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ORIGINAL PAPER

Consumption of krill protein concentrate prevents early renal injury and nephrocalcinosis in female Sprague–Dawley rats

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Abstract Female Sprague–Dawley rats provide an animal model for studying the role of nutrition in renal health due to their sensitivity to diet-induced alterations in kidney function. Nephrocalcinosis, a common renal abnormality found in rats, has been implicated in subsequent renal failure. Simple dietary manipulations, such as changing the source of dietary protein, may influence nephrocalcinosis. This study evaluates the consumption of krill protein concentrate (KPC), a novel and high-quality protein, on renal and bone health. Young female Sprague–Dawley rats ($n = 10$/group) were individually housed in metabolic cages and fed ad libitum diets consisting of 10% crude protein supplied as KPC or casein for 4 weeks. Diets were isocaloric, isonitrogenous, and matched for calcium (Ca) and phosphorus (P). Urinary *n*-acetyl glucosaminidase (NAG) was measured and kidney histology performed to assess kidney damage. Biomarkers of kidney function were determined by calorimetric assays. Ca and P balance and bone concentrations were measured using inductively coupled plasma mass spectrometry. Femoral strength was determined by three-point bend testing. Rats fed KPC had lower ($P = 0.005$) urinary NAG levels and minimal microtubular Ca deposition compared to rats fed casein. There was a tendency ($P < 0.06$) for higher glomerular filtration rates and lower proteinuria, and higher ($P = 0.03$) urinary output in rats fed KPC compared to casein. There were no differences in Ca and P balance or bone measurements of total bone mineral content, Ca, P or strength between rats fed KPC and casein. Based on the study results, KPC prevented early renal injury leading to nephrocalcinosis and potential bone loss.

Keywords Krill protein concentrate · Casein · Nephrocalcinosis · Femur · Safety

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Introduction

Diet-induced alterations in kidney health are common in female rats, with renal calcification (nephrocalcinosis) being a prime example [1–3]. The transition of nephrocalcinosis to kidney stones (nephrolithiasis) has been implicated in nephropathy and subsequent renal failure in rats [4]. Diet-induced alterations in kidney function have been attributed to manipulating various components of the diet, including the dietary calcium: phosphorus ratio [5–9], and the dietary protein content [10–12]. Diets rich in phosphorus (P) or leading to increased P excretion have been reported as being an underlying cause of nephrocalcinosis [13]. However, Meyer et al. [10] reported that changing the source of dietary protein fed to female Wistar rats reduced calcium (Ca) deposition in the kidneys to a greater extent than manipulating dietary minerals. Together the study results suggest that the amount, as well as the source, of dietary protein influences the development of nephrocalcinosis and nephrolithiasis [11, 12]. Additionally, different protein sources are associated with different fatty acids. The role of lipids in nephrocalcinosis and nephrolithiasis should be considered because fatty acids have been shown to differentially influence inflammatory pathways, and inflammation has also been implicated as an initiating factor in tissue calcification [14–16].

Nephrocalcinosis is commonly associated with increased urinary Ca excretion (hypercalcuria), and dietary proteins

also influence the excretion of Ca. It has been proposed that higher consumption of dietary protein increases intestinal Ca absorption and this may increase urinary Ca excretion [17, 18]. The consumption of dietary protein has also been correlated with an increase in urinary acid excretion [19–21], which has been shown to reduce renal Ca reabsorption and possibly promote hypercalciuria [22]. These potential changes in kidney function may influence mineral balance and, in turn, bone health. Calvo et al. [23] reported hypercalciuria in male Sprague–Dawley rats fed high protein diets; however, there was no effect on bone mineral content (BMC). Female rats display a higher sensitivity to alterations in renal handling of Ca [5] and therefore, in the present study, female rats were used since they may provide a more sensitive model for mineral and subsequent bone loss.

In our study, female Sprague–Dawley rats fed protein derived from a pelagic source were compared to rats fed casein, a standard protein source used in commercial semi-purified rodent diets. Fish contain high-quality protein as well as other nutrients with potential health benefits. However, depletion of existing fish stocks and environmental contaminants has raised public concern regarding fish consumption. A potential alternative is to promote consumption of under-utilized species such as krill. Based on body size, krill has the largest amount of protein (over 65% dry weight) among all organisms worldwide. Analysis of lipid content showed that ~27% of total fatty acids in Antarctic krill (*Euphausia superba*) consists of the health benefiting omega-3 polyunsaturated fatty acids (ω-3 PUFAs) [24]. Krill offers a promising high quality and sustainable source of protein for human consumption. Therefore, the objective of this study was to evaluate the effects of consuming krill protein concentrate (KPC) in regard to renal and bone health.

Materials and methods

Diets and animal feeding study

All animal procedures were conducted in accordance with the guidelines set forth by the Institute of Laboratory Animal for the Care and Use of Laboratory Animals [25], and were approved by the Animal Care and Use Committee at West Virginia University.

Immature (age 28 days) female Sprague–Dawley rats ($n = 20$) were purchased from Taconic Farms (Rockville, MD). Female rats were used due to their higher susceptibility to nephrocalcinosis compared to male rats [3]. Upon arrival, rats were individually housed in metabolic cages. All animals were maintained in a room at 21°C with a 12 h light/dark cycle. During a 14-day acclimation period, animals were given ad libitum access to deionized distilled water (ddH_2O) and AIN-93G diet (Harklan Teklad, IN). Following the 14-day acclimation period, rats were randomly assigned ($n = 10$ per group) to be fed ad libitum a low protein diet consisting of 10% crude protein supplied as casein or KPC for 4 weeks.

Table 1 Diet composition

Ingredients (g/kg diet)	10% Casein	10% KPC[a]
Casein	115	0
DL-Methionine	1.5	0
KPC	0	128
Sucrose	531.8	609.8
Corn starch	200	229.4
Corn Oil	53.5	49.9
Cellulose	52	59.6
Vitamin mix[b]	10	11.5
Ethoxyquin	0.01	0.01
Mineral mix[b]	13.4	15.3
Calcium phosphate	20.2	20.9
Calcium carbonate	2.6	3.6
Gross energy (kcal/g)	4.3	4.3

KPC krill protein concentrate

[a] Diet formulated for 872 g of diet + 128 g addition of KPC

[b] Based on the AIN-93G vitamin and mineral mixes [7]

KPC was isolated from whole frozen Antarctic krill (*E. superba*) purchased from Krill Canada (Langley, BC, Canada) using an isoelectric solubilization/precipitation method previously described in Gigliotti et al. [26]. The recovered KPC was 77.7% crude protein, 8.1% total lipid, and 4.4% total ash on a dry basis by proximate analysis. Freeze-dried KPC was stored at −20°C until mixed into the diet. Replacement of the protein as either KPC or casein at a level of 10% in AIN-93G diet was corrected for protein and lipids so that the diets were isocaloric and isonitrogenous. Ca and P contents of the diets were also matched to provide a 1:2 ratio (Table 1). Diets were kept stored at 5°C. Food consumption and ddH_2O intake were measured and replaced with fresh ddH_2O and assigned diet every 2 days.

Kidney mineral content

At the end of 4 weeks, rats were euthanized by CO_2 inhalation, and both kidneys excised. Kidneys were trimmed, decapsulated, and weighed separately. No statistical differences were found between the right and left kidney weights. Therefore, the left kidneys were assayed for mineral content and the right kidneys used for histological evaluation.

To determine kidney mineral concentrations, the left kidneys were dried overnight at 110°C in an oven and then ashed at 550°C in a muffle furnace (Lindberg 515A2, WI)

for 24 h. To determine specific minerals, ashed samples were dissolved in 70% nitric acid (2 ml) and neutralized in ddH_2O (5 ml). Samples were then filtered through Whatman no. 1 paper, diluted to 50 ml with ddH_2O, and renal Ca and P content in the kidneys were measured using inductively coupled plasma spectrometry (ICP) (model P400, Perkin Elmer, Shelton, CN).

Kidney histology

The right kidneys ($n = 4$) were dissected and immediately fixed in 10% formalin solution overnight and then transferred to 70% ethanol. Tissues were dehydrated through a series of increasing ethanol concentrations (70–100% in ddH_2O) to xylene, embedded in paraffin, and serially cut in 8-μm thick sections using a microtome (American Optical Company, NY). Paraffin-embedded sections were placed on moist glass slides and dried for mounting. Mounted sections were then deparaffinized in xylene, and rehydrated through a series of decreasing ethanol concentrations (100–70%) prior to staining.

Renal morphology and microtubular calcification was examined by standard hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining. PAS staining was performed following a commercially available protocol (Sigma-Aldrich, MO). Briefly, hydrated slides were incubated in periodic acid solution for 5 min. After rinsing in ddH_2O, slides were immersed in Schiff's reagent for 15 min, washed in water, and then counterstained in Gill Hematoxylin solution for 90 s and cover slipped. All slides were analyzed under a Nikon TE 2000-S light microscope (Nikon Instruments, NY) by three trained individuals, and images captured using a PC interfaced with Q-Capture imaging software (Quantitative Imaging Corporation, BC, Canada).

Urinary assays of renal function and damage

Rats were individually housed in metabolic cages to collect urine for determination of renal function and damage. Urine samples were collected and centrifuged at 1,500*g* for 10 min at 4°C to remove any debris. Urine samples were aliquoted into fresh tubes and stored at −80°C until assayed. Baseline urine consisted of pooled samples collected over 7 days of the first week of treatment. Final urine consisted of pooled samples collected over the final 7 days of the 4-week feeding study.

To determine renal function, urinary total protein and creatinine were determined using commercially available colorimetric assays (Cayman Chemical, MI). Serum creatinine concentrations were determined enzymatically using a commercially available Vet-16 rotor (Hemagen Diagnostics Inc., Columbia, MD). Glomerular filtration rate (GFR) was determined based on creatinine clearance (CrC) calculated as: $CrC = (UC/SC) \times V$, where UC is urinary creatinine concentration, V the volume of measured urine excreted per minute, and SC is serum creatinine concentration. Renal damage was determined by measuring urinary *n*-acetyl glucosaminidase (NAG) activity, an early indicator of renal damage, using a commercially available colorimetric kit (Diazyme, CA). The optical densities of the urinary samples were determined at wavelength 505 nm in a Beckman DU530 spectrophotometer (Beckman Coulter, CA). Urinary sodium (Na) was determined by diluting the urine 1:40 (v/v) in ddH_2O and measuring the mineral concentration using the ICP. Urinary pH was determined using a standard pH meter (Beckman, CA).

Mineral balance

Mineral balance studies were performed by individually housing rats in metabolic cages to determine food intake and to collect feces and urine samples. Ca apparent absorption was calculated as (Ca intake − fecal Ca excretion)/Ca intake × 100. Calcium retention was determined by calculating Ca intake − (fecal Ca excretion + urinary Ca excretion). P apparent absorption was calculated as (P intake − fecal P excretion)/P intake × 100 and P retention was determined by calculating P intake − (fecal P excretion + urinary P excretion).

Ca and P intake was calculated by multiplying the amount of diet consumed and the dietary concentration of Ca (6 mg/g diet) or P (5 mg/g diet) as provided by diet manufacturer and confirmed by ICP. Urine and fecal samples consisted of pooled samples collected during the final 7 days of the 4-week feeding study. To determine urinary minerals, samples were diluted 1:40 in ddH_2O and Ca and P concentrations measured by ICP. To determine fecal minerals, the fecal samples were freeze-dried for 48 h. Feces were ashed overnight in a muffle furnace (Lindberg 515A2, WI), dissolved in 70% nitric acid, diluted to 50 ml in ddH_2O and then Ca and P concentration determined by ICP.

Bone measurements

Both femurs were dissected and defleshed with care being taken not to damage the periosteum. Each femur was wrapped in saline soaked gauze, and frozen at −20°C until assayed. For analysis, each bone was brought to room temperature. Bone morphology was determined by measuring length, width, and depth using a vernier caliper (Bel-Art Products, NJ). Bones were dried at 110°C for 48 h and dry weights measured using an analytical balance (Mettler Toledo, OH). The dried femurs were ashed at 600°C in a muffle furnace (Lindberg 515A2, WI) for 24 h and total

BMC determined by the weight of the bone ash. Ashed femur samples were dissolved in 70% nitric acid, diluted to 50 ml with ddH_2O and femoral Ca and P content measured by ICP.

Femoral strength was determined on a TA.HDi Texture Analyzer (Texture Technologies Corp, NY) outfitted with a three-point bending apparatus. The femur was placed on supports (1 mm width at tip) and bent until broken by lowering a centrally placed blade (1 mm width) at constant crosshead speed (0.1 mm/s). The load–deflection data collected by a PC interfaced with the TA.HDi Texture Analyzer were used to determine the bone strength measurement of peak force. Bone morphometry, BMC, Ca, P, and bone strength results of the bone pairs were averaged after no differences between right and left femurs were determined by paired *t* test with significance level set at $P < 0.05$.

Statistical analysis

The animal feeding study was a completely randomized design, with $n = 10$ rats per diet treatment. The *t* test was used to compare differences between treatment groups, and Mann–Whitney Rank Sum test was performed on data not normally distributed. The *t* test and the Mann–Whitney Rank test were both performed using Sigma Stat 3.1 (Systat Software Inc., San Jose, CA). Differences were considered significant at $P < 0.05$.

Results

Kidney mineral content

Figure 1 shows the relative weights (g/g body weight × 100) of the kidneys, kidney total mineral (ash), Ca, and P content of rats fed KPC relative to rats fed casein. Kidney weights of rats fed KPC were lower ($P < 0.001$) compared to the kidney weights of rats fed casein. Rats fed KPC had decreased ($P < 0.001$) kidney total mineral content compared to rats fed casein. Measurements of specific minerals showed that rats fed KPC also had lower ($P = 0.002$) kidney Ca concentrations, and lower ($P < 0.001$) kidney P concentrations compared to rats fed casein.

Kidney histology

H&E and PAS stains were used to observe renal calcium deposits, with more than 115 renal tissue sections of $n = 4$ rats/diet used. All slides containing the cortico-medullary junction from rats fed casein displayed extensive microtubular calcification (Fig. 2a, b, top). Interestingly, only one (Fig. 2c) of the four rats fed KPC (Fig. 2c, d) used for histological evaluation showed renal calcification in the same region of renal tissue and this was mild compared to casein-fed rats. Focal areas of tubular atrophy were also observed; however, no distinction between the diets could be made. There were no indications of fibrosis or other glomerular abnormalities in rats fed casein or KPC.

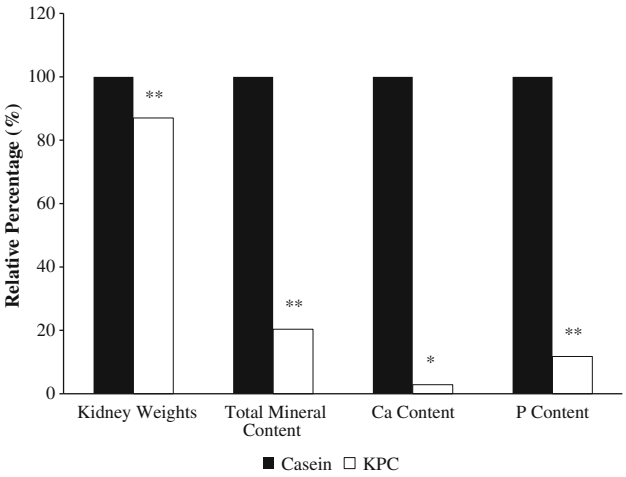

Fig. 1 Weights, and total mineral, Ca, and P content of kidneys from rats fed 10% protein diets consisting of either casein or KPC for 4 weeks. Data presented as percent relative to casein. *Symbols* indicate significant difference of $*P = 0.002$ or $**P < 0.001$ by *t* test (*KPC* krill protein concentrate)

Urinary assays of renal function and damage

To determine kidney damage and function, various biomarkers were determined in urine collected during the final week of the 4-week study. Table 2 shows that NAG concentrations were lower ($P = 0.005$) in rats fed KPC compared to casein. Although not significant, rats fed KPC tended to have higher CrC ($P = 0.055$) and lower total urinary protein ($P = 0.057$) than rats fed casein. Rats fed KPC had a more basic ($P = 0.004$) urinary pH values compared to rats fed casein. The urine of rats fed KPC also had higher urinary Na ($P < 0.001$) concentrations compared to rats fed casein (Table 2).

Mineral balance

Table 3 shows that rats fed KPC had higher a ($P = 0.02$) urinary output after the initiation of the diet. Despite the higher urinary output, there was no difference in urinary Ca excretion between rats fed casein and KPC. There was no effect on Ca homeostasis indicated by no significant differences in Ca intake, absorption, retention, or fecal excretion between rats fed KPC or casein at the initiation of the diet. During the final week, rats fed KPC had greater ($P < 0.001$) Ca intake than the rats fed casein. However, there were no differences in measures of total Ca intake, absorption,

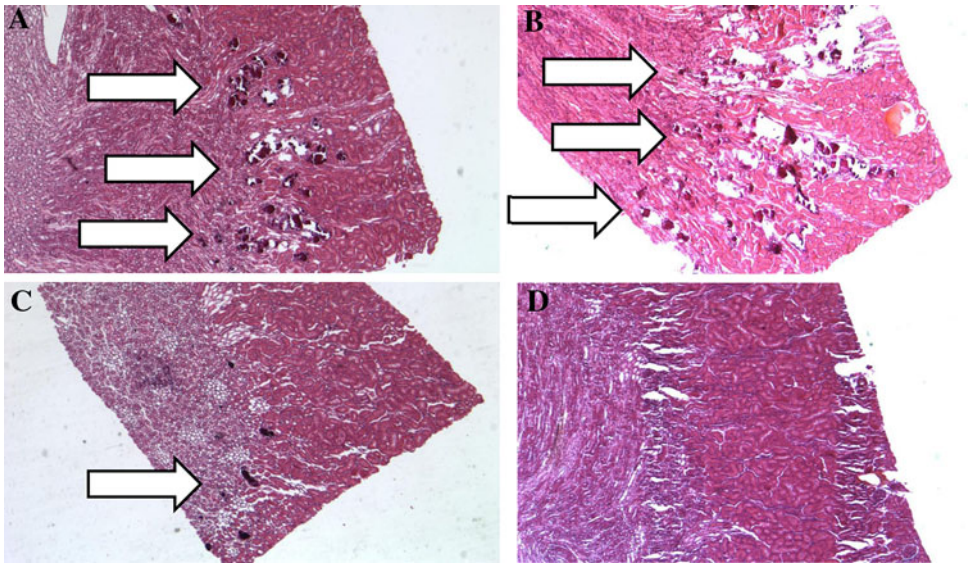

Fig. 2 Hematoxylin and eosin (H&E) stained renal tissue from rats fed 10% protein diets consisting of either casein (**a**, **b**) or KPC (**c**, **d**) for 4 weeks. *Arrows* highlight mineral deposition observed at ×4 (*KPC* krill protein concentrate)

Table 2 Urinary assays for kidney damage and function in female Sprague–Dawley rats fed different dietary sources of protein

Biomarkers[a]	Casein[b]	KPC[b]	*P* value
Urinary NAG (IU/L)	22.9 ± 6.9	3.9 ± 1.0	0.005
CrC (ml/min)	1.7 ± 0.7	5.5 ± 2.1	0.055
Total urinary protein (μg/ml)	0.6 ± 0.02	0.5 ± 0.03	0.057
Urinary pH	7.5 ± 0.5	9.0 ± 0.02	0.004
Urinary Na (mg/7 days)	81.3 ± 10.4	246.2 ± 40.1	<0.001

CrC creatinine clearance, *NAG n*-acetyl glucosaminidase, *Na* sodium

[a] Rats were individually housing in metabolic cages. Urine samples consisted of pooled samples collected during the final 7 days of the 4-week feeding study

[b] Values are expressed as the mean ± SEM of $n = 10$ rats/group. The *t* test was used to compare differences between treatment groups, Differences were considered significant at $P < 0.05$

Table 3 Calcium balance of female Sprague–Dawley rats fed different dietary sources of protein

Measurements[a]	Casein	KPC	*P* value
Total Ca intake	3.16 ± 0.08	3.23 ± 0.08	0.5
First week			
Urinary output (ml/day)	6.05 ± 3.5	13.9 ± 3.4	0.02
Fecal output (mg/day)	1.9 ± 0.1	2.03 ± 0.1	0.7
Ca intake (mg/day)	82.0 ± 2.7	82.6 ± 3.7	0.9
Ca apparent absorption (%)	40.2 ± 4.4	46.6 ± 3.7	0.6
Fecal Ca excretion (mg/d)	43.0 ± 5.6	44.1 ± 3.7	0.7
Urinary Ca excretion (mg/d)	0.67 ± 0.2	1.01 ± 0.3	0.4
Ca retention (mg/day)	22.3 ± 2.8	26.3 ± 2.4	0.6
Final week			
Urinary output (ml/day)	6.7 ± 2.9	13.6 ± 3.6	0.03
Fecal output (mg/day)	2.2 ± 0.134	2.2 ± 0.113	0.9
Ca intake (mg/day)	111.0 ± 4.4	133.0 ± 2.7	<0.001
Ca apparent absorption (%)	23.8 ± 5.1	31.1 ± 6.4	0.7
Fecal Ca excretion (mg/day)	83.7 ± 4.3	79.9 ± 11.4	0.7
Urinary Ca excretion (mg/day)	3.3 ± 0.7	3.6 ± 1.7	0.3
Ca retention (mg/day)	19.1 ± 4.1	31.4 ± 6.6	0.2
Serum Ca (mg/dl)	11.5 ± 0.4	11.4 ± 0.8	0.6

[a] Values are expressed as the mean ± SEM of $n = 10$ rats/group. The *t* test was used to compare differences between treatment groups. Differences were considered significant at $P < 0.05$

retention, or fecal Ca concentrations between rats fed KPC and rats fed casein (Table 3).

Table 4 shows that consumption of KPC had no effect on P homeostasis at the initiation of the diet as indicated by no differences in P intake, absorption, retention, urinary, or fecal output between rats fed KPC and rats fed casein. During the final week, there was no effect on P homeostasis as indicated by no differences in P intake, absorption, retention, urinary or fecal P concentrations between rats fed KPC and rats fed casein.

Bone measurements

Table 5 shows there were no significant differences in femur morphometry measurements of weight, length, width or depth between rats fed KPC and rats fed casein. There were no significant differences in femur total BMC of rats fed KPC compared to rats fed casein. Similarly, measurements of the major bone minerals indicated no significant difference in either the femoral Ca or P content of rats fed KPC compared to rats fed casein. Measurement of bone strength as determined by peak force in the femur showed no significant difference between rats fed KPC and rats fed casein.

Table 4 Phosphorus balance of female Sprague–Dawley rats fed different dietary sources of protein

Measurements[a]	Casein	KPC	*P* value
Total P intake (mg/28 days)	2.44 ± 0.06	2.50 ± 0.06	0.5
First week			
P intake (mg/day)	89.7 ± 3.0	90.9 ± 4.1	0.8
P apparent absorption (%)	71.3 ± 2.4	74.8 ± 1.7	0.5
Fecal P excretion (mg/day)	25.1 ± 1.9	23.1 ± 2.0	0.8
Urinary P excretion (mg/day)	18.2 ± 3.7	13.7 ± 1.1	0.3
P retention (mg/day)	30.3 ± 3.4	37.9 ± 2.5	0.3
Final week			
P intake (mg/day)	87.6 ± 3.4	91.3 ± 1.9	0.4
P apparent absorption (%)	47.4 ± 3.9	51.2 ± 4.6	0.8
Fecal P excretion (mg/day)	45.1 ± 2.3	42.2 ± 3.3	0.4
Urinary P excretion (mg/day)	15.6 ± 1.4	13.5 ± 0.8	0.2
P retention (mg/day)	19.9 ± 3.6	25.5 ± 3.2	0.4
Serum P (mg/dl)	11.3 ± 0.4	10.7 ± 0.5	0.4

[a] Values are expressed as the mean ± SEM of $n = 10$ rats/group. The *t* test was used to compare differences between treatment groups. Differences were considered significant at $P < 0.05$

Table 5 Bone measures in female Sprague–Dawley rats fed different dietary sources of protein

Measurements[a]	Casein	KPC	*P* value
Femoral weight (g)	0.53 ± 0.02	0.55 ± 0.02	0.4
Femoral length (mm)	27.2 ± 0.22	27.3 ± 0.33	0.9
Femoral width (mm)	3.8 ± 0.09	3.7 ± 0.14	0.5
Femoral depth (mm)	3.00 ± 0.03	2.98 ± 0.04	0.7
Femoral BMC (g)	0.54 ± 0.03	0.52 ± 0.01	0.2
Femoral Ca (mg/g)	41.4 ± 4.6	39.0 ± 1.7	0.7
Femoral P (mg/g)	55.0 ± 8.8	67.5 ± 7.9	0.5
Femoral strength (N)	159.0 ± 6.5	174.6 ± 9.1	0.2

[a] Values are expressed as the mean ± SEM of $n = 10$ rats/group. The *t* test was used to compare differences between treatment groups. Differences were considered significant at $P < 0.05$

Discussion

Protein quality of KPC is similar to that of casein, a standard protein source, used in commercial semi-purified rodent diets [26]. However, female rats fed KPC had lower ($P < 0.001$) kidney weights compared to rats fed casein. To investigate the effect of KPC compared to casein on kidney health, NAG was measured as an early marker of renal injury. Despite being provided a low (10% g/kg diet) protein diet, urinary NAG concentrations were higher ($P = 0.005$) in rats fed casein compared to KPC, indicating that protein source is an important factor to consider in renal health. Similarly, NAG excretion was greater in male Munich-Wistar rats consuming casein compared to a soy protein based diet. The authors suggested that casein induced renal injury through increased acidification of the kidney filtrate [27]. Ritskes-Hoitinga et al. [28] reported that high urinary pH values were associated with low degree of nephrocalcinosis indicated by a negative correlation ($r = 0.68$, $P < 0.05$) between urinary pH and the severity of nephrocalcinosis. Therefore, in our study, higher ($P = 0.004$) urinary pH in rats fed KPC compared to rat fed casein may be responsible for preventing early renal injury.

The pathogenesis of nephrolithiasis has been proposed to involve renal injury followed by deposition of calcium crystals to the site of injury [29]. In our study, kidney mineral content ($P < 0.01$), Ca ($P < 0.001$), and P ($P < 0.001$) concentrations were lowered in rats fed KPC compared to casein. Furthermore, histological evaluation of the kidneys showed that rats fed casein had extensive renal calcification, whereas mild to absent renal calcification was observed in the kidneys of rats fed KPC. Similarly, Zhang and Benyen [12] reported that female Wistar rats fed cod meal had lower kidney Ca content compared to rats fed casein or soybean protein isolate. Together, the study results suggest that pelagic protein sources may provide protection against mineral deposition in the kidneys.

After an initial renal injury, kidney function often declines [30]. Determination of urinary total protein excretion provides an estimation of kidney filtrating ability with proteinuria considered a risk factor for progressive loss of kidney function. CrC provides an estimation of GFR with reduced CrC used as a clinical diagnosis for renal disease [31]. Although not significant, rats fed KPC for 4 weeks tended to have lower ($P = 0.057$) urinary total protein excretion and higher ($P = 0.055$) CrC compared to rats fed casein. Similarly, male Wistar rats fed soy protein had lower urinary total protein and higher CrC compared to rat fed casein [32]. These study results indicate that the protein source influences renal function. In the current study, urinary output was higher ($P = 0.02$) in rats fed KPC compared to casein-fed rats. This may have been the result of a tendency ($P = 0.10$) for rats fed KPC to consume more ddH_2O (970.3 ml/28 days) than rats fed casein (620 ml/28 days). Increased urinary output in rats fed KPC may have contributed to the observed decreased kidney mineral content and deposition. Promoting increased urinary output is a common recommendation for individuals suffering from kidney mineralization [33]. However, in a study comparing different dietary proteins and development of nephrocalcinosis, Zhang and Benyen [12] reported that the rats with the lowest kidney mineral content did not have the highest urinary outputs. Therefore, decreased mineralization of the kidneys in rats fed KPC in the current study was likely not the result of an increased urinary output alone.

The tendency for higher GFRs and lower urinary total protein excretion, accompanied by lower kidney calcification, in KPC compared to casein-fed rats suggested that consumption of KPC had beneficial effects on kidney function. Furthermore, lower urinary NAG concentration and renal tissue Ca deposition suggested that KPC consumption prevented kidney injury. However, these beneficial effects may not be due to the protein alone, since KPC also contains minerals and lipids that may affect the kidneys.

KPC is composed of 4.4% minerals by weight [26]. Alterations in dietary Ca:P ratio have been reported to play an important role in the development of nephrocalcinosis [6]. Increasing the Ca:P ratio to greater than 1.0 has been reported to prevent nephrocalcinosis [7]. In our study the KPC and casein diets were formulated to have matching Ca and P concentrations which resulted in a Ca:P ratio of 1.2. Therefore, it appears that decreased kidney mineralization in rats fed KPC was not due to differences in dietary Ca and P content.

Common urinary abnormalities associated with nephrocalcinosis and nephrolithiasis are hypercalciuria and/or hyperphosphouria. In our study, there were no differences in urinary Ca and P concentrations between rats fed KPC and casein. Unexpectedly, rats fed KPC had a fourfold increase in urinary Na compared to rats fed casein. Subsequent analysis of diets showed that KPC had twice the Na content of the casein diet. Increased Na was likely due to the processing method used to obtain KPC. As a result, rats fed KPC consumed twice the amount of Na compared to rats fed casein. Higher Na consumption in rats fed KPC may explain their tendency ($P = 0.10$) to consume more ddH_2O, and, in turn, their increased urinary output. High urinary Na has been suggested to interfere with tubular Ca reabsorption leading to hypercalciuria, a risk factor for nephrocalcinosis and nephrolithiasis [34, 35]. In the current study, the increased urinary Na in rats fed KPC did not appear to affect urinary Ca excretion compared to rats fed casein. Furthermore, Mattson et al. [36] concluded that dietary composition significantly influenced the development of renal disease in salt sensitive rodent models independent of the NaCl content. Taken together these data suggest that the mineral content of the diet is not the sole determinant of urinary mineral excretion, a risk factor for developing nephrocalcinosis. This finding is supported by Meyer et al. [10] who reported that changing the source of protein in semi-synthetic diets reduced mineral deposits, and this exceeded the effects of changing the mineral composition of the diet.

KPC also contains a lipid component that may benefit renal health. Gigliotti et al. [26] reported that KPC consists of 8.1% lipid by weight. The lipids in KPC are rich (~27%) in the ω-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA have anti-inflammatory properties that potentially contributed to renal health. Rats fed cod meal, a potential source of EPA and DHA, was reported to have reduced kidney Ca concentrations compared to rats fed casein or soy isolate [12]. Furthermore, Schlemmer et al. [37] reported that rats supplemented with ω-3 PUFAs had decreased calcification of the renal tubules. Consuming KPC as a source of protein provides a high-quality protein with the added benefit of ω-3 PUFAs, whereas other high-quality protein foods tend to be high in saturated fatty acids.

In addition to renal injury, changes in renal function may negatively affect Ca and P balance, which can lead to compromised bone health [38]. Protein source appears to affect Ca and P absorption and retention. Van Dael et al. [39] reported that replacing casein with whey protein diets resulted in higher absorption of Ca and higher absorption and retention of P. In our study, there were no differences between Ca and P absorption and retention in rats fed KPC compared to casein. Furthermore, bone measurements showed no differences in femoral total BMC, Ca, P or bone strength between rats fed KPC and casein. Similarly, the lower urinary pH observed in rats fed casein had no effect on bone mass or strength measurements. It is likely that extracellular pH was not lowered sufficiently to activate bone resorption in rats fed casein, a proposed mechanism for protein-induced bone loss [40]. Studies addressing bone loss due to lower pH commonly use high protein diets of up to 50% protein. It was unlikely that our diet consisting of 10% protein would promote the metabolic acid load required to affect bone health. Recent evidence suggests that dietary protein may be as important as dietary Ca in preventing bone loss [41, 42]. Bone tissue is formed by the mineralization of the collagen, a protein-derived connective tissue. Thus, dietary protein may contribute to bone density and strength by promoting complete bone matrix formation.

In conclusion, early renal injury and Ca deposits were influenced by the protein source fed to young female Sprague–Dawley rats, even with a low protein diet (10% g/kg diet). Therefore, it is important not only to consider the amount, but also the source of dietary protein. Compared to rats fed casein, the consumption of KPC prevented early renal injury. This was indicated by the lower urinary NAG, reduced kidney mineralization, and a tendency for higher GFRs and lower proteinuria. Other nutrients associated with KPC, particularly the ω-3 PUFA-rich lipids, may also have contributed to reduced kidney injury and Ca deposition. In addition to the beneficial effect on the kidney, KPC consumption had no effect on bone mass or strength. Based on the study results, KPC is a novel and sustainable protein source which appears to attenuate the development of diet-induced alterations in renal health and associated bone loss in rats.

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