

Original Research

# Betaine and Soluble Fiber Improve Body Composition and Plasma Metabolites in Cats with Chronic Kidney Disease

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## Abstract

**Background:** Chronic kidney disease (CKD) is a common condition in cats and cachexia (loss of lean body mass) is a concern. A nutrition-based intervention was investigated in cats with CKD for its effects on body composition, the plasma metabolome, and possible implications on health. **Methods:** After a 4-week prefeed period with the control food, cats with CKD (N = 24) were randomized to one of six groups to consume a control food; a food supplemented with 0.5% betaine, 0.39% oat beta-glucan, and 0.27% short-chain fructooligosaccharides (scFOS, test food 1); and a food supplemented with 0.5% betaine, 0.59% oat beta-glucan, and 0.41% scFOS (test food 2) in a William's Latin Square design, each for 10 weeks. Body composition was assessed via dual-energy X-ray absorptiometry measurements, and the plasma metabolome was characterized. **Results:** Despite no significant differences in daily intake among the three foods, significant increases in total body mass, lean body mass, and lean plus bone mineral composition were observed when cats with CKD consumed test food 1 compared with the control food; numerical increases were seen with test food 2 versus the control food. Plasma metabolomics indicated increased one-carbon metabolism following consumption of test food 1 and/or 2, with significant increases in sarcosine and numerical increases in methionine. Lower levels of plasma trans-4-hydroxyproline and N-methylproline following consumption of test foods 1 and 2 indicates reduced collagen breakdown and perhaps reduced fibrosis. Several acylcarnitines and branched-chain fatty acids associated with CKD were also reduced when cats ate test food 1 or 2 versus the control food. Higher plasma levels of sphingomyelins with consumption of test food 1 or 2 may reflect less severe CKD. **Conclusions:** Consumption of foods with supplemental betaine and fibers by cats with CKD led to improvements in body composition and changes in the plasma metabolome that correspond to better kidney health.

**Keywords:** betaine; fiber; feline; chronic kidney disease; plasma; metabolomics; body composition

## 1. Introduction

Chronic kidney disease (CKD) is common in cats, with a reported overall prevalence in primary care practice estimated at 1.2%, with an increase to 3.6% in cats aged  $\geq 9$  years [1]. The actual prevalence may be a lot higher, as another study, which used complete blood count, serum chemistry, and urine analysis as diagnostic criteria, found the prevalence of CKD to be about 40% in cats aged 0–14 years and 80% in cats aged  $\geq 15$  years, all in cats recruited for non-CKD clinical trials [2]. The differences between these findings are likely due to the fact that clinical signs of CKD are not apparent until a great amount of kidney function has been lost. Nevertheless, CKD affects a large number of cats with increasing prevalence with age.

Treatment recommendations for the early stages of CKD in cats focus on slowing progression in order to retain the remaining kidney function [3,4]. Renal diets low in phosphorus and protein are recommended for cats with CKD [3,4] and are commonly used to treat CKD as seen in primary care practice [1].

The gut microbiome and its associated metabolites have been an area of extensive research in recent years, as

they can affect and provide clues regarding the health of the host. In turn, the nutrition intake of the host as well as underlying health issues can alter the gut microbiome and/or its metabolites [5]. In the case of CKD, many uremic toxins are produced by the gut microbiota [6,7]. Differences between cats with CKD and healthy controls have been observed in plasma and fecal metabolites and in the fecal microbiome [8–11].

A nutritional approach to modifying the metabolites produced by the gut microbiota is an attractive method to decrease the effects and slow the progression of CKD. Although cats are carnivores, the microbial phylogeny and gene content of their gut microbiomes are similar to those of omnivores [12]. Fiber is added to commercial cat foods [12], and gut microbiome-mediated fiber fermentation likely confers benefits to cats [13]. In fact, increased dietary fiber is associated with lower odds of chronic renal failure in cats [14]. Healthy cats and those with CKD respond differently to dietary supplementation with fermentable fibers [8]. Short-chain fructooligosaccharides (scFOS) are a more readily fermentable fiber [15] and are thus preferred over apple pomace in cats with CKD [8].



Consumption of prebiotic fermentable fibers has shown favorable effects on oxidative stress, inflammation, gut barrier integrity, and gut-derived uremic toxins in rodent models of CKD [16].

Betaine, or trimethylglycine, serves many useful cellular functions. It is a methyl group donor for methylation reactions, an essential biochemical process across all life, and has anti-inflammatory and antioxidant properties [17]. In addition, betaine protects against osmotic stress, in part by stabilizing proteins [17]. In the kidney medulla, betaine is an osmoprotectant, where it preserves cell volume and function in the presence of the high osmolarity needed for urine concentration and water reabsorption [18]. Plasma levels of betaine decrease with CKD progression [19], but oral administration of betaine in hyperuricemic mice resulted in significantly reduced serum uric acid and increased markers of improved kidney function [20]. Foods high in betaine have been reported to increase the abundance of beneficial gut bacteria [21].

Some prior work has tested the effects of betaine and fiber on the metabolome. Several beneficial changes in the plasma metabolome, including decreases in uremic toxins, were observed following consumption of foods containing betaine, oat beta-glucan, and scFOS by dogs with early renal disease [22]. A small study of cats with CKD (N = 7) that consumed a food with betaine, oat beta-glucan, and scFOS for 8 weeks had higher levels of plasma metabolites involved in one-carbon metabolism compared with the control food [23].

The present study expands upon the results seen with the betaine, oat beta-glucan, and scFOS food in cats with CKD, here in a different base food and in a greater number of cats fed for 10 weeks. In addition, a food with added betaine but lower levels of oat beta-glucan and scFOS was tested, and body composition and plasma metabolites are reported.

## 2. Materials and Methods

### 2.1 Study Foods

The control food was a complete and balanced dry food designed to aid in the management of renal disease with no added betaine or fiber from scFOS or beta-glucan. The two test foods were of similar formulation to the control food except that each was supplemented with 0.5% betaine; the test food with a lower amount of added fiber (test food 1) also had 0.39% oat beta-glucan and 0.27% scFOS, and the test food with a greater amount of added fiber (test food 2) had 0.59% oat beta-glucan and 0.41% scFOS. To compensate for the increased fiber inclusion in test food 2, 0.34% whole red wheat was added to test food 1. All foods contained fiber from brown rice, whole wheat, and barley, with the amount of whole wheat adjusted to accommodate for the added betaine, scFOS, and oat beta-glucan. All foods were manufactured at Hill's Pet Nutrition in dry form and met the Association of American Feed Control Officials (AAFCO)

maintenance nutrition requirements for adult cats. Proximate analysis were carried out using AOAC methods as previously described [23].

### 2.2 Animals and Experimental Design

Twenty-four spayed or neutered cats owned by Hill's Pet Nutrition with International Renal Interest Society stage 1 CKD (based on the results of annual physical examinations, complete blood count, serum biochemistries, and urinalysis; blood creatinine <1.6 mg/dL and symmetric dimethylarginine <18  $\mu$ g/dL), nonazotemic kidney stones (n = 5), or small kidney (identified on physical examination and ultrasonographic imaging; n = 1), were included in this study. The mean  $\pm$  standard deviation age of the cats was  $11.9 \pm 2.2$  years. All cats were maintained in individual housing at the Hill's Pet Nutrition Center. Cats were fed once daily and had unlimited access to water. Cats were to be removed from the study if they would benefit from removal as determined by the colony veterinarian.

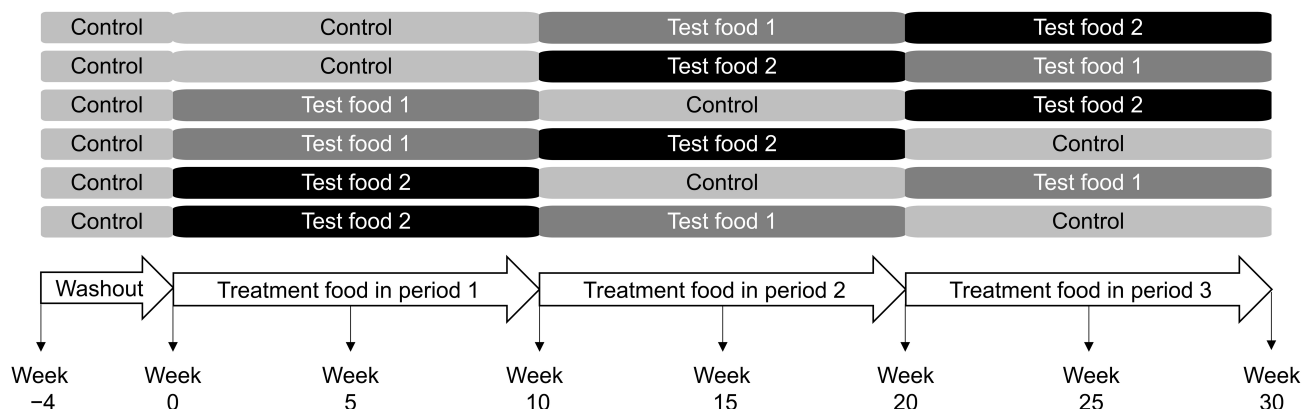
The study protocol was approved by the Hill's Institutional Animal Care and Use Committee (IACUC) (Permit number: 830) and Animal Welfare Committee. This study complied with the US National Research Council guide for the care and use of laboratory animals [24]. After a 4-week prefeed period on the control food, cats were assigned into six subgroups to receive the control food, test food 1, or test food 2, each for 10 weeks, in a Williams Latin Square design sequence [25] with age and sex balanced among groups (Fig. 1). The amounts of food given were to maintain body weight, and intake was recorded for each cat. Blood samples were collected from fasted cats midway and at the end of each treatment period.

### 2.3 Dual-Energy X-Ray Absorptiometry and Metabolite Analysis

Dual-energy X-ray absorptiometry (DEXA) measurements were conducted using the DXA-QDR-4500 system (Hologic, Waltham, MA, USA) at baseline and the end of each treatment period. A chemistry screen was used to assess circulating blood creatinine and blood urea nitrogen (BUN) after fasting [26]. Analysis of plasma metabolomics was performed by Metabolon (Morrisville, NC) as previously described [27].

### 2.4 Statistical Analysis

JMP Pro software (JMP® Pro 15S, AS Institute, Cary, NC, USA) was used for data analyses. Metabolomics data was log-transformed before statistical analysis. Mean units for metabolites were scaled to a median of 1. Mixed model analysis was used to compare the treatment effect during the treatment period using animal identification as the random designate. In all analyses, statistical significance was considered to be  $p \leq 0.05$ .



**Fig. 1. Study design.** Sequence and timeline in which cats consumed the control and test foods in a Williams Latin Square design. Test food 1 contained 0.5% betaine, 0.39% oat beta-glucan, and 0.27% scFOS, and test food 2 contained 0.5% betaine, 0.59% oat beta-glucan and 0.41% scFOS. scFOS, short-chain fructooligosaccharides.

### 3. Results

#### 3.1 Food Analysis

Results of the proximate analysis of the foods are shown in Table 1. The three foods had similar nutrient profiles except that both test foods 1 and 2 contained higher amounts of soluble fiber than the control food. The concentration of soluble fiber in test food 1 was greater than that of test food 2 due to the added whole wheat.

**Table 1. Proximate analysis of the study foods.**

Proximate analysis parameter	Control food	Test food 1	Test food 2
Moisture	5.76	5.83	5.74
Ash	4.5	4.6	4.6
Crude protein	29.1	29.6	29.7
Crude fat	20.7	22.3	21.4
Crude fiber	1.0	1.0	0.9
Neutral detergent fiber	2.8	3.1	2.9
Soluble fiber	1.0	2.4	1.7
Total dietary fiber	7.4	6.9	6.3
Lysine	1.45	1.45	1.42
Methionine	0.65	0.64	0.64
Cystine	0.44	0.45	0.45
Phosphorus	0.56	0.58	0.58
Potassium	0.78	0.79	0.79
Chloride	0.54	0.51	0.52
Calcium	0.72	0.73	0.72
Magnesium	0.08	0.09	0.09
Betaine	0.04	0.58	0.58
Food (kcal/kg)	4160.7	4158.8	4157.8

Reported values are percentages unless otherwise indicated. Test food 1 contained 0.5% betaine, 0.39% oat beta-glucan, and 0.27% scFOS, and test food 2 contained 0.5% betaine, 0.59% oat beta-glucan and 0.41% scFOS. scFOS, short-chain fructooligosaccharides.

#### 3.2 Study Animals, Food Intake, and Body Composition

Twenty-two of the 24 cats completed the study; one cat with kidney stones was removed due to low food intake, and another cat with renal failure was removed due to the severity of the disease.

The inclusion of fiber did not appear to affect average daily food intake since there was no significant difference ( $p > 0.50$ ) among the three foods (Table 2). There were significant increases in total body mass, lean body mass, and lean plus bone mineral composition when cats were fed test food 1 compared with the control food (Table 2). Total body mass tended to be higher when cats were fed test food 2 compared to the control food ( $p = 0.06$ ). There were no additional significant differences in body composition among the treatment groups.

#### 3.3 Blood Chemistry Parameters and Plasma Metabolomics

There was no treatment effect on the blood chemistry parameters of creatinine or BUN following consumption of each food, with respective values of 1.49, 1.49, and 1.57 mg/dL for the control food, test food 1, and test food 2 for creatinine ( $p = 0.806$ ) and 23.4, 24.0, and 24.4 mg/dL for BUN ( $p = 0.879$ ).

Consumption of both test foods led to significant increases in markers of methylation such as betaine and sarcosine (significantly changed metabolites are shown in Table 3). Methionine tended to be higher after cats ate the test food 1 ( $p = 0.070$  vs control food), while S-methylmethionine was significantly increased by both test foods. The methylated amino acid N,N-dimethylalanine, markers of collagen breakdown (trans-4-hydroxyproline and N-methylproline), the xenobiotics homostachydrine and stachydrine, and the branched-chain fatty acids 2-hydroxy-3-methylvalerate and 2-hydroxybutyrate/2-hydroxyisobutyrate were reduced after cats consumed either test food. In addition, branched-chain

**Table 2. Mean food intake and body composition.**

Parameter	Control food	Test food 1	Test food 2	<i>p</i> value, test food 1 vs control food	<i>p</i> value, test food 2 vs control food	<i>p</i> value, test food 1 vs test food 2
Food intake, g/day	34.7 ± 19.2	37.6 ± 21.2	38.3 ± 20.7	0.620	0.498	0.834
Total body mass, g	4992.4 ± 242.8	5097.7 ± 242.8	5071.1 ± 243	0.014	0.063	0.529
Lean body mass, g	3507.4 ± 124.8	3549.6 ± 124.8	3529.8 ± 124.8	0.031	0.244	0.312
Total fat mass, g	1486.1 ± 114.9	1550 ± 115	1540 ± 115	0.220	0.335	0.967
Bone mineral composition, g	137.7 ± 4.3	138.5 ± 4.3	138.9 ± 4.3	0.483	0.242	0.882
Lean body mass + bone mineral composition, g	3645 ± 128.1	3688.2 ± 128.1	3668.7 ± 128.2	0.027	0.216	0.317

Actual means are shown. Test food 1 contained 0.5% betaine, 0.39% oat beta-glucan, and 0.27% scFOS, and test food 2 contained 0.5% betaine, 0.59% oat beta-glucan and 0.41% scFOS. scFOS, short-chain fructooligosaccharides.

amino acids and dipeptides including isovalerylglycine, 2-hydroxy-3-methylvalerate, alpha-hydroxyisocaproate and gamma-glutamylalanine were significantly reduced after cats were fed test food 1 (Table 3). There were only a few metabolites that significantly differed between the two test foods; these included higher levels of betaine, N6-methyllysine, alpha-hydroxyisocaproate, and 3-hydroxyisobutyrate and lower levels of N,N-dimethylalanine with test food 2 compared with test food 1.

Certain sphingomyelins were increased while carnitine-conjugated lipids were reduced after cats ate both treatment foods (Table 4). Several long-chain mono/polyunsaturated fatty acids including myristoleate and docosatrienoate (22:3n6) were significantly increased after cats consumed test food 1 with non-significant increases in EPA and DHA. None of the lipid metabolites were statistically different between the two treatment foods (Table 4).

#### 4. Discussion

In this study, body composition parameters of total body mass, lean body mass, and lean body mass plus bone mineral composition were significantly higher in cats with CKD following consumption of test food 1 versus the control food. Similarly, though not statistically significant, consumption of test food 2 also led to numerically higher values for these body composition parameters. This greater body composition was observed despite similar daily food intakes among the three study foods. These results are consistent with prior work that found that cats with CKD had a higher body mass after consuming a food similar to test food 2 compared with a control food even though food intakes were not significantly different [23]. Together, the data from that study and this one indicate that betaine and these fermentable fibers increase the ability of cats with CKD to utilize food. These results are especially noteworthy given that loss of body weight is a concern in cats with CKD since it is associated with a shorter survival time [28].

There is additional evidence that supplementation with betaine can increase body mass. In Huang Jiang mini-

pigs, dietary supplementation with betaine for 60 days increased final body weight, average daily gain, and lean percentage while decreasing average backfat thickness compared with a control food [29]. Also, a study of men in a strength training program found that betaine supplementation for 6 weeks improved body composition by lowering body fat and fat mass and increasing lean body mass compared with placebo [30]. However, meta-analyses of clinical trials in humans found that dietary betaine supplementation did not affect body mass [31,32], similar to healthy cats [23]. Body fat was lowered with betaine supplementation [32], so the effects of betaine on body mass may depend on the species, kidney function status, and/or high metabolic demand. Betaine may exert its effect on increasing body mass by increasing protein synthesis [33] and the cell proliferation rate in regulating the cellular response to hyperosmolarity [34].

In this study, the main difference between the nutrient profiles of the test foods and the control food was the inclusion of betaine, as the amounts of crude and total fiber were the same among all foods. The observation of improved body composition after cats with CKD consumed test food 1 warrants further research to understand the mechanism by which betaine and prebiotics deliver benefits toward preventing cachexia in cats with CKD. In addition, it would be useful to evaluate the independent effects of betaine and soluble fiber on body composition since test food 1 had the highest amounts of soluble fiber among the foods tested here.

Not surprisingly, supplementation of the test foods with betaine resulted in higher plasma concentrations of betaine relative to the control food. This allowed for increased one-carbon metabolism, as evidenced by the higher levels of sarcosine, a downstream metabolite of betaine, following consumption of either test food. Also consistent with the present study, higher levels of plasma sarcosine and betaine were observed following consumption of test food 2 compared with the control food [23]. Betaine supplementation significantly lowers plasma homocysteine levels in healthy adult humans, likely due to increased methionine formation due to betaine-mediated methylation of homocysteine [35].

**Table 3. Plasma metabolites of amino acid metabolism and xenobiotics significantly influenced by treatment.**

Plasma metabolite	Control food	Test food 1	Test food 2	<i>p</i> value, test food 1 vs control food	<i>p</i> value, test food 2 vs control food	<i>p</i> value, test food 1 vs test food 2
Increased following test foods						
Amino Acid Metabolism						
Betaine	0.79	1.09	1.16	<0.001	<0.001	0.012
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)	0.98	1.05	1.09	0.031	0.003	NS
4-imidazoleacetate	1.04	1.27	1.18	0.002	0.004	NS
Imidazole lactate	1.0	1.15	1.18	NS	0.007	NS
Imidazole propionate	0.95	1.14	1.18	0.002	0.002	NS
1-methyl-4-imidazoleacetate	1.05	1.18	1.28	NS	0.009	NS
N-acetylserine	0.96	1.06	1.06	0.020	0.046	NS
Sarcosine	0.93	1.23	1.11	<0.001	0.002	NS
S-methylmethionine	0.88	1.26	1.39	<0.001	<0.001	0.070
Succinoyltaurine	1.05	1.11	1.11	0.035	0.022	NS
Decreased following test foods						
Amino Acid Metabolism						
Alpha-hydroxyisocaproate	1.16	1.03	1.13	0.007	NS	0.014
Gamma-glutamylalanine	1.1	1.0	1.0	0.047	NS	NS
Glutamine	1.04	0.99	1.0	0.043	NS	NS
Glutarylcarntine (C5-DC)	1.04	0.94	0.97	0.015	NS	NS
2-hydroxybutyrate/2-hydroxyisobutyrate	1.75	1.37	1.47	<0.001	0.013	0.069
2-hydroxy-3-methylvalerate	1.2	1.11	1.13	0.009	NS	NS
3-hydroxyisobutyrate	1.08	1.0	1.12	0.066	NS	0.03
Isovalerylglycine	1.12	0.98	1.24	0.024	NS	NS
N-acetylglutamate	1.12	0.98	1.05	0.012	NS	NS
N6-methyllysine	1.14	1.03	1.12	0.004	NS	0.01
N-methylproline	1.13	0.96	0.91	0.016	0.001	NS
N,N-dimethylaniline	1.41	0.81	0.55	0.002	<0.001	0.028
Trans-4-hydroxyproline	1.18	0.90	0.93	<0.001	<0.001	NS
Vanillactate	0.9	0.78	0.88	0.032	NS	NS
Xenobiotics						
Homostachydrine	1.43	0.84	0.81	<0.001	<0.001	NS
Stachydrine	1.38	0.89	0.89	<0.001	<0.001	NS

Values were rescaled to a median value of 1. There was no difference by sequence of treatment ( $p > 0.100$ ). Test food 1 contained 0.5% betaine, 0.39% oat beta-glucan, and 0.27% scFOS, and test food 2 contained 0.5% betaine, 0.59% oat beta-glucan and 0.41% scFOS. scFOS, short-chain fructooligosaccharides. NS, not significant.

Homocysteine, a normal product of the methionine cycle, is a known risk factor for the loss of cognitive function [36]. It is also a gut microbiota-derived, protein-bound uremic toxin [6], and high plasma homocysteine is a marker for increased risk of cardiovascular disease and stroke [37]. Although not significant, plasma methionine levels were numerically higher with the test foods in this study compared with the control food; homocysteine was not detected in the metabolite screen.

Betaine may be the ingredient responsible for the decrease in N-methylproline seen here with the test foods, as healthy dogs that consumed a food supplemented with only betaine exhibited lower levels of plasma N-methylproline compared with the control food [38]. In addition, cats with different single nucleotide polymorphisms in the gene encoding alanine-glyoxylate aminotransferase 2, which puts them at different risk levels for calcium oxalate kidney stone formation, showed lower levels of trans-4-

hydroxyproline after consuming a food with added betaine and botanicals compared with the control food [39].

Renal fibrosis involves the remodelling and turnover of the extracellular matrix, specifically the degradation of collagen by matrix metalloproteases. Thus, collagen degradation reflects the extent of renal fibrosis in CKD [40]. Trans-4-hydroxyproline is a major component of collagen, with N-methylproline a more minor one. The lower levels of plasma N-methylproline and trans-4-hydroxyproline observed here with the test foods compared with the control food may indicate reduced collagen breakdown and by extension, reduce the likelihood of renal fibrosis. Levels of plasma N-methylproline and/or trans-4-hydroxyproline were decreased relative to baseline in healthy cats, cats with CKD, and in dogs with early renal disease that consumed foods with supplemental betaine, oat beta-glucan, and scFOS [8,22,23].



**Table 4. Plasma lipid metabolites significantly influenced by treatment.**

Plasma metabolite	Control food	Test food 1	Test food 2	<i>p</i> value, test food 1 vs control food	<i>p</i> value, test food 2 vs control food	<i>p</i> value, test food 1 vs test food 2
Increased following test foods						
Docosatrienoate (22:3n6)	1.04	1.38	1.16	0.047	NS	0.059
Glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	1.03	1.14	1.11	0.027	0.047	NS
Heptadecanedioate (C17-DC)	0.93	1.11	0.97	0.003	NS	0.051
Hexadecanedioate (C16-DC)	0.95	1.16	1.02	<0.001	0.084	0.065
Myristoleate (14:1n5)	1.03	1.21	1.1	0.049	NS	NS
N-palmitoyl-sphinganine (d18:0/16:0)	1.03	1.11	1.12	0.039	0.034	NS
Octadecadienedioate (C18:2-DC)	0.95	1.09	1.02	0.010	NS	NS
Octadecanedioate (C18-DC)	0.97	1.12	1.02	0.006	NS	NS
Octadecenedioate (C18:1-DC)	0.89	1.22	1.04	<0.001	NS	NS
Sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)	0.98	1.06	1.03	0.004	0.014	NS
Sphingomyelin (d18:2/24:1, d18:1/24:2)	0.98	1.04	1.05	0.044	0.010	NS
Decreased following test foods						
Adipoylcarnitine (C6-DC)	1.11	0.94	1.04	0.011	NS	NS
Branched-chain, straight-chain, or Cyclopropyl 10:1 fatty acid (1)	1.26	0.89	0.81	<0.001	<0.001	NS
Cerotoylcarnitine (C26)	1.11	0.91	0.92	<0.001	0.001	NS
Erucoylcarnitine (C22:1)	1.0	0.86	0.92	0.033	NS	0.08
3-hydroxyoleoylcarnitine	1.14	1.04	0.98	0.028	0.013	NS
Lignoceroylcarnitine (C24)	1.09	0.94	1.03	0.010	NS	NS
Nervonoylcarnitine (C24:1)	1.10	0.95	0.96	0.005	0.021	NS
1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	1.19	1.03	1.36	0.006	NS	NS
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	1.12	0.98	1.02	0.035	0.070	NS
Pentadecanoylcarnitine (C15)	1.15	1.09	0.93	0.032	<0.001	0.09
Stearoylcarnitine (C18)	1.08	0.10	0.99	0.031	0.062	NS
Suberoylcarnitine (C8-DC)	1.07	0.90	0.95	0.011	NS	NS
Ximenoylcarnitine (C26:1)	1.17	0.94	0.92	0.002	0.001	NS

Values were rescaled to a median value of 1. There was no difference by sequence of treatment ( $p > 0.100$ ). Test food 1 contained 0.5% betaine, 0.39% oat beta-glucan, and 0.27% scFOS, and test food 2 contained 0.5% betaine, 0.59% oat beta-glucan and 0.41% scFOS. scFOS, short-chain fructooligosaccharides. NS, not significant.

Sphingomyelins are found in animal cell membranes; in addition to providing structure to the membrane, some also participate in signalling functions. A number of sphingomyelin metabolites were at higher plasma levels in both dogs fed supplemental betaine [38] and in the present study in which cats with CKD consumed the foods with supplemental betaine, oat beta-glucan, and scFOS. Levels of plasma sphingomyelins were lower in humans on hemodialysis compared with patients with lower stages of CKD [41]. Thus, the significant increases in various sphingomyelins observed in cats that ate test food 1 or 2 compared with the control food may be more reflective of less severe CKD and are perhaps beneficial in delaying CKD progression.

Acylcarnitines transfer long-chain fatty acids from the cytoplasm to the mitochondria for fatty acid oxidation. Here, a number of acylcarnitines were found at lower levels following cats' consumption of the test foods compared with the control food. Similarly, plasma levels of several types of carnitines were lower in both dogs fed supplemental betaine [38]. These observed decreases in circulating carnitines may be the result of their shift to the

liver and muscle [42], also supported by the observation of the significant increase in lean body mass in the present study. Lower levels of carnitines may also be beneficial since glutarylcarnitine is inversely associated with eGFR in humans [43] and is at higher levels in plasma from people with diabetic kidney disease compared with those with type 2 diabetes alone [44]. Several acylcarnitines, including pentadecanoylcarnitine, stearoylcarnitine, and erucoylcarnitine, were at higher levels in the brains of aged mice compared with younger mice [45]. Accumulation of stearoylcarnitine also impairs the tricarboxylic acid cycle in beta cells, thus depleting these cells of energy during the progression of type 2 diabetes [46]. A subset of individuals with elevated levels of trimethylamine N-oxide (TMAO), a gut microbial metabolite that is elevated in CKD [47], also had elevated plasma levels of several carnitines, including glutarylcarnitine, stearoylcarnitine, ximenoylcarnitine, lignoceroylcarnitine, and nervonoylcarnitine [48], all of which were at lower plasma levels after cats consumed the test foods compared with the control food in the present study.

A few plasma metabolites containing imidazole moieties were increased compared with the control food following the consumption of the test foods. Imidazole lactate and imidazole propionate were also found to be increased in plasma with food similar to test food 2 in healthy cats [23]. These imidazole metabolites are found at higher levels in urine from patients with end-stage renal disease on hemodialysis compared with controls [49]. Despite this, imidazole propionate, a metabolite of histidine produced by the gut microbiota, has been found to protect against cardiopulmonary toxicity induced by radiation for cancer therapy [50]. In addition, several imidazole-containing compounds exhibit a variety of anticancer activities [51].

The branched-chain fatty acids 2-hydroxybutyrate/2-hydroxyisobutyrate and 2-hydroxy-3-methylvalerate were at lower levels in plasma following consumption of the test foods in this study compared with the control food. Prior work showed that BCFAs were at higher levels in feces of cats with CKD compared with healthy controls, and levels were even higher in cats with muscle atrophy [52]. Branched-chain fatty acids are also associated with type 2 diabetes, obesity, and some types of cancer [53]. Further, the reduction of branched-chain fatty acids was associated with an increase in saccharolytic metabolism and enhanced markers of renal health in cats [54]. Thus, lower circulating BCFA levels may be beneficial to health.

Several branched-chain or methylated amino acids that were at significantly lower plasma levels following consumption of test food 1 and/or 2 compared with the control food in this study have been associated with adverse health effects. Lower kidney clearance of isovaleryl-glycine was identified as one of six solutes associated with significantly higher risks of progression of CKD [55] and cognitive decline [56] in a large clinical study of over 3000 patients. In addition, N-methylproline and gamma-glutamylalanine were among the metabolites negatively associated with physical function in a clinical study of older adults [57]. Alpha-hydroxyisocaproate was one of a few serum metabolites that were predictive of acute ischemic stroke in males [58].

## 5. Conclusions

In summary, cats with CKD that consumed food supplemented with betaine, oat beta-glucan, and scFOS displayed benefits in lean body mass, likely due to enhanced energy use. A number of changes in plasma metabolites that likely indicate health benefits were also observed, including lower levels of collagen breakdown components, acyl-carnitines associated with CKD, and branched-chain fatty acids as well as higher levels of sphingomyelins and sarcosine, the latter indicating increased one-carbon metabolism. Thus, supplemental betaine and fiber are an aid in the nutritional management of renal disease in cats.

## Abbreviations

CKD, chronic kidney disease; scFOS, short-chain fructooligosaccharides; TMAO, trimethylamine N-oxide.

## Availability of Data and Materials

The datasets analyzed during the study are available from the corresponding author on reasonable request.

## Author Contributions

These should be presented as follows: EE and DEJ designed the research study. EE performed the research, analyzed the data, and wrote the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript.

## Ethics Approval and Consent to Participate

The study protocol was approved by the Hill's Institutional Animal Care and Use Committee (IACUC) (Permit number: 830) and Animal Welfare Committee.

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## Conflict of Interest

Although the funder (Hill's Pet Nutrition, Inc.) provided support in the form of salaries for the authors, the funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The research was performed at the Pet Nutrition Center, Topeka, Kansas, where these authors currently work (E.E.) or formerly worked (D.E.J.).

## 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<sup>TM</sup> study. *The Veterinary Record*. 2019; 184: 526.
- [2] Marino CL, Lascelles BDX, Vaden SL, Gruen ME, Marks SL. Prevalence and classification of chronic kidney disease in cats randomly selected from four age groups and in cats recruited for degenerative joint disease studies. *Journal of Feline Medicine and Surgery*. 2014; 16: 465–472.
- [3] International Renal Interest Society. IRIS treatment recommendations for CKD in cats (2019). 2019. Available at: <http://iris-kidney.com/guidelines/recommendations.html> (Accessed: 10 August 2022).
- [4] Sparkes AH, Caney S, Chalhoub S, Elliott J, Finch N, Ganayake I, *et al.* ISFM Consensus Guidelines on the Diagnosis and Management of Feline Chronic Kidney Disease. *Journal of Feline Medicine and Surgery*. 2016; 18: 219–239.
- [5] Wernimont SM, Radosevich J, Jackson MI, Ephraim E, Badri

- DV, MacLeay JM, *et al.* The Effects of Nutrition on the Gastrointestinal Microbiome of Cats and Dogs: Impact on Health and Disease. *Frontiers in Microbiology*. 2020; 11: 1266.
- [6] Graboski AL, Redinbo MR. Gut-Derived Protein-Bound Uremic Toxins. *Toxins*. 2020; 12: 590.
- [7] Beker BM, Colombo I, Gonzalez-Torres H, Musso CG. Decreasing microbiota-derived uremic toxins to improve CKD outcomes. *Clinical Kidney Journal*. 2022; 15: 2214–2219.
- [8] Hall JA, Jackson MI, Jewell DE, Ephraim E. Chronic kidney disease in cats alters response of the plasma metabolome and fecal microbiome to dietary fiber. *PLoS ONE*. 2020; 15: e0235480.
- [9] Hall JA, Jewell DE, Ephraim E. Changes in the Fecal Metabolome Are Associated with Feeding Fiber Not Health Status in Cats with Chronic Kidney Disease. *Metabolites*. 2020; 10: 281.
- [10] Summers SC, Quimby JM, Isaiah A, Suchodolski JS, Lunghofer PJ, Gustafson DL. The fecal microbiome and serum concentrations of indoxyl sulfate and p-cresol sulfate in cats with chronic kidney disease. *Journal of Veterinary Internal Medicine*. 2019; 33: 662–669.
- [11] Summers SC, Quimby J, Blake A, Keys D, Steiner JM, Suchodolski J. Serum and Fecal Amino Acid Profiles in Cats with Chronic Kidney Disease. *Veterinary Sciences*. 2022; 9: 84.
- [12] Barry KA, Middelbos IS, Vester Boler BM, Dowd SE, Suchodolski JS, Henrissat B, *et al.* Effects of dietary fiber on the feline gastrointestinal metagenome. *Journal of Proteome Research*. 2012; 11: 5924–5933.
- [13] Rochus K, Janssens GPJ, Hesta M. Dietary fibre and the importance of the gut microbiota in feline nutrition: a review. *Nutrition Research Reviews*. 2014; 27: 295–307.
- [14] Hughes KL, Slater MR, Geller S, Burkholder WJ, Fitzgerald C. Diet and lifestyle variables as risk factors for chronic renal failure in pet cats. *Preventive Veterinary Medicine*. 2002; 55: 1–15.
- [15] Stewart ML, Timm DA, Slavin JL. Fructooligosaccharides exhibit more rapid fermentation than long-chain inulin in an in vitro fermentation system. *Nutrition Research*. 2008; 28: 329–334.
- [16] Melekoglu E, Samur FG. Dietary strategies for gut-derived protein-bound uremic toxins and cardio-metabolic risk factors in chronic kidney disease: A focus on dietary fibers. *Critical Reviews in Food Science and Nutrition*. 2021; 1–15.
- [17] Zhao G, He F, Wu C, Li P, Li N, Deng J, *et al.* Betaine in Inflammation: Mechanistic Aspects and Applications. *Frontiers in Immunology*. 2018; 9: 1070.
- [18] Kempson SA, Vovor-Dassu K, Day C. Betaine transport in kidney and liver: use of betaine in liver injury. *Cellular Physiology and Biochemistry*. 2013; 32: 32–40.
- [19] Alvarenga L, Ferreira MS, Kemp JA, Mafra D. The Role of Betaine in Patients With Chronic Kidney Disease: a Narrative Review. *Current Nutrition Reports*. 2022; 11: 395–406.
- [20] Liu YL, Pan Y, Wang X, Fan CY, Zhu Q, Li JM, *et al.* Betaine reduces serum uric acid levels and improves kidney function in hyperuricemic mice. *Planta Medica*. 2014; 80: 39–47.
- [21] Koistinen VM, Kärkkäinen O, Borewicz K, Zarei I, Jokkala J, Micard V, *et al.* Contribution of gut microbiota to metabolism of dietary glycine betaine in mice and in vitro colonic fermentation. *Microbiome*. 2019; 7: 103.
- [22] Ephraim E, Jewell DE. Effect of Added Dietary Betaine and Soluble Fiber on Metabolites and Fecal Microbiome in Dogs with Early Renal Disease. *Metabolites*. 2020; 10: 370.
- [23] Hall JA, Jewell DE, Ephraim E. Feeding cats with chronic kidney disease food supplemented with betaine and prebiotics increases total body mass and reduces uremic toxins. *PLoS ONE*. 2022; 17: e0268624.
- [24] National Research Council Committee Update of the Guide for the Care and Use of Laboratory Animals: The National Academies Collection: Reports funded by National Institutes of Health. National Academies Press (US), National Academy of Sciences: Washington (DC). 2011.
- [25] Wang BS, Wang XJ, Gong LK. The construction of a Williams Design and randomization in cross-over clinical trials using SAS. *Journal of Statistical Software*. 2009; 29: 1–10.
- [26] 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 foods enriched with fish oil, L-carnitine, and medium-chain triglycerides. *Veterinary Journal*. 2014; 202: 588–596.
- [27] Ephraim E, Jackson MI, Yerramilli M, Jewell DE. Soluble fiber and omega-3 fatty acids reduce levels of advanced glycation end products and uremic toxins in senior dogs by modulating the gut microbiome. *Journal of Food Science and Nutrition Research*. 2020; 3: 18–33.
- [28] Freeman LM, Lachaud MP, Matthews S, Rhodes L, Zollers B. Evaluation of Weight Loss Over Time in Cats with Chronic Kidney Disease. *Journal of Veterinary Internal Medicine*. 2016; 30: 1661–1666.
- [29] Zhong Y, Yan Z, Song B, Zheng C, Duan Y, Kong X, *et al.* Dietary supplementation with betaine or glycine improves the carcass trait, meat quality and lipid metabolism of finishing minipigs. *Animal Nutrition*. 2021; 7: 376–383.
- [30] Cholewa JM, Wyszczelska-Rokiel M, Glowacki R, Jakubowski H, Matthews T, Wood R, *et al.* Effects of betaine on body composition, performance, and homocysteine thiolactone. *Journal of the International Society of Sports Nutrition*. 2013; 10: 39.
- [31] Ashtary-Larky D, Bagheri R, Tinsley GM, Asbaghi O, Salehpour S, Kashkooli S, *et al.* Betaine supplementation fails to improve body composition: a systematic review and meta-analysis. *The British Journal of Nutrition*. 2022; 128: 975–988.
- [32] Gao X, Zhang H, Guo XF, Li K, Li S, Li D. Effect of Betaine on Reducing Body Fat-A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients*. 2019; 11: 2480.
- [33] Chen S, Lu XT, He TT, Yishake D, Tan XY, Hou MJ, *et al.* Betaine Delayed Muscle Loss by Attenuating Samtor Complex Inhibition for mTORC1 Signaling Via Increasing SAM Level. *Molecular Nutrition & Food Research*. 2021; 65: e2100157.
- [34] Petronini PG, De Angelis EM, Borghetti P, Borghetti AF, Wheeler KP. Modulation by betaine of cellular responses to osmotic stress. *The Biochemical Journal*. 1992; 282: 69–73.
- [35] McRae MP. Betaine supplementation decreases plasma homocysteine in healthy adult participants: a meta-analysis. *Journal of Chiropractic Medicine*. 2013; 12: 20–25.
- [36] Miller AL. The methionine-homocysteine cycle and its effects on cognitive diseases. *Alternative Medicine Review*. 2003; 8: 7–19.
- [37] Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. *Nutrition Journal*. 2015; 14: 6.
- [38] Jewell DE, Jackson MI. Dietary Betaine and Fatty Acids Change Circulating Single-Carbon Metabolites and Fatty Acids in the Dog. *Animals*. 2022; 12: 768.
- [39] Hall JA, Panickar KS, Brockman JA, Jewell DE. Cats with Genetic Variants of AGXT2 Respond Differently to a Dietary Intervention Known to Reduce the Risk of Calcium Oxalate Stone Formation. *Genes*. 2022; 13: 791.
- [40] Papasotiropoulos M, Genovese F, Klinkhammer BM, Kunter U, Nielsen SH, Karsdal MA, *et al.* Serum and urine markers of collagen degradation reflect renal fibrosis in experimental kidney diseases. *Nephrology, Dialysis, Transplantation*. 2015; 30: 1112–1121.
- [41] Duranton F, Laget J, Gayraud N, Saulnier-Blache JS, Lundin U, Schanstra JP, *et al.* The CKD plasma lipidome varies with disease severity and outcome. *Journal of Clinical Lipidology*. 2019;



13: 176–185.e8.

- [42] Pekkinen J, Olli K, Huotari A, Tiihonen K, Keski-Rahkonen P, Lehtonen M, *et al.* Betaine supplementation causes increase in carnitine metabolites in the muscle and liver of mice fed a high-fat diet as studied by nontargeted LC-MS metabolomics approach. *Molecular Nutrition & Food Research*. 2013; 57: 1959–1968.
- [43] Goek ON, Döring A, Gieger C, Heier M, Koenig W, Prehn C, *et al.* Serum metabolite concentrations and decreased GFR in the general population. *American Journal of Kidney Diseases*. 2012; 60: 197–206.
- [44] Liu JJ, Ghosh S, Kovalik JP, Ching J, Choi HW, Tavintharan S, *et al.* Profiling of Plasma Metabolites Suggests Altered Mitochondrial Fuel Usage and Remodeling of Sphingolipid Metabolism in Individuals With Type 2 Diabetes and Kidney Disease. *Kidney International Reports*. 2016; 2: 470–480.
- [45] Loppi SH, Tavera-Garcia MA, Becktel DA, Maiyo BK, Johnson KE, Nguyen TVV, *et al.* Increased fatty acid metabolism and decreased glycolysis are hallmarks of metabolic reprogramming within microglia in degenerating white matter during recovery from experimental stroke. *Journal of Cerebral Blood Flow and Metabolism*. 2023. (online ahead of print)
- [46] Aichler M, Borgmann D, Krumsiek J, Buck A, MacDonald PE, Fox JEM, *et al.* N-acyl Taurines and Acylcarnitines Cause an Imbalance in Insulin Synthesis and Secretion Provoking  $\beta$  Cell Dysfunction in Type 2 Diabetes. *Cell Metabolism*. 2017; 25: 1334–1347.e4.
- [47] Stubbs JR, House JA, Ocque AJ, Zhang S, Johnson C, Kimber C, *et al.* Serum Trimethylamine-N-Oxide is Elevated in CKD and Correlates with Coronary Atherosclerosis Burden. *Journal of the American Society of Nephrology*. 2016; 27: 305–313.
- [48] Manor O, Zubair N, Conomos MP, Xu X, Rohwer JE, Krafft CE, *et al.* A Multi-omic Association Study of Trimethylamine N-Oxide. *Cell Reports*. 2018; 24: 935–946.
- [49] Mair RD, Sirich TL, Plummer NS, Meyer TW. Characteristics of Colon-Derived Uremic Solutes. *Clinical Journal of the American Society of Nephrology*. 2018; 13: 1398–1404.
- [50] Chen Z, Wang B, Dong J, Li Y, Zhang S, Zeng X, *et al.* Gut Microbiota-Derived l-Histidine/Imidazole Propionate Axis Fights against the Radiation-Induced Cardiopulmonary Injury. *International Journal of Molecular Sciences*. 2021; 22: 11436.
- [51] Sharma P, LaRosa C, Antwi J, Govindarajan R, Werbovetz KA. Imidazoles as Potential Anticancer Agents: An Update on Recent Studies. *Molecules*. 2021; 26: 4213.
- [52] Summers S, Quimby JM, Phillips RK, Stockman J, Isaiah A, Lidbury JA, *et al.* Preliminary evaluation of fecal fatty acid concentrations in cats with chronic kidney disease and correlation with indoxyl sulfate and p-cresol sulfate. *Journal of Veterinary Internal Medicine*. 2020; 34: 206–215.
- [53] Cuomo P, Capparelli R, Iannelli A, Iannelli D. Role of Branched-Chain Amino Acid Metabolism in Type 2 Diabetes, Obesity, Cardiovascular Disease and Non-Alcoholic Fatty Liver Disease. *International Journal of Molecular Sciences*. 2022; 23: 4325.
- [54] Badri DV, Jackson MI, Jewell DE. Dietary Protein and Carbohydrate Levels Affect the Gut Microbiota and Clinical Assessment in Healthy Adult Cats. *The Journal of Nutrition*. 2021; 151: 3637–3650.
- [55] Chen Y, Zelnick LR, Wang K, Hoofnagle AN, Becker JO, Hsu CY, *et al.* Kidney Clearance of Secretory Solutes Is Associated with Progression of CKD: The CRIC Study. *Journal of the American Society of Nephrology*. 2020; 31: 817–827.
- [56] Lidgard B, Bansal N, Zelnick LR, Hoofnagle A, Chen J, Coilaizzo D, *et al.* Association of Proximal Tubular Secretory Clearance with Long-Term Decline in Cognitive Function. *Journal of the American Society of Nephrology*. 2022; 33: 1391–1401.
- [57] Lustgarten MS, Price LL, Chale A, Fielding RA. Metabolites related to gut bacterial metabolism, peroxisome proliferator-activated receptor- $\alpha$  activation, and insulin sensitivity are associated with physical function in functionally-limited older adults. *Aging Cell*. 2014; 13: 918–925.
- [58] Poupore N, Chosed R, Arce S, Rainer R, Goodwin RL, Nathaniel TI. Metabolomic Profiles of Men and Women Ischemic Stroke Patients. *Diagnostics*. 2021; 11: 1786.