DOI 10.26873/SVR-1515-2023
UDC 636.8.09:616.61:613.24:637.54:616.152/.153
Pages: 25–35

Original Research Article

The Effect of a Specific Chicken Based Renal Diet as Monotherapy on Clinical, Biochemical, Urinary and Serum Oxidative Stress Parameters in Cats With CKD Stage 1 and 2

Key words

clinical parameters,
symmetric dimethylarginine,
oxidative stress,
renal diet,
cats,
chronic kidney disease,
urinary protein electrophoresis

Martina Krofič Žel[1], Alenka Nemec Svete[1], Breda Jakovac Strajn[2], Katarina Pavšič Vrtač[2], Tomaž Vovk[3], Nataša Kejžar[4], Darja Pavlin[1]*

[1]Small Animal Clinic, [2]Institute of Food Safety, Feed and Environment, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, [3]Chair of Biopharmacy and Pharmacokinetics, Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, [4]Institute for Biostatistics and Medical Informatics, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia

***Corresponding author:** darja.pavlin@vf.uni-lj.si

Abstract: The aim of the study was to investigate the effect of a therapeutic renal diet on selected clinical, biochemical, and urinary parameters and on selected parameters of oxidative stress in cats with early stages of chronic kidney disease (CKD). A prospective study of a 3-month duration was conducted to evaluate the effect of renal diet on selected clinical and laboratory parameters in client-owned cats with early stages of CKD. Of a total of 29 enrolled client-owned cats, nineteen (19) cats completed the study, ten receiving renal diet and nine receiving a diet of the owner's choice. A clinical examination was performed, and blood and urine samples were collected on the day of presentation and at regular check-ups after 3-4, 7-8, and 10-12 weeks. Serum creatinine and symmetric dimethylarginine (SDMA) concentrations and selected parameters of oxidative stress (plasma glutathione peroxidase (GPX) activity and plasma malondialdehyde (MDA) and serum selenium concentrations), were measured and electrophoresis of urinary proteins was performed. At inclusion, a significant positive correlation ($p < 0.001$) was found between serum selenium concentration and plasma GPX activity (Pearson correlation coefficient 0.83 (95% CI: [0.65 - 0.92]) and a significant negative correlation ($p < 0.001$) between serum SDMA and urine specific gravity (Pearson correlation coefficient -0.70 (95% CI: [-0.87 - (-0.38)]). At the end of the 3-month feeding trial no significant difference was found in SDMA and creatinine concentrations.

Received: 11 July 2022
Accepted: 6 February 2023

Introduction

Chronic kidney disease (CKD) in cats has an overall prevalence of 2-3% of the feline population (1, 2). The prevalence is higher in older cats: 10% in cats older than ten years (3) and 28% in cats older than 12 years (4). A prevalence of 28% and a 67% is reported in the overall population of cats and in those older than 18 years old, respectively, based on the serum concentration of symmetric dimethylarginine (SDMA) (5). Chronic kidney disease is characterized by progressive loss of functional renal tissue, leading to renal fibrosis, which can cause uremic crisis and death (6). Early recognition of CKD is essential for prompt management of such patients, leading to a better long-term prognosis (7,8).

Proteinuria is an important and independent predictor of worsening of CKD (9,10,11). In contrast to dogs, chronic interstitial nephritis is predominantly found in cats. Therefore, tubular proteinuria is more common than glomerular proteinuria (12).

Oxidative stress aids progression of CKD in human patients (13). Plasma glutathione peroxidase (GPX), synthesized by renal tubular cells, is the major reactive oxygen species scavenger in the kidneys (14). In human CKD patients, plasma GPX activity decreases as the disease progresses and its activity is already reduced in patients with mild chronic uremia (15,16). On the other hand, a significantly higher plasma GPX activity was found in cats with CKD IRIS stage 4 compared with healthy cats (17).

Plasma malondialdehyde (MDA) is one of the most popular and reliable markers of the extent of lipid peroxidation and thus oxidative stress (18). Selenium is an integral part of selenoproteins, one of which is plasma GPX. This microelement is present in protein-rich foods, and its excess is excreted via the kidneys (19). Unlike human uremic patients, uremic cats do not have a selenium deficiency (17).

According to evidence-based veterinary medicine, renal diet is the therapy of choice in both feline and canine CKD patients from the International Renal Interest Society (IRIS) stage 2 (20,21,22). Hall and colleagues (23) reported that cats with IRIS CKD stage 1 and 2 benefit from a diet with increased caloric density and enhanced concentrations of carnitine and essential amino acids. The biomarkers of kidney function, body weight and lean muscle mass were stable in cats consuming such a diet. However, a recent study reported that feeding a highly phosphorus-restricted diet to cats with early-stage CKD may lead to hypercalcemia and urolithiasis, while a diet moderately restricted in protein and phosphorus may be beneficial (24).

The authors are aware of only a few studies that deal with oxidative stress in feline CKD (17, 25, 26, 27, 28, 29, 30). However, the results of these studies are inconclusive about the role of oxidative stress in the pathophysiology of CKD. Furthermore, there is a lack of data on the effects of renal diet on oxidative stress parameters in CKD cats.

The aim of the present study was to investigate/explore the effect of a therapeutic renal diet on selected clinical, biochemical, and urinary parameters and on selected parameters of oxidative stress in cats with early stages of CKD and to provide credible insight with monitoring of clinical and laboratory parameters.

Materials and methods

This prospective study was conducted on client-owned cats with early stages of CKD and lasted for three months. The inclusion criterion was CKD stage 1 or 2, according to the IRIS guidelines (31), with no previous treatment recorded.

Cats with acute kidney injury, prerenal or postrenal azotemia, nephropathy of toxic or infectious origin within the last 28 days, urinary tract obstruction, acute systemic inflammation, liver disease, chronic heart failure, cancer or serologically positive for feline leukemia or feline immunodeficiency were excluded from the study.

All owners signed a consent form before enrolling the cats in the study. All procedures complied with the relevant Slovenian governmental regulations (Animal Protection Act, Official Gazette of the Republic of Slovenia, No. 43/2007).

The cats were randomly divided into two groups: cats in the control group, which received a regular diet and cats in the experimental group, which received a renal diet. Simple randomization method with sequentially numbered sealed envelopes was used to group the patients (32).

The cats in both groups received their diet *ad libitum* according to their habitual regime. Clinical examination including body weight monitoring, blood pressure measurement, routine hematological and biochemical analyses, and urinalysis with UPC (urine protein to creatinine ratio) were performed on the day of presentation and regular check-ups after 3-4, 7-8, and 10-12 weeks.

In addition to routine laboratory parameters, measurements of SDMA concentration, and selected parameters of oxidative stress (GPX, MDA, selenium) were also performed at each check-up and will be described below.

Composition of the diet

The cats in the experimental group were fed Vet Life Feline Renal Formula (Farmina Pet Foods, Naples, Italy). The composition of the renal diet is shown in Table 1. The renal diet used in the study had the same lot number for all cats. The cats in the control group continued to receive the maintenance diet to which they were accustomed to prior to participation in the study.

Blood and urine sample collection, processing, and analysis

Blood samples were taken from the jugular vein and transferred into serum separator tubes (Vacuette, Greiner Bio-One, Kremsmunster, Austria) for the determination of serum biochemical profiles, including SDMA, and antigen detection of feline leukemia virus (FeLV) and specific antibody against feline immunodeficiency virus (FIV). The tubes were stored for 30 minutes at room temperature to clot and then centrifuged at 1300 × g for 10 minutes at room temperature to separate the serum. Serum samples for the determination of routine biochemical parameters (urea, creatinine, alanine aminotransferase, alkaline phosphatase, total proteins, albumins, total calcium, inorganic phosphate, electrolytes (sodium, potassium, chloride)) were analysed on the day of blood collection. For measurement of SDMA concentration in serum, an aliquot of the serum sample was prepared and immediately stored at -80°C until analysed in batch.

Table 1: The composition of the renal diet

Raw protein	26.00%
Raw oils and fats	20.00%
Raw fiber	2.40%
Raw ashes	7.30%
Calcium	0.80%
Phosphorus	0.60%
Sodium	0.35%
Potassium	0.90%
Magnesium	0.07%
Omega 3 fatty acids	0.40%
Omega 6 fatty acids	3.90%
EPA	0.10%
DHA	0.15%
Energy value	3965 kcal/kg – 16.6 MJ/kg
Nitrogen-free extract/1000 kcal	11.77 g/1000 kcal
Selenomethionine	60 mg per kg corresponding to 13.5 mg selenium/kg dry matter)

Legend: EPA eicosapentaenoic acid; DHA docosahexaenoic acid

Composition: pea starch, potatoes, chicken fat, hydrolyzed fish proteins, dehydrated whole eggs, hydrolyzed chicken proteins, dehydrated chicken meat, quinoa seed extracted, dehydrated fish, fish oil, calcium carbonate, inulin, fructooligosaccharides, mannanoligosaccharides, potassium chloride, sodium chloride, glucosamine (500 mg/kg), Marigold extract (source of lutein)

Additives per kg

Nutritional additives: Vitamin A 15000 IU; Vitamin D3 600 IU; Vitamin E 550 mg; niacin 125 mg; pantothenic acid 42 mg; Vitamin B2 17 mg; Vitamin B6 7 mg; Vitamin B1 8 mg; Vitamin H 1.3 mg; folic acid 1.3 mg; Vitamin B 12 0.08 mg; choline chloride 2500 mg; beta-carotene 1.5 mg; zinc chelate of the analogous methionine hydroxylase 725 mg; manganese chelate of the analogous methionine hydroxylase 385 mg; ferrous chelate of glycine hydrate 185 mg; copper chelate of the analogous methionine hydroxylase 54 mg; selenomethionine 60 mg; calcium iodate anhydrous 2.4 mg; taurine 2000 mg; DL methionine 5000 mg; L-lysine HCl 2000 mg; L-tryptophan 2000 mg; L-carnitine 250 mg. Technological additives: potassium citrate 3000 mg.

Antioxidants: tocopherol-rich extracts of natural origin 10 mg.

Blood samples for hematological analysis were collected into 0.5 ml EDTA-containing tubes (BD Microtainer Tubes, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA).

Urine samples were collected by cystocentesis and analyzed within 1 to 2 hours.

Biochemical profiles (urea, creatinine, alanine aminotransferase, alkaline phosphatase, total proteins, albumins, total calcium, inorganic phosphate), except electrolytes and SDMA, were determined with an automated biochemistry analyser RX Daytona (Randox, Crumlin, UK). The electrolytes were determined with an Ilyte electrolyte analyzer (Instrumentation Laboratory, Lexington, Massachusetts, USA). Hematological analyses were performed with an automated laser hematology analyzer ADVIA 120 (Siemens, Munich, Germany) using species-specific software.

ELISA for the detection of antigen against FeLV and specific antibody against FIV were carried out according to the instructions of the manufacturer (IDEXX, Lenexa, Kansas, USA) on the day of collection.

Determination of the MDA concentration

Blood samples for the determination of plasma MDA concentration were collected into 2 ml EDTA-containing tubes (Vacuette, Greiner Bio-One, Kremsmunster, Austria). All samples were immediately centrifuged at 1500 × g for 15 min at 4°C. The plasma was separated and immediately frozen at -80°C until analysis.

The total plasma concentration of MDA was determined by a gentle alkaline saponification and derivatization method (33). MDA was derivatized with 2,4-dinitrophenylhydrazine to a pyrazole derivative and determined with an Agilent 1200 series high performance liquid chromatography system (Agilent, Waldbronn, Germany). The derivatized samples were separated on an Agilent Eclipse XBD-C18 column by gradient elution with acetonitrile, water and acetic acid and the MDA derivative was detected with the diode array detector. The plasma MDA concentration was expressed as µmol per L (µmol/L).

Determination of the SDMA concentration

All serum SDMA concentrations were measured in batch at the end of the study by IDEXX Laboratories in Germany (IDEXX SDMA Test, IDEXX Laboratories INC., Leipzig, Germany).

Determination of GPX activity

Plasma GPX activity was measured spectrophotometrically with an automated biochemistry analyzer RX-Daytona (Randox, Crumlin, UK) using the commercial Ransel kit (Randox Laboratories, Crumlin, UK) which is based on the

Paglia and Valentine method (34). The activity of plasma GPX was expressed as units per L (U/mL).

Determination of the selenium concentration

The microwave digestion of serum samples was performed with a Start D Microwave Acceleration Reaction System (Milestone, Sorisole, Italy). 0.4 to 1 mL of the samples were transferred into a 100 mL Teflon vessel and 3 mL 65% nitric acid, 0.5 mL 30% hydrogen peroxide and 4.5 mL Milli-Q water were added. The samples were digested in a closed 10-vessel microwave system at 200°C for 30 min. After cooling to room temperature, the solutions were diluted with Milli-Q water, and the concentrations of selenium were determined by inductively coupled plasma mass spectrometry (Varian 820-MS, Mulgrave, Australia). Argon was used as the carrier gas, and the isotope ^{78}Se was selected as the analytical mass in ICP-MS normal sensitivity mode. For measurements of selenium, a Collision Reaction Interface (CRI) was used to reduce common polyatomic interferences.

Urinalysis

Urinalysis included the measurement of specific gravity with a refractometer, the use of a standard multitest urine dipstick (Multistix 10SG, Siemens, Munich, Germany) and microscopic examination of the urine sediment. The urine samples were centrifuged at 800 × g for 10 minutes at room temperature. Urine supernatants were used to determine protein and creatinine concentrations to calculate the UPC. Protein and creatinine concentrations were measured with an automated biochemistry analyzer RX Daytona (Randox, Crumlin, UK) using the pyrogallol red and picric acid methods, respectively. Protein concentrations were not determined if the urine samples were grossly contaminated with blood. Gel electrophoresis was performed routinely by a commercial laboratory Euregio Laboratory Services (Kerkrade, Netherlands) in batch at the end of the study.

Statistical analysis

Based on the sample size, five keynote parameters were selected for statistical analysis (body weight, creatinine, SDMA, MDA and GPX). The differences between the first and the last (4^{th}) measurement time-point were compared. The remaining parameters were presented in the form of descriptive statistics (median and interquartile range (IQR)) and with boxplots over time (Supplementary material).

Basic characteristics and baseline measurements of systolic blood pressure selected hematological, biochemical, oxidative stress, and urinalysis parameters in both groups were compared using the Fisher exact test (categorical) and the Mann-Whitney U test (numerical variables). Since a slight deviance towards older cats in the experimental group was observed, the comparison of the difference in keynote parameters was adjusted for age by the use of linear regression. The P-values for the group comparison from the linear models were corrected by Holm procedure (5%).

The Pearson product-moment correlation was used to investigate the possible correlation between the parameters (scatter plots are presented in the Supplementary material).

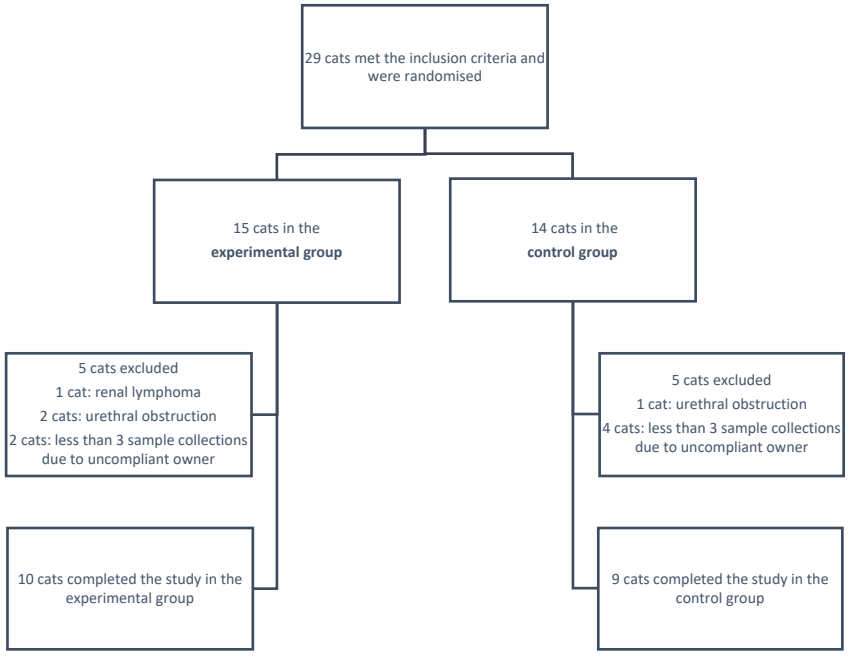

Figure 1: The flow diagram of cats with CKD during the study

Results

Patients' baseline characteristics

A total of 29 cats of different breeds were enrolled, all of which were neutered.

Of 29 cats enrolled in the study, 19 cats (ten cats in the experimental group and nine cats in the control group) completed the study. They represented the sample that we used for all analyses. The flow diagram of cats included in the study is presented in Figure 1.

Blood and urine samples were collected at all four scheduled check-ups from most cats enrolled. The missing blood and urine samples were not obtained due to problems with owners' compliance. In all animals the first and the last (10-12 weeks later) check-up were performed.

Baseline characteristics (demographic data) are shown in Table 2. At the point of randomization in terms of age, sex, and body weight, there were no significant differences between the experimental group and the control group; however, the cats in the experimental group that completed the study were slightly older.

Clinical signs and baseline clinical and laboratory parameters

Clinical signs at presentation were mild in all included cats; the owners usually reported polyuria/polydipsia. At the end of the study, most owners of cats in the experimental group reported an improvement in the clinical status of the cats with reduced vomiting. At inclusion, all cats of both groups were reported to have normal appetite, which remained unchanged during the whole observation period.

On each occasion, the owners were asked to evaluate their cats' appetite and acceptance; the information on the appetite and acceptance of the diet was recorded at each check-up. The cats were given the amount of the diet that was appropriate for their weight as recommended by the manufacturer. The new diet was accepted 100 % by all cats in the experimental group; and the diet change was achieved in two weeks.

During the first two weeks, the new diet was mixed with the usual diet and the amount of the new diet was gradually increased until the meal consisted only of the new diet. The owners reported that the entire amount of the meal was eaten by all included cats both during the transition period and during the study. Furthermore, the amount of the diet eaten during the study remained the same as before being enrolled into the study.

Medians (IQR) for baseline and final (4^{th}) measurement of laboratory parameters are presented in Supplementary material. The distributions of none of the parameters measured at baseline significantly differed between groups. Time monitoring of all measured parameters is also presented graphically in Supplementary material.

Serum creatinine concentration

During the study, median serum creatinine concentrations decreased in both the experimental and control groups (Supplementary material). The age adjusted difference in mean serum creatinine concentration decrease between both groups was not significant (Figure 2 A, Table 3). With one exception, all the included cats had stable CKD and remained at the same IRIS stage during the study. However, one cat in the experimental group was reclassified from IRIS 2 to 3 at the end of the study. The exact cause of the increase in serum creatinine concentration in that cat was not found. Seven out of 19 cats with elevated serum SDMA and creatinine concentrations, abnormal renal imaging findings and pathological urinary sediment, consistent with CKD, that were classified to IRIS stage 2, retained their urine concentration ability (Supplementary material).

Serum SDMA concentration

After the 3-month feeding trial, the median serum SDMA concentration decreased numerically in the

Table 2: Baseline demographic and laboratory characteristics of cats in the experimental and the control group

Group	Control group (n = 9)	Experimental group (n = 10)	p value
F/M	4/5	3/7	0.65
Age (months) Median (IQR)	78 (64–107)	116 (94–166)	0.066
Body weight (kg) Median (IQR)	5.9 (3.3–6.5)	4.9 (3.6–6.2)	0.842
Creatinine Median (IQR)	140.2 (130.0–178.8)	168.9 (161.1–178.8)	0.441
UPC (unitless) Median (IQR)	0.14 (0.12–0.19)	0.17 (0.11–0.26)	0.755

Legend: F-female cats; M-male cats; IQR-interquartile range

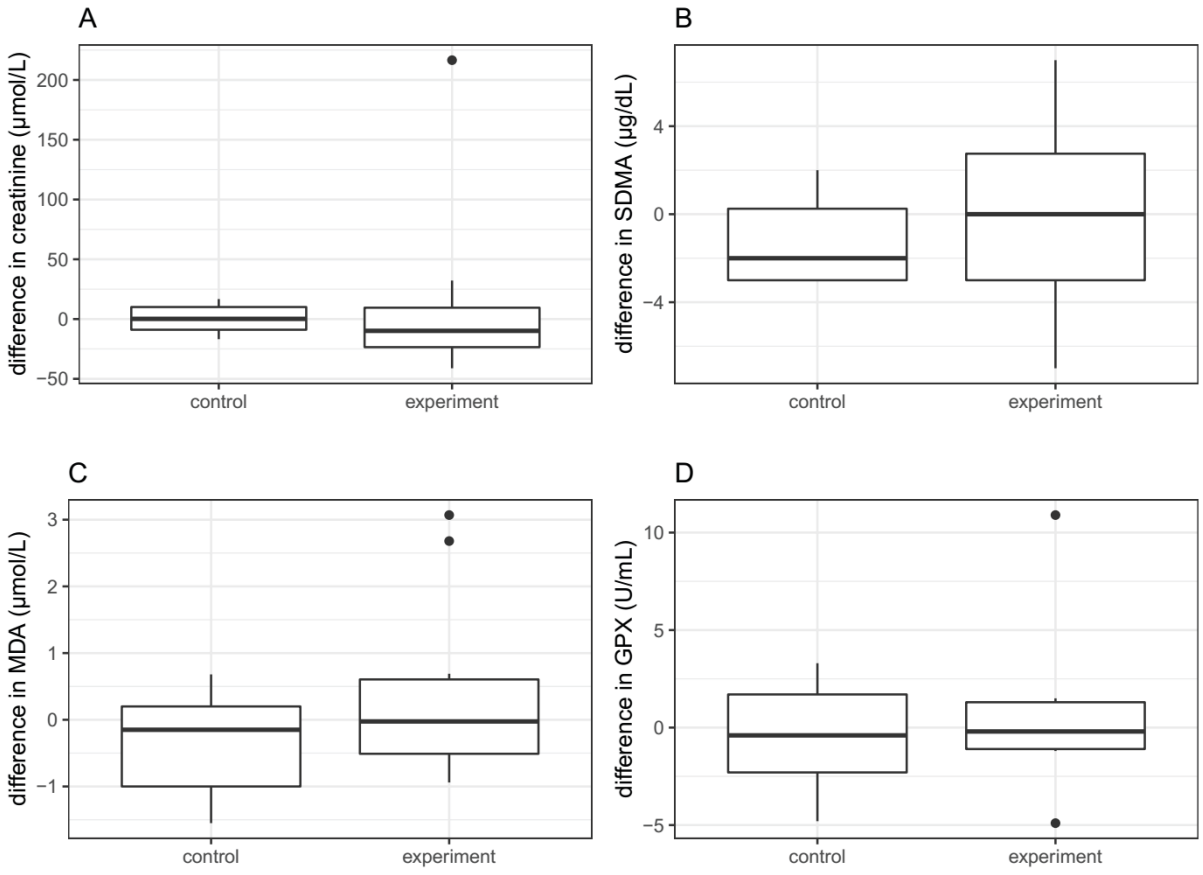

Figure 2: The changes in the concentrations of serum creatinine, serum SDMA, plasma MDA, and plasma GPX activity from the beginning to the end of the study in cats receiving renal diet in comparison to the control group

A – change in serum creatinine concentration (P = 0.92), B – change in serum SDMA (symmetric dimethylarginine) concentration (P = 0.85), C – change in plasma MDA (malondialdehyde) concentration (P = 0.18), D – change in plasma GPX (glutathione peroxidase) activity (P = 0.65); P-values stand for comparisons of age-adjusted differences

experimental group but remained the same in the control group (Supplementary material).

There was no significant difference in age-adjusted decrease of serum SDMA concentration between both groups (Table 3).

Plasma MDA concentration

At inclusion there was no significant difference in median plasma MDA concentrations between the sampled cats in the control group and those in the experimental group (Supplementary material). After the 3-month feeding trial, the mean age-adjusted difference in MDA was negligible in both groups (Table 3).

Serum selenium concentration and plasma GPX activity

In sampled cats receiving renal diet, median values of plasma GPX activity and serum selenium concentration did not differ from the median values in the control group (Supplementary material). There was a negligible mean age-adjusted difference in plasma GPX activity after 3-month feeding trial in both groups (Table 3).

A significant positive correlation (Pearson correlation coefficient 0.83 (95% CI: [0.65 - 0.92]) was found between the selenium concentration and plasma GPX activity at the beginning of the study.

Urinary Protein electrophoresis

At the beginning of the study, four cats in the control group and three cats in the experimental group had microalbuminuria. Furthermore, more cats in the experimental group had non-zero fractions of protein in the urinary sample. The leading protein fractions at the beginning of the study were alpha 1 and albumin in the control group and beta in the experimental group. At the end of the study, beta fraction predominated in both groups. The median per cent fraction of the urinary protein at the beginning of the study in comparison to the end of the study is shown in Table 4.

Moreover, at the beginning of the study, a significant negative correlation (Pearson correlation coefficient -0.70 (95%

Table 3: Results of five linear regression models, modelling difference (measurement 4 – measurement 1) between control and experimental group; the model controls for the age of cats

	Linear regression coefficient at group	P value	Adjusted P value
Weight (kg)	0.401	0.054	0.272
Creatinine (µmol/L)	-2.907	0.919	> 0.999
SDMA (µg/dL)	0.373	0.854	> 0.999
MDA (µmol/L)	0.891	0.175	0.699
GPX (U/mL)	0.875	0.654	> 0.999

Legend: Adjusted P-values are adjusted by Holm procedure for multiple comparisons; SDMA-Serum symmetric dimethylarginine; MDA-Plasma malondialdehyde; GPX-Plasma glutathione peroxidase

CI: [-0.87 - (-0.38)]) between SDMA and USG in the sampled cats were found.

Discussion

Cats with CKD IRIS stage 1 and 2 were monitored during a prospective 3-month feeding trial. All cats tolerated the diet change well in the experimental group and had normal appetite. During the study, the SDMA concentrations did not significantly change in any of the groups studied. Previously published data in cats with CKD IRIS stage 1 and 2 report a gradual increase in serum SDMA concentration regardless of the diet used (23). In the mentioned study, serum SDMA concentration increased from baseline at the first check-up after one month and continued to increase after three months in both the experimental and control groups (23). According to the published data, SDMA has a lower index of individuality than creatinine in cats (35) and dogs (36). Furthermore, consecutive measurements were performed in our study, and an individual value for each cat determined. A gradual increase in SDMA in successive measurements can therefore be due to a gradual decrease in kidney function (35,37). However, the difference in results of the mentioned studies might be ascribed to different renal pathologies of the patients that were included in both studies. Since the IRIS classification is applied to all patients suffering from CKD regardless of their cause, a variety of patients can be included. The progression of CKD and its response to treatment may be more variable at early stages than later when the majority of nephrons are lost. Although the serum SDMA concentration is correlated with the glomerular filtration rate (GFR) (38), further studies are warranted to assess the effect of the renal diet on the GFR.

Serum selenium concentration and plasma GPX activity measured in the present study were generally consistent with previously reported values (13, 39). No cat was selenium deficient at the beginning nor at the end of the 3-month feeding trial. In addition, a significant positive correlation between serum selenium concentration and plasma GPX activity was found, which contrasts with previously published data in cats (39). The study mentioned above found a correlation between serum selenium concentration and plasma GPX activity only in the case of selenium deficiency. When selenium concentrations continued to increase, plasma GPX activity reached its plateau, which was not observed in our study. Unlike in human patients, selenium is not a limiting factor in feline CKD (16, 17, 39). In addition, the correlation between the above-mentioned parameters is inconsistent in human CKD patients (16).

The median plasma MDA concentrations measured in our study were slightly higher, but in general agreement with the previously published values in healthy and CKD cats (40). We found no significant difference in mean change (final measurement – baseline) of plasma MDA concentration or

Table 4: Median per cent fraction of the urinary protein at the beginning of the study in comparison to the end of the study (after 3 months)

		albumin	Alpha 1	Alpha 2	Beta	gamma
Beginning	Control group	4.0	5.8	0	2.4	0
	Experimental group	3.1	4.1	0	14.7	0
After 3-month diet	Control group	3.8	7.3	0	54.1	0
	Experimental group	1.9	9.4	0	12.7	0

plasma GPX activity between the experimental group and the control group. Our results suggest that the renal diet had no significant effect on the parameters of oxidative stress measured in our study.

In contrast to previously published studies (39, 41), we found that three out of ten cats staged to IRIS 1 with elevated serum SDMA concentration, abnormal renal imaging findings as well as pathological urinary sediment, consistent with CKD, had a normal urinary specific gravity. The loss of the ability to concentrate urine is one of the first clinical signs of CKD and occurs when two-thirds of the nephrons are not functional. Apparently, the serum SDMA concentration increased before the ability of the kidneys to concentrate urine was impaired and proved valuable in the clinical evaluation of feline CKD patients at risk of developing CKD.

Furthermore, it was observed that some cats (seven out of 19) with elevated serum SDMA and creatinine concentrations, abnormal renal imaging findings and pathological urinary sediment, consistent with CKD, that were classified to IRIS stage 2, retained their urine concentration ability. The USG in these cats was up to 1.070 without showing clinical signs of heart failure, dehydration or hypovolemia. The literature data on this topic are scarce; Watson (42) reported that in contrast to dogs, USG values may remain normal (up to 1.045) in some cats with CKD and azotemia and that kidney disease may therefore still be suspected in a cat if these values are accompanied by persistent azotemia. Furthermore, cats often retain some concentrating ability in IRIS stages 2 and 3 CKD (43). The authors assume that the high USG in the CKD cats included in the present study could partially be caused by eating dry food. Further research is warranted to address this topic.

Cats of both groups had early kidney disease. Most of them exhibited borderline proteinuria, some were non-proteinuric. Except for one cat in the experimental group, the kidney disease was stable and did not deteriorate during the study. Two cats in the experimental group ended the study with a marked decrease of UPC. However, in one of these cats the serum creatinine and SDMA concentration rose to such extent that the cat was restaged from IRIS 2 to 3. The reason for this progression remained unknown. As serum SDMA and creatinine concentrations are negatively correlated to glomerular filtration, it might be assumed that subclinical dehydration, lower glomerular filtration rate as well as lower glomerular pressure may have led to a decreased UPC in that cat.

Though not expected, we found a negative correlation between SDMA and USG in the sampled cats. A similar finding has already been reported in dogs with decreased glomerular filtration rate (44). Cats with CKD have and impaired GFR; SDMA in such patients is increased (38). With a concurrent impairment of urine concentration ability, a decrease in USG is observed. A negative correlation between SDMA and USG in the sampled cats that all had CKD might therefore reflect the pathogenesis of CKD or it might be a consequence of a stochastic chance.

At the beginning of the study, four cats in the control group and three cats in the experimental group had microalbuminuria. According to Giraldi and colleagues (45), microalbuminuria is found in cats at risk for developing CKD. Overall, urinary protein electrophoresis and the UPC values indicate that tubular processes rather than glomerular disease were present in the cats that were enrolled in the study. Furthermore, we observed more cats in the experimental group to have non-zero fractions of protein in the urinary sample which might be partially ascribed to a higher systolic blood pressure or to a different predominant renal pathology. At the end of the study, beta fraction predominated in cats of both groups of our sample. However, when compared to the beginning of the study, there is an increase in the median percent beta fraction in cats in the control group, while it remained similar in the experimental group. Furthermore, the per cent albumin fraction decreased in cats in the experimental group while it remained similar in the control group. The presence of a leading beta fraction at the end of our study in both groups suggests tubular kidney damage, although the cats were non-azotemic, non-proteinuric or borderline proteinuric. Thus, we may assume that the tubular inflammatory processes progressed in both groups of cats, but to a different extent, although the UPC values remained grossly unchanged (45).

Furthermore, the results of our sample show no effect of renal diet on USG and UPC. The results are similar to the study published by Hall and colleagues (23), where no change in UPC or USG are reported. Therefore, we may conjecture that the tested renal diet had no effect either on electrophoretic pattern of urinary proteins or on halting the progression or development of proteinuria. Urine protein electrophoresis seems to be a valuable tool in assessing the progression of CKD. In order to provide better insight into the dynamics of CKD, we suggest urine protein electrophoresis to be added into monitoring scheme of feline CKD. Furthermore, recent recommendations in dogs with CKD include urinary electrophoresis, especially in those where renal biopsy is not indicated or not possible to be performed. The same recommendations may also be proposed in cats (46).

The main limitation of our study was the small number of patients who completed the study. In addition, the study lasted for a relatively short period of time, which may be an additional reason for the lack of significant differences in the measured parameters between the groups. Further studies with greater number of animals and with the assessment of GFR and urinary protein typization including the LMW (low molecular weight) spectrum are needed to get a thorough insight of the effect of renal diet on renal pathology.

Another limitation of the study is the fact that the diet of the cats in the control group was not standardized. As some cats do not tolerate any changes in their feeding regime including the diet change, the data gathered in this study give insight into the natural progression of early CKD where no medical intervention is possible. Moreover, some nutritional studies in human medicine follow similar design, where only experimental group receives diet, and the control group consists of individuals who continue with their habitual diet (47).

The study was performed on client-owned cats. Due to this fact, some cats were not brought to every scheduled check-up and some samples could therefore not be collected. The compliance of the owners in clinical studies like the present one tends to be a common problem.

Conclusions

After the 3-month feeding trial, no significant change in difference of body weight, serum creatinine or serum SDMA concentrations between experimental and control group was observed. Renal diet did not significantly increase the level of lipid peroxidation and decrease the activity of GPX, indicative of increased oxidative stress. Furthermore, our study demonstrated a significant positive correlation between serum selenium concentration and plasma GPX activity and a significant negative correlation between SDMA and USG in all CKD cats at inclusion.

Acknowledgements

The study was supported by Farmina Pet Foods. The funders were not involved in the study design, sample collection, analysis, data interpretation, or writing the manuscript.

References

1. Conroy M, Brodbelt DC, O'Neill D, Chang YM, Elliott J. Chronic kidney disease in cats attending primary care practice in the UK: a VetCompass (TM) study. Vet Rec 2019; 184(17): e526. Available from: doi: 10.1136/vr.105100
2. O'Neill DG, Church DB, McGreevy PD, et al. Prevalence of disorders recorded in cats at-tending primary-care veterinary practices in England. Vet J 2014; 202: 286–91.
3. Lulich JP, Osborne CA, O'Brien TD, Polzin DJ. Feline renal failure: questions, answers, questions. Compend Contin Educ Pract Vet 1992; 14(2): 127–52.
4. Bartlett PC, Van Buren JW, Bartlett AD, Zhou C. Case-control study of risk factors associated with feline and canine chronic kidney disease. Vet Med Int 2010; 2010: e957570. doi: 10.4061/2010/957570
5. SDMA impacts how veterinarians diagnose and manage kidney disease in dogs and cats. IDEXX, c2015 [homepage on the internet] http://www.idexx.com.jp/pdf/jp/smallanima/reference-laboratories/sdma-data-white-paper-pages.pdf.
6. Polzin DJ. Chronic kidney disease. In: Ettinger SJ, Feldman EC, eds. Textbook of veterinary internal medicine. 7th ed. St Louis, MO: Saunders, 2010: 2036–67.
7. Ross SJ, Osborne CA, Kirk CA, et al. Clinical evaluation of dietary modification for treatment of spontaneous chronic kidney disease in cats. J Am Vet Med Assoc 2006; 229: 949–57.
8. Plantinga EA, Everts H, Kastelein AM, et al. Retrospective study of the survival of cats with acquired chronic renal insufficiency offered different commercial diets. Vet Rec 2005; 157: 185–7.
9. Syme HM, Markwell PJ, Pfeiffer D, et al. Survival of cats with naturally occurring chronic renal failure is related to severity of proteinuria. J Vet Intern Med 2006; 20: 528–35.
10. King JN, Tasker S, Gunn-Moore DA, et al. Prognostic factors in cats with chronic kidney disease. J Vet Intern Med 2007; 21: 906–16.
11. Kuwahara Y, Ohba Y, Kitoh K., Kuwahara N, Kitagawa H. Association of laboratory data and death within one month in cats with chronic renal failure. J Small Anim Pract 2006; 47: 446–50.
12. McLeland SM, Cianciolo RE, Duncan CG, Quimby JM. A Comparison of biochemical and histopathologic staging in cats with chronic kidney disease. Vet Pathol 2015; 52(3): 524–34.
13. Daenen K, Andries A, Mekahli D, Van Schepdael A, Jouret F, Bammens B. Oxidative stress in chronic kidney disease. Pediatr Nephrol 2019; 34: 975–91.
14. Galle J. Oxidative stress in chronic renal failure. Nephrol Dial Transplant 2001; 16: 2135–7.
15. Ceballos-Picot I, Witko-Sarsat V, Merad-Boudia M, et al. Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. Free Radic. Biol Med 1996; 21(6): 845–53.
16. Zachara B, Gromadzinska J, Wasowicz W, Zbrog Z. Red blood cell and plasma glutathione peroxidase activities and selenium concentration in patients with chronic kidney disease: a review. Acta Biochim Pol 2006; 53(4): 663–77.
17. Krofič Žel M, Tozon N, Nemec Svete A. Plasma and erythrocyte glutathione peroxidase activity, serum selenium concentration, and plasma total antioxidant capacity in cats with IRIS stages I-IV chronic kidney disease. J Vet Intern Med 2014; 28(1): 130–6.
18. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014; 2014: e360438. doi: 10.1155/2014/360438
19. Holben DH, Smith AM. The diverse role of selenium with selenoproteins: a review. J Am Diet Assoc 1999; 99: 836–43.
20. Polzin DJ. Evidence-based step-wise approach to managing chronic kidney disease in dogs and cats. J Vet Emerg Crit Care 2013; 23(2): 205–15.
21. Elliott J, Rawlings JM, Markwell PJ, et al. Survival of cats with naturally occuring chronic renal failure: effect of dietary management. J Small Anim Pract. 2000; 41: 235–42.
22. Ross SJ, Osborne CA, Kirk CA, Lowry SR, Koehler LA, Polzin DJ. Clinical evaluation of dietary modification for treatment of spontaneous chronic kidney disease in cats. J Am Vet Med Assoc 2006; 229: 949–57.
23. Hall JA, Fritsch DA, Jewell DE, Burris PA, Gross KL. Cats with IRIS stage 1 and 2 chronic kidney disease maintain body weight and lean muscle mass when fed food having increased caloric density, and enhanced concentrations of carnitine and essential amino acids. Vet Rec 2018; 184(6): 190. doi: 10.1136/vr.104865

24. Schauf S, Coltherd JC, Atwal J, et al. Clinical progression of cats with early-stage chronic kidney disease fed diet s with varying protein and phosphorus contents and calcium to phosphorus ratios. J Vet Intern Med 2021; 35(6): 2797–811. doi: 10.1111/jvim.16263.

25. Keegan RF, Webb CB. Oxidative stress and neutrophil function in cats with chronic renal failure. J Vet Intern Med 2010; 24(3): 514–19.

26. Timmons R, Webb CW. Vitamin E supplementation fails to impact measures of oxidative stress or the anemia of feline chronic kidney dis-ease: a randomised, double-blinded placebo control study. Vet Med Sci 2016; 2: 117–24. doi: 10.1002/vms3.21

27. Whitehouse W, Quimby J, Wan S, Mona-ghan K, Robbins R, Trepanier LA. Urinary F 2 -isoprostanes in cats with international renal interest society stage 1–4 chronic kidney disease. J Vet Intern Med 2017; 31(2): 449–56. doi: 10.1111/jvim.14634

28. Yu S, Paetau-Robinson I. Dietary Supple-ments of vitamins E and C and β-carotene reduce oxidative stress in cats with renal insufficiency. Vet Res Commun 2006; 30: 403–13.

29. Granick M, Leuin AS, Trepanier LA. Plasma and urinary F2-isoprostane markers of oxidative stress are increased in cats with early (stage 1) chronic kidney disease. J Feline Med Surg 2021; 23(8): 692–9.

30. Valle E, Prola L, Vergnano D, et al. Investigation of hallmarks of catbonyl stress and for-mation of end products in feline chronic kidney disease as markers of uraemic toxins. J Feline Med Surg 2019; 21(6): 465–74.

31. Intenational Renal Interest Society. IRIS staging of CKD 2019 [internet] http://www.iris-kid-ney.com/pdf/IRIS_Staging_of_CKD_modified_2019.pdf.

32. Ferreira JC, Patino CM. Randomization: beyond tossing a coin. J Bras Pneumol 2016; 42 (5): 310. doi: 10.1590/S1806-37562016000000296

33. Czauderna M, Kowalczyk J, Marounek M. The simple and sensitive measurement of malondialdehyde in selected specimens of biological origin and some feed by reversed phase high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2011; 879(23): 2251–8.

34. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158–69.

35. Prieto JM, Carney PC, Miller ML, et al. Biologic variation of symmetric dimethylarginine and creatinine in clinically healthy cats. Vet Clin Pathol 2020; 49(3): 401–6.

36. Kopke MA, Burchell RK, Ruaux CG, Burton SE, Lopez-Villalobos N, Gal A. Variability of symmetric dimethylarginine in apparently healthy dogs. J Vet Intern Med 2018; 32(2): 736–42. doi: 10.1111/jvim.15050

37. Mack RM, Hegarty E, McCrann DJ, Michael HT, Grauer GF. Longitudinal evaluation of symmetric dimethylarginine and concordance of kidney biomarkers in cats and dogs. Vet J 2021; 276: e105732. doi: 10.1016/j.tvjl.2021.105732.

38. Braff J, Obare E, Yerramilli M, Elliott J, Yerramilli M. Relationship between serum symmetric dimethylarginine concentration and glomerular filtration rate in cats. J Vet Intern Med 2014; 28: 1699–701.

39. Foster DJ, Thoday KL, Arthur JR, et al. Selenium status of cats in four regions of the world and comparison with reported incidence of hyperthyroidism in cats in those regions. Am J Vet Res 2001; 62(6): 934–7.

40. Todorova I, Simeonova G, Kyuchukova D, et al. Reference values of oxidative stress parameters (MDA, SOD, CAT) in dogs and cats. Comp Clin Pathol 2005; 13(4): 190–4.

41. Hall JA, Yerramilli M, Obare E, Yerramilli M, Yu S, Jewell DE. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in healthy geriatric cats fed reduced protein food s enriched with fish oil, L-carnitine, and medium-chain triglycerides. Vet J 2014; 202: 588–96.

42. Watson ADJ, Lefebvre HP, Elliott J. Using urine specific gravity, 2015 [internet] http://www.iris-kid-ney.com/education/urine_specific_gravity.html

43. Bradley R, Tagkopoulos I, Kim M, et al. Predicting early risk of chronic kidney disease in cats using routine clinical laboratory tests and machine learning. J Vet Intern Med 2019; 33(6): 2644–56.

44. Pelander L, Häggström J, Larsson A, et al. Comparison of the diagnostic value of symmetric dimethylarginine, cystatin C, and creatinine for detection of decreased glomerular filtration rate in dogs. J Vet Intern Med 2019; 33(2): 630–9. doi: 10.1111/jvim.15445

45. Giraldi M, Paltrinieri S, Scarpa P. Electro-phoretic patterns of proteinuria in feline spontaneous chronic kidney disease. J Feline Med Surg 2019; 22(2): 114–21. doi: 10.1177/1098612X1

46. Dornelas L, Orozco A, Ramírez López C, et al. Application of serum and urinary electrophoresis as an aid tool for evaluating dogs at different stages of chronic kidney disease. Comp Clin Pathol 2021; 30: e1–11. doi: 10.1007/s00580-021-03231-2

47. Markhus MW, Kvestad I, Midtbø LK, et al. Effects of cod intake in pregnancy on iodine nutrition and infant development: study protocol for Mommy's Food - a randomized controlled trial. BMC Nutr 2018; 4: e7. doi: 10.1186/s40795-018-0215-1