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Research Article

Clinical and Paraclinical Assessment of the Efficacy of Calcium Carbonate, Vitamin D_3 and Chitosan in the Management of Chronic Renal Failure in Dogs

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Abstract

Chronic kidney disease (CKD) is the most commonly diagnosed renal condition in dogs, having a gradual and irreversible course. In this context, the present study aimed to evaluate the efficacy of a nutritional supplement containing calcium carbonate, vitamin D_3, and chitosan, administered over a 60-day period to a group of 20 mixed-breed dogs (10 females and 10 males), aged between 8 and 12 years, diagnosed with CKD stages 1, 2 and 3, according to the IRIS classification. Supplement administration resulted in significant changes in hematological and biochemical parameters**:** a 4.14% increase in red blood cell count, a 34.18% increase in total serum protein concentration and a 55.18% increase in albumin levels. Concurrently, marked decreases were observed in key renal dysfunction markers: phosphorus (-53.7%), creatinine (-75.54%), SDMA (-48.1%), and urea (-71.7%). Pulsed Doppler ultrasound revealed a 48.6% reduction in the renal resistive index (RRI), suggesting improved renal perfusion. In addition to these favorable biochemical effects, clinical improvements were also reported, including reduced polyuria and polydipsia, increased appetite, and enhanced general health status. These findings suggest that the tested supplement may play a beneficial role in slowing CKD progression and supporting the metabolic and clinical condition of affected dogs, indicating promising therapeutic potential as an adjuvant in the management of this disease.

Keywords: Calcium carbonate, Chitosan, Chronic kidney disease, Dogs, Doppler ultrasound, Vitamin D_3

Introduction

Chronic kidney disease (CKD) is the most frequently diagnosed renal disorder in dogs, characterized by its irreversible and progressive nature. The onset and progression of CKD are associated with a gradual decline in glomerular filtration rate (GFR), accumulation of metabolic waste products, and disruption of fluid, electrolyte, and acid–base homeostasis. This condition predominantly affects geriatric dogs, with an estimated prevalence ranging from 0.5% to 1.0% in the general canine population, increasing to approximately 7% among elderly animals [1]. Clinical manifestations commonly include polyuria, polydipsia, weight loss, muscle atrophy, and gastrointestinal signs such as vomiting, anorexia, and halitosis [2].

Therapeutic strategies are aimed at slowing disease progression, maintaining metabolic homeostasis, and improving the animal's quality of life through nutritional modifications, pharmacological interventions, and metabolic support [3]. Among the frequent complications of CKD, hyperphosphatemia plays a critical role, being directly associated with disease progression, increased risk of secondary hyperparathyroidism, and the development of renal osteodystrophy [4].

Calcium carbonate is widely used as a phosphate-binding agent, acting at the gastrointestinal level by forming insoluble complexes with dietary phosphorus, thereby preventing its absorption and promoting fecal excretion [4]. Calcium-based phosphate binders have demonstrated efficacy in maintaining serum phosphorus within normal limits, thereby reducing the risk of bone-related complications [5].

Another key component of CKD therapy is vitamin D_3, whose active metabolism is impaired due to reduced renal conversion to calcitriol. Supplementation with vitamin D_3 has been shown to enhance intestinal calcium absorption, suppress parathyroid hormone (PTH) secretion, and prevent the development of metabolic bone disorders [6]. However, careful monitoring is essential to avoid potential adverse effects such as hypercalcemia and ectopic calcification, which may worsen the clinical course of CKD.

Chitosan, a polysaccharide derived from chitin, has gained recent attention for its nephroprotective potential. Studies have reported that chitosan administration can lead to reductions in serum creatinine and urea levels, thereby exerting a favorable effect on renal function and disease progression [7]. Furthermore, chitosan exhibits phosphate-binding properties, contributing synergistically with calcium carbonate to control serum phosphorus concentrations [8].

An integrated nutritional approach that combines calcium carbonate, vitamin D_3, and chitosan may offer an effective strategy for managing early to intermediate stages of CKD. Such interventions could help delay the onset of severe complications and reduce the need for more aggressive treatments in advanced stages [9].

Accurate and early assessment of renal function is essential for the effective management of chronic kidney disease (CKD). Among the biochemical markers used for this purpose, dimethylarginines (DMA) -specifically asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA)- have emerged as important indicators. SDMA, predominantly eliminated via renal excretion and unaffected by muscle mass, offers a more precise reflection of glomerular filtration rate (GFR) compared to traditional markers such as serum creatinine [10]. Its capacity to reveal early renal impairment, prior to detectable changes in creatinine or urea levels, underscores its value in guiding clinical decision-making in CKD [10].

The present study investigates the therapeutic effects of a nutritional supplement (Renal Vet), composed of calcium carbonate, vitamin D_3, and chitosan, in dogs diagnosed with chronic kidney disease (CKD) at stages 1, 2, and 3 according to the IRIS classification system, which reflects the progressive severity of renal impairment. Biochemical and hematological parameters were evaluated with respect to the severity of the IRIS stages, focusing in the early stages (IRIS 1 and 2) on sensitive biomarkers of early renal function such as symmetric dimethylarginine (SDMA), alongside serum creatinine and urea monitoring, to enable early detection of renal dysfunction and timely therapeutic adjustment. In the intermediate stage (IRIS 3), emphasis was placed on parameters associated with metabolic imbalances secondary to progressive renal failure, including serum phosphorus levels, which influence the risk of secondary hyperparathyroidism and renal osteodystrophy, as well as relevant hematological indicators for assessing the overall clinical condition of the patient. The primary scientific objective was to determine the capacity of this combined nutritional regimen to effectively modulate stage-specific biomarkers, with the aim of slowing CKD progression, maintaining metabolic homeostasis, and improving clinical and functional parameters, thereby providing a differentiated and stage-adapted therapeutic approach according to disease severity.

Material and Methods

Ethical Statement

This study received ethical approval from the Local Ethics Committee for Animal Experimentation at the University of Life Sciences "King Mihai I" from Timişoara, under approval certificate no. 137/26.09.2022.

Study Design and Sample Collection

The study was conducted on a group of 20 mixed-breed dogs (n=20), consisting of 10 females and 10 males, aged between 8 and 12 years (mean age: 10.53 ±1.31 years). Clinically, the dogs included in the stud exhibited recurrent dyspeptic syndrome (n=13), characterized by episodes of vomiting (n=8), soft stools (n=5), decreased appetite (n=13), alopecia in the dorsolombar region (n=12), mild weight loss (n=20), and lethargy (n=11).

The research was conducted over a 60-day period. All subjects had been previously diagnosed with chronic kidney disease (CKD), and their disease severity was classified according to the staging system established by the International Renal Interest Society (IRIS), based on clinical assessments and laboratory findings.

Among the 20 dogs diagnosed with chronic kidney disease (CKD), 13 were classified as Stage 1, 4 as Stage 2, and 3 as Stage 3, according to the severity of the disease.

Upon reassessment at 30 and 60 days, all dogs included in the study remained alive.

The study population consisted of dogs from Timiş County, Romania, specifically from the commune of Cerneteaz, housed in an authorized veterinary shelter. Each dog was kept in an individual pen, providing approximately 4 m^2 of indoor space and 2.5 m^2 of outdoor space.

Water was provided *ad libitum* throughout the study period, and dogs were fed a renal-specific diet, Purina Pro Plan Veterinary Diets NF Renal Function, administered twice daily in individualized portions according to the manufacturer's guidelines. This veterinary diet

is specifically formulated for dogs with chronic kidney disease and comprises carefully selected ingredients, including corn, rice, corn gluten meal, animal fat, pea fiber, dehydrated salmon protein, dried egg powder, calcium carbonate, monocalcium phosphate, fish oil, potassium chloride, sodium chloride, as well as essential vitamins (Vit-E, Vit-C, and Vit-B-complex) and trace minerals (iron, zinc, and selenium). The formulation aims to support renal function, reduce metabolic burden on the kidneys, and maintain overall nutritional balance in affected animals.

A control group was not included due to the nature of the shelter, where healthy animals were periodically adopted. The exclusion of a control group aimed to prevent disruption to the adoption process and to ensure animal welfare. Paraclinical assessments were conducted at baseline, 30 days and 60 days, with the initial evaluation serving as a reference for comparison.

The nutritional and therapeutic management of dogs with chronic kidney disease (CKD) was performed following the IRIS treatment guidelines for stages 1, 2, and 3. The treatment regimen included dietary sodium (Na) restriction and administration of isotonic and polyionic fluids, such as lactated Ringer's solution, Ringer's solution, and 5% glucose, to maintain fluid and electrolyte balance. Antiemetic medications, including maropitant and ondansetron, were used to control nausea and vomiting. Gastric protection was provided through H2-receptor antagonists (e.g., famotidine) or proton pump inhibitors (e.g., pantoprazole, omeprazole). Vitamin supplementation included B-complex vitamins, specifically thiamine (B_1), pyridoxine (B_6), and cobalamin (B_{12}).

Throughout the study, the dogs received an oral nutritional supplement, Renal Vet, containing calcium carbonate, chitosan, and vitamin D_3, administered according to the manufacturer's instructions. Dogs weighing up to 10 kg received one capsule per day, while those over 10 kg were given one capsule per 10 kg of body weight daily.

The clinical presentation of the dogs included recurrent vomiting episodes, reduced appetite, soft stools, moderate weight loss, lethargy, muscle weakness and dorsolumbar alopecia. Given the inherent subjectivity of clinical evaluation, a series of hematological and biochemical tests were performed to support the diagnosis.

The assessed parameters included:

• *Erythrocyte profile:* total red blood cell count (RBC), hematocrit, hemoglobin, mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV).

• *Leukocyte profile:* total white blood cell count (WBC), neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

• *Renal biochemical profile:* total protein, albumin, creatinine, symmetric dimethylarginine (SDMA), urea, phosphorus, and calcium.

The study was conducted in two phases, with clinical, hematological, and biochemical evaluations performed at baseline (before treatment initiation), after 30 days and after 60 days of supplement administration.

Blood samples were collected from the cephalic vein following standard veterinary procedures. Sampling was repeated on days 30 and 60. The blood samples were stored in BD Vacutainer® K2 EDTA tubes (Becton Dickinson, USA) for hematological analysis and transported to the Bioclinca SA laboratory for processing. Before analysis, the samples were homogenized for 3 min at 40 rpm using a roller mixer.

Hematological and biochemical analyses were performed using the automated Exigo EosVet analyzer (Boule Medical AB, Stockholm, Sweden).

For abdominal ultrasound evaluations, two imaging systems were used: a stationary device (My Lab XVet) and a portable unit (Chisson 2 Vet), both equipped with sector transducers operating at frequencies between 5.5 and 6.5 MHz. To enhance imaging quality, the hair over the examination area was clipped, and ultrasound gel was applied before scanning. Each kidney was assessed in both longitudinal (dorsal and sagittal) and transverse planes, with additional evaluation of the renal resistive index (RRI) using pulsed-wave Doppler ultrasound [11,12].

Statistical Analysis

Hematological and biochemical data were systematically recorded in Microsoft Excel and statistically analyzed using SPSS software, version 26.0 (SPSS Inc., Chicago, IL, USA). The normality of data distribution was assessed using the Shapiro-Wilk test, and results were expressed as mean ± standard deviation (SD). To evaluate differences across the three time points -T0 (baseline, before administration - BA), T1 (30 days after administration - AA_30d), and T2 (60 days after administration - AA_60d) - a Linear Mixed Model (LMM) was applied, allowing for the appropriate analysis of repeated measures and intra-subject variability. Statistical significance was set at $P<0.05$. Graphs were generated using GraphPad Prism, version 10.

Results

The graphical representation of the mean hematological parameter values, as determined according to the experimental protocol in dogs treated with calcium carbonate, vitamin D_3 and chitosan is presented in *(Fig. 1)*.

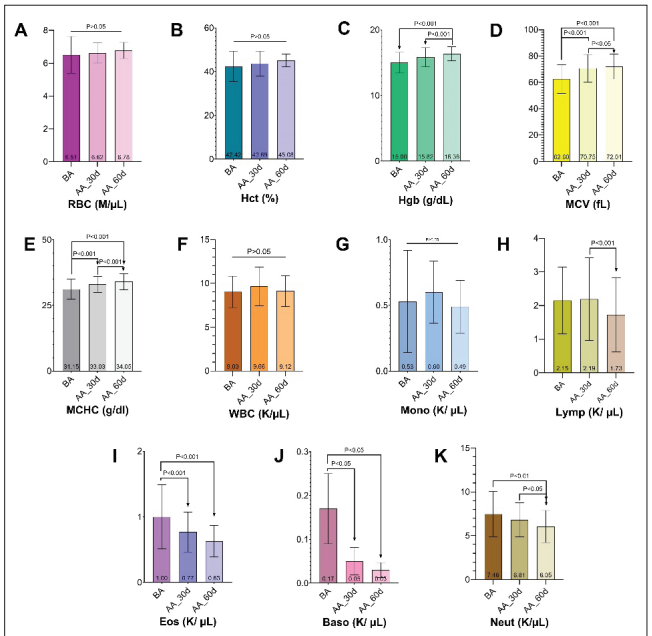

Fig 1. Graphical representation of hematological parameters (A-K) recorded in dogs before and after the administration of calcium carbonate, vitamin D_3, and chitosan. **A-** Red Blood Cells, **B-** Hematocrit, **C-** Hemoglobin, **D-** Mean Corpuscular Volume (MCV), **E-** Mean Corpuscular Hemoglobin Concentration (MCHC), **F-** White Blood Cells, **G-** Monocytes, **H-** Lymphocytes, **I-** Eosinophils, **J-** Basophils, **K-** Neutrophils. Normally distributed data are expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using a Linear Mixed Model (LMM), with the significance threshold denoted on the corresponding graph BA: Before administration, AA_30d: After 30 days of administration, AA_60d: After 60 days of administration

The administration of a dietary supplement containing calcium carbonate, vitamin D_3, and chitosan (Renal Vet) to dogs diagnosed with stage 1-3 chronic kidney disease (CKD) was associated with moderate improvements in several hematological parameters [13].

The mean erythrocyte count (A) increased over the course of the study, from 6.51 M/µL at baseline to 6.62 M/µL after 30 days (1.68%) and 6.78 M/µL after 60 days (4.14%); however, these changes were not statistically significant (P>0.05). Erythrocyte indices remained within physiological limits during the study.

Hematocrit values (B) increased by a total of 6.27%, from an initial mean of 42.42 g/dL to 43.69 g/dL at 30 days and 45.08 g/dL at 60 days, with no statistically significant differences observed between time points (P>0.05). In contrast, the mean hemoglobin concentration (C) showed a statistically significant increase throughout the study, rising from 15.0% at baseline to 15.82% after 30 days and 16.36% after 60 days (P<0.001).

The mean corpuscular volume (MCV) (D) increased from 62.6 fL to 70.75 fL after 30 days and 72.01 fL after 60 days (15.03%), while the mean corpuscular hemoglobin concentration (MCHC) (E) rose from 31.15 g/dL to 33.03 g/dL and 34.05 g/dL, respectively. Both MCV and MCHC showed statistically significant differences across the evaluation points (P<0.001).

Leukocyte dynamics (F) revealed a non-significant increase of 6.97% at 30 days, followed by a slight decrease to 9.12 K/µL at 60 days (P>0.05). These variations were considered nonspecific and unlikely to impact disease progression.

Monocyte levels (G) showed a slight increase from an initial value of 0.53% to 0.60% at 30 days, followed by a decrease to 0.49% at 60 days. These changes were not statistically significant (P>0.05).

Lymphocytes (H) showed a slight increase of 1.86% after 30 days, followed by a significant reduction of 21% after 60 days compared to baseline (P<0.001).

Eosinophil counts (I) decreased by 23% at 30 days and by 37% at 60 days, while remaining within the physiological reference range; statistically significant differences were observed at each evaluation point (P<0.001).

Basophils (J) exhibited a marked decrease of 70.58% after 30 days and 82.35% after 60 days, with statistically significant differences between the two stages of the study (P<0.05).

Supplementation with calcium carbonate, chitosan, and vitamin D_3 resulted in a significant reduction in neutrophil counts (K): a 15.41% decrease at 30 days and 18.9% at 60 days compared to baseline (P<0.001), with an additional 4.12% reduction between days 30 and 60 (P<0.05), suggesting a potential systemic anti-inflammatory effect.

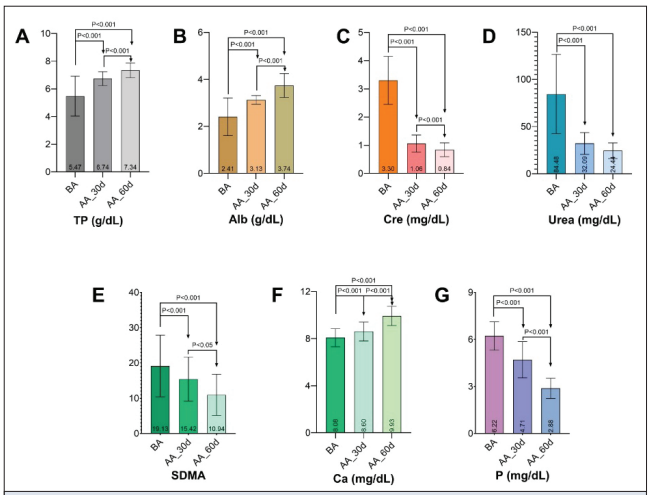

Fig 2. Graphical representation of the values of blood biochemical parameters **(A-G)** recorded in dogs before and after the administration of calcium carbonate, vitamin D_3, and chitosan. **A-** Total protein (TP), **B-** Albumine (Alb), **C-** Creatinin (Cre), **D-** Urea, **E-** Symmetric dimethylarginine (SDMA), **F-** Calcium (Ca), **G-** Phosphor (P). Normally distributed data are expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using a Linear Mixed Model (LMM), with the significance threshold denoted on the corresponding graph BA: Before administration, AA_30d: After 30 days of administration, AA_60d: After 60 days of administration

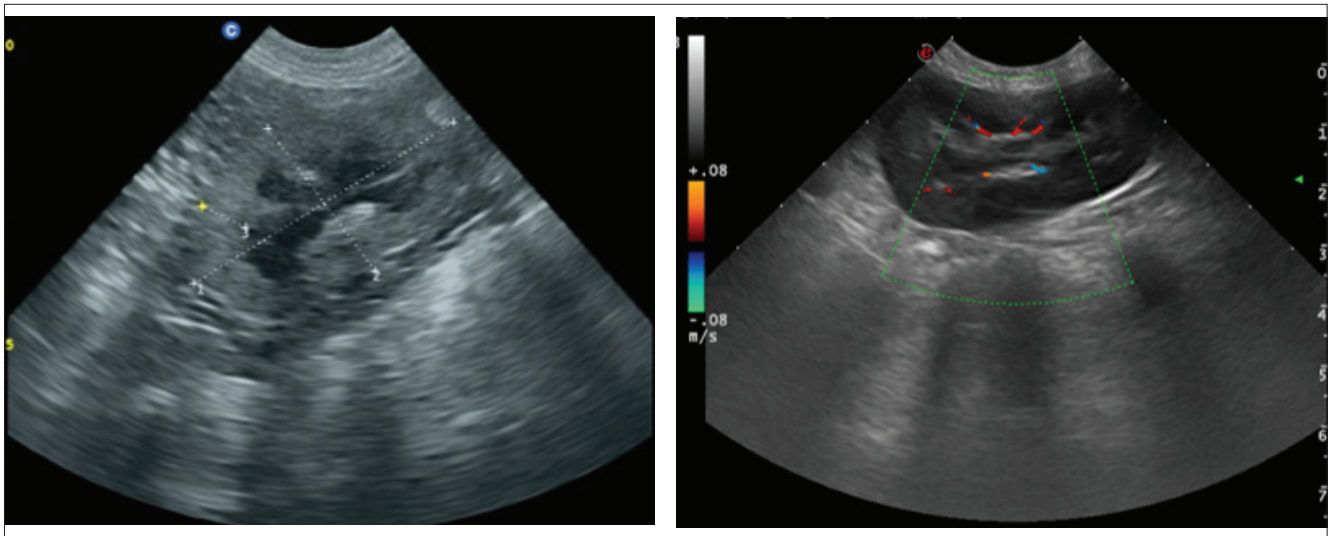

Fig 3. Ultrasound image at the level of the left kidney, in a dog. **A**- sagittal section, mode B and **B**- color Doppler at renal arcuate arteries' level, before treatment

The graphical representation of serum levels of total proteins, albumin, creatinine, symmetric dimethylarginine (SDMA), urea, calcium, and phosphorus in dogs with chronic kidney disease from the current study is presented *(Fig. 2)*.

The serum levels of total proteins (A) and albumin (B), initially below physiological limits (5.47 g/dL and 2.41 g/dL, respectively), increased significantly after 30 days of supplementation (total proteins: by 17.4%; albumin: by 6.99%; P<0.001). After 60 days, the mean total protein concentration reached 7.34 g/dL, reflecting a 34.18% increase compared to baseline, while albumin rose to 3.74 g/dL (an increase of 55.18%). Statistically significant differences were observed between all study phases (P<0.001).

Creatinine (C) and urea levels (D), initially elevated (3.3 mg/dL and 84.48 mg/dL, respectively), showed a marked decline following supplementation. After 30 days, creatinine decreased to 1.06 mg/dL (a 67.87% reduction), and urea to 32.09 mg/dL (a 62.01% reduction). By day 60, creatinine further declined to 0.84 mg/dL (a 74.54% decrease from baseline), while urea dropped to 24.44 mg/dL (a 71.7% reduction), both values approaching or falling within the physiological reference range. These reductions were statistically significant (P<0.001).

Symmetric dimethylarginine (SDMA) (E), a sensitive biomarker of early renal dysfunction, had an initial mean value of 19.13 µg/dL, exceeding the reference threshold (<18 µg/dL). After 30 days, the mean SDMA concentration decreased to 15.42 µg/dL (a 19.39% reduction), and by day 60 it further declined to 10.94 µg/dL, representing a 42.81% decrease from baseline. The changes were statistically significant (P<0.001).

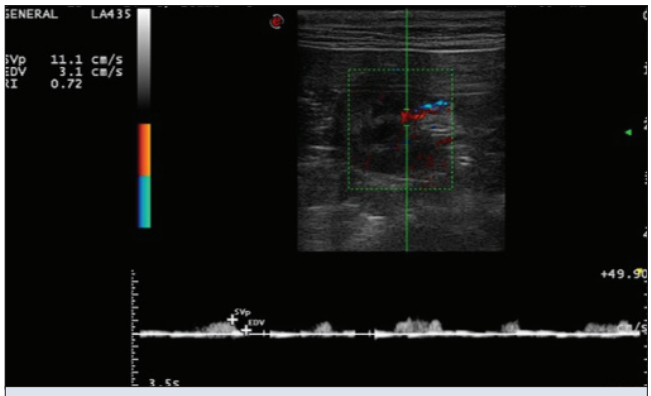

Fig 4. Ultrasound image at the level of the kidney, in a dog, sagittal section, pulsed Doppler at the level of the renal interlobar artery, before treatment

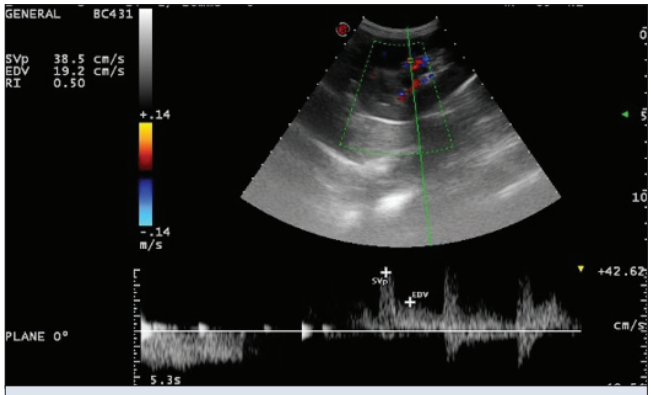

Fig 5. Ultrasound image at the level of the kidney, in a dog, sagittal section, pulsed Doppler at the level of the renal interlobar artery, after 60 days treatment

Serum calcium (F), initially below the physiological limit (8.08 mg/dL), increased to 8.6 mg/dL after 30 days, and normalized to 9.93 mg/dL by day 60, representing a 22.89% increase compared to baseline. These changes were statistically significant (P<0.001).

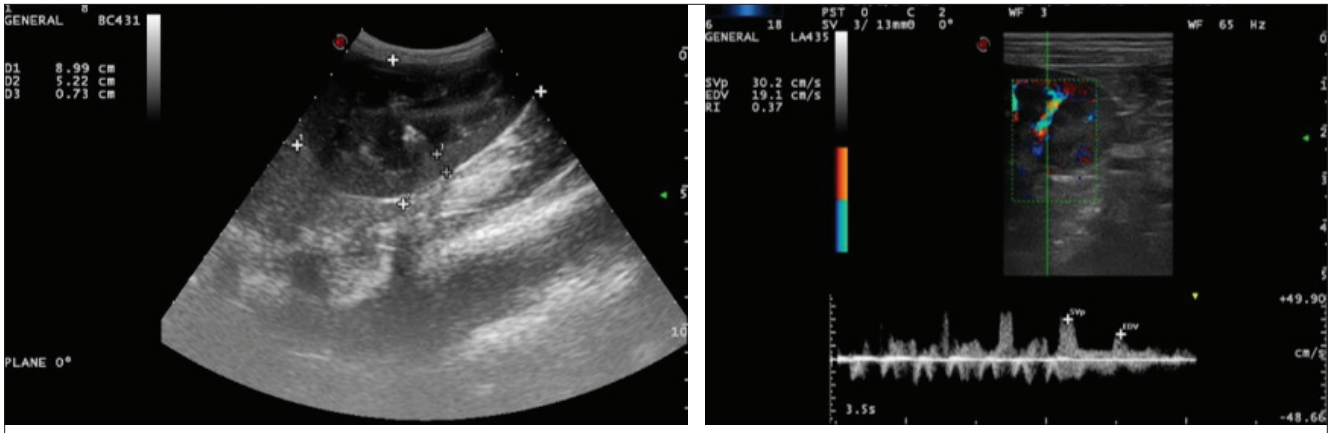

Fig 6. Ultrasound image of the left kidney, in a dog. **A**- sagittal section, B-mode and **B**- pulsed Doppler, 60 days after treatment

Phosphorus levels (G), initially elevated at 6.22 mg/dL, decreased to 4.71 mg/dL after 30 days (a 24.27% reduction), and further declined to 2.88 mg/dL at 60 days (a 53.7% reduction), returning to the physiological reference range. Statistically significant differences were recorded between all three study phases ($P<0.001$).

In the dogs diagnosed with stage 3 chronic kidney disease, ultrasonographic examination revealed degenerative changes in both the renal cortex and medulla, characterized by increased cortical echogenicity, reduced kidney size (longitudinal and transverse dimensions), and an elevated parenchymal index *(Fig. 3)*. Color Doppler ultrasound showed significantly decreased blood flow in the interlobar and interlobular arteries.

At baseline, the renal resistive index (RRI), measured using pulsed Doppler ultrasound, was elevated (0.72), indicating moderate vascular resistance and renal hypoperfusion *(Fig. 4)*. After 60 days of dietary supplementation, a notable improvement in renal hemodynamics was observed. The RI decreased significantly by 30.55% points at intermediate assessment and by 48.6% points by the end of the study, reaching 0.37 *(Fig. 5, Fig. 6)*. Concurrently, a reduction in cortical echogenicity was noted, suggesting improved renal perfusion and function.

Discussion

The clinical signs observed in the studied dogs were consistent with those reported in the literature, supporting the external validity of the findings [14]. Hematological analysis at treatment onset showed red blood cell counts within physiological limits, indicating that significant hematological changes are not typical in the early stages of chronic kidney disease (CKD).

By the end of the study, a slight increase in red blood cell count was noted, likely due to improved renal oxygenation. This may be attributed to the effects of calcium carbonate, vitamin D_3, and chitosan, which reduce oxidative stress and enhance erythropoietin bioavailability at the juxtaglomerular level.

Martello et al.[15] demonstrated that long-term supplementation with calcium carbonate, calcium lactate-gluconate, chitosan, and sodium bicarbonate in dogs with stage 3 CKD led to increased erythrocyte counts, reduced phosphorus levels, and improved urinary and biochemical markers. Similarly, a 90 days study using a comparable supplement with added *Lactobacillus acidophilus, Olea europaea* extract, and prebiotics showed effective control of uremia, phosphate, acid-base balance, inflammation, and oxidative stress, along with a mild increase in diuresis [16].

Literature emphasizes the role of renal microcirculation and juxtaglomerular function in chronic kidney disease (CKD) management, with supportive therapies proving essential in slowing disease progression [17]. Hematological changes such as non-regenerative anemia due to reduced erythropoietin and immune alterations like neutrophilia, lymphopenia, or monocytosis reflect CKD-related inflammation [18]. Early-stage lymphocytosis may represent a compensatory immune response, later evolving into immunosuppression, often exacerbated by chronic subclinical infections like pyelonephritis [19].

The progressive decrease in neutrophil levels during the administration of the supplement suggests a systemic anti-inflammatory effect, which is relevant in the context of chronic inflammation associated with renal disease. The active compounds -chitosan, vitamin D_3, and calcium carbonate- may influence immune response and mineral metabolism, thereby contributing to the normalization of neutrophil counts. These findings support the potential role of nutritional adjuvants as complementary therapy in the management of chronic kidney disease in dogs.

Monocytosis observed in the study, indicative of chronic inflammation, represents a significant hematological alteration in dogs with chronic kidney disease (CKD), being associated with macrophage activation and increased production of pro-inflammatory cytokines that contribute to progressive renal damage and systemic inflammation [13]. Monitoring these changes is essential for early detection and disease management. Correlating hematological data with biochemical and imaging analyses provides a more accurate understanding of disease progression and can guide therapeutic decisions, thereby impacting patient outcomes [20].

Lymphocytosis and monocytosis reflect the complex immune-inflammatory interplay characteristic of CKD. These parameters indicate immune activation and may inform therapeutic strategies aimed at modulating the immune response and improving prognosis [15,19]. The decrease in lymphocyte counts observed after 60 days of treatment may signal a relevant immunological shift, suggesting an adaptive or regulatory phase following at least two months of dietary supplement administration.

Continuous monitoring of hematological profiles is crucial for identifying complications associated with chronic kidney disease (CKD). Specifically, eosinopenia and basopenia require exclusion of iatrogenic causes such as glucocorticoid use, which may suppress bone marrow production and redistribute eosinophils to tissues via activation of the hypothalamic-pituitary-adrenal axis and increased cortisol secretion [19].

Dogs with CKD are predisposed to secondary infections and persistent inflammatory responses, contributing to decreased peripheral eosinophil counts. Basophils are also sensitive to elevated cortisol levels, and their reduction in circulation may be further influenced by oxidative stress and systemic inflammation [21]. Furthermore, secondary hyperparathyroidism, a common CKD complication, may adversely affect basophil numbers [22].

The decreased percentages of eosinophils and basophils may represent a compensatory mechanism aimed at limiting excessive inflammation and fibrotic progression in renal tissue. However, these changes require careful monitoring, as they may indicate underlying immune dysfunction and oxidative imbalances.

The observed increase in total protein and albumin levels in dogs with chronic kidney disease (CKD) may reflect an improvement in renal function or a reduction in proteinuria, suggesting a favorable response to the administered treatments. These fluctuations can also be modulated by systemic inflammation, with anti-inflammatory interventions potentially supporting the elevation of protein parameters. Supplements containing calcium and vitamin D_3 may enhance protein balance by reducing losses and supporting nephron function.

Recent studies highlight the efficacy of dietary supplementation with calcium carbonate, calcium lactate-gluconate, chitosan, and sodium bicarbonate in advanced CKD management, showing reductions in serum phosphorus, improved acid–base status, and better uremic control [15,16]. Notably, this supplement also demonstrated anti-inflammatory and antioxidant effects, aligning with findings that showed decreased serum creatinine at 30 days and reduced urea levels at 60 days, indicative of improved renal clearance [7,23].

These clinical and biochemical changes suggest effective with chronic kidney disease (CKD) management, marked by improvements in both hematologic and renal biomarkers. Early CKD diagnosis is crucial, as traditional markers like creatinine rise only after significant nephron loss. In contrast, support symmetric dimethylarginine (SDMA) is a more sensitive biomarker that increases earlier in the disease course, enabling prompt detection and monitoring of renal dysfunction.

Sargent et al.[24] support symmetric dimethylarginine (SDMA) as a screening tool in line with IRIS guidelines, although further studies are needed to clarify non-renal influences. Michael et al.[25] confirmed SDMA as a sensitive indicator of early GFR decline, often rising before creatinine. They recommend concurrent SDMA and creatinine evaluation for accurate disease staging and monitoring. Similarly, other studies underscore the value of tracking SDMA dynamics in with chronic kidney disease (CKD) progression, showing strong correlations with renal function and higher diagnostic relevance than creatinine alone [26].

Although SDMA levels are elevated in both acute kidney injury (AKI) and CKD, the SDMA/creatinine ratio is significantly higher in CKD, albeit with overlap between groups, indicating limited specificity for differentiating disease types. Therefore, SDMA should be interpreted alongside other diagnostic tools to achieve a comprehensive assessment [2].

Chronic kidney disease progression is closely linked to disturbances in calcium-phosphorus metabolism. As glomerular filtration rate (GFR) declines, phosphate excretion decreases, leading to hyperphosphatemia a key driver of renal osteodystrophy and secondary hyperparathyroidism (SHPT). At the end of the supplementation period, calcium levels normalized, with a progressive increase from baseline, reflecting restored calcium homeostasis. Statistically significant changes confirmed the supplement's impact on mineral regulation.

Initial hyperphosphatemia reflected impaired renal phosphate excretion. Post-supplementation, phosphorus

levels declined significantly, correlating with improved clinical status and restored phosphocalcic balance. These findings support the supplement's role in correcting mineral imbalances and slowing chronic kidney disease (CKD) progression.

Calcium and phosphorus homeostasis is regulated by kidney-bone-intestine interactions, modulated by parathyroid hormone (PTH), calcitriol, and fibroblast growth factor 23 (FGF-23) [27]. In chronic kidney disease (CKD), dysregulation of these mediators contributes to mineral and bone disorders. Managing phosphorus retention through dietary restriction and phosphate binders is essential. In SHPT, vitamin D receptor activators, phosphate binders, and calcimimetics are first-line; refractory cases may require parathyroidectomy, which has shown benefits in reducing symptoms and cardiovascular risks [28].

Supplementation with calcium carbonate, chitosan, and vitamin D_3 has demonstrated moderate increases in serum calcium and reductions in phosphorus after 30 days, indicating improved mineral balance and reduced calcification risk [29]. Hyperphosphatemia contributes to soft tissue calcification, impairing cardiovascular and renal functions.

Vitamin D_3 supplementation and calcium carbonate have proven effective in controlling hyperphosphatemia and enhancing renal function. Studies on calcifediol show improved vitamin D status, calcium homeostasis, and mineral metabolism in CKD, further supporting its therapeutic relevance [30,31].

A study assessing dietary supplements in advanced stages of CKD in dogs reported notable improvements in uremia control, phosphorus regulation, acid-base balance, blood pressure, and reductions in inflammation and oxidative stress [32]. These findings support the role of nutritional interventions in slowing CKD progression and enhancing clinical status.

Management of mineral imbalances in chronic kidney disease (CKD) relies on dietary phosphorus restriction, phosphate binders, and supplementation with vitamin D_3 and calcium. Monitoring serum calcium, phosphorus, parathyroid hormon (PTH), and the Ca/P ratio is essential for preventing complications and improving prognosis [33,34]. Calcium and vitamin D_3 supplementation can support calcium homeostasis and attenuate inflammation, though protocols must be individualized and monitored by veterinarians to ensure safety and efficacy [35].

Renal ultrasonography remains the gold standard for non-invasive morphological evaluation of kidney architecture and hemodynamics. In chronic kidney disease (CKD) typical sonographic findings include increased cortical echogenicity, diminished corticomedullary differentiation, reduced renal size, and irregular contours hallmarks of advanced disease [11,36].

The Renal Resistivity Index (RRI), derived from Doppler ultrasonography, provides insight into renal vascular resistance, especially within interlobar arteries. An RRI >0.8 suggests increased intrarenal vascular resistance, often associated with hypertension, renal artery stenosis, or CKD, while lower values may indicate vasodilation.

In our study, dogs with CKD exhibited cortical and medullary degeneration, evidenced by increased echogenicity and reduced renal dimensions. These alterations correlated with a higher parenchymal index, indicating structural damage. Color Doppler revealed diminished flow in interlobar and interlobular arteries, consistent with renal hypoperfusion.

Following 60 days of treatment with the dietary supplement, ultrasonographic re-evaluation showed reduced cortical echogenicity and improved perfusion, alongside a significant decrease in RRI, suggesting enhanced renal circulation and reduced vascular resistance. These findings imply a beneficial effect of the supplement on renal hemodynamics, with improved function and diuresis.

The reduction in both echogenicity and RRI post-treatment highlights improved renal perfusion and function, supporting the supplement's therapeutic potential in CKD management.

Chetboul et al.[10] emphasized the utility of renal resistivity index (RRI) in detecting subclinical renal lesions in dogs with MMVD, showing strong correlation with renal hemodynamic alterations. Compared to biochemical markers such as Symmetric Dimethylarginine (SDMA) and Cystatin C, Renal Resistivity Index (RRI) provides the advantage of a non-invasive, repeatable assessment of renal circulation and serves as an early indicator of renal dysfunction. Integrating RRI into clinical monitoring may thus enhance early detection and management of renal complications in Myxomatous Mitral Valve Disease (MMVD).

In human nephrology, renal venous blood flow assessment has gained relevance, while in dogs, predictive use of arterial indices such as RRI and PI remains standard. However, renal venous flow velocity remains unexplored in canine medicine [37].

Age-related variations in RRI were highlighted by a recent study [38], which observed a trend toward lower values in older dogs and a weak correlation between RRI and plasma renin activity in juveniles. These findings underscore the influence of hormonal modulation on renal hemodynamics and the necessity of age-adjusted

interpretation when using RRI in clinical settings.

The Renal Resistivity Index (RRI) is a valuable parameter in the evaluation of Acute Kidney Injury (AKI) in dogs. An increased RRI correlates with the severity of renal impairment, while a reduction in this index following treatment indicates an improvement in renal function and a favorable prognosis. Therefore, RRI can be employed as a reliable, non-invasive tool for monitoring disease progression and therapeutic response, although further studies are required to validate its routine use in AKI diagnosis and prognosis [39]. Data obtained through pulsed Doppler ultrasonography further support its role in establishing accurate renal prognosis.

Imaging analyses revealed improvements in cortical echogenicity and renal perfusion, suggesting a partial restoration of renal structure and function. The progressive decrease in Symmetric Dimethylarginine (SDMA) confirms the efficacy of supplementation in slowing the progression of chronic kidney disease (CKD). These findings are consistent with the literature, which links chronic renal hypoperfusion with characteristic ultrasonographic changes, such as increased cortical echogenicity, and supports the use of supplements that improve renal circulation through vasodilation or enhancement of renal hemodynamics [40].

In conclusion, calcium carbonate, vitamin D_3, and chitosan show promising therapeutic potential in CKD management. However, larger cohort studies and longer follow-up periods are necessary to confirm the long-term clinical benefits of this supplementation strategy.

Declarations

Availability of Data and Materials: The authors declare that the results of the investigations and other data related to the article are available at the correspondent author and at first author.

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