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Effects of dietary protein content on renal parameters in normal cats

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This study evaluates the effect of dietary protein content on renal parameters in 23 healthy spayed female cats. The objective was to determine if cats eating diets high in protein will have higher serum urea nitrogen (UN) and creatinine values without a detectable change in kidney function, as assessed by urinalysis. A single random cross-over design was used. Cats were fed a standard maintenance diet for at least 1 month prior to the dietary trial. They were fed in two phases. For the first phase, cats were randomly assigned to receive either a high protein [HP = 46% metabolizable energy (ME)] or low protein (LP = 26% ME) diet. For the second phase, cats were fed whichever diet they were not fed during the phase I period. Blood and urine samples were collected at 2-week intervals for the duration of the study (10 weeks). UN, albumin, alanine aminotransferase and urine specific gravity were significantly higher, and creatinine and phosphorus were significantly lower ($P < 0.05$) when cats were fed the HP diet as compared to when they were fed the LP diet, although none of the mean values were found to be outside of the corresponding reference interval. Dietary intake can result in clinically significant changes in UN and statistically significantly changes in several other biochemical analytes, although all analytes are likely to remain within normal reference intervals. Therefore, an accurate dietary history is necessary to help determine if renal parameters are being influenced by diet in a particular patient.

It is recognized that high dietary protein levels for cats are not only essential in kittens during growth, but are also beneficial throughout all life stages, and the ramifications of protein deficiencies have been demonstrated. Excellent examples are the undeniable effects of particular amino acid deficiencies including (but not limited to) arginine, taurine, methionine, and cysteine.[1–6] Dietary protein content is not only of importance in feline health, but in many disease states as well. Diseases such as diabetes mellitus and obesity,[7,8] as well as upper and lower urinary tract disease have specific dietary protein recommendations.[9,10] In particular, the prevalence of obesity and diabetes has greatly increased in cats in the last 20 years,[11,12] and an increasing awareness of the benefits of high protein (HP) diets [>45% metabolizable energy (ME)] in the management of both of these diseases has led to an increase

in use of these diets therapeutically. Feeding obese diabetic cats diets high in protein and low in carbohydrate content yields improved glycemic control and enhanced insulin sensitivity.[13,14] Further, there has been an increasing consumer desire to feed cats higher protein diets that more closely mimic the cat's natural diet. Thus, diets higher in overall protein content are being fed with more frequency. In HP diets, meat is typically the major source of the protein and can contribute to increased creatine and creatinine intake, which can result in increased serum creatinine concentrations.[15,16]

Some have wondered whether these increases are an indication of declining renal function, and some question whether a HP diet might lead to decreased renal function despite several studies demonstrating that dietary protein content does not appear to cause progression of renal pathology.[17,18] However, premature placement of these cats on a reduced protein/renal appropriate diet could have deleterious effects.

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The objective of this study was to determine the effect of high levels of dietary protein on selected renal parameters in healthy adult cats.

Materials and methods

Twenty-four adult neutered female cats, 2—3 years of age with normal body condition (5 out of 9) that had been housed as a colony in a research facility at Texas A&M University were used as the study subjects. All cats were determined to be clinically normal via physical exam, complete blood count (CBC), serum chemistry profile, urinalysis, urine culture, and urine protein/creatinine ratio (UPC), and they tested negative for feline leukemia virus/feline immunodeficiency virus (FeLV/FIV) prior to onset of the study. Any cats with concurrent azotemia (creatinine > 2.0 mg/dl, 176.8 μmol/l) and decreased urine specific gravity (USG < 1.035) using a cystocentesis sample were excluded from the study. Only cystocentesis samples were used for evaluation of USG. This study was performed in accordance with the Texas A&M Institutional Animal Care and Use Committee.

Study design

The effect of a HP diet on various serum analytes and USG was determined utilizing a two treatment, two sequence, four period fully balanced cross-over study with 'run-in' and 'wash-out' periods. Baseline measurements were determined before the start of periods I and III. The cats were fed a dry adult commercially available feline maintenance diet (Science Diet Feline Adult Maintenance, Topeka, KS) for 1 month prior to commencement of the study ('run-in' period), after which the cats were then randomly divided into two groups each receiving one of the two treatment diets. The treatment diets were then fed for 4 weeks, and blood was collected after 2 weeks (period I) and 4 weeks (period II). The maintenance diet was then fed for 2 weeks during the 'wash-out' period. After the 'wash-out' period the two diets were reversed for the treatment groups during periods III and IV; however, feeding duration and blood collection followed the pattern from periods I and II. Blood and urine samples were collected every 2 weeks throughout the duration of the study starting at BL1 (baseline 1). A full CBC, biochemical profile, urinalysis, and UPC were performed at the beginning and end of the study, whereas a complete urinalysis and selected biochemical parameters [UN, creatinine, phosphorus, albumin, and alanine aminotransferase (ALT)] were measured during weeks 2, 4, 6, and 8. Results for the significant variables for each sequence were averaged for the two periods. The cats were routinely fed once daily. The blood samples were collected prior to feeding.

Diets

The diets were dry extruded commercial products designed to vary in their protein content, but they were complete and balanced to meet all the known dietary needs of young adult spayed, female cats. Group 1 was fed the HP diet (46% ME) (Nestlé Purina DM, St Louis, MO) for the first 4-week trial period, and group 2 was fed the low protein diet (LP, 26% ME) (Nestlé Purina NF, St Louis, MO) for that period. Batches from a single diet were mixed together prior to the dietary trials to eliminate interbatch variations. After the first 4-week trial, the cats were switched to a feline maintenance diet (30—32% ME protein) (Science Diet Feline Adult Maintenance, Topeka, KS) for a 2-week wash-out period, then crossed over to the other respective diet for the second 4-week trial period. The LP diet had 6 g/100 g diet of corn oil top dressed onto the diet to make the diets isocaloric and to render the total fat concentration of the diets equivalent. This addition made the caloric density of each diet 412 kcal/100 g. By assuring that the diets were isocaloric and that fat concentrations were equivalent, the diets differed mainly in their protein and carbohydrate content (Table 1). Cats were fed to maintain a normal body condition score (BCS 5). Consumption was recorded daily and animals weighed weekly. The calorie requirements for individual cats were calculated according to Mitsuhashi et al.[19]

Analytical methods

CBCs were performed with a CELL-DYN 3700 (Abbott Laboratories, Abbott Park, Ill) using Ethylenediaminetetraacetic acid (EDTA) whole blood. Leukocyte differential counts were determined manually. Heparinized plasma was used for chemistry analysis using a Vitros 250 (Ortho-Clinical Diagnostics, Rochester, NY). Urine sediment and dipstick (Multistix, Bayer corp) evaluation were performed. For the sediment examination, 3 ml urine (voided or cystocentesis) was centrifuged at $500 \times g$ for 5 min. The supernatant was removed and the sediment evaluated microscopically. The UPC was determined on the urine supernatant using a Vitros 250. Specific gravity was determined using a refractometer.

Statistical analyses

Effect of diet on serum analytes (UN, creatinine, phosphorous, albumin, and ALT) and USG was analyzed as a two-treatments four periods cross-over analysis of covariance with a random effect for subject nested within sequence using PROC MIXED of SAS (v9.2, SAS Institute). All models for each analyte included terms for treatment, sequence, period, first-order carry-over, and baseline. Statistical significance was declared at $P < 0.05$. Results are presented as least-squared means and their standard errors.

Results

Twenty-four spayed female cats were randomly assigned to two sequences. In sequence 1, cats were fed the HP diet followed by the LP diet, and this order was switched for sequence 2. One cat assigned to

Table 1. Nutrient composition of diets fed to 23 healthy, adult cats in order to assess their effect on selected blood and urine parameters.

	HP[†]	LP[‡] plus corn oil	Maintenance[*]
	(g/100 kcal ME)		
Protein (g)	12.94	6.97	8.2
Fat (g)	4.01	4.28	4.7
Carbohydrate (g)	3.35	11.49	9.1
Fiber (g)	0.28	0.26	0.2
Calcium (mg)	0.35	0.15	0.21
Phosphorous (mg)	0.34	0.10	0.18
Potassium (mg)	0.28	0.21	0.20
Sodium (mg)	0.13	0.05	0.096
Chloride (mg)	0.19	0.14	NA
Magnesium (mg)	0.03	0.02	0.02
Taurine (mg)	0.08	0.04	0.06

*Ingredients: Maintenance (Science Diet Feline Adult Maintenance, Topeka, KS) − Chicken by-product meal, whole grain corn, brewers rice corn gluten meal, animal fat (preserved with mixed-tocopherols and citric acid), chicken liver flavor, choline chloride, potassium chloride, calcium sulfate, DL-methionine, vitamin E supplement, iodized salt, vitamins (L-ascorbyl-2-polyphosphate (source of vitamin C), vitamin E supplement, niacin, thiamine mononitrate, vitamin A supplement, calcium pantothenate, riboflavin, biotin, vitamin B12 supplement, pyridoxine hydrochloride, folic acid, vitamin D3 supplement), taurine, minerals (ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), preserved with mixed-tocopherols and citric acid, phosphoric acid, beta-carotene, rosemary extract.

†Ingredients: HP (Nestlé Purina DM, St Louis, MO) − Poultry by-product meal, soy protein isolate, corn gluten meal, soy flour, animal fat preserved with mixed-tocopherols (form of vitamin E), corn starch, animal liver flavor, calcium carbonate, phosphoric acid, fish oil, potassium chloride, L-lysine monohydrochloride, DL-methionine, choline chloride, taurine, powdered cellulose, salt, vitamin E supplement, zinc sulfate, ferrous sulfate, niacin, manganese sulfate, vitamin A supplement, calcium pantothenate, thiamine mononitrate, copper sulfate, riboflavin supplement, vitamin B_{12} supplement, pyridoxine hydrochloride, folic acid, vitamin D_3 supplement, calcium iodate, biotin, menadione sodium bisulfite complex (source of vitamin K activity), sodium selenite.

‡Ingredients: LP (Nestlé Purina NF, St Louis, MO) − Brewers rice, whole grain corn, corn gluten meal, soybean meal, animal fat preserved with mixed-tocopherols (form of vitamin E), animal digest, fish meal, calcium carbonate, potassium citrate, phosphoric acid, potassium chloride, L-lysine monohydrochloride, fish oil, calcium phosphate, choline chloride, salt, taurine, zinc sulfate, ferrous sulfate, vitamin E supplement, manganese sulfate, niacin, vitamin A supplement, calcium pantothenate, thiamine mononitrate, copper sulfate, riboflavin supplement, vitamin B_{12} supplement, pyridoxine hydrochloride, folic acid, vitamin D_3 supplement, calcium iodate, biotin, menadione sodium bisulfite complex (source of vitamin K activity), sodium selenite.

sequence 1 was excluded because her serum chemistry panel and USG indicated stage 3 renal failure according to the International Renal Interest Society (IRIS) classification system. Mean values obtained from cats fed each diet for UN, creatinine, phosphorus, albumin, ALT and USG are presented in Table 2. UN, albumin, ALT and USG were significantly higher, and creatinine and phosphorus were significantly lower ($P < 0.05$) when cats were fed the HP diet as compared to when they were fed the LP diet. UPC was within normal limits at measured time points.

Discussion

Cats are obligate carnivores, and as such, appropriate amounts of dietary protein and essential amino acids

are a required component of their daily diet.[1,2,20] Dietary protein is necessary not only for the provision of essential amino acids, but of dispensable amino acids that are used for growth, maintenance, gluconeogenesis, and other metabolic pathways such as the urea cycle.[2] Cats are unique as compared with omnivores as they require dietary protein for energy and they minimally use carbohydrates as an energy source.[21] Cats use hexokinase for glucose metabolism that has been shown to work at a slower rate than glucokinase, which is the predominant means of glucose metabolism in dogs.[35] Cats are so well adapted to carnivorous diets that protein metabolism is maintained even with protein deficient diets, which might be at the expense of lean muscle mass.[20] They not only have protein requirements that must be met, but they also have unique amino acid requirements. When fed diets

Table 2. Comparison of results for selected serum chemistry and urine parameters when 23 healthy, adult cats were fed a HP (DM) diet as compared with a LP (NF) diet.

Analyte	Diet sequence	n	Run-in		1	2	'Wash-out'		3	4
			Mean ± SE			Mean ± SE			Mean ± SE	
BUN	1	12	16.73 ± 0.73	DM	23.09 ± 1.00	22.36 ± 1.11	17.36 ± 0.77	NF	19.18 ± 1.26	18.82 ± 1.07
(mg/dl, mmol/l)	2	11	18.08 ± 0.68	NF	19.08 ± 1.37	19.17 ± 1.13	18.5 ± 0.62	DM	22.75 ± 1.07	23.25 ± 1.09
Creatinine	1	12	1.74 ± 0.06	DM	1.55 ± 0.05	1.48 ± 0.06	1.66 ± 0.07	NF	1.68 ± 0.08	1.53 ± 0.07
(mg/dl, μmol/l)	2	11	1.75 ± 0.05	NF	1.62 ± 0.10	1.48 ± 0.09	1.53 ± 0.06	DM	1.43 ± 0.051	1.49 ± 0.06
Phosphorus	1	12	4.93 ± 0.14	DM	4.74 ± 0.12	4.58 ± 0.14	4.58 ± 0.14	NF	4.76 ± 0.14	4.6 ± 0.17
(mg/dl, mmol/l)	2	11	4.98 ± 0.16	NF	5.07 ± 0.14	4.81 ± 0.16	4.53 ± 0.15	DM	4.72 ± 0.13	4.72 ± 0.10
Albumin	1	12	3.25 ± 0.09	DM	3.22 ± 0.03	3.38 ± 0.05	3.25 ± 0.05	NF	3.15 ± 0.05	3.2 ± 0.06
(g/dl, g/l)	2	11	3.21 ± 0.08	NF	3.13 ± 0.05	3.08 ± 0.06	2.98 ± 0.06	DM	3.16 ± 0.07	3.28 ± 0.08
ALT (U/l)	1	12	66.09 ± 6.80	DM	73.36 ± 9.49	91.09 ± 17.76	68.82 ± 9.09	NF	79.27 ± 22.87	73.82 ± 19.42
	2	11	57.17 ± 5.01	NF	66.5 ± 9.59	74.00 ± 20.89	42.5 ± 5.68	DM	45.58 ± 3.58	52.08 ± 5.06
USG*	1	—	1.053 ± 0.005 (n = 9)	DM	1.059 ± 0.003 (n = 11)	1.062 ± 0.003 (n = 9)	1.049 ± 0.006 (n = 7)	NF	1.050 ± 0.004 (n = 8)	1.057 ± 0.003 (n = 7)
	2	—	1.053 ± 0.004 (n = 11)	NF	1.055 ± 0.003 (n = 9)	1.062 ± 0.007 (n = 11)	1.056 ± 0.003 (n = 11)	DM	1.059 ± 0.024 (n = 10)	1.062 ± 0.002 (n = 11)

Sequence 1: 'Run-in' − DM − DM − 'Wash-out' − NF − NF. Sequence 2: 'Run-in' − NF − NF − 'Wash-out' DM − DM.
*Only cystocentesis samples were included for evaluation.

deficient in arginine, for example, they rapidly become hyperammonemic with resultant neurologic signs and potential mortality.[1,20] Protein is not only required for energy metabolism in cats, but it is now being demonstrated as an integral component of glucose and lipid metabolism as well as a key component in the management of disorders of insulin sensitivity: diabetes mellitus, obesity and idiopathic hepatic lipidosis.[7,13,22−24,36] Further, the benefits of dietary protein modification have been recognized in many other disease states in cats. Harte et al demonstrated the benefits of protein restriction in stage 3 naturally occurring kidney disease that has been reinforced by Elliott and Ross.[9,25,26]

Due to the high prevalence of these diseases and increasing awareness of the benefits to feline health of feeding higher protein diets, these diets are being fed with more frequency. With these changes in dietary strategy comes the need to interpret the biochemical effects of increased levels of dietary protein. The results of the present pilot study demonstrated statistically significant effects on UN, creatinine, phosphorous, albumin, ALT, and USG when cats were fed different levels of dietary protein for only 1 month, and a clinically relevant effect was observed for UN. Further studies of longer duration and using tools to better assess renal function [eg, glomerular filtration rate (GFR)] and effects on metabolism are needed to fully elucidate the changes that occur in biochemical analytes during HP feeding.

Plasma creatinine is the end product of creatine degradation. Creatinine is formed non-enzymatically in an irreversible reaction from creatine, and its generation is dependent on the total muscle mass of the individual since the primary store of creatine is skeletal muscle.[27] Creatinine is freely filtered through the glomerulus, and it is neither reabsorbed nor secreted by the renal tubules in cats. It is, therefore, used as a rough estimate of GFR. Creatinine in conjunction with UN, USG, UPC, electrolytes and minerals are used to diagnose kidney disease. Parameters proposed by the IRIS for staging chronic kidney disease (CKD) are based on increased plasma creatinine levels due to renal failure and the presence or absence of proteinuria and hypertension. Creatinine was selected as it is influenced by fewer extrarenal factors than urea nitrogen (UN). Extrarenal parameters that can influence plasma creatinine concentrations include: age (young adult animals have a higher serum creatinine concentration than geriatric animals), diet/feeding, exercise (in greyhound dogs), and muscle mass.[28] Dietary supplementation with creatine has been shown to increase the serum creatinine concentration in dogs, and it increases urinary creatinine excretion in people, reflecting an increased rate of muscle creatine degradation to creatinine.[29,30] However, the main quantities that differ between the diets to create the defining high and low protein qualities of the respective diets are poultry by-product meal, soy protein isolate, corn gluten meal, and soy flour in the HP diet, and whole grain corn, brewers rice, and fish meal in the LP diet. The ramifications of these variables on dietary creatine content have not been established. Fish is one of the highest sources of creatine and methionine, which is a necessary component of creatine synthesis. Although the LP diet is overall of a lesser protein content, the presence of fish meal may be a confounding variable in the evaluation.

An important feature to note regarding the cats used in this research project is that they were all of good body condition with stout muscle mass likely due to their constant activity (climbing, jumping, etc) in their large housing areas. Were the evaluation for renal disease to be based solely on a one-time reading of creatinine levels, a number of these cats would be classified as having stage II CKD according to IRIS standards. This emphasizes the need to assess multiple serum creatinine concentrations in cats before diagnosing CKD and applying the IRIS staging system. In this particular case, the authors theorize that the relatively high creatinine concentrations observed in these cats can be attributable to their solid muscle mass and is not reflective of underlying kidney insufficiency given that these cats had highly concentrated urine. However, because DEXA (dual energy X-ray absorptiometry) scans were not performed to accurately assess body composition, this is speculation. Creatinine concentrations in these cats were significantly lower ($P < 0.05$) when the cats were fed the HP diet. This is in contrast to what the authors expected, and it may be secondary to a higher GFR as increased dietary protein content has been demonstrated to increase glomerular filtration.[31] Additionally, it could be speculated that the observed changes reflect a transient response to the HP diet, and an increase may be observed with prolonged feeding if it were to result in increased muscle mass as has been previously demonstrated.[32,33] However, contrarily, Frantz and Yamka found that feeding a HP diet to cats for 180 days did not appear to change lean muscle mass.[18]

The majority of UN is synthesized by the liver from ammonia as a waste product of protein catabolism.[34] The kidney is the most important route of excretion. However, even at the highest urine flow rate, approximately 40% of the filtered urea is reabsorbed making this a poorer estimation of GFR when compared to creatinine. The major pre-renal causes for elevation in UN are: increased protein catabolism (ie, small bowel hemorrhage, necrosis, starvation, prolonged exercise, etc), dehydration, and HP diets. As expected, UN was significantly higher when cats were fed the HP diet as compared to when they were fed the LP diet. While this value was not outside the reference interval, the difference was clinically noticeable (approximately 50% higher than baseline values), and further increases may have been observed with time. This illustrates the need to be cautious when evaluating UN as a biomarker of renal disease in cats consuming a HP diet.

Diets formulated for animals in renal failure are restricted in protein, phosphorous, calcium, and sodium. These dietary restrictions show a clear benefit to survival in cats with stages II−IV CKD.[17,26,35] All other things being equal, the maintenance of normal

phosphorous levels is dependent on dietary intake and renal excretion. Although the values were similar to each other and well within the reference interval, a significant ($P < 0.05$) difference in phosphorous was found between the two trial diets, with lower serum phosphorus observed when cats were fed the HP diet. This is in contrast to what was anticipated as the LP diet is restricted in phosphorus content. As phosphorus is primarily controlled by renal excretion, primary consideration for this finding is an increased GFR due to increased protein intake when cats were fed the HP diet.

A significant difference in albumin concentration was also noted in this study. Albumin in these cats was higher when fed the HP diet and lower when fed the LP diet as compared with the maintenance diet. The higher albumin observed with the HP diet may be due to increased production. Alternatively, there may have been decreased production of albumin when cats were fed the LP diet due to decreased protein availability. It is important to note that albumin values obtained during feeding of both diets were still well within the reference interval and differences observed were not clinically relevant.

USG was significantly higher when cats were fed the HP diet. The significance of this finding is unknown, and urine obtained while feeding both diets was highly concentrated (>1.050). It is important to note that USG was only evaluated when collected by cystocentesis. Although cats were held overnight in metabolism cages to aid in urine collection, cats frequently spilled their water and thus contaminated any voided urine. This decreased the number of observations used in the analysis by 22. Although not assessed in the utilized diets, a potential cause for increased USG in the HP diet could be higher creatinine excretion.

ALT is a cytosolic enzyme mostly associated with hepatocellular injury, and a significant difference between diets was not anticipated. However, ALT was significantly increased when cats were fed the HP diet, although the mean was still within the reference interval. It could be speculated that the increase in activity with the HP diet is due to the activity of aminotransferases associated with protein catabolism. However, its significance at this time is unclear, and further investigation may be necessary to determine whether this finding has any clinical significance.

The intent of this pilot study was to evaluate the need for further investigation of the effects of dietary protein on renal parameters in otherwise healthy cats. Limitations of this study are intrinsic to pilot studies and include small sample size, the short duration of the study, and lack of more accurate measures of GFR and muscle mass. The differences were significant for those analytes measured at each time interval (UN, creatinine, phosphorous, albumin, ALT and USG), which supports the importance of diet on laboratory results. However, it is important to note that these values were still within the normal reference interval and the difference between the values obtained with the two diets was not clinically significant, with the possible exception of UN. Given enough time, perhaps more pronounced trends would become evident, and follow-up studies will evaluate this more thoroughly. Further, it illustrates the need to obtain an accurate dietary history in feline patients in order to account for dietary influences on renal parameters, particularly regarding UN.

Acknowledgements

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