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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

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A prospective, randomised, 6-month feeding trial was performed in 28 adult cats with International Renal Interest Society (IRIS) stage 1 and 2 chronic kidney disease (CKD). All cats were assigned to either a control food: Royal Canin Renal Support A Feline, dry or a test food: Hill's Prescription Diet k/d Feline with chicken, dry. Food intake was recorded daily; body weight weekly; and serum, urine, dual-energy x-ray absorptiometry (DEXA) and body condition assessments were performed at 0, 1, 3 and 6 months. Twenty cats (9 control, 11 test group) completed the study according to protocol. Cats consuming control food had significant loss of body weight (n=14; mean, −13.0 per cent, P<0.0001) and lean body mass (LBM; mean, −11.1 per cent, P<0.0001) over the 6-month feeding period, whereas cats consuming test food had a significant increase in body weight (n=14; mean, 5.8 per cent, P=0.003) and no change in LBM (P=0.42). Cats consumed 23 per cent more calories (P=0.05) when fed test food (mean, 207.1 kcal/day) compared with cats fed control food (mean, 168.0 kcal/day). Serum creatinine increased at a faster rate (P=0.0004) in cats consuming control food compared with cats consuming test food. Cats consuming test food had increased caloric and essential amino acid intake, increased body weight, stable biomarkers of kidney function and maintained LBM compared with cats consuming control food.

Introduction

Chronic kidney disease (CKD) is an irreversible disease, which results in progressive loss of renal function, and may eventually lead to a uraemic crisis and death.[1] In a recent study of randomly selected cats from a single feline-only practice, the prevalence of CKD in cats 5– 15 years of age was >40 per cent, with a higher prevalence (80 per cent) among cats >15 years.[2] Data from IDEXX Laboratories in the USA, from approximately 1.1 million cats with serum symmetric dimethylarginine (SDMA) concentrations measured since July 2015, indicate that overall prevalence of CKD is 28 per cent, and prevalence increases with age (67 per cent in cats >18 years).[3] In addition, over two times the number of cats can be diagnosed with CKD using serum SDMA concentrations as opposed to serum creatinine concentrations alone.[3] This is because serum SDMA detects CKD earlier when serum creatinine is still normal,[4] and because it is not negatively impacted by loss of lean body mass (LBM) with ageing.[5] Serum creatinine declines with age because of muscle wasting, whereas SDMA increases as glomerular filtration rate (GFR) declines with age.[5]

Cats with CKD are staged according to guidelines developed by the International Renal Interest Society (IRIS) and accepted by the American and European Societies of Veterinary Nephrology and Urology. The IRIS stages range from no azotemia (IRIS stage 1) to the most severe azotemia (IRIS stage 4). Staging guidelines are helpful for making diagnostic, prognostic and therapeutic recommendations for CKD.

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Currently, feeding a renal food to cats with IRIS stage 2 CKD or higher is considered the standard of care with strong evidence supporting this recommendation.[6 7] In one study, feeding a renal food was better than an adult maintenance food for minimizing uraemic episodes and kidney-related deaths in cats with IRIS stage 2 or 3 CKD.[8] In another study, cats fed any of seven commercial foods designed for cats with CKD had longer median survival times (16 months) compared with cats fed conventional foods (7 months).[9]

Typical renal protective foods have reduced protein, phosphorus and sodium content, increased caloric density, increased buffering capacity and increased soluble fibre, B-complex vitamins, antioxidants and (n-3) fatty acids (FA).[6] Feline kidney foods are supplemented with potassium.[6] Such foods meet the Association of American Feed Control Officials (AAFCO, 2017 Official Publication) minimum recommendations for protein.[10] The goals of nutritional therapy are to slow progressive loss of kidney function, reduce clinical and biochemical consequences of CKD and maintain adequate nutrition.[6]

Nutritional management of CKD is also recommended if persistent proteinuria, for example, urine protein-to-creatinine (UPC) ratio >0.4, or hyperphosphataemia (>4.6 mg/dl) exists in stage 1 CKD.[6] However, in a recent clinical study, client-owned geriatric cats with IRIS stage 1 CKD (without proteinuria or hyperphosphataemia) that were fed a food designed to promote healthy ageing were more likely to demonstrate stable renal function compared with cats fed owner's-choice foods over a 6-month period.[11] Cats fed owner's-choice foods were more likely to demonstrate progressive renal insufficiency.[11] Using the serum SDMA test, it is now possible to identify these cats with CKD before azotemia develops. These data suggest that renal foods are beneficial for cats with IRIS stage 1 CKD.

Although renal therapeutic foods have been recommended for decades with the expectation that they would alleviate clinical signs of uraemia and slow progression of CKD, their clinical effectiveness and nutritional impact recently have been challenged.[12] Some veterinarians have even suggested that cats with CKD should be fed high-protein foods as a strategy to preserve muscle mass.[13] A major concern is that dietary interventions that benefit renal function in non-azotemic cats with reduced GFR, for example, IRIS stage 1 CKD, have not been well studied. In addition, guidelines extrapolated from data derived from dialysis-dependent people consuming less than their protein requirements, as well as studies in other species such as dogs and rodents, may not be relevant to cats.[13]

The purpose of this study was to provide prospective, clinical trial data on dietary management of cats with CKD using commercially available therapeutic renal support foods. The goal was to determine if a therapeutic renal food with controlled protein and phosphorus, increased caloric density, an enhanced essential amino acid profile, added L-carnitine, fish oil, antioxidants and enhanced palatability[14] could help cats maintain body weight and muscle mass over a 6-month period compared with cats fed a control food having a similar protein content, but different composition. Test food was a high-quality protein food

Table 1 Analysed chemical composition* of control and test foods, with AAFCO minimum recommendations

Nutrient†	Control food‡	Test food§	AAFCO minimum¶
Protein	6.650	6.693	6.5
Fat	4.441	5.005	2.25
Energy**, kcal/kg	4.078	4.569	NA
Crude fibre	1.194	0.208	NA
Ash	1.545	1.048	NA
Arginine	0.436	0.372	0.26
Carbohydrate (NFE)††	10.8	8.9	NA
Histidine	0.132	0.138	0.078
Isoleucine	0.228	0.256	0.13
Leucine	0.530	0.744	0.31
Lysine	0.240	0.361	0.208
Methionine	0.216	0.153	0.05
Phenylalanine	0.294	0.346	0.105
Threonine	0.206	0.274	0.183
Tryptophan	0.064	0.068	0.04
Valine	0.270	0.300	0.155
Taurine	0.057	0.061	0.025
L-carnitine, ppm as fed	6	591	NA
Linoleic acid (18:2; n-6)	0.797	0.858	0.14
α-Linolenic acid (18:3; n-3)	0.042	0.046	NA
Arachidonic acid (20:4; n-6)	0.020	0.020	0.005
Eicosapentaenoic acid (20:5; n-3)	0.059	0.072	NA
Docosahexaenoic acid (22:6; n-3)	0.056	0.050	NA
Calcium	0.226	0.201	0.15
Phosphorus	0.118	0.105	0.125
Potassium	0.250	0.223	0.15
Sodium	0.086	0.077	0.05
Chloride	0.174	0.155	0.05
Σ (n-6) FA‡‡	0.837	0.915	NA
Σ (n-3) FA§§	0.196	0.193	NA
(n-6):(n-3) ratio	4.28	4.74	NA

*Ingredient label in order of preponderance for control food and test food is as follows:
Control food: brewers rice, corn, chicken fat, wheat gluten, corn gluten meal, chicken by-product meal, soy protein isolate, powdered cellulose, natural flavours, wheat, chicory, fish oil, calcium carbonate, sodium silico aluminate, potassium chloride, DL-methionine, psyllium seed husk, potassium citrate, L-arginine, fructooligosaccharides, salt, choline chloride, vitamins (DL-alpha tocopherol acetate (source of vitamin E), L-ascorbyl-2-polyphosphate (source of vitamin C), niacin supplement, biotin, riboflavin supplement, D-calcium pantothenate, pyridoxine hydrochloride (vitamin B_6), vitamin A acetate, thiamine mononitrate (vitamin B_1), vitamin B_{12} supplement, folic acid, vitamin D_3 supplement), taurine, trace minerals (zinc oxide, ferrous sulfate, manganous oxide, copper sulfate, calcium iodate, sodium selenite), L-lysine, marigold extract (*Tagetes erecta* L.), rosemary extract, preserved with mixed tocopherols and citric acid.
Test food: brown rice, corn gluten meal, chicken, pork fat, whole grain wheat, cracked pearled barley, wheat gluten, chicken liver flavour, pea protein, egg product, fish oil, lactic acid, potassium citrate, calcium sulfate, L-lysine, L-arginine, choline chloride, calcium carbonate, vitamins (vitamin E supplement, L-ascorbyl-2-polyphosphate (source of vitamin C), niacin supplement, thiamine mononitrate, vitamin A supplement, calcium pantothenate, pyridoxine hydrochloride, biotin, vitamin B_{12}supplement, riboflavin supplement, folic acid, vitamin D_3supplement, menadione sodium bisulfite complex (source of vitamin K)), L-threonine, taurine, iodised salt, DL-methionine, minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), potassium chloride, L-carnitine, L-tryptophan, mixed tocopherols for freshness, natural flavours, beta-carotene.
†All analytical values are expressed as g/100 kcal, unless otherwise indicated.
‡Control food was Feline Renal Support A dry cat food (Royal Canin Veterinary Diet, St. Charles, Missouri, USA).
§Test food was Hill's Prescription Diet k/d Feline with chicken, dry cat food (Hill's Pet Nutrition, Topeka, Kansas, USA).
¶AAFCO values are the adult maintenance minimum recommendations, expressed as g/100 kcal.
**Energy calculated using the modified Atwater factors as described.[31]
††Carbohydrate composition was determined by calculation.
‡‡Sum of the (n-6) fatty acids.
§§Sum of the (n-3) fatty acids.
AAFCO, Association of American Feed Control Officials; NFE. nitrogen-free extract.

Table 2 Baseline demographics*

Variable	Control food† (n=14)	Test food‡ (n=14)	P value
Age, years	8.99±0.71	9.55±0.62	0.56
Sex	Males=7, females=7	Males=7, females=7	1.00
Body weight, g	5334±264	5803±290	0.24
IRIS stage CKD	Stage 1=12; stage 2=2	Stage 1=11; stage 2=3	0.64
Body condition score§	3.36±0.63	3.71±0.91	0.24
Lean body mass, g	3885±158	3825±167	0.80
Fat body mass, g	1351±682	1761±877	0.18
Serum creatinine, mg/dl	1.40±0.09	1.35±0.06	0.64
Serum SDMA, µg/dL	15.5±0.5	15.4±0.5	0.84
USG	1.043±0.003	1.043±0.003	0.99
UPC ratio	0.21±0.06	0.21±0.03	0.86

*Values are means±SEM.
†Control food was Feline Renal Support A dry cat food (Royal Canin Veterinary Diet, St. Charles, Missouri, USA).
‡Test food was Hill's Prescription Diet k/d Feline with chicken, dry cat food (Hill's Pet Nutrition, Topeka, Kansas, USA).
§Body condition score scale ranged from 1 to 5.
CKD, chronic kidney disease; IRIS, International Renal Interest Society; SDMA, symmetric dimethylarginine; UPC, urine protein-to-creatinine ratio; USG, urine-specific gravity.

that contained less protein than maintenance foods but was fortified with essential amino acids, thus exceeding the AAFCO minimum recommendation for protein and amino acids (table 1), low phosphorus, antioxidant-enriched food. The hypothesis of this study was that cats consuming test food would maintain or show improved status of serum renal biomarkers (creatinine, blood urea nitrogen (BUN) and SDMA concentrations) and urinalysis parameters (urine-specific gravity (USG) and UPC ratio) across time compared with cats fed control food. In addition, it was hypothesised that cats fed test food would consume more calories, and maintain body weight and LBM compared with cats fed control food over the 6-month study period.

Materials and methods

Procedures were designed to avoid or minimise discomfort, distress and pain. Cats were monitored for any signs of disease. If an adverse event occurred, the cat's health took precedence over continuation in the trial.

Cats and study design

This was a prospective, randomised, blocked and controlled clinical trial of 6 months duration involving adult cats in the Hill's Pet Nutrition colony with IRIS stage 1 or 2 CKD. Cats were eligible if they were between 2 and 16 years of age. Both males and females were included (all gonadectomised); all breeds were eligible and there were no initial body weight restrictions. Fifty-seven cats were screened for CKD (physical examination, blood work and urinalysis) of which 28 cats were enrolled in the study. Cats had to be in good general health based on physical examination and laboratory analyses, with a body condition score (BCS) of 2/5 or greater, and could only consume dry food during the trial. Cats had to have increased SDMA concentration (>13.5 µg/dl), and could have one or more of the following: increased serum creatinine concentration (>1.6 mg/dl), abnormal kidney on palpation, dilute urine with USG<1.035 or UPC ratio >0.4. Cats were not eligible for the study if diagnosed with a systemic illness other than CKD, were having planned surgery, were pregnant or likely to become pregnant during the study period or had fractious behaviour. After screening, cats were blocked into groups based on serum SDMA and creatinine concentrations, LBM, age, body weight, sex, USG and UPC. Each block of cats was randomly assigned to receive either control or test food. Cats were maintained in either group or individual housing at Hill's Pet Nutrition Center.

The IRIS stage of CKD was determined according to IRIS guidelines,[15] where IRIS stage is assigned based on serum creatinine and SDMA concentrations and whether an abnormal kidney on palpation, dilute urine or elevated UPC ratio are present and persistent over a period of at least 3 months. Cats were considered to have early stage 1 CKD (non-azotemic) based on one or more of the following: serum creatinine increasing within the reference range (<1.6 mg/dl), persistently increased serum SDMA (>13.5 µg/dl) or persistent renal proteinuria (UPC >0.4). Cats were considered to have stage 2 CKD if both SDMA (>13.5 µg/dl) and serum creatinine (1.6–2.8 mg/dl) were elevated, and USG was <1.035.

Cats were removed from the study if the Clinical Research Veterinarian determined the cat was unable to continue in the study because of progressive kidney disease or if other concurrent medical conditions were diagnosed that warranted additional medical treatment, if an adverse reaction or injury warranted treatment or surgical intervention, or if the cat died spontaneously or was euthanased. In the event that a cat refused to eat two or more consecutive meals within a 36-hour period, they were dismissed from the study.

All eligible cats that entered the study were fed test or control food for 6 months. Twenty cats completed the study according to protocol (9 cats fed control food and 11 cats fed test food). Cats had a physical examination by the Clinical Research Veterinarian at 1, 3 and 6 months postscreening, and blood was drawn to assess CBC and serum chemistries. A routine urinalysis was performed and UPC ratio determined.

Food composition

The control food was commercially available Feline Renal Support A dry cat food (Royal Canin Veterinary Diet, St. Charles, Missouri, USA). The test food was commercially available Hill's Prescription Diet k/d Feline with chicken, dry cat food (Hill's Pet Nutrition, Topeka, Kansas, USA), a food appropriate for adult cats (>1 year). Analysed chemical food composition, expressed as g/100 kcal, is shown in table 1, along with the AAFCO minimum recommendations for protein and

the essential amino acids. Proximate, vitamin, amino acid, fatty acid and mineral analyses were performed using certified official compendial methods by Eurofins Nutrition Analysis Center (Des Moines, Iowa, USA), an ISO accredited commercial laboratory. Methods for crude protein (990.03), fat (973.18), crude fibre (962.09), ash (942.05), amino acids (982.30, 994.12, 988.15), (n-6) and (n-3) FA (Ce 2–66, Ce 1e-91) and minerals (985.01, 969.10) were performed according to the Association of Official Analytical Chemists (AOAC 2000). L-carnitine was determined as previously reported.[16]

Each cat was offered an equivalent amount of calories based on the presumed resting energy requirement (RER) calculated by the formula: RER=70×(body weight in kg)$^{0.75}$. An activity factor was assigned as a multiplier of the RER, which was determined from the metabolic rate established for each individual cat before this study. Thus, the RER was multiplied by an activity factor (mean=1.1±0.3) to meet maintenance energy requirements, with additional instructions to increase or decrease food offering as needed to maintain body weight.

The threonine recommended intake was calculated using AAFCO minimum recommendations in g/100 kcal and expected caloric intake for each cat based on the National Research Council (NRC) estimates of caloric need.[17] Actual threonine intake was calculated for each cat based on the threonine concentration in the food the cat was eating and food intake. This enabled cats to be divided into those that met or exceeded the threonine intake based on AAFCO minimum recommendations and those that did not. Then, LBM data were compared between these two groups based on whether or not cats consumed the recommended amounts of threonine.

Serum and urine analyses

Blood was collected from each cat (following an overnight fast) at each time period to assess CBC and serum chemistries. Serum creatinine concentrations were determined using enzymatic colorimetric methods and BUN concentrations were determined using a kinetic test, by the in-house laboratory (Hill's Pet Nutrition Center). All serum SDMA concentrations were determined retrospectively, after the feeding trial ended, by a commercial laboratory (IDEXX Laboratories, North Grafton, Massachusetts, USA).

Urine was collected by cystocentesis at each time point for routine urinalysis and UPC. USG was determined using a Vet360 Temperature Compensated Hand Held refractometer (Reichert, Depew, New York, USA). Urine creatinine concentration was used as an internal reference and measured with the same assay as serum creatinine. Urine protein concentrations were determined using the urine supernatant (benzethonium chloride turbidometric method). The UPC ratio calculations are reported as mg/dl protein:mg/dl creatinine.

Body mass and composition

Changes in body mass and composition were assessed by dual-energy x-ray absorptiometry (DXA-QDR-4500, Hologic, Waltham, Massachusetts, USA) scan analysis at baseline and after consuming foods for 1, 3 and 6 months. Total, fat and lean body mass were determined using software supplied by the manufacturer.

Statistical analysis

The number of cats targeted for this study was 14 cats in each group. This number of cats was estimated to be sufficient to detect a statistically significant difference in body weight, LBM and serum SDMA and creatinine concentrations between the treatment groups at an 80 per cent power. This estimate was based on a previous feline CKD study that collected similar biological data.[11]

Body weight, BCS, body mass and composition and blood and urinalysis data were analysed using a linear mixed-model for repeated measures with diet, month and diet×month as fixed effects. The Kenwood-Roger adjustment was used to calculate the denominator degrees-of-freedom for the fixed effects. To model the correlation between the repeated measures (months), compound symmetry, unstructured, first-order autoregressive and antedependence covariance structures were modelled and the best fit was selected using AICC and BIC-fit statistics. The diet×month interactions are reported using orthogonal polynomials for unequally spaced intervals.

Daily caloric intake data were averaged for weekly intervals and analysed using a linear mixed-model for repeated measures with diet, week and diet×week as fixed effects. The Kenwood-Roger adjustment was used to calculate the denominator degrees-of-freedom for the fixed effects. To model the correlation between the average weekly caloric intake, compound symmetry and first-order autoregressive covariance structures were modelled and the best fit was selected using AICC and BIC-fit statistics. The two foods were compared at weekly intervals using the SLICEDIFF option in PROC GLIMMIX. This option produces F-tests that test the simultaneous equality of week means for each diet.[18] The overall effect of diet×week interaction is reported.

The authors evaluated the relationship between total energy, total protein and threonine intake and change in LBM in a multiple linear regression using PROC MIXED. All data were included based on intent to treat. All analyses were performed using SAS V.9.2 (SAS Institute, Cary, North Carolina, USA). Statistical significance was declared at $P \leq 0.05$.

Results

Cats

Fifty-seven cats were screened, 28 cats were enrolled in the study and 20 completed the 6-month study according to protocol (9 cats fed control food and 11 cats fed test food). Of the 28 cats enrolled in the study,

12 cats in the control food group were categorised as IRIS stage 1 CKD and 2 cats as IRIS stage 2 CKD. In the test food group, 11 cats were categorised as IRIS stage 1 CKD and 3 cats as IRIS stage 2 CKD. Seven cats had elevated UPCs at baseline (all UPC ≤0.5). There were 2 cats with UPC=0.4 and 1 cat with UPC=0.5 placed on control food, and 2 cats with UPC=0.4 and 2 cats with UPC=0.5 placed on test food. Baseline demographic data are shown in table 2. There were no significant differences between control food and test food groups at baseline in age, sex, reproductive status, LBM, BCS, body weight or IRIS stage of CKD.

There were eight dismissals during the 6-month trial. Two cats were dismissed during the first 10 days for palatability issues, one control food (IRIS stage 1 CKD) and one test food (IRIS stage 2 CKD). Thus, 93 per cent (13/14) of cats successfully transitioned onto either the control or test food for the clinical trial. One cat was euthanased after 27 days on test food for hypertrophic cardiomyopathy (IRIS stage 1 CKD). Another cat was dismissed after 41 days on test food for hypertrophic cardiomyopathy and hypertension (IRIS stage 1 CKD). Four cats on control food were dismissed for body condition deterioration: one for progressive renal disease after 28 days (IRIS stage 2 CKD), and three for weight loss and poor appetite after 50, 68 and 68 days, respectively (all IRIS stage 1 CKD). Thus, 26 cats completed 1 month on the feeding trial (13 cats in each control and test food groups) and 20 cats completed 3 and 6 months (9 control cats and 11 test food cats) according to protocol.

Food consumption

The amount of food offered was determined by MER calculations for each cat. A similar number of calories was offered to the group consuming the control food (mean, 263.2 kcal/day) compared with the group fed test food (mean, 261.6 kcal/day) during the 6-month feeding period (P=0.93). Cats consuming control food voluntarily consumed 64 per cent of calories offered; cats consuming test food voluntarily consumed 79 per cent of calories offered. Thus, cats voluntarily consumed more calories (P=0.05) when offered test food (mean, 207.1 kcal/day) compared with cats offered control food (mean, 168.0 kcal/day) during the 6-month feeding period (figure 1). Similar trends in diet×week interaction were observed in the intent-to-treat cats (P=0.005) compared with those cats that completed the study according to protocol (P=0.082).

Body weights and body condition scores

Cats consuming control food had significant loss of body weight (mean, −13.0 per cent; P<0.0001) during the 6-month feeding period, whereas cats consuming test food had significant increase in body weight (mean, 5.8 per cent; P=0.003; figure 2) based on DEXA analysis. The difference between groups was significant at P<0.0001. Weekly body weight measurements indicated that 11 of 14 cats consuming control food lost more than 5 per cent of their body weight during the study, whereas only 1 of 14 cats gained more than 5 per cent of its body weight. None of the cats consuming test food lost more than 5 per cent of its body weight, and 7 of 14 cats consuming test food gained more than 5 per cent of their body weight. Similar results were noted in the intent-to-treat cats and cats that completed the study according to protocol.

Cats consuming control food had a significant decrease in BCS (mean: −0.5; P=0.002), whereas cats consuming test food had no change in BCS (P=0.76) (data not shown). The difference between groups was significant at P=0.03. Similar results were noted in the

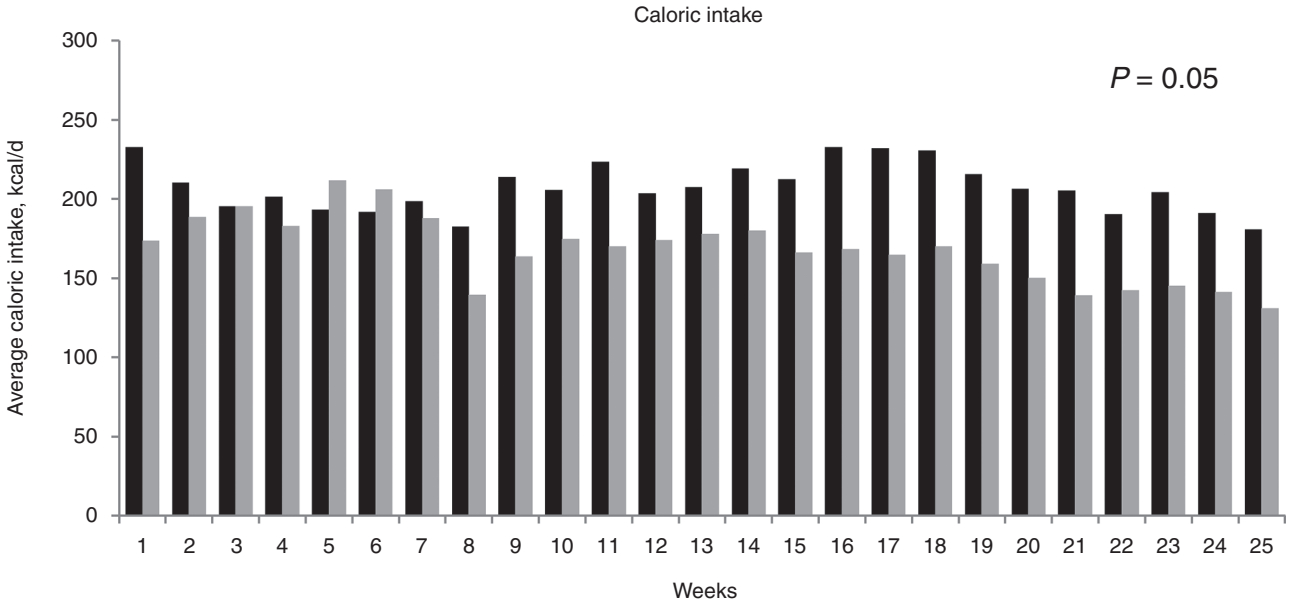

Figure 1 Caloric intake (means) for each week are plotted for all cats (n=28) at all time points for which data were available. A similar number of calories was offered to cats fed test food (black bars; 261.6 kcal/day) and control food (grey bars; 263.2 kcal/day) during the 6-month feeding period (P=0.93). Cats voluntarily consumed 23 per cent more calories when offered test food (mean, 207.1 kcal/day) compared with cats offered control food (mean, 168.0 kcal/day) during the 6-month feeding period (P=0.05).

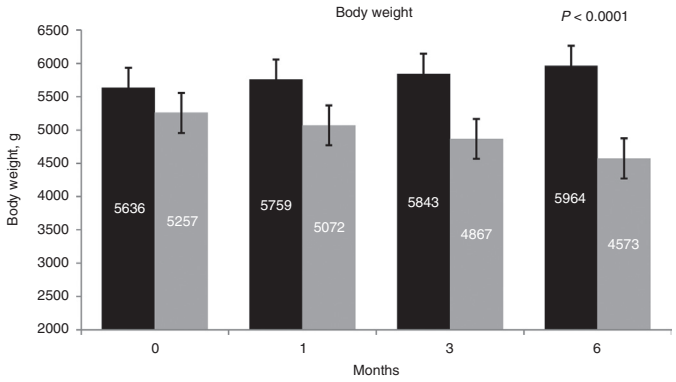

Figure 2 Body weights (means±SEM) at baseline and 1, 3 and 6 months are plotted for all cats (n=28) at all time points for which data were available. Cats consuming control food (grey bars) had significant loss of body weight (mean, −13.0 per cent; P<0.0001) during the 6-month feeding period, whereas cats consuming test food (black bars) had significant increase in body weight (mean, 5.8 per cent; P=0.003) based on dual-energy x-ray absorptiometry analysis. The difference between groups was significant at P<0.0001 (diet×time interaction).

intent-to-treat cats and cats that completed the study according to protocol.

Lean body mass

The LBM of cats was determined by DEXA scan analysis at each examination time point. Cats consuming control food had significant loss of LBM (mean: −11.1 per cent; P<0.0001) over the 6-month feeding period, whereas cats consuming test food had no change in LBM (P=0.42; figure 3). Similar results were noted in the intent-to-treat cats and cats that completed the study according to protocol. The difference between groups was significant at P<0.0001.

To further explore factors that may have influenced change in LBM, energy intake, protein intake and threonine intake were evaluated as to their association with change in LBM. Threonine intake was positively associated with change in LBM (P<0.001). Total protein intake (P=0.13) and total energy intake (P=0.04) were negatively associated with change in LBM.

Essential amino acid consumption

Based on analysed chemical composition (table 1), both control and test foods met the AAFCO minimum

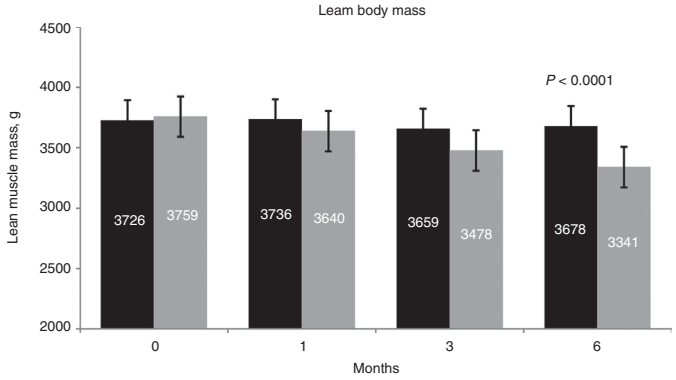

Figure 3 Lean body mass (means±SEM) at baseline and 1, 3 and 6 months are plotted for all cats (n=28) at all time points for which data were available. Cats consuming control food (grey bars) had significant loss of lean body mass (mean: −11.1 per cent; P<0.0001) over the 6-month feeding period, whereas cats consuming test food (black bars) had no change in lean body mass (P=0.42). The difference between groups was significant at P<0.0001 (diet×time interaction).

Table 3 Total threonine intake for cats over the 6-month feeding period vs change in LBM

Cats grouped according to total threonine intake*	LBM at baseline, g†	LBM at end of 6 months, g‡	Change in LBM, g§	Total threonine intake, g¶
Low	3661±175	3369±177	−292±46	73.1±18.9
Adequate	3790±155	3786±179	−4±49	114.7±11.0

*Cats that completed the study (6 months) according to protocol whose threonine intake was less than the AAFCO minimum recommendation (low) or above the AAFCO minimum recommendation (adequate). The estimated threonine intake needed to maintain LBM for cats was calculated using the NRC estimate for energy intake and the AAFCO minimum recommendation for threonine intake. The estimated minimum threonine intake was 97.4 g for cats consuming control food and 102 g for cats consuming test food.
†LBM at beginning of 6-month feeding period, mean±SEM.
‡LBM at end of 6-month feeding period, mean±SEM.
§Change in LBM from baseline to end of 6-month feeding period, mean±SEM. Low vs adequate groups are different at P<0.001.
¶Measured food intake over the 6-month feeding period was multiplied by threonine concentration in food to calculate threonine intake over the 6-month feeding period for each cat, mean±SEM. Low vs adequate groups are different at P<0.001.
AAFCO, Association of American Feed Control Officials; LBM, lean body mass.

recommendations for essential amino acid content. The most limiting essential amino acid (determined by dividing amino acid concentration in g/100 kcal by the AAFCO value in g/100 kcal for that amino acid) was threonine; control food was 13 per cent higher than AAFCO minimum recommendations, and test food was 50 per cent higher than AAFCO minimum recommendations.

Measured food intake over the 6-month feeding period was used to calculate threonine intake over the 6-month feeding period for each cat (table 3). Threonine intake was then compared with change in LBM. The estimated threonine intake needed to maintain LBM for cats was calculated using the NRC estimate for energy intake and the AAFCO minimum recommendation for threonine intake. Estimated minimum threonine intake was 97.4 g for cats consuming control food and 102 g for cats consuming test food. Cats that consumed less than the estimated minimum recommendation for threonine intake had significant loss of LBM, whereas cats consuming at or above the estimated minimum maintained LBM. Those cats consuming less than the AAFCO minimum recommended dietary threonine intake experienced a mean loss of 292±46 g in LBM over 6 months (P<0.001), whereas those cats consuming the minimum or more had no significant change in LBM (average loss 4±49 g; P=0.95). None of the cats consuming control food (n=9) met the AAFCO minimum recommended dietary threonine intake, whereas 7 of 11 cats consuming test food met the recommendation.

CBC, serum renal biomarkers and serum chemistries

Mean values for 16 CBC analytes (red blood cells, reticulocytes, immature reticulocyte fraction, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelets) were

Table 4 Renal function biomarkers, serum chemistries and urinalysis parameters for cats with IRIS stage 1 and 2 CKD fed control or test food at baseline and after consuming foods for 1, 3 and 6 months (mean±SEM)

Analytes	Food fed*	Baseline	Month 1	Month 3	Month 6	P value†
Renal function biomarkers and serum metabolites‡						
Urea nitrogen, mg/dl	Test	25.8±1.3	25.0±1.4	23.3±1.0	24.5±1.6	0.86
(16.3–39.0 mg/dl)	Control	27.1±1.3	25.3±1.4	24.0±1.0	25.6±1.6	
Creatinine, mg/dl	Test	1.35±0.08	1.37±0.08	1.49±011	1.53±0.10§	0.0004
(<1.6 mg/dl)	Control	1.39±0.08	1.61±0.08	1.70±0.11	1.64±0.10§	
SDMA, µg/dl	Test	12.5±0.8	18.1±1.2	20.0±1.0	18.5±1.2	0.43
(<14 µg/dl)	Control	13.1±0.8	20.3±1.1	21.9±1.0	21.0±1.2	
Calcium, mg/dl	Test	9.0±0.1	9.2±0.2	9.3±0.2	9.7±0.2§	0.0002
(8.6–10.7 mg/dl)	Control	9.2±0.1	9.5±0.2	10.3±0.2	11.0±0.2§	
Sodium, mmol/dl	Test	153.0±0.4	151.1±0.4	152.6±0.4	151.3±2.1	0.007
(149–156 mmol/dl)	Control	151.9±0.4	151.7±0.4	153.3±0.4	147.3±2.4	
Phosphorus, mg/dl	Test	4.4±0.2	4.5±0.2	4.2±0.2	4.5±0.2	0.03
(3–6 mg/dl)	Control	4.2±0.2	4.4±0.2	4.8±0.2	4.4±0.2	
Albumin, mg/dl	Test	3.08±0.07	3.13±0.08	3.21±0.08	3.00±0.08	0.47
(2.5–3.8 mg/dl)	Control	3.20±0.07	3.22±0.08	3.27±0.08	3.01±0.08	
Globulin, mg/dl	Test	3.54±0.12	3.57±0.13	3.74±0.13	3.70±0.13§	0.01
(2.5–5.3 mg/dl)	Control	3.41±0.12	3.12±0.13	3.51±0.13	3.29±0.13	
Total protein, mg/dl	Test	6.6±0.1	6.7±0.1	7.0±0.1	6.7±0.1	0.001
(5.6–8.3 mg/dl)	Control	6.6±0.1	6.3±0.1	6.8±0.1	6.3±0.1§	
Urinalysis parameters						
Urine-specific gravity	Test	1.044±0.003	1.047±0.004	1.042±0.002	1.043±0.003	0.37
	Control	1.044±0.003	1.044±0.003	1.044±0.002	1.042±0.003	
Urine creatinine, mg/dl	Test	348±28	402±30	370±30	381±30	0.03
	Control	333±28	455±29	451±31	475±32§	
Urine protein:creatinine ratio	Test	0.26±0.04	0.22±0.14	0.17±0.08	0.19±0.03	0.69
	Control	0.21±0.04	0.31±0.13	0.23±0.08	0.16±0.04	

*Test food was Hill's Prescription Diet k/d Feline with chicken, dry cat food (Hill's Pet Nutrition, Topeka, Kansas, USA). Control food was Feline Renal Support A dry cat food (Royal Canin Veterinary Diet, St. Charles, Missouri, USA).
†P values are shown for diet×time interaction.
‡Normal reference intervals are shown in parentheses.
§For those analytes with diet×time interaction P<0.05, indicates mean values at 6 months that were different from baseline at P≤0.05.
CKD, chronic kidney disease; IRIS, International Renal Interest Society; SDMA, symmetric dimethylarginine.

within the reference intervals for all time points in both treatment groups. There were no significant diet×time interactions, thus, no CBC results are shown.

Mean values for serum renal biomarkers (creatinine, BUN and SDMA concentrations) are shown in table 4. There was a significant diet×time interaction only for serum creatinine (P=0.0004). Although mean creatinine concentrations for both groups of cats were within the reference interval at baseline, mean creatinine was >1.6 mg/dl for cats consuming control food at 1, 3 and 6 months. Serum creatinine concentrations increased over time in cats consuming both control (P=0.001) and test (P=0.01) foods, but increased at a faster rate in cats fed control food. Serum SDMA concentrations also increased over time in cats fed both control and test foods (both P<0.0001); however, mean SDMA concentrations were <25 µg/dl at all time points.

There were significant diet×time interactions (table 4) for serum calcium (P=0.0002), sodium (P=0.007) and phosphorus (P=0.03), although baseline concentrations were within the normal reference intervals. Mean values for sodium and calcium concentrations were outside the reference interval at 6 months in cats fed control food. Mean calcium concentration was above the normal reference interval and mean sodium concentration was below the normal reference interval at 6 months for cats fed control food. Mean concentrations at 6 months were different from baseline concentrations only for calcium (higher for cats fed both control and test foods; P<0.01).

Changes in mean concentrations across time for albumin, globulin, total protein and phosphorus are also shown in table 4. There were significant diet×time interactions for serum globulins (P=0.01) and total protein (P=0.001). Concentrations across time remained within reference intervals. Mean concentrations for globulins at 6 months were increased from baseline concentrations in cats fed test food (P=0.03), whereas mean concentrations for total proteins at 6 months were decreased from baseline concentrations in cats fed control food (P=0.03).

There were significant diet×time interactions for four additional serum chemistry analytes (alkaline phosphatase, potassium, chloride and cholesterol). However, mean values for these analytes were within the reference interval for all time points in cats fed both control and test foods; therefore, changes over time were not considered to be biologically relevant (data not shown). Mean concentrations for alkaline phosphatase at 6 months were decreased from baseline concentrations in cats fed control food (P<0.0001),

for chloride were decreased from baseline in cats fed both control and test foods (P<0.0001) and for cholesterol were increased from baseline in cats fed test food (P<0.0001). Similar serum chemistry results were observed in both intent-to-treat cats and cats that completed the study according to protocol.

Urinalysis parameters

No significant diet×time interactions were noted for cats fed test food compared with cats fed control food for USG or UPC ratio. However, there was a significant diet×time interaction for urine creatinine concentration. This was because urine creatinine concentration in cats consuming control food increased (P<0.0001) across time compared with baseline, whereas urine creatinine concentration of cats fed test food did not change compared with baseline (P=0.22). There was a trend for UPC ratio to be lower in cats fed test food across time compared with baseline (P=0.055). Similar urinalysis results were noted in both intent-to-treat cats and cats that completed the study according to protocol.

Discussion

Two renal-support foods were compared in a 6-month, prospective clinical trial on dietary management of IRIS stage 1 and 2 CKD in cats. The palatability of both control and test foods were very good; only one cat in each group was dismissed in the first 10 days of the study for poor food consumption. Thus, 93 per cent of cats in each group successfully transitioned onto foods fed in this study. However, cats consuming control food had a significant loss of mean body weight (−13.0 per cent) during the 6-month feeding period, whereas cats consuming test food had a significant increase in mean body weight (5.8 per cent). Cats consuming control food also had a significant reduction in mean BCS (−0.5), whereas cats consuming test food had no change in BCS. In addition, cats consuming control food had a significant loss in LBM (−11.1 per cent) over the 6-month feeding period, whereas cats consuming test food had no change in LBM.

Both control and test food contained similar crude protein (6.65 and 6.69 g/100 kcal, respectively) and phosphorus (1.18 and 1.05 g/100 kcal, respectively) concentrations, but lower concentrations compared with industry averages.[10] Both renal foods met AAFCO recommendations for protein and essential amino acid concentrations in cat food. In addition, if food and caloric consumption were normal based on NRC estimates,[17] both control and test foods should have provided adequate intake of essential amino acids. However, control cats did not consume enough food to maintain body weight and, therefore, also had reduced intake of all essential amino acids. Threonine was the most limiting amino acid in the control food (13 per cent overage vs 50 per cent overage in test food compared with AAFCO minimum recommended dietary threonine intake). Both foods had adequate threonine concentration when compared with AAFCO and NRC recommendations on an energy basis (minimums or adequate intakes, respectively). But, because there was reduced food intake in cats with renal disease, which was especially apparent in cats consuming control food, there was a greater need for amino acid fortification of foods. The greater dietary intake of essential amino acids in test food (a combination of increased consumption and higher concentration) resulted in all cats consuming test food having adequate intake of all amino acids, including threonine.

Consequently, the authors hypothesised that insufficient consumption of essential amino acids was correlated with change in LBM noted in these CKD cats. The authors found that consumption of the AAFCO recommended concentrations of threonine, or greater, resulted in maintenance of LBM, whereas there was a significant loss of LBM in cats that did not consume the recommended amounts of threonine (P<0.001). Furthermore, only threonine intake was positively correlated with change in LBM; both total protein and total energy intake were negatively correlated with change in LBM. These data suggest that it is beneficial to increase amino acid fortification (relative to AAFCO minimums) in foods designed for pets with compromised appetite, for example, in early stages of CKD. Although threonine was the first limiting amino acid in this study, it is likely that inadequate intake of any of the essential amino acids would have resulted in loss of LBM.[19] Thus, adequate essential amino acid intake, not total dietary protein, was correlated with maintenance of LBM in CKD cats.

Food portions were determined by RER calculations for each cat. A similar number of calories were offered to cats fed control food (263 kcal/day) compared with cats fed test food (262 kcal/day) throughout the 6-month feeding period. However, cats voluntarily consumed 23 per cent more calories when fed test food (mean, 207.1 kcal/day) compared with cats fed control food (mean, 168.0 kcal/day). A majority of cats fed control food did not consume sufficient calories to maintain body weight, as 11 of 14 cats lost >5 per cent of their baseline body weight. Weight loss among cats fed control food impacted their overall health. Four cats fed control food were dismissed for severe weight loss and BCS deterioration. Although cats fed test food also had substantial fluctuations in daily caloric intake, they still consumed sufficient calories to maintain body weight. In fact, 7 of 14 cats fed test food gained more than 5 per cent of their baseline body weight such that mean body weight was significantly increased by the end of the study.

Cats with CKD often exhibit a reduced appetite, and their daily caloric intake may vary widely from day to day.[20] Weight loss is a common clinical finding in cats with CKD, and is often accompanied by loss of LBM

and decreased BCS in more advanced stages of the disease[20].[5] .[520] Most of the cats enrolled in this study were in early stages of CKD (table 2), such that finding a significant loss of LBM during the 6-month feeding study was unexpected. Therefore, it was surprising that cats fed control food on average lost 11.1 per cent of LBM, whereas cats consuming test food had no loss of LBM.

There are several possible explanations for differences in mean body weights, BCS and LBM results between the two groups of cats. Test food may have had enhanced palatability compared with control food, thus increasing food intake. Test food was shown in a previous study to improve appetite in cats with CKD.[14] Test food also had 12 per cent higher caloric density compared with control food. Test food contained higher concentrations of most essential amino acids (>140 per cent of AAFCO minimum recommendations) compared with control food, which was limited in threonine because of decreased food intake. Lysine in the control food was the second most limiting amino acid (15 per cent overage vs 74 per cent overage in test food compared with the AAFCO minimum recommended dietary lysine). In addition, it is known that total lysine digestibility can be inaccurate when applied to commercially available cat foods.[21]

Threonine and lysine play central hub and top controller roles, respectively, in the inter-relations between plasma amino acid concentrations and metabolism in essential amino acid-deficient rats.[22] Test food also contained 100× the concentration of L-carnitine present in control food. L-carnitine is responsible for facilitating transport of FA into the mitochondria for beta-oxidation and energy production.[23] In another study, adult cats consuming food supplemented with L-carnitine gained LBM over a 6-month feeding period compared with cats fed identical food without carnitine supplement (unpublished data). It is possible that any one or more of these test food attributes were responsible for differences in body weight changes when comparing control and treatment groups of cats. This study suggests that therapeutic renal foods with excellent palatability, increased caloric density and enhanced concentrations of essential amino acids and L-carnitine may help cats with CKD maintain body weight and LBM, particularly if they are finicky eaters or have fluctuating or poor appetites.

Regarding renal biomarkers, serum creatinine increased at a significantly faster rate in cats consuming control food compared with cats consuming test food. Simultaneously, urine creatinine concentrations increased in cats fed control food over the 6-month feeding period. Serum SDMA increased from baseline at a similar rate for both groups of cats; however, mean SDMA was below 25 µg/dl at all time points. There was a trend for UPC to be lower in cats fed test food at 6 months compared with baseline (P=0.055), whereas USG remained stable in both groups of cats over the 6-month feeding period. Higher monthly serum and urine creatinine concentrations in cats fed control food compared with cats fed test food, may reflect catabolism of body tissues to meet energy requirements.[12]

The goals of nutritional management of CKD are to meet the patient's nutrient and energy requirements, as well as to alleviate clinical signs and slow the progression of CKD. Restriction of dietary protein has been demonstrated to slow the rate of progression of renal damage in rats and people.[24 25] However, the effect of protein restriction on the progression of renal damage in dogs and cats remains controversial and no definitive study exists on this matter.[7 12 13] At constant quality and digestibility, feeding more protein will result in more peptides and amino acids reaching the colon for fermentation by the colonic microbiota. Metabolites originating from bacterial fermentation of proteins in the large intestine appear to play a key role in the progression of CKD. Both serum indoxyl sulfate and p-cresyl sulfate concentrations have been shown to negatively correlate with GFR in humans.[26] Indoxyl sulfate likely causes renal tubular damage through oxidative stress, promoting tubulointerstitial fibrosis, glomerular sclerosis and progression of CKD in rats.[27]

CKD is associated with malnutrition, inflammatory and oxidative stress, and cachexia.[28] Cachexia is a wasting syndrome associated with increased resting energy expenditure, weight loss, muscle atrophy, fatigue and loss of appetite. Although mechanisms are not completely understood, pro-inflammatory cytokines are thought to disturb the concentrations and activities of leptin (an anorexigenic peptide) and ghrelin (an orexigenic peptide), which act on hypothalamic neurons to decrease energy intake and increase energy expenditure. Both renal-support foods that were fed in this study contained high concentrations of long-chain (n-3) polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). At sufficiently high dietary concentrations, these (n-3) PUFA have been shown to decrease the production of pro-inflammatory eicosanoids and cytokines, ROS and the expression of adhesion molecules.[29]

In conclusion, cats with CKD readily transition to renal food. The data suggest that renal foods with high concentrations of carnitine and essential amino acids, and of higher energy density, are critical for cats with IRIS stage 1 and 2 CKD to maintain body weight, BCS and LBM as these cats may have decreased appetite and fluctuations in daily food intake. Attributes of renal therapeutic foods considered important for managing major metabolic disorders associated with cachexia (increased resting energy requirements, decreased appetite and muscle atrophy) and for maintaining LBM are enhanced palatability, high caloric density, high concentrations of all essential amino acids, L-carnitine supplementation and added fish oil as a source of (n-3)

highly PUFA. As in dogs,[30] these results support the recommendation that feeding a renal-support diet to cats with IRIS stage 1 and 2 CKD should be considered the standard of care.

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