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Effect of the association of diet, omega-three, and antioxidants in dogs with chronic kidney disease

Efeito da associação da dieta, do ômega três, e de antioxidantes em cães portadores de doença renal crônica

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Abstract

To evaluate the contribution of the drug combination of omega-3, vitamin E, sodium selenite, copper gluconate, zinc gluconate, chondroitin sulfate, and glucosamine (Gerioox®), 12 dogs with chronic kidney disease (CKD) in outpatient clinical care at the veterinary hospital of a federal institution of higher education were studied. Complete blood counts, urinalyses, measurements of the serum concentration of calcium, phosphorus, urea and creatinine, and calculations of the urinary protein to creatinine ratio (UPC) and glomerular filtration rate (GFR) were performed before starting the experiment (T0) and after 30 (T1), 90 (T2), and 120 days (T3). There was a significant negative correlation ($P<0.05$) between the GFR and UPC and between the GFR and serum urea and creatinine (when the GFR was high, the UPC, serum urea and creatinine were decreased). Improvement was observed in the clinical status of the patients studied, as reported by their owners, who indicated improved vitality and appetite, and by clinical observation, which showed improvement in the overall health status, coat, and analyzed parameters. The combination of omega-3, vitamin E, sodium selenite, copper gluconate, zinc gluconate, chondroitin sulfate, and glucosamine found in Gerioox® proved to be an important adjuvant in the conservative treatment of dogs with CKD, causing an increase in the GFR with a decrease in proteinuria. This result indicates that there was an improvement in the quality of excretion, not an increase in the excretion itself, which is a result of the undesired effect of increased glomerular pressure.
Key words: Polyunsaturated fatty acids, kidneys, canine

Resumo

Com o objetivo de avaliar a contribuição da associação medicamentosa de ômega-3, vitamina E, selenito de sódio, gluconato de cobre, gluconato de zinco, sulfato de condroitina e glucosamina (Gerioox®), 12 cães oriundos do atendimento clínico ambulatorial do hospital veterinário de uma instituição federal de ensino superior, portadores de doença renal crônica (DRC), foram estudados. Foram realizados exames de hemograma, medida de concentração sérica de cálcio, fósforo, ureia e creatinina, urinálise, razão

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proteína creatinina urinária (RPC) e taxa de filtração glomerular (TFG) antes de iniciado o experimento (T0), após 30 (T1), 90 (T2) e 120 dias (T3). Houve correlação negativa significativa (P<0,05) entre a TFG e a RPC e a TFG e uréia e creatinina sérica (quando a TFG apresentava-se elevada, a RPC, assim como ureia e a creatinina séricas encontravam-se diminuídas). Houve melhora do estado clínico dos pacientes estudados observado pela sinalização dos tutores informando melhora da vitalidade e apetite e pelas observações clínicas de melhora do estado geral, da pelagem e dos parâmetros analisados. A associação de ômega-3, vitamina E, selenito de sódio, gluconato de cobre, gluconato de zinco, sulfato de condroitina e glucosamina revelou ser um importante coadjuvante no tratamento conservador da DRC de cães, causando aumento da TFG com diminuição da proteinúria, significando que houve uma melhora na qualidade da excreção, e não um aumento da excreção, por um efeito indesejado de aumento da pressão glomerular.
Palavras-chave: Ácidos graxos poli-insaturados, rins, canino

Introduction

Chronic kidney disease (CKD) is a condition in which animals have kidneys with abnormal anatomical-functional characteristics. These abnormalities, which are caused by genetic or congenital malformations or by acquired processes, lead to an inability to exercise one or more renal functions, which can be manifested both mildly and severely in animals. This condition is degenerative, progressive and irreversible and decreases the animal's lifespan (BROWN, 1999; POLZIN, 2011; BARTGES, 2012).

Protocols for the clinical management of older dogs and cats with a CKD diagnosis aim at maximizing the residual renal function, delaying the progression of the disease and alleviating the symptoms of uremia. These effects are achieved by improved excretion and correction of electrolyte, acid-base, endocrine and nutritional balance (HOSKINS, 2008; BARTGES, 2012).

Dietary therapy remains the basis of conservative medical treatment for these animals. The main objectives of nutritional support for patients with CKD are to maintain lean muscle mass and an ideal body condition and to control protein, phosphorus and sodium as well as other compounds, such as vitamins and other minerals (BROWN, 1999; POLZIN, 2011; BARTGES, 2012).

Although standard protocols have been proposed for treating CKD, the use of adjuvant therapies with renoprotective effects has been widely discussed. These effects have been observed with the use of omega-3 (BROWN et al*.,* 2000; HERNÁNDEZ et al*.,* 2005; VEADO et al., 2005).

Recently, a drug has become available on the Brazilian market that consists of a combination of omega-3, vitamin E, sodium selenite, copper gluconate, zinc gluconate, chondroitin sulfate, and glucosamine (Gerioox®). This drug was initially developed for the treatment of geriatric dogs and cats and has antioxidant and chondroprotective properties. It was hypothesized that this combination improves the glomerular filtration rate (GFR), reducing the primary cause of CKD progression, to wit, proteinuria (BARTGES; POLZIN, 2011).

Slowing the progression of CKD is a critical part of the care of affected dogs and cats. Renal oxidative stress is a factor previously unrecognized in the progression of CKD in small animals. The use of drugs, such as antihypertensive inhibitors of angiotensin-converting enzyme (ACE), which is responsible for converting Angiotensin I into Angiotensin II, and calcium channel antagonists as well as the use of omega-3 fatty acids and other antiproteinuric therapies, are commonly recommended for these patients. A reduction in oxidative stress of the kidney would be expected with the use of these therapeutic measures, decreasing the production of reactive oxygen species (ROS) (BARTGES; POLZIN, 2011).

Traditionally, the term oxidative stress describes the imbalance between the oxidant and antioxidant

defense mechanisms. In research involving human patients with CKD, an increase in lipid peroxidation and a decrease in antioxidant enzymes and vitamins and trace elements have been shown (Selênio, Zinco) (MAFRA et al., 1999). The same reactions most likely occur in dogs because animals with CKD often have concurrent conditions that increase the generation of ROS, such as advanced age, activation of the renin-angiotensin-aldosterone system and several systemic disorders (SCOTT, 2008; BARTGES; POLZIN, 2011). Furthermore, Carciofi et al. (2002) emphasized the importance of vitamin E when reporting that the consumption of polyunsaturated fatty acids (PUFA) over an extended period can also increase lipid peroxidation; therefore, PUFA consumption should be compensated with a higher supplementation of vitamin E.

Studies have been investigating the effects of omega-3 on healthy, under assault, and diseased kidneys. In this context, the combination of omega-3, vitamin E, sodium selenite, copper gluconate, zinc gluconate, chondroitin sulfate, and glucosamine (Gerioox®) has not been thoroughly evaluated as to its true beneficial effects on kidneys (VEADO et al., 2013).

This study aimed to evaluate the action of omega-3 in association with antioxidants as an adjuvant treatment in dogs with CKD. For this, a clinical study was performed with the drug combination of omega-3, vitamin E, sodium selenite, copper gluconate, zinc gluconate, chondroitin sulfate, and glucosamine (Gerioox®).

The statistical analyses of the data were performed using descriptive statistics and correlation statistics.

Materials and Methods

Animals

This study was approved by the Ethics Committee for Animal Experimentation of a federal institution of higher education and was conducted under protocol number 267/2012.

The owners of all of the animals who participated in this study were required to sign an informed consent form.

Twelve dogs recruited from the outpatient clinical care unit of a Veterinary Hospital specializing in veterinary nephrology were used: seven five males and five females (three Poodles, three Shih Tzu, one Lhasa Apso, one Maltese, one Bichon Frise, one German Shepherd, and two dogs of undefined breed), with ages ranging from 3 to 12 years and body weights between 5.0 and 25 kilograms.

In this study, all animals showed sonographic findings consistent with CKD, i.e., kidneys with decreased size that were hyperechoic with decreased corticomedullary differentiation. During the experiment, the animals were kept in their homes, but on four separate occasions, they were hospitalized for 24 hours at the veterinary hospital for evaluation and testing.

Before animals were allowed to participate in this experiment, serological tests (ELISA and RIFI) were conducted to exclude animals with leishmaniasis. Furthermore, all animals had undergone updated vaccinations and an annual deworming. Additional therapy for CKD beyond the specified diet and Gerioox® was not provided during this study, although the use of gastric mucosal protectants, such as omeprazole, was allowed for patients presenting signs of uremia.

The patients were classified according to the criteria proposed by the International Renal Interest Society (IRIS – IRIS Staging System of CKD, 2009) for CKD staging. Seven animals belonged to stage 1, three animals to stage 2, one animal to stage 3, and one animal to stage 4. The animals in stage 3 and 4 were already being followed by a veterinary nephrologist and were therefore already hydrated upon being included in the study.

Additionally, the proposed classification considers sub-stages related to proteinuria and to systemic arterial hypertension. These are considered independent factors of CKD progression

that interfere in prognosis and that require specific therapeutic intervention.

To confirm the persistence of proteinuria before the beginning of the experiment, two UPC tests and one urinalyse were performed at intervals of 15 days, as recommended by IRIS. However, the blood pressures were not measured because of the difficulty in handling some dogs and the subsequent controversial blood pressure results due to the agitation of the animals.

Diet

The animals were fed a specific diet for patients with nephropathy[5]. The diets were adjusted at the time the animals were admitted to the study for obtaining the best energy balance as well as the best levels of minerals, vitamins and proteins. The diet was introduced gradually, and the transition to the new diet occurred within one week.

The amount of food given to the experimental animals was estimated by the calculation of maintenance energy requirements, using the following formula:

$$MER = 140 \times BW^{0.75} \text{ kcal}$$

MER = Maintenance Energy Requirement

BW = Body weight in kilograms

$BW^{0.75}$= Metabolic weight

Gerioox® [6]

The drug combination, developed as a geriatric medicine for dogs and cats with immunomodulatory, antioxidant, and chondroprotective actions, acts to improve the vital functions of the body through the action of omega-3 in combination with antioxidants in different organs of the body (GERIOOX, 2005).

Each tablet is composed of oil rich in omega-3 fatty acids (flaxseed, fish, borage oil, or rose oil) (0.2 mL); D-Glucosamine (0.140 g); Chondroitin Sulfate "A" (0.150 g); Copper gluconate (0.003 g); Zinc gluconate (0.20 g); Sodium selenite (0.005 mg); Vitamin E (0.05 g); and Excipients q.s. 1.800 g.

Study protocol

For 30 days, the animals received only the specified diet, followed by a 60-day period during which they received a half a tablet of Gerioox® every 12 hours. At the end of the 60 days, Gerioox® was prescribed for another 30 days at a dose of half a tablet per day. This dosage is proposed by the manufacturer and is used by clinicians who prescribe this medication.

Analysis of patients

The dogs were selected based on the characteristic signs of CKD observed by ultrasonography. The ultrasound evaluation consisted of determining the kidney size, contour, echogenicity, echotexture, and corticomedullary differentiation. Animals whose kidneys were decreased in size, hyperechoic and with decreased corticomedullary differentiation by ultrasound were classified as having CKD. All of the animals were normohydrated.

After inclusion in the experiment, the animals were clinically evaluated based on the classic semiological parameters and through measurements of the heart rate, respiratory rate, and rectal temperature. Laboratory tests of the blood count, renal profile (serum urea and creatinine), serum calcium and phosphorus, urinalysis, and UPC as well as clinical evaluations were performed at four time-points: before starting the experiment (T0) and after 30 (T1), 90 (T2), and 120 days (T3) (Table 1). T0 corresponds to the beginning of the experiment; T1 corresponds to 30 days using only the specified diet; T2 corresponds to 90 days using the specified diet and 60 days of supplementing with Gerioox® at a dose of half a tablet every 12 hours; T3 corresponds to 120 days using the specified diet and 30 days of supplementation with Gerioox® at a dose of half a tablet per day.

[5] Renal Feed – Farmina Vetlife®

[6] Gerioox – Labyes Especialidades Veterinárias®

Table 1. Time-points of patient evaluations and tests.

Before Time-point 0 (T0)	After 30 days Time-point 1 (T1)	After 90 days Time-point 2 (T2)	After 120 days Time-point 3 (T3)
Farmina Vet life® Renal Feed	After 30 days of Renal Feed	After 60 days of Renal Feed + Gerioox® loading dose	After 90 days of Renal Feed + Gerioox® maintenance dose

Four treatments were considered for the statistical analysis, which corresponded to the four assessment time-points.

All of the data and test results of these animals were recorded in medical files and organized in a descriptive manner.

Collection of materials

After each time interval studied, the dogs were subjected to a 24-hour stay at the Veterinary Hospital for clinical evaluation and the collection of test material. Blood samples were collected by venipuncture of the jugular vein after fasting the animals for eight hours, beginning at the time of arrival. The dogs were housed in individual metabolic cages (external width and depth of 800 mm and height of 1350 mm), with a screen base above a tray for urine collection to calculate the urine volume for the evaluation of GFR.

Evaluation of GFR

An endogenous creatinine marker was used to evaluate GFR (FINCO, 1995; GRAUER, 2010; POLZIN, 2011). Serum samples obtained from the animals at the time of arrival to the hospital and the urine samples collected during the 24-hour hospital stay were used to obtain the serum and urine creatinine concentrations, respectively. The volume (in mL) of urine collected over this period was then divided by 1,440 (the number of minutes in 24 hours) and multiplied by body weight to calculate the GFR per minute. The data (urine volume, serum, and urinary creatinine) were used in the following formula to calculate GFR (FINCO, 1995; GRAUER, 2010):

$$GFR = \frac{Cr_u\ (mg/dL) \times Vol_u\ (mL)}{Cr_{ser}\ (mg/mL) \times T\ (min) \times BW\ (Kg)}$$

where

GFR = glomerular filtration rate

Cr_u = urinary concentration of creatinine

Vol_u = urine volume

Cr_{ser} = serum concentration of creatinine

T = time in minutes (1,440 minutes)

BW = Body weight

Results and Discussion

Clinical observations

According to the reports from those responsible for the studied animals and the clinical observations, there was significant improvement in the overall health status of the patients due to systemic effects caused by the use of Gerioox®. Signs of increased appetite with a consequent improvement in quality of life, as indicated by greater vitality, were reported by owners, who said that their animals were livelier and showed greater agility and happiness. Upon clinical examination, this improvement could be

perceived through better appearance of the skin and coat and the nutritional status of these patients (HOSKINS, 2008). Because the animals did not exhibit low weight conditions, there was no change in their body condition scores during the studied period.

Hoskins (2008) has reported that cognitive, physical, and medical changes impact animal behavior and that any drugs used to control a primary medical problem may also influence behavior.

Hematological changes

Although it is commonly found in patients with CKD, anemia was only detected in one animal from the present study. This finding is easily explained by the demographics of the group studied because only one animal belonged to stage 4 of the IRIS classification. The animal that was classified as stage 3 did not present with anemia, which shows that complications of CKD are inherent to each patient and each stage of IRIS classification.

Regarding the evaluation of leukocytes, only the animal belonging to stage 4 in the IRIS classification system presented leukopenia. According to Jaber et al. (2001), uremic toxins produced in CKD can affect leucocytes, in particular neutrophils, making them more susceptible to apoptosis and strengthening the hypothesis that dogs with renal disease have compromised innate immune systems.

Changes in serum biochemistry

Azotemia, a common complication that occurs in patients with renal failure, is characterized by increased non-protein nitrogen waste, such as urea and creatinine, in the blood (DIBARTOLA, 2004; POLZIN, 2011). Ten animals in the study showed serum urea levels greater than the reference values, and of those animals, only four had serum creatinine levels above the reference value at T0. The reference values used for dogs in this study were 15 to 40 mg/dL for urea and 0.5 to 1.5 mg/dL for creatinine (GRAUER, 2010).

Seven of the 12 animals studied showed improvement in the serum urea levels, and two maintained their original levels throughout the study. Of the seven who showed improvement, six also showed improvement in the serum creatinine levels; one animal had no change in creatinine levels despite some improvement in urea levels.

A decrease in the mean creatinine levels can be observed in Table 2, especially when comparing T0 with T2.

Table 2. Mean values of urea, creatinine, phosphorus, UPC, and GFR for dogs that received the drug combination of omega-3, vitamin E, sodium selenite, copper gluconate, zinc gluconate, chondroitin sulfate, and glucosamine, analyzed at 0, 30, 90, and 120 days of treatment.

	Mean values				
	Urea	Creatinine	Phosphorus	UPC	GFR
T 0	91.26	1.74	6.30	0.87	2.37
T 1	71.03	1.76	5.20	0.81	2.00
T 2	80.75	1.34	6.12	0.69	2.98
T 3	79.33	1.56	5.88	0.58	2.65

Comparing the mean serum urea and creatinine levels in Table 2, it can be observed that there was a more evident decrease in urea levels. The diet used in this experiment consists of protein with a high biological value, which helps to decrease the formation of urea, and this feature may explain the reduction in the level of this catabolite. Additionally, the diet contains beet fibers that stimulate the growth

of nitrogen-dependent bacteria, further decreasing the production of urea. Because serum creatinine did not show a decrease between T0 and T1, unlike serum urea, it is believed that the decrease in urea levels was due more in part to the decreasing urea production than to improved excretion. However, when assessing the mean urea and serum creatinine levels, the results show a decrease in both levels between the time-points T0 and T3. Brown et al. (1998) and Wong et al. (2010) also reported a decrease in serum creatinine in studies of dogs and humans whose diets were supplemented with omega-3.

Hyperphosphatemia is also a common finding in dogs with CKD, as it is caused by reduced phosphate excretion by the kidneys and is also a result of the reduction in the synthesis of calcitriol (GRAUER, 2010).

The normal range for serum phosphorus is considered to be 2.5 to 5.5 mg/dL for dogs (GRAUER, 2010). Evaluating the trend of the mean serum phosphorus levels in Table 2, it can be observed that the T0 levels are above the normal range and that the T1 levels are already within normal range, showing that diet has an important influence on the normalization of serum phosphorus concentrations. This trend is changed again when Gerioox® is introduced, as an increase in serum phosphorus levels is observed for the first 60 days, only to decrease in the 90-day evaluation, with a tendency, however, toward the normal range. Eight animals had serum phosphorus levels greater than normal at time T0; of these, six animals showed a significant decrease in those levels by T3. Four of these 6 animals, which showed a decrease in serum phosphorus levels, showed their lowest phosphorus levels at T2, and the remaining 2 animals showed their lowest phosphorus levels at T3.

The total serum calcium can be found at high, low or normal levels in uremic patients. The normal values for serum calcium in dogs are considered to be 9.0 to 11.3 mg/dL (GRAUER, 2010). All of the animals showed calcium levels within the normal range throughout the study.

Changes in urinalysis and urine biochemistry

Although urine specific gravity varies widely inter- and intra-individuals in healthy conditions, this is a practical, sensitive parameter for assessing renal function because a decreased urinary concentrating ability is often a result of renal injury (BROWN et al., 1997; GRAUER, 2010). The presence of organic solutes, such as proteins, amino acids and glucose, can cause an increase in urine specific gravity. It is expected that patients with CKD will present values of urine specific gravity at a range of variation known as "isosthenuria" (1.008 to 1.012). In this study, the animals evaluated had a mean urinary specific gravity with values close to the lower end of the normal range for dogs (1.015 to 1.045) (DIBARTOLA, 2004).

The urinary pH of dogs varies from 5.5 to 7.5. In the present study, all animals had a pH within this range (DIBARTOLA, 2004).

Proteinuria has received much attention in small animal nephrology in recent years and is considered the leading cause of CKD progression. It has been shown that proteinuria may decrease with ACE inhibitor treatment, in both humans and dogs (LEES et al., 2005; GRAUER, 2010; POLZIN, 2013). A small amount of protein in the urine is considered normal. This protein originates from low-molecular weight plasma proteins that pass through the glomerulus or originate in the tubules or urogenital tract. Urine may also contain proteins actively secreted into the tubular lumen, such as Tamm-Horsfall mucoprotein and immunoglobulin A (FINCO, 1995; REGO et al., 2001).

Furthermore, there can also be proteins present in urine due to pyuria resulting from a urinary tract infection. Therefore, it is important to emphasize that the results must always be interpreted in conjunction with the analysis of urinary sediment

(GRAUER, 2010). The proteinuria type of relevance to this study is persistent significant albuminuria. It is currently held that the most practical, reliable way to quantify albuminuria is by measuring the UPC (LEES et al., 2005) (Table 2).

According to Grauer (2010), proteinuria should be quantified by measuring the UPC. This test shows a strong statistical relationship to the determination of 24-hour proteinuria. The advantages of using the UPC include the fact that only a small urine sample obtained at any time of day is needed for the test, which does not interfere significantly with the results. In addition, this test was also sensitive enough to detect mild glomerular disease (ARAÚJO, 2007). The UPC should show results under 0.2 to be considered normal, whereas values between 0.2 and 0.5 are considered the cutoff in dogs. However, if these animals are azotemic, they are already considered proteinuric if they are within this variation range (POLZIN, 2011).

Seven animals had UPC over 0.4 and were considered proteinuric. Eleven dogs displayed a decrease in the value of UPC during the study (Table 2).

The decrease of UPC values over the time studied is suggestive of decreased albuminuria. The animals that participated in this study have in common the fact that they all have glomerular hypertension. This hypertension causes stress on the vascular endothelium, triggering inflammatory reactions, glomerulosclerosis and proteinuria. These consequences of glomerular hypertension are considered the main causative factors of CKD progression (BRENNER et al., 1982). Controlling proteinuria is therefore an important objective for conservative treatment of patients with kidney disease and is one of the most modern concepts used to control the progression of this disease. In the present study, the reduction of proteinuria is a result that already justifies the use of this drug combination because the control of proteinuria represents the control of one of the most important factors of CKD progression (BROWN et al., 1997).

There is a consensus in human and veterinary nephrology that GFR is the best indicator of renal excretory function. The estimation of GFR is simply a special process for measuring the removal of a substance from the body (GRAUER, 2010). According to DiBartola (2004), creatinine is not metabolized and is excreted by the kidneys almost entirely by glomerular filtration. In a state of equilibrium, the creatinine excretion rate is relatively constant, and the serum creatinine concentration varies inversely with GFR; thus, the determination of creatinine clearance is a good way to estimate GFR.

Population-based studies are needed to define the cutoff values for GFR. The reference values most often reported for dogs and cats are between 2 and 4 mL/min/kg (HEIENE; LEFEBVRE, 2007; POLZIN, 2011).

Six animals had GFR values under 2 mL/min/Kg at the beginning of the experiment, but throughout the experiment, all of the animals showed some degree of increase, as was reflected in the mean GFR of the group shown in Table 2. When analyzing the results of GFR and serum creatinine, GFR was observed to decrease, and the serum creatinine increased between T2 and T3. This result can be explained by the proposed treatment between T2 and T3, which was to reduce the dose of the drug combination by 50%. This decrease caused a worsening in excretion, as indicated by the change in behavior of the GFR and serum creatinine.

Kidneys in dogs with CKD have few nephrons. These remaining nephrons hypertrophy due to poor distribution of blood in the renal parenchyma. The same volume of blood enters the diseased kidneys by the renal artery as would enter a normal kidney, even though the diseased kidneys have few glomeruli to receive it. This leads to glomerular hypertension and, consequently, to an increase in the GFR of each nephron (BRENNER et al., 1982). The animals in this experiment showed a trend of

improved excretion (as indicated by the urea and creatinine results), decreased proteinuria and mildly increased GFR. This improvement in GFR also reflects an improvement in creatinine clearance, which is associated with improved quality of the vascular endothelium and not with a greater filtration effort, as observed by Brown (2002). If the increase in GFR was due to a greater filtration effort, there would be an increase in proteinuria, an obvious consequence of increased glomerular filtration in these patients; however, this was not observed in the experiment. A further increase in GFR would be an undesired improvement because the filtration effort would increase and, consequently, so too would the stress on the glomerulus, thus drastically reducing its lifespan. This improvement in the quality of the glomerular endothelium is expected as a consequence of the anti-inflammatory effect of omega-3, which suppresses the formation of pro-inflammatory mediators (BROWN, 2002). Furthermore, omega-3 participates in maintaining the integrity of endothelial cells and generates prostaglandins that increase glomerular filtration by means of its vasodilator effect (BROWN et al., 2000). Chondroitin sulfate, vitamin E, sodium selenite, copper gluconate and zinc gluconate are able to reduce biological damage and the generation of free radicals in cases of oxidative stress, increasing the lifespan and improving the quality of glomerular cells (SANTOS, 2009; BARTGES; POLZIN, 2011).

Evaluation of correlation between the tests performed

Correlation tests applied in this experiment were valuable for indicating consistent trends between evaluations, which could confirm the effectiveness of the proposed treatment. The correlation analysis was required to show important changes in some of the elements analyzed.

When evaluating the result of a correlation, the change of a particular analyte is directly or inversely related to the alteration of another component.

The potential correlation between GFR, urea, creatinine, serum phosphorus, and UPC were considered for this experiment. The potential correlation between these parameters was evaluated by Pearson's correlation coefficient.

The correlations in this experiment that had a level of significance lower than or equal to 0.05 are presented in Table 3. Correlations between GFR and serum phosphorus are also shown, which, despite having a level of significance greater than 0.05, demonstrate the interference of one variable with another and also show their similar behaviors. The other correlations were found to be of low magnitude in this study and, thus, were not highlighted.

There was a significant negative correlation ($P<0.05$) between GFR and UPC. This result reveals that an increase in GFR was accompanied by a decrease in UPC. If the increase in GFR was related to an increase in glomerular pressure, this increase being undesirable, there would also be an increase in UPC. The correlation would then be positive. The observed increase in GFR and decrease in proteinuria is one of the more important results of the experiment, as it shows that the combination of omega-3, vitamin E, sodium selenite, copper gluconate, zinc gluconate, chondroitin sulfate, and glucosamine appears to have contributed to what could be called an "improved kidney environment", inhibiting vasoconstriction and platelet aggregation with a consequent reduction in inflammatory reactions in the glomerular endothelium and in other renal vessels. Because of the changes observed in the results of this experiment, such as improved GFR and decreased proteinuria, one might suspect, as other authors have observed, that one or more of the following changes may have occurred: decreased glomerular pressure, decreased glomerular wall thickness, decreased blood viscosity, improved prostaglandin production, decreased serum concentrations of triglycerides, reversal of dyslipidemia, limitation of intrarenal calcification preventing the deterioration of renal function and preserving the renal structure

with a consequent reduction of glomerulosclerosis, and reduction in renal oxidative stress (CLARK et al., 1991; BROWN et al., 1998; BROWN, 2002; ANDRADE; CARMO, 2006; POLZIN, 2011; BARTGES, 2012); many other potential benefits are also expected when using one or all of the compounds tested.

Table 3. Correlations between the evaluations of GFR and UPC, GFR and serum urea, GFR and serum creatinine, and GFR and serum phosphorus, analyzed at time-points 0, 30, 90, and 120 days of treatment.

Variables	Correlations	
	Correlation (r)	Significance (P)
GFR x UPC	-0.56	<0.0001
GFR x serum urea	-0.52	0.0002
GFR x serum creatinine	-0.45	0.0014
GFR x serum phosphorus	-0.12	0.4334

This negative correlation was also observed between GFR and serum urea and creatinine. In other words, when the patient showed increased GFR, serum urea and creatinine were found in low concentrations. This result confirms that in addition to the feed having controlled and reduced the serum concentrations of urea, Gerioox® improved the excretion of substances.

Despite the negative correlation found between GFR and serum phosphorus, this correlation showed a level of significance greater than 0.05, indicating that the difference was not statistically significant. While serum phosphorus concentrations remain within the normal range, a significant reduction of its levels in the bloodstream is not to be expected, even if GFR improves. This trend was observed in this experiment. The slight increase in GFR was not enough to significantly modify phosphorus excretion.

The ideal dose of omega-3 is difficult to determine because there are no studies indicating the conversion rate of linolenic acid (omega-3) to eicosapentaenoic acid and of linoleic acid (omega-6) to dihomo-gamma linolenic acid and arachidonic acid (CARCIOFI et al., 2002). However, it is likely that a possible synergistic effect of these substances results in a lower dosage requirement when combined.

Conclusions

The 90-day study period appears to have been sufficient to assess the beneficial effects of the drug combination tested on the kidneys, with regard to improvement of the excretory function. The diet used in the study helped to homogenize the group of animals studied, decreasing the concentrations of some compounds whose excess was undesirable, maintaining body condition scores and nutritional status and reducing the possibility of uremia onset.

The increase in GFR with a decrease in proteinuria indicates an improvement in the quality of excretion, not an increase in excretion itself due to an undesired increase of glomerular pressure.

The combination of omega-3, vitamin E, sodium selenite, copper gluconate, zinc gluconate, chondroitin sulfate, and glucosamine proved to be an important adjuvant in the conservative treatment of dogs with CKD.

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