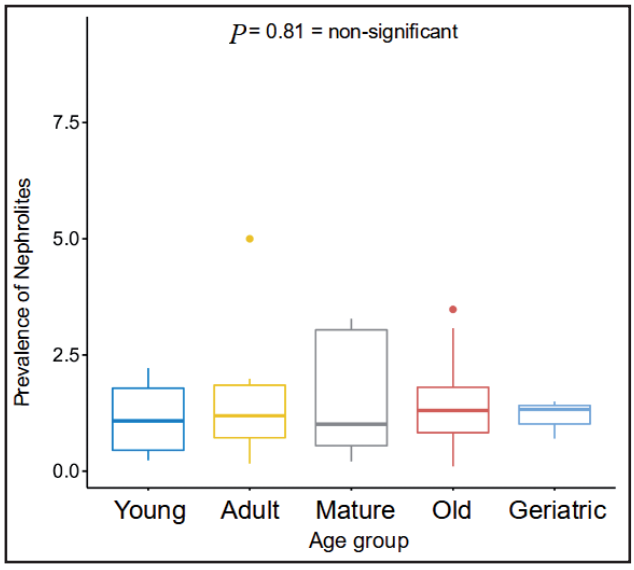

Figure 1. Box-plot of the analysis of variance between the prevalence of nephrolites and age groups. Dots on the graph indicate outliers.

Treatment safety assessment

The hematological variables remained within the reference value in all of the cats throughout the study. In the evaluation of the urinalysis of both groups, only 6% of the EG (3/51) presented with CaC2O4 crystals and concomitant acid pH, before the treatment. However, at the end of 30 days, crystals were no longer observed in 2 cats, and one cat persistently had crystalluria until 60 days after treatment. At 90 days, none of the cats had crystalluria.

The correlation between the variation in the diameter of the nephrolites and variations in the serum concentrations of magnesium, creatinine, and urea was similar; that is, there was no significant reduction or increase in the serum concentrations of these compounds during the treatment related to the size of the lithiasis (Figure 2).

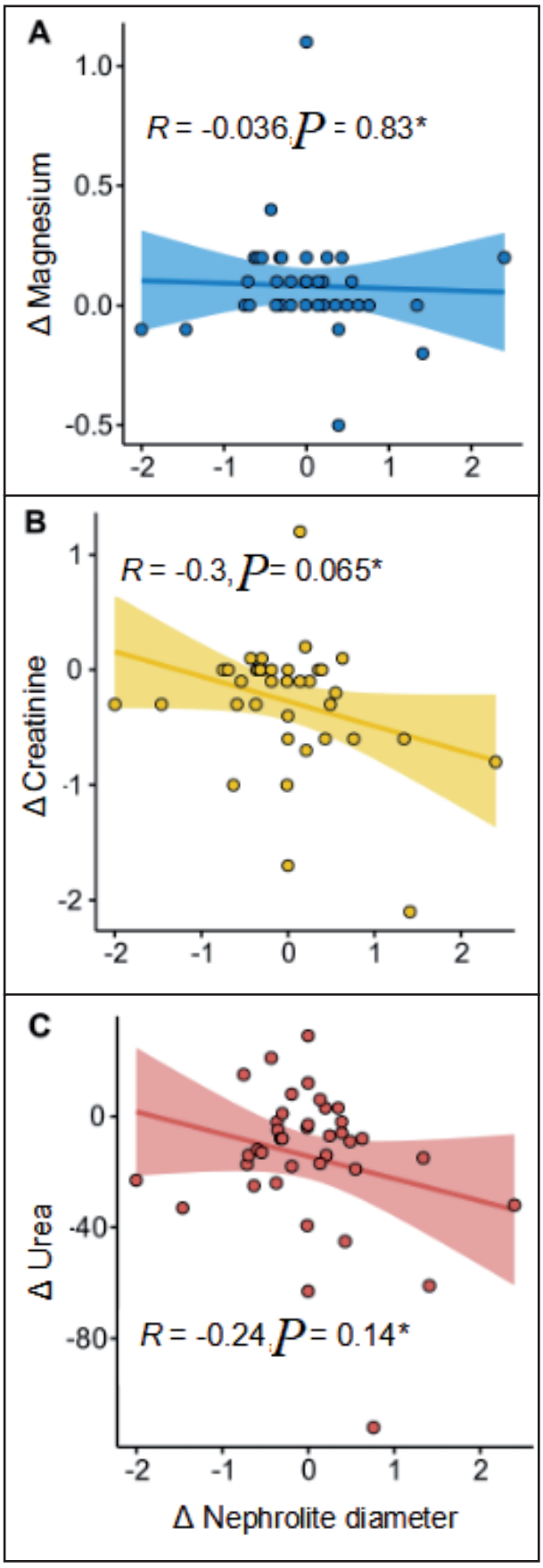

Figure 2. Pearson correlations between the variation in the nephrolite diameter and variations. A- Magnesium. B- Creatinine. C- Urea. *not significant.

During the use of the nutraceutical formulation by the EG and use of placebo by the CG, there were no clinical and/or laboratory manifestations of side effects.

Evaluation of treatment effectiveness

It was possible to observe a significant reduction in the diameter of nephrolites between both groups (Figure 3) [$P = 0.0046$] and throughout treatment (Figure 4) [$P = 0.003$], indicating the therapeutic efficacy of the nutraceutical compound (Figures 5 & 6). No significant difference was detected between nephrolite diameters and their presence in the right and left kidneys (Figure 7), indicating that there was no evidence of greater incidence and/or diameter in one kidney over the other.

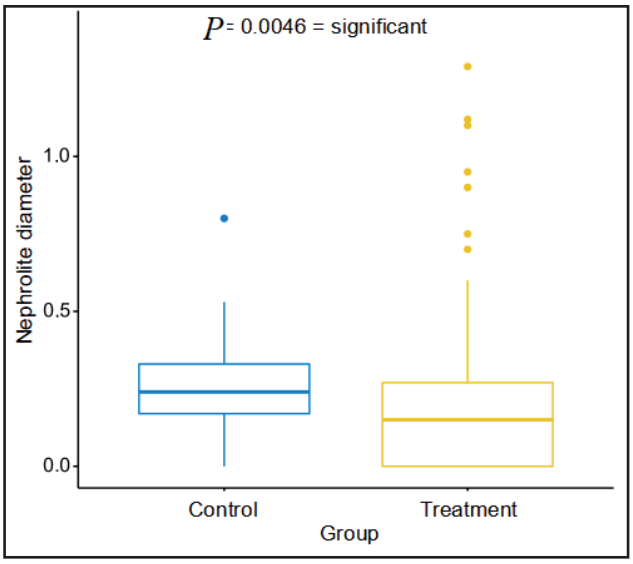

Figure 3. Box-plot analysis of variance between the nephrolite diameter and treatment and control groups. Dots on the graph indicate outliers.

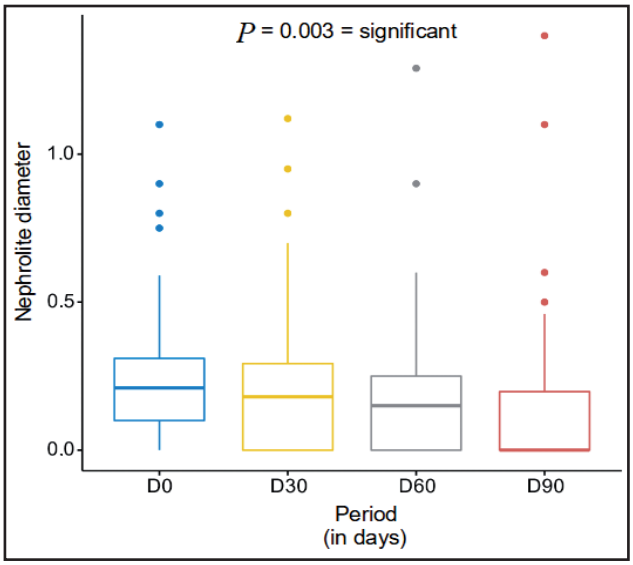

Figure 4. Box-plot analysis of variance between the nephrolite diameter and treatment period. D0= day zero; D30= day 30; D60= day 60; and D90= day 90. The dots on the graph indicate outliers.

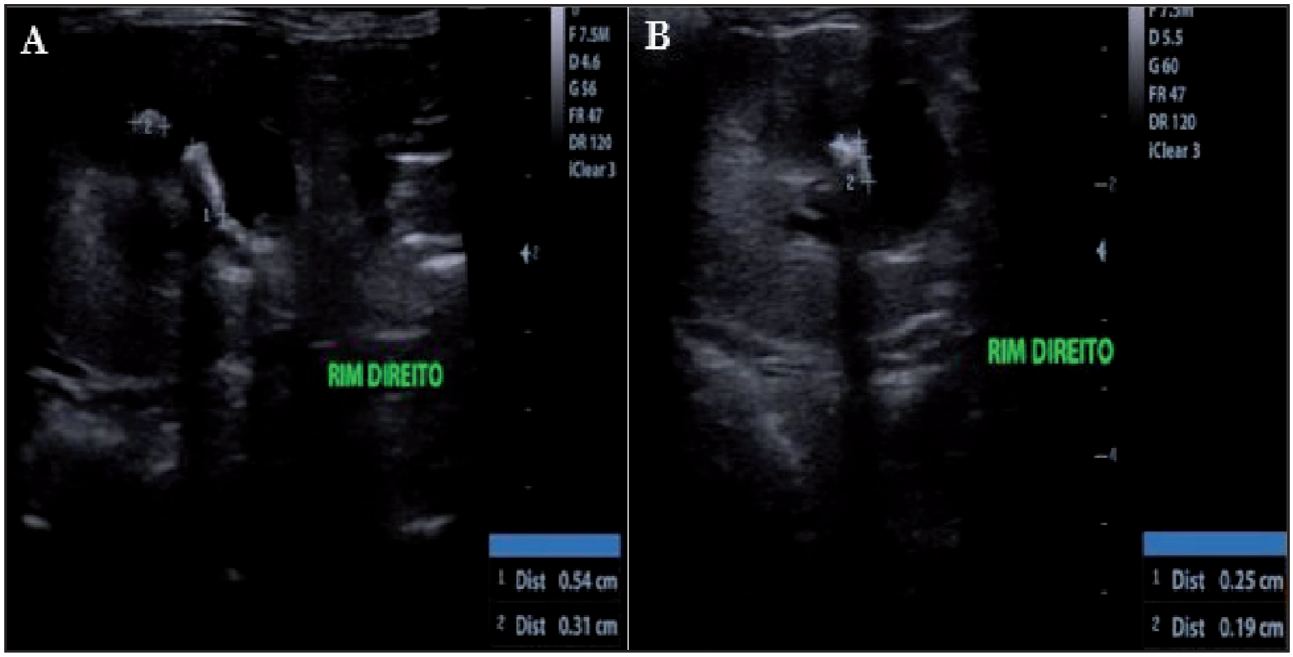

Figure 5. Diagnosis of nephrolithiasis in one right kidney. A- Day 0: Presence of 2 uroliths measuring 0.54 cm and 0.31 cm, respectively. B- After 30 days of treatment: reduction in diameter of both uroliths (0.25 cm and 0.19 cm, respectively).

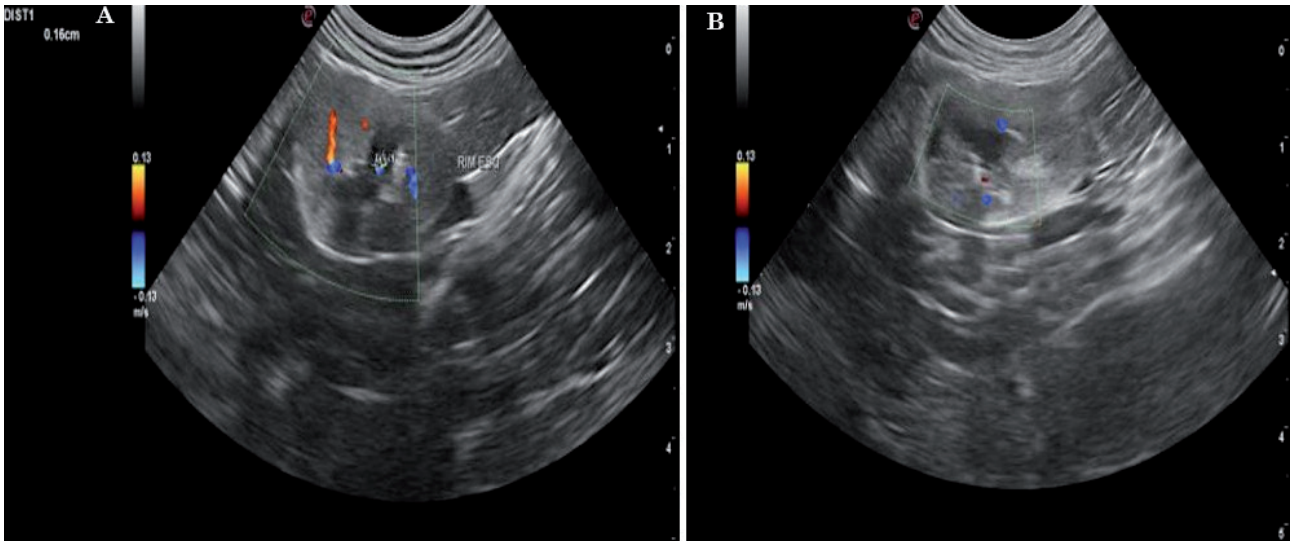

Figure 6. Diagnosis of nephrolithiasis in one right kidney. A- Day 0: Presence of 1 urolith measuring 0.16 cm. B- After 30 days of treatment: absence of its visualization.

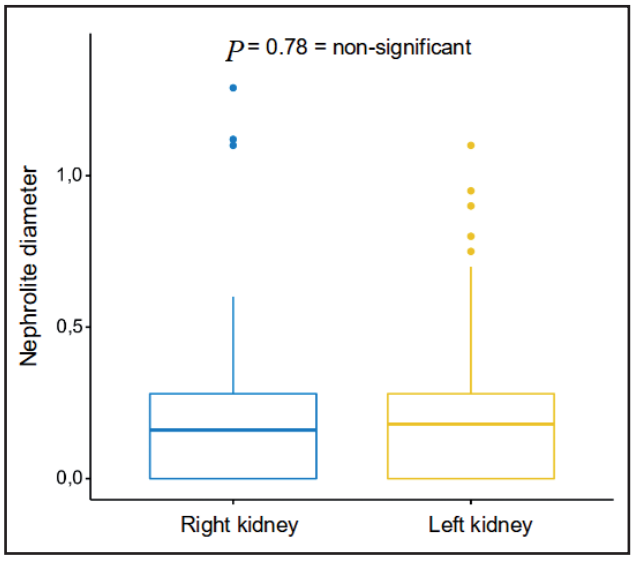

Figure 7. Box-plot analysis of variance between the nephrolite diameter and presence of nephrolites in the right and left kidneys. Dots on the graph indicate outliers.

DISCUSSION

Regarding the sexual distribution of both groups, the results obtained were similar to those described in literature [4,16,21], in which the number of affected females was higher than that of males. However, no study to date has proven a sexual predisposition [9].

The results of the evaluation of hematological variables were not surprising since patients with nephrolithiasis do not usually present with hematological alterations in the absence of complications secondary to their presence [15].

The low occurrence of crystalluria in both groups reinforced the findings of one research [16], that reported only a small percentage of cats with CaC_2O_4 nephroureterolites presented with CaC_2O_4crystalluria. In cats, it is associated with CaC_2O_4 crystalluria due to a magnesium-restricted diet to control struvite lithiase formation [2,3]. However, this fact cannot be attributed to this study as 3 of the cats had serum magnesium levels within the reference value [6], although measuring urinary concentrations is more highly recommended than measuring serum to assess the occurrence of deficiency [13].

The fact that only 5.8% of the cats (3/51) had an acidic urinary pH reinforces that the role of pH in the formation of CaC_2O_4 is controversial as it can develop at both acidic and basic pH [5].

Azotemia has been reported as a common laboratory alteration in felines with active nephrolites, which is not consistent with the findings of this study in which all of the cats had inactive nephrolites. Thus, we can observe that in azotemic cats, chronic kidney disease (CKD) is confirmed, revealing a positive association between nephrolithiasis and CKD. However, it cannot be said that lithiasis is the trigger of CKD or vice versa [14].

Although hypermagnesemia is related to a worsening of kidney disease [17], no patient presented an increase in magnesium concentration after tak-

ing supplements during the treatment, showing that it was safe for the animals to consume this mineral. Despite this result, it is worth emphasizing the ability of the remaining nephrons to adapt and to maintain the glomerular filtration rate in patients with kidney disease [10].

However, the execution time of this study may not have been sufficient for the occurrence of serum elevation of this mineral. It is interesting to note that there were no differences between the variation in the diameter of nephrolites and serum concentrations of total magnesium, urea, and creatinine, even pointing toward a negative correlation, suggesting that the reduction in nephrolite diameter is positively correlated with the reduction of the aforementioned serum concentrations. This is possibly a result of the renoprotective action of other nutraceuticals that make up the compound, acting on oxidative stress and damage to renal epithelial cells promoted by the presence of CaC_2O_4 crystals [9,15].

The dissolution of nephrolites was correlated with the synergism promoted by the combination of nutraceuticals and not with the individual beneficial action of each nutraceutical since nutraceuticals have individually been used in isolation for a long time in veterinary medicine without having yielded the same benefit [7,18]. Those undissolved were attributed to a different type of mineral composition than the one associated with the nutraceutical compound in this study, such as struvite or ammonium urate, of mixed composition or comprising solidified dried blood, which has shown an increasing increase in use in recent years [8,14].

The undissolved nephrolites were attributed to the nephrolites that obtained partial dissolution at the end of 90 days, in that they possibly needed a longer time to obtain complete dissolution or that they present another mineral composition in their nucleus; that is, it is a compound-type urolith [11].

The right kidney presented with the largest dissolution of nephrolites. It was not possible to attribute this fact to the receipt of bioactive compounds of nutraceuticals in higher concentrations in the right than in the left kidney since neither the vascular architecture nor hemodynamic aspects of both kidneys, as detected via Doppler mapping, changed before or during this study [12,19].

Such results demonstrate a promising alternative in the clinical management of feline nephrolithiasis as nutraceuticals have the advantage of being low-cost, non-invasive, and without side effects. These criteria are desirable when treatment approaches are discussed with patients who have a high prevalence of occurrence of functional renal disorders [22].

CONCLUSION

The nutraceutical compound proved to be effective in dissolving nephrolites in felines, and the time of use showed no negative influence on the clinic and/or laboratory tests during the entire period of treatment. Additional and prolonged studies are needed to determine (on average) the time of use of this compound, time interval between use and relapses of nephrolites, whether there will be systemic repercussions of chronic use, and its effects on the serum levels of urea, creatinine, and magnesium.

MANUFACTURERS

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***Ethical approval*.** This research was approved by the Committee for Ethics on the Use of Animals (CEUA) of the Veterinary Institute of the Federal Rural University of Rio de Janeiro (UFRRJ) - under protocol n. 4278041119.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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