Effect of a Product Containing the Dietary Phosphate Binders Calcium and Magnesium Carbonate Associated with Other Reno-protectant Substances (Pronefra®) on Blood Parameters and Mineral Balance in Adult Cats

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ABSTRACT

This study investigated the effects of a combination of calcium and magnesium carbonate intestinal phosphate binders, associated with natural reno-protectant substances (the uraemic toxin binder chitosan, vasoactive peptides and an extract of *Astragalus membranaceus*) (Pronefra®, Virbac, France) on blood parameters and mineral balance in cats. Ten cats, 2 to 5 years old at day 0, were given the supplement with their normal food for 12 weeks at 0.5ml/kg/d. Plasma creatinine and phosphorus levels decreased significantly during the study period (p=0.016, p=0.002 respectively). Fractional excretion of phosphorus was significantly lower at the end of the study than at the beginning (p=0.02), and a significant correlation (p=0.002) was found between changes in blood creatinine and changes in urinary fractional excretion of phosphorus between days 0 and 84. All calcium and magnesium blood levels remained within normal ranges throughout the study. Pronefra®, a new palatable oral supplement, was shown to be safe over 12 weeks of continuous use, and not only efficiently reduced blood phosphorus levels, but also creatinaemia. This is likely to be an indirect indicator reflecting an overall improvement of renal function. Pronefra® may, therefore, represent an interesting new supportive option in cats suffering from

renal disorders from the earliest stages after diagnosis.

INTRODUCTION

Feline chronic kidney disease (CKD) and the ensuing renal failure is the most common kidney disease in domestic carnivores,[1] diagnosed in 2-20% of all cats regardless of age[2] and in up to 31% of geriatric cats older than 15 years.[3] Because CKD cannot be diagnosed until significant lesions are present (loss of more than 75% of functional nephrons),[1] it is not yet possible to identify and to prevent the early changes in this disease. Furthermore, the renal lesions in CKD are irreversible,[1] and so prevention of progression after the earliest possible diagnosis is the key objective in the management of this difficult condition.

The clinical management of chronic kidney disease in cats has progressed a lot over the past 15 years, and new standards in diagnosis, understanding, and treatment of this pathological condition have been set. Dietary and medical management can ameliorate the clinical signs of uraemia, minimize disturbances associated with excesses or losses of electrolytes, and modify progression of renal failure in order to increase the quality of life of the animal and increase life expectancy.[4-8]

Hyperphosphatemia has been recognized as a serious and frequent complication of CKD in cats. It is an important factor related to progression[9] and death from pathological renal conditions in cats.[4] Other important prognostic and diagnostic factors have also been recognized over the past 30 years, including serum levels of creatinine (SCr) and proteinuria.[4,10,11]

SCr and blood urea nitrogen (BUN) concentrations are commonly used as screening tests[5] in veterinary practices. Their levels are negatively correlated with the glomerular filtration rate (GRF).[12] Creatinine is a breakdown product of creatine phosphate in mammalian muscle and, excepting significant changes in lean body mass, it is usually produced by the body at a constant rate. It is removed from the body by the kidney via the urine in a passive manner, without any interaction, and is not reabsorbed. SCr levels, therefore, depend on the GFR and the muscle mass.[4] Thus although SCr levels are not a precise indicator of GFR, they can still provide an approximate indication of renal filtration capacity and renal health,[2] and form the basis of the IRIS staging system.[13]

SCr levels are strongly associated with survival in CKD cats.[2,10,11] Precise GFR assessment gives a much more accurate estimation of renal function, but is not easy to perform in the clinic for practical reasons.[4] BUN is a soluble protein breakdown product that is normally excreted by the kidneys but which accumulates in the blood of cats suffering from CKD. However this accumulation is not specific to kidney disease, and increased levels are also seen following a protein-rich meal or with dehydration. Nevertheless, BUN concentrations tend to correlate with clinical signs of uraemia,[1] and for practical purposes BUN may thus also be viewed as a marker of retained uremic toxins.

Dietary phosphorus is absorbed from the gastrointestinal tract. It is freely filtered at the glomerulus, and could also be considered as a marker of GFR.[4] In healthy animals, blood phosphorus levels will remain stable if the dietary intake of phosphorus remains constant. In animals with renal disease, a decline in the GFR will lead to phosphorus retention and hyperphosphatemia and consequently to renal hyperparathyroidism. Effectively controlling this rise in phosphorus levels in cats affected by CKD can dramatically reduce disease progression and extend life expectancy.[4-8,14]

Hyperphosphatemia can be managed by altering dietary availability of phosphorus: by dietary restriction,[7,9] by administration of intestinal phosphate binders (IPB),[5,6,9] or both.[15] Thus dietary modification has been demonstrated to be a mainstay of efficient disease management.[3-5,7,14] Although many cats in IRIS stage 2 will have phosphorus levels within the normal range, phosphate restriction could also be indicated in these

animals[14] because of the risk of renal secondary hyperparathyroidism. Unfortunately, specific phosphorus-restricted diets designed for cats with CKD are not always well accepted by the cats, or could be inappropriate for other reasons. Additionally, in the later stages, they may not be sufficient alone. In these cases, IPBs, administered with food or at least within 2 hours of feeding, become a key management tool to decrease dietary phosphorus availability.[14] However, it has been previously demonstrated that dietary restriction or IPBs administered alone have no apparent effect on BUN, SCr, GFR, renal plasma flow, or proteinuria,[6] so they should be used as part of a global management of this condition.

Unfortunately, many IPBs also have significant problems of palatability, and so use is currently low in the field.[16] As a result of all of these factors, there is now a clear need for an IPB that is both sufficiently palatable to be accepted willingly by cats, and will ideally support general renal function from the earliest stages after diagnosis. Such a tool could simplify the management of CKD to the benefit of cats and their owners.

The goal of this study was to assess the ability of Pronefra®, a new palatable oral suspension for dogs and cats suffering from CKD, to reduce serum phosphorus levels and to follow its impact on other renal biochemical parameters and on blood calcium and magnesium levels.

MATERIALS AND METHODS

Animal Selection

Ten adult cats living in a closed colony, five males and five females, aged between 2 and 5 years, were enrolled in the study after examination by a veterinarian. They were considered as sufficiently healthy for enrolment if no clinical signs raising a suspicion of renal failure, cardiovascular disease, cancer, or hyperthyroidism were detected during the clinical examination or in their clinical records. Specific biological screening or special tests were not performed before the start of the study. Animals were fed with a standard feline maintenance diet that they had received continuously for at least the past 12 months. All animals had exactly the same living conditions and level of physical activity throughout the study duration as well as during the 12 months prior to the study.

Tested Product

Pronefra® is a palatable oral suspension in the form of a palatable liquid suspension containing a 3.75 to 1 ratio of two IPBs: calcium carbonate and magnesium carbonate, associated with other natural reno-protectant substances (the uraemic toxin binder chitosan, vasoactive peptides, and an extract of *Astragalus membranaceus*, described as having antifibrotic proprieties).[17]

The product was administered orally for 12 weeks at the normal dose (0.5 ml/kg/day). The product was given in one single administration in the morning immediately before the meal.

Biological and Clinical Analyses

Blood and urine sampling, as well as clinical examinations and body weight measurements, were performed in the morning before feeding. Biological analyses were performed at four time-points (day (D)0, D7, D42, D84). The following variables were assessed:

- BUN
- Serum Creatinine (SCr)
- Serum inorganic phosphorus (P)
- Serum magnesium (Mg)
- Serum calcium (Ca
- Urinary fractional excretion of phosphorus
- Urinary specific gravity (USG), and
- Weight in kg.

Statistical Analysis

Statistical analysis was performed using SAS 9.2.

The biochemical parameters were compared between D0 and D84 using a paired t-test or a Wilcoxon signed ranks test at the 5% significance threshold. In order to test correlation between the parameters urea, creatinine, phosphorus, and weight, Pearson

Figure 1. *Mean serum calcium concentrations (with standard error of the mean) over the course of the study in all cats (n=10).*

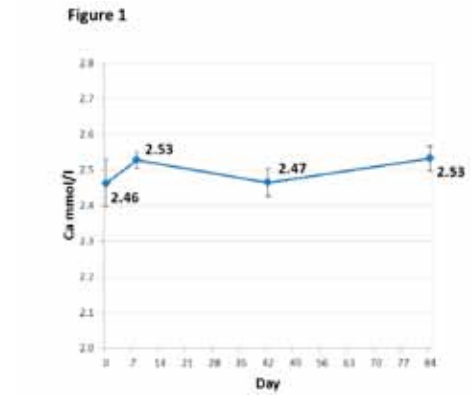
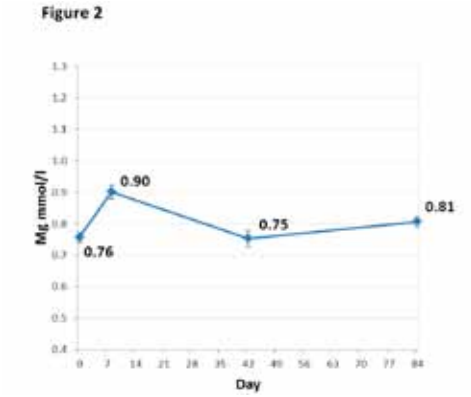


Figure 2. *Mean serum magnesium concentrations (with standard error of the mean) over the course of the study in all cats (n=10).*



coefficients of correlation were computed and tested at the 5% significant threshold.

As blood analyses revealed values for BUN and SCr that were significantly elevated compared to the normal range for cat number 9 on Day 0 (with no detectable clinical signs), the statistical analysis for BUN, SCr and P were performed for two groups of cats: "All cats" (all cats included initially in the study, N=10) and "healthy cats" (where cat number 9, which was suffering from a mild renal disorder, was excluded, N=9).

RESULTS

Product Safety

Over the period of the study, all Ca and Mg blood levels remained within normal ranges in all animals supplemented with the tested composition (Figures 1 and 2). The USG remained normal in all cats throughout the study, and no cat experienced a decrease of USG over the course of the study.

Product Efficacy

Over the study period (day 0 compared with day 84), supplementation with Pronefra® resulted in a statistically significant decrease of P levels. The reduction was significant whether all cats (p=0.002) or only healthy cats (p=0.006) were considered (see figures 3a and 3b). Supplementation with Pronefra® also resulted in a statistically significant decrease of SCr. Once again this reduction was significant, whether all cats (p=0.016) or only healthy cats (p=0.029) were considered (see figures 4a and 4b). BUN results were not statistically different between the beginning and the end of the study (see figures 5a and 5b). Fractional excretion of phosphorus was significantly reduced by supplementation. The reduction remained significant whether all cats (p=0.02) or only healthy cats (p=0.03) were considered.

Statistical Correlation

A significant correlation was found between changes in SCr values and the changes in values of urine fractional excretion of phosphorus over the course of the study. The correlation remained significant whether all cats (p=0.002) or only healthy cats (p=0.01) were considered (See figures 6a and 6b). Changes over the course of the study in SCr and BUN levels were also found to be statistically correlated, but only when all animals were considered (p=0.038) suggesting a major impact of the decrease in BUN level in the cat with renal failure.

No statistical correlation was found between changes in other parameters, including changes in body weight and SCr levels over course of the study (p=0.63 for all cats, and p=0.90 for the healthy cats). Likewise there was no correlation between SCr and initial bodyweight whether all cats (p=0.21) or only healthy cats (p=0.93) were consid-

Figures 3a, 3b and 3c. *Mean serum inorganic phosphorus concentrations (with standard error of the mean) over the course of the study in all cats (n=10, Fig. 3a) and in healthy cats only (n=9, Fig. 3b) (* $p<0.05$ compared to the beginning of the study at day 0), and serum inorganic phosphorus concentrations in cat number 9 with a mild renal disorder (Fig.3c).*

Figures 4a, 4b and 4c. *Mean SCr concentrations (with standard error of the mean) over the course of the study in all cats (n=10, Fig. 4a) and in healthy cats only (n=9, Fig. 4b) (* $p<0.05$ compared to the beginning of the study at day 0), and SCr concentrations in cat number 9 with a mild renal disorder (Fig.4c).*

Figure 3a

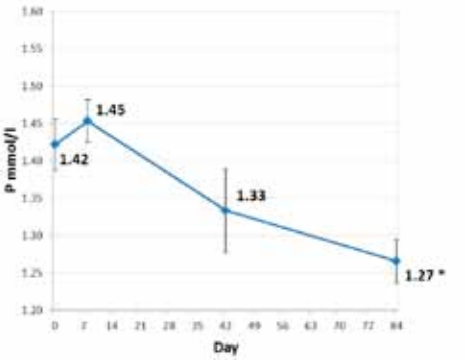


Figure 4a

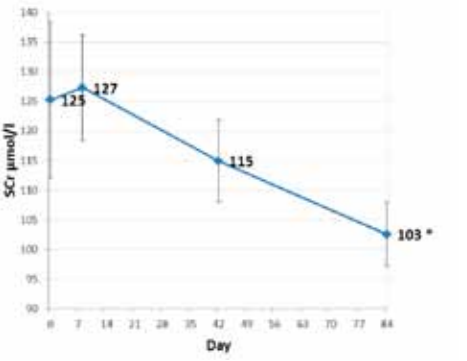


Figure 3b

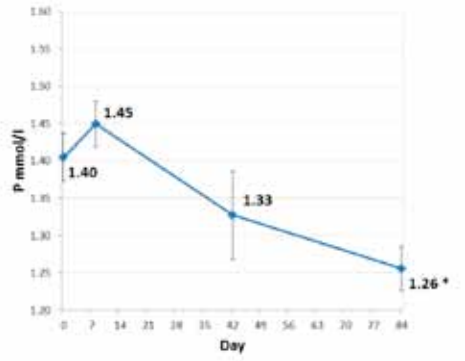


Figure 4b

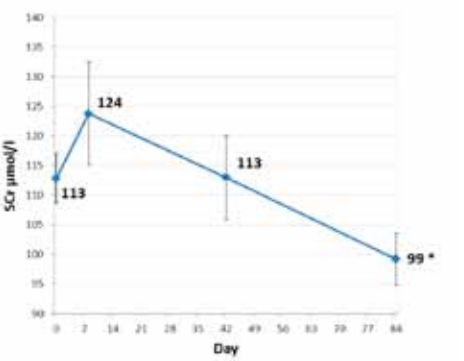


Figure 3c

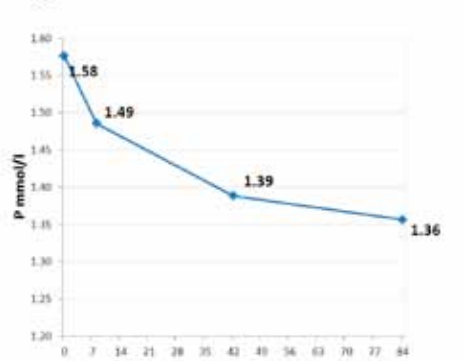


Figure 4c

Figures 5a,5b and 5c. *Mean BUN concentrations (with standard error of the mean) over the course of the study in all cats (n=10, Fig. 5a) and in healthy cats only (n=9, Fig. 5b), and BUN concentrations in cat number 9 with mild renal disorder (Fig.5c)*

Figure 5a

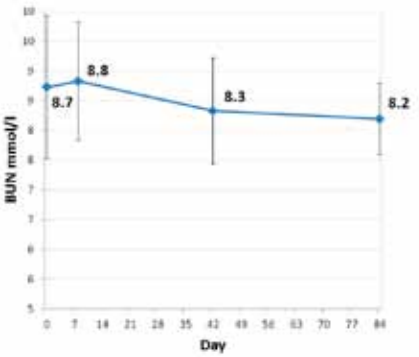


Figure 5b

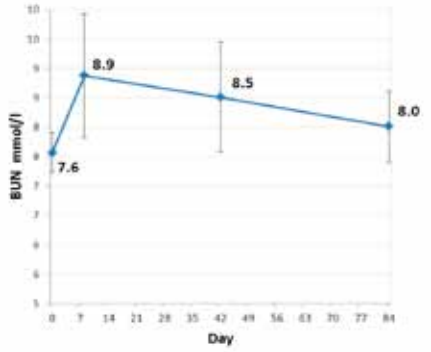
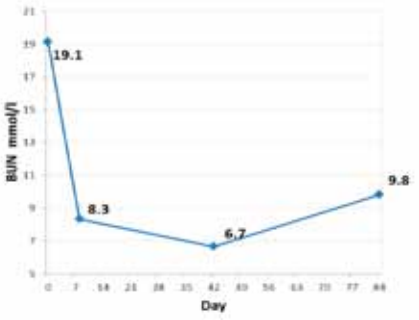


Figure 5c



ered (see figures 7a and 7b). This confirms that the reduction in SCr is not the result of any impact of the animals' bodyweight, but is more likely to be due to the support of renal function.

DISCUSSION

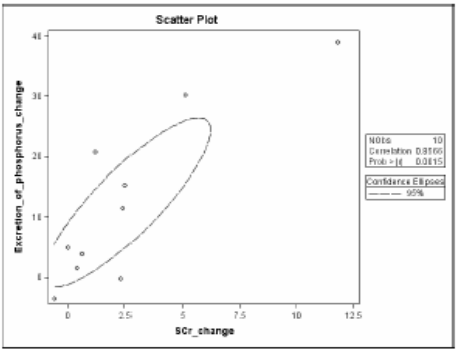
This study provided data on the safety and efficacy of a new palatable oral suspension (Pronefra®) containing an association of two IPBs with other natural reno-protectant substances.

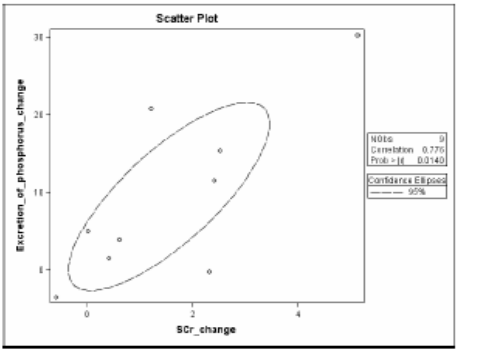
Although the diet fed to the cats had a phosphorus content higher than that typically recommended in specific diets for CKD patients, the dietary levels of phosphorus and protein were normal for cats of this age group, and all cats were clinically healthy and had been maintained on the same diet for at least 1 year.

The low phosphorus content necessary in renal diets is dictated by the increased retention and subsequent overload of phosphorus as a consequence of a decrease in renal function in CKD patients, and constitutes a nutritional controversy.[18,19] Proteins being rich in phosphorus,[9,18] the low level of phosphorus in specific feline diets for CKD is often achieved through restriction of protein.[18] However, as protein is a key appetent factor for cats, this further decreases compliance in animals already known to have reduced appetite.[20] Moreover, many cats object to nutritional changes. and the use of a diet with a very different profile can lead to refusal of the meal.[21]

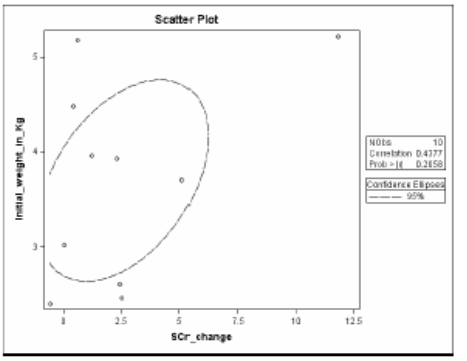
It is, however, of major importance that cats with CKD maintain an adequate intake of nutrients and energy, and so the necessary dietary modifications have to be carefully weighed against the risk of malnutrition. Thus, phosphorus restriction through intestinal phosphate binding with simultaneous maintenance of the standard protein levels provided by normal cat food seems to be sufficient (and even desirable) in the early stages of CKD,[14,15,20] and often adequately controls phosphorus levels. In the later stages of CKD, a change to specific diet in addition to IPBs may be required to achieve

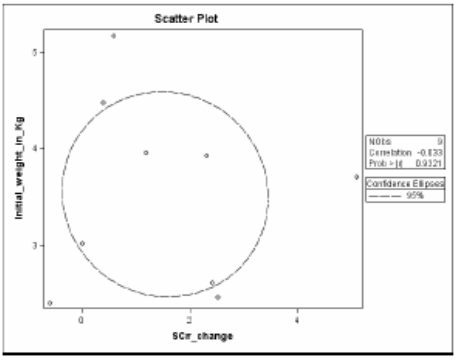
Figures 6a and 6b. *Correlation between changes in serum creatinine levels and changes in urinary fractional excretion of phosphorus in all cats (n=10, Fig. 6a, p=0.002) and healthy cats only (n=9, Fig. 6b, p=0.01)*





Figures 7a and 7b. *Absence of correlation between changes in serum creatinine levels and bodyweight in all cats (n=10, Fig. 7a, p=0.21) and healthy cats only (n=9, Fig. 7b, p=0.93)*





adequate control of phosphorus levels.[14]

The first objective of this study was to investigate the ability of the product to reduce serum phosphorus levels. On D0, all cats (including the cat with a mild renal disorder) had serum phosphorus levels within commonly accepted normal ranges (0.9-2.2 mmol/l). These levels can be considered as physiologically normal and, therefore usually non-susceptible to significant changes. However, use of Pronefra® resulted in a statistically significant decrease in serum phosphorus levels of more than 10%, whether all cats or only healthy cats were considered, thus proving a high efficiency of intestinal phosphate binding for this type of product. Increasingly it is recognised that cats, and possibly also other species with early stage CKD, often have secondary hyperparathyroidism, even when they have phosphorus levels within the normal range.[15,22,23] It is therefore highly desirable to reduce serum phosphorus levels even before they reach the upper threshold of the normal range to manage this situation.[15] For this reason, it was particularly interesting to observe the ability of the product to reduce blood phosphorus levels in animals with values in the normal range. The fact that fractional excretion of phosphorus was also significantly decreased over the study period supports this evidence for the efficacy of the IPBs deployed in this study. The inverse relation between intestinal phosphate binding and urinary excretion of phosphorus has been reported previously.[20]

A second objective of this study was to assess the safety of the product. In particular, due to a theoretical concern regarding the possible induction of hypercalcaemia or hypermagnesaemia, specific evaluations of blood Ca and Mg levels were performed during this study. During the whole study, Ca and Mg blood levels remained within normal ranges in all animals supplemented with Pronefra,® which was well tolerated in all animals.

To the authors' knowledge, this is the first time that an association of IPBs with other reno-protectant substances not only generated a decrease in phosphataemia, but also produced a statistically significant decrease of creatinaemia unrelated to body weight or activity changes. It has been shown that use of IPBs alone does not change levels of SCr.[5,6] SCr levels are used in practice for GFR estimation and an increase in creatinine reflects a decline of GFR.[12] However SCr is relatively insensitive to the early changes of GFR in near-normal renal function, where even large decreases in GFR will result in only slight increases of SCr.[12] In this study, a decrease in SCr was noticeable and remained statistically significant whether all cats or only healthy cats were assessed. Moreover, it was not correlated with body weight (figures 7a and 7b), and the fact that no cat experienced a decrease of USG over the course of the study excludes any potential diuretic explanation for this effect.

Additionally it should be noted that all cats taking part of the study were observed daily, and no animal experienced changes in the level of physical activity which could potentially impact SCr levels. This result is, therefore, probably related to an overall improvement of renal functional ability and most likely reflects an improvement of GFR. On the other hand, we did not perform specific GFR measurements in this study, and this limits our ability to conclude definitively that this is the reason for the decreased creatinaemia. Other limits of the present study are the relatively small number of included animals, a lack of biochemical pre-screening before the study (resulting in the inadvertent inclusion of one cat which had a mild renal disorder) and the fact that urinary infections were not specifically checked for and excluded.

In addition to the IPBs, Pronefra® contains chitosan, vasoactive peptides, and *Astragalus membranaceus* extract. In the course of this study, we did not attempt to assess the long-term effects of the use of the product on the preservation of renal structure and function in cats. However, according to multiple results with *Astragalus membranaceus* in laboratory animals and in humans, previously described, analysed, and critically evaluated,[24,25] it could be considered to be a beneficial ingredient for renal function that could potentially reduce progression of histologically observable pathological changes to the kidneys, reduce proteinuria, and modify fibrogenic renal mechanisms.[26,27] The anti-fibrotic and anti-inflammatory action of *Astragalus*, due to specific polysaccharides,[28] has been demonstrated in rats with induced nephrotic syndrome[17] and in induced glomerulonephritis.[28]

One study in humans reported decreased SCr and improvement of creatinine clearance with use of an *Astralagus* supplement.[24,25] The potential diuretic effects noted in the literature for some forms of *Astragalus*[29] were not noted at the dosage, and with the galenic form used in this study. This excludes any impact on other parameters. However, the vasoactive peptides of Pronefra® are believed to have moderate anti-hypertensive properties[30] which may therefore contribute to maintenance of balanced blood pressure and maintenance of normal renal function.

The benefits of simultaneous use of chitosan and phosphate binders in cats suffering from CKD have been shown previously[5] and are an accepted part of the medical management of this condition. It has been suggested that chitosan can reduce intestinal ammonia absorption in CKD cats,[5] thus providing a benefit in cats with high BUN levels. There

are a few reports suggesting also some phosphate binding effect in different species,[5,31] but to the authors' knowledge, no changes in creatinemia have ever been shown with the simultaneous use of chitosan and phosphate binders in cats.[5] In this study, we did not find a statistically significant decrease in BUN levels, which is not surprising given the fact that all the animals began the study with BUN levels within normal ranges. In order to assess this particular aspect of the product's activity, it would be necessary to use azotaemic animals in the later stages of kidney failure.

The results of this study suggest that Pronefra® is efficient at reducing phosphataemia and creatinaemia, even in cats whose values are initially still within normal ranges, and could therefore be a useful option for cats suffering from CKD from the earliest stages.

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