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Analytical Methods

Determination of the nutritional value, protein quality and safety of krill protein concentrate isolated using an isoelectric solubilization/precipitation technique

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ABSTRACT

Despite its abundance, krill is not utilized for human consumption due to the lack of proper technology for protein recovery. The objectives of this study were to isolate krill protein concentrate (KPC) and to determine the nutritional value, health benefits, and safety of KPC for human consumption. Proximate analysis of protein recovered by isoelectric solubilzation/precipitation indicated that KPC was composed of approximately 78% protein and 8% fat (dry weight). *In vivo* analysis of protein quality indicated that protein digestibility corrected for amino acid score and protein efficiency ratio was equal to casein. KPC safety was indicated by the absence of differences in clinical measures of kidney function compared to casein. Fatty acid analysis of KPC showed that approximately 27% were omega-3 polyunsaturated fatty acids (ω-3 PUFAs). Based on our study, KPC appears to be a promising protein source for human consumption with the advantage of being a rich source of ω-3 PUFAs.

1. Introduction

The term "krill" refers to approximately 85 species of pelagic crustaceans belonging to the order *Euphausiacea*. Krill are estimated to have the largest multi-cellular biomass on the planet, with estimates as high as 500 million tonnes (Nicol, James, & Pitcher, 1987). Despite this large biomass, only about 12% of the total krill catch is consumed by humans (Ichii, 2000). Due to its abundance and under-utilization, krill offers a relatively untapped potential food source for humans.

Whole krill are composed of 60–80% protein, 7–26% lipid, and 12–17% ash on a dry weight basis (Grantham, 1977). The protein derived from krill is considered high quality based on chemical analysis showing krill protein contains all nine essential amino acids in sufficient quantities to meet the FAO/WHO/UNU requirements for human adults (Chen, Tou, & Jaczynski, under review). In addition to the amino acid content, bioavailability also must be assessed to determine protein quality. Iwantani, Obtake, and Tamura (1977) reported that rats fed whole krill gained less weight and had reduced protein quality measurements of protein efficiency ratio, biological value, and net protein utilization compared to rats fed egg protein. The authors suggested that the decreased digestibility of krill protein may have been due to the presence of the exoskeleton. Removal of the exoskeleton is important because indigestible polysaccharides such as chitin can impede digestion and absorption (Ikegamie et al., 1990). Another challenge to the use of krill as a food source for humans is the presence of hydrolytic enzymes. Upon the demise of the krill, hydrolytic enzymes are released into the surrounding tissue which results in rapid tissue liquefication and spoilage.

To overcome these obstacles, various methods have been used to isolate krill protein (Heinz, Henk, & Kesting, 1981; Rys & Koreleski, 1979). Many techniques used to isolate proteins employ protein hydrolysis; however, this decreases the functional characteristics of recovered proteins. Chen and Jaczynski (2007a) using an isoelectric solubilization/precipitation technique, isolated protein from trout byproducts with functional characteristics that allow for a broad range of use in the food industry. Chen and Jaczynski (2007b) also successfully applied the isoelectric solubilization/precipitation to whole krill. They demonstrated that the protein recovered from krill retained gel-forming ability. Therefore, we used the isoelectric solubilization/precipitation technique to recover protein from Antarctic krill (*Euphausia superba*) in order to assess the nutritional value of krill protein *in vivo*.

In addition to being a promising source of high quality protein, krill also offers the advantage of being high in the omega-3 polyunsaturated fatty acids (ω-3 PUFAs) (Tou, Jaczynski, & Chen, 2007). The ω-3 PUFAs have been reported to have various beneficial health effects that include reducing the risk of cardiovascular disease

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(CVD) (Simopoulos, 2002). Conversely, the safety of krill protein for human consumption also needs to be considered. Previous research has found that changes in dietary proteins may have negative effects on kidney function (Zhang & Beynen, 1992). Meyer, Blom, and Sondergaard (1982) found that changing the source of dietary proteins fed to rats had negative effects on the kidneys. Replacing casein with lactalbumin had greater effects on kidney calcification than fluxuations in the mineral composition. Therefore, the effect of krill protein concentrate (KPC) on kidney function needs to be assessed.

Widespread acceptance of krill as part of the human diet will depend on the consumer's perception of krill as a nutritious, healthy, and safe food. Therefore, the objectives of this study were to evaluate the nutritional value, potential health benefits, and safety of KPC isolated using an isoelectric solubilization/precipitation technique.

2. Materials and methods

2.1. Krill protein isolation

Whole, frozen Antarctic krill (*E. superba*) were purchased from Krill Canada (Langley, BC, Canada). The krill blocks were transported overnight to our laboratory in heavily insulated industrial strength boxes filled with dry ice. Upon arrival, the Antarctic krill was immediately stored at −80 °C.

Protein was isolated from whole krill using an isoelectric point solubilization/precipitation method according to Chen and Jaczynski (2007b). Briefly, 428 g of frozen Antarctic krill was blended in a 1:6 (w/v) krill/deionized distilled water (ddH_2O) mixture. The krill ddH_2O mixture was homogenized at a temperature of 4 °C (PowerGen 700 Homogenizer, Fairlawn, NJ), and the pH adjusted to 11.5 using NaOH. The krill homogenate was centrifuged at 10,000*g* for 10 min and the supernatant collected. The pH of the supernatant was adjusted to 5.5 using HCl then centrifuged at 10,000*g* for another 10 min. The resultant sediment was collected as KPC, freeze-dried, and stored at −80 °C.

2.2. Proximate analysis

The proximate composition of frozen, whole Antarctic krill and freeze-dried KPC was determined according to standard AOAC (Association of Official Analytical Chemists, 1995) methods. Analysis of samples were performed in triplicates. Moisture content of Antarctic krill and KPC was determined by placing samples (2 g) in an aluminum dish and oven-drying at 100 °C. Samples were weighed at regular intervals until the weight was constant (18 h). Ash content was determined by ashing the KPC and whole Antarctic krill at 550 °C for 24 h in a muffle furnace (Lindberg 515A2, Watertown, WI). Total crude protein of whole Antarctic krill and KPC was determined by the Kjeldahl method (Kjeltec Auto 1030 Analyzer, Foss North America Inc., MN). Total lipid content of the KPC and whole Antarctic krill was determined by soxhlet extraction according to the Association of Official Analytical Chemists (1995).

2.3. Amino acid profile

The analysis of essential and non-essential amino acids was conducted according to the AOAC method 982.30 E (a, b, c) by the Agriculture Experiment Station Chemical Laboratories at the University of Missouri-Columbia. Antarctic krill and freeze-dried KPC were hydrolyzed with 6 N HCl for 24 h. Amino acids were quantified using the Beckman Amino Acid Analyzer (Model 6300, Beckman Coulter Inc., CA) employing sodium citrate buffers as step gradients with the cation exchange post-column ninhydrin derivatization method.

To determine whether the amino acid content of KPC met the human amino acid requirements, whole Antarctic krill and KPC was compared to FAO/WHO/UNU (1985) amino acid requirements for human adults and infants.

2.4. Animal feeding study

All animal procedures were conducted in accordance with the guidelines set forth by the Institute of Laboratory Animal Resources Commission on Life Sciences for the Care and Use of Laboratory Animals (1996), and approved by the Animal Care and Use Committee at West Virginia University.

Immature (age 28 d), female Sprague–Dawley rats were individually housed in a metabolic cage to determine food intake and to collect urine and feces throughout the experiment. Rats were kept in rooms maintained at 21 °C with a 12 h light/dark cycle. During a 14 d acclimation period, animals were given *ad libitum* access to ddH_2O and AIN-93G diet (Harklan Teklad; Indianapolis, IN). The AIN-93G meets all the nutrient requirements for growing rats as defined by the National Research Council (1995).

Following the 14 d acclimation period, rats ($n = 30$) were randomly assigned to be fed ad libitum, one of three diets consisting of: (1) 10% crude protein supplied as KPC for 4 weeks ($n = 10$), (2) 10% crude protein supplied as casein for 4 weeks ($n = 10$), or (3) 10% casein diet for 2 weeks followed by a protein-free diet for the final 2 weeks ($n = 10$). 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. Calcium (Ca) and phosphorus (P) contents of the diets were also matched (Table 1). Diets containing KPC were prepared weekly and kept stored at 4 °C. The assigned diets and ddH_2O were measured and replaced with fresh diet every 2 d. Body weights of all animals were measured weekly.

Urine and fecal samples were collected and measured weekly. Ascorbic acid (0.1%) was added to the urine collection tube as a preservative along with 1 ml of mineral oil to prevent evaporation. Collected urine samples were centrifuged at 1500*g* for 10 min at 4 °C then aliquoted into fresh tubes and stored at −20 °C until assayed for nitrogen content. Fecal samples were freeze-dried for 48 h then stored at −20 °C until assayed for nitrogen content.

Table 1
Diet composition

Ingredients (g/kg diet)	10% Casein	10% KPC[b]	Protein free
Casein	115	0	0
DL-methionine	1.5	0	0
KPC	0	128	0
Sucrose	531.8	609.8	631.8
Corn Starch	200	229.4	200
Corn Oil	53.5	49.9	54.6
Cellulose	52	59.6	66.4
Vitamin Mix[a]	10	11.5	10
Ethoxyquin	0.01	0.01	0.01
Mineral Mix[a]	13.4	15.3	13.4
Calcium Phosphate	20.2	20.9	23.7
Calcium Carbonate	2.6	3.6	0.04
Gross Energy (kcal/g)	4.3	4.3	4.2

KPC, krill protein concentrate.
[a] Based on the AIN-93G vitamin and mineral mixes (Reeves, Rossow, & Lindauf, 1993).
[b] Diet formulated for 872 g of diet + 128 g addition of KPC.

2.5. Protein quality measurements

Nitrogen measurements were obtained using the Kjeldahl method, and the following protein quality measurements determined: true digestibility (D), true biological value (BV), net protein utilization (NPU), protein digestion corrected for amino acid score (PDCAAS) and protein efficiency ratio (PER). Protein quality measurements were calculated according to Pellet and Young (1980). BW is body weight gain, I is nitrogen intake, FN is fecal nitrogen excreted, EFN is endogenous fecal nitrogen excreted by the protein-free group, UN is urinary nitrogen excreted, EUN is endogenous urinary nitrogen excreted by the protein-free group.

$$\text{True Digestibility(D)} D = [I - (\text{FN} - \text{EFN})]/I$$

$$\text{True Biological Value(BV)} BV = [I - (\text{FN} - \text{EFN}) - (\text{UN} - \text{EUN})] / [I - (\text{FN} - \text{EFN})]$$

$$\text{Net Protein Utilization(NPU)} NPU = [I - (\text{FN} - \text{EFN}) - (\text{UN} - \text{EUN})]/I$$

Protein Digestion Corrected for Amino Acid Score(PDCAAS)

PDCAAS = (the amount of limiting amino acid in the test protein (KPC)/the measured amount of limiting amino acid in reference protein(casein)) × True Digestibility

$$\text{Protein Efficiency Ratio(PER)} PER = \text{BW}/(I \times 6.25).$$

2.6. KPC fatty acid analysis

Extraction of lipids from KPC was performed according to Bligh and Dyer (1959), and all samples were analyzed in duplicates. To quantify fatty acids, 48 μl of *cis*-10-heptadecaenoic acid were added as a standard during the initial weighing of the samples. Powdered freeze-dried KPC was mixed in a chloroform/methanol/acetic acid (2:1:0.015, v/v/v) solution. Following centrifugation at 900*g* for 10 min at 10 °C, the chloroform layer was collected. The collected chloroform was then filtered through 1-phase separation filters to remove any remaining water and precipitated material. The extracted lipid was then transmethylated following the procedure described by Fritsche and Johnston (1990). Briefly, extracted fatty acids were methylated by adding 4% H_2SO_4 in anhydrous methanol to the collected chloroform layer followed by incubation in a 90 °C water bath for 60 min. Samples were dried under nitrogen gas then dissolved in 3 μl iso-octane. The fatty acid methyl esters were analyzed by gas chromatography (CP-3800, Varian, CA) programmed to an initial temperature of 140 °C. The initial temperature was held for 5 min with an increase of 1 °C per min to a final temperature of 220 °C. Nitrogen was used as the carrier gas over a total separation time of 110 min. Peak area and amount of each fatty acid was calculated by Star GC Workstation computer software (Varian Inc., CA).

Fatty acid oxidation of KPC was determined by measuring thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides. All samples were measured in duplicates. TBARS were determined according to Yu and Sinnhuber (1957) by mixing powdered, freeze-dried KPC with thiobarbituric acid and trichloroacetic acid and incubating in a 100 °C water bath for 30 min. The concentration of TBARS was determined by recording the absorbance at 535 nm by spectrophotometry (model Du Series 500, Beckman Instruments, Fullerton, CA). TBARS were expressed as mmol malondialdehyde (MDA) equivalents/kg KPC. Lipid hydroperoxide concentrations were measured using a commercially available Lipid Hydroperoxide Assay Kit (Cayman Chemical, Ann Arbor, MI).

2.7. Serum lipid and lipoprotein measurements

At the end of the 4 weeks feeding study, rats were euthanized by CO_2 inhalation. Trunk blood was collected and centrifuged at 1500*g* for 10 min at 4 °C. Serum was collected and stored at −80 °C until analyzed. Serum cholesterol, triglyceride, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) were determined by enzymatic reactions using a commercially available Lipid Analytical Test Rotor and absorbance read at 630 nm by the Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc., Columbia, MD).

2.8. Organ weights and clinical blood measurements

Following euthanasia, the major organs (i.e. brain, heart, liver, and kidneys) were excised, trimmed, blotted, and weighed as indicators of toxicity. The adrenals were weighed as an indicator of chronic stress (data not shown). The retroperitoneal and gonadal fat pads were also excised and weighed.

To analyze kidney mineral content, kidneys were ashed in a muffle furnace for 24 h at 600 °C. Kidney concentrations of Ca and P were determined according to Tsanzi, Light, and Tou (2008). Briefly, ashed samples were dissolved in 1 ml of 70% nitric acid. The acidified samples were neutralized in 5 ml of ddH_2O and filtered through Whatman no. 1 paper. Samples were then diluted to volume 50 ml in a volumetric flask and kidney Ca and P concentrations measured by inductively coupled plasma optical emission spectrometry (model P400, Perkin–Elmer, Shelton, CN).

Kidney function was assessed by measuring serum total protein, albumin, blood urea nitrogen (BUN), creatinine, Ca, and P. BUN and creatinine were determined by enzymatic reactions, whereas total protein, albumin, Ca and P concentrations were determined by colorimetric assays using a commercially available Vet-16 rotor (Kania-Korwel et al., 2004) with absorbance measured by Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc., Columbia, MD). Total urinary output was also measured.

2.9. Statistical analysis

The animal feeding study was a completely randomized design, with $n = 10$ rats per diet treatment. The *t*-test was used to determine differences between casein and KPC diets and one-way ANOVA with Tukey's multiple comparison test was used to determine differences among the three diets. The *t*-test and one-way ANOVA were performed using Sigma Stat 3.1 (Systat Software Inc., San Jose, CA). To correct organ weight for body size, a covariate analysis was performed using GLM procedure. Repeated Measures in a mixed model procedure was used to analyze body weight gain during the 4-week feeding study. Both the covariate and repeated measure calculations were performed using SAS 9.1 (SAS Institute Inc., Cary, NC). All differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Krill protein isolation and proximate analysis

The composition of whole Antarctic krill was 81.2% moisture and on a dry basis 76.5% crude protein, 12.1% total lipid, and 17.4% total ash. Values for whole krill are in agreement with other studies reporting proximate analysis of krill on a dry basis of 45–80% crude protein, 7–30% total lipid, and 10–20% total ash (Grantham 1977; Savage & Foulds 1987; Sidhu, Montgomery, Holloway, Johnson, & Walker 1970).

Protein recovered from krill by the isoelectric solubilization/precipitation technique resulted in KPC with a protein recovery yield of approximately 46% (dry basis). The proximate composition of KPC recovered by the isoelectric solubilization/precipitation technique after freeze-drying was 3.3% moisture, and on a dry basis 77.7% crude protein, 8.1% total lipid, and 4.4% total ash.

The isoelectric solubilization/precipitation technique resulted in an approximate 33% reduction in total lipid content while the

protein content remained relatively constant compared to whole krill. Total ash was reduced approximately 75% due to removal of the exoskeleton, which is the predominate source of minerals in crustaceans. Removal of the exoskeleton is important because indigestible polysaccharides such as chitin can impede digestion (Ikegamie et al., 1990).

Based on results of the proximate analysis, the isoelectric solubilization/precipitation technique allowed successful isolation of krill protein and removal of the exoskeleton. Next, the protein quality of the KPC recovered by the isoelectric solubilization/precipitation procedure was assessed to determine its suitability as a protein source for human consumption.

3.2. Amino acid profile

The amino acid content of KPC was compared to whole Antarctic krill, and to the FAO/WHO/UNU amino acid requirements for human adults and infants (Table 2). The isoelectric solubilization/precipitation method effectively concentrated the essential and non-essential amino acid content of KPC compared to whole krill (Table 2). The total essential amino acids for KPC were 531.5 mg/g protein compared to 212.1 mg/g for whole krill. The presence of all nine essential amino acids in sufficient amounts to meet FAO/WHO/UNU requirements for human adults indicates that KPC is a high quality protein for human consumption.

Products where krill protein may substitute for other proteins are: imitation meat products, sports drinks, infant formulas, and milk substitutes. For use as a protein source in foods for infants, KPC was lower in leucine and tryptophan concentration than the FAO/WHO/UNU requirements for human infants. However protein quality is determined not only by the essential amino acid composition, but also by its digestibility. Therefore, an animal feeding study was performed to determine the bioavailability of KPC.

Table 2
Amino acid composition of whole krill, krill protein concentrate, and the FAO/WHO/UNU amino acids requirements for human adults and infants

Amino acid	KPC (mg/g protein)	Whole Antarctic krill (mg/g protein)	FAO/WHO/UNU (1985) adult (mg/g protein)	FAO/WHO/UNU 1985 infants (mg/g protein)
Essential amino acids				
Isoleucine	57.1	25.4	13	46
Leucine	88.4	39.9	19	93
Lysine	92	43.7	16	66
Cysteine + Methionine	48.5	24	17	42
Phenylalanine + Tyrosine	97.6	50	19	72
Threonine	46.4	21.5	9	43
Tryptophan	16.8	7.3	5	17
Valine	58.1	26	13	55
Histidine	26.6	11.4	16	26
Arginine	62.6	37.8	–	–
Total EAA (mg/g protein)	531.5	212.1	127	460
Non-essential amino acids				
Cysteine	14.9	8.5		
Tyrosine	46.5	27.9		
Alanine	54.4	29.4		
Aspartate	106.3	53.4		
Glutamate	116.7	66.9		
Glycine	41.3	33.5		
Proline	31.8	22.9		
Serine	37.6	19.1		
Taurine	4.2	15.5		
Total NEAA (mg/g protein)	516.3	314.9		

KPC, krill protein concentrate, Total EAA, total essential amino acids, Total NEAA, total non-essential amino acids.

3.3. Protein quality measurements

Fig. 1 shows the body weights of growing female rats fed KPC and casein. The ability of KPC to support growth equal to that of rats fed casein indicated that KPC protein is a complete protein. Table 3 shows protein quality measurements of digestibility, BV, NPU, PDCAAS and PER. Lower ($P < 0.001$) BV and NPU for KPC than casein indicated that there was less nitrogen retention by the rats fed KPC compared to casein-fed rats. However, the casein diet was supplemented with DL-methionine whereas the diet with KPC was not (Table 1). The addition of limiting amino acids such as DL-methionine can increase protein quality values (Sarwar, 1997). This in turn, may explain the lower NPU and BV in rats fed the KPC compared to the casein diet. The NPU and BV measurements also have limitations. Primarily, they do not account for the amount of essential amino acids present in the test protein.

Currently, the PDCAAS is the preferred measurement for protein quality (Schaafsma, 2000) and the protein information on food labels are assessed using the PDCAAS. The PDCAAS takes into consideration the amino acid content as well as the digestibility of the protein of interest. The results of our study showed that PDCAAS scores were equal for KPC and casein (Table 3).

The PER, a ratio of body weight gain per gram protein ingested, is used to determine the quality of protein sources to be added to foods for infants (Gropper, Smith, & Groff, 2005). Young rats are routinely used to determine the quality of proteins to be used in infant formulas. Fig. 1 shows that KPC was equal to casein for supporting the growth of young female rats. The Food and Drug Administration specifies the use of casein as the reference protein

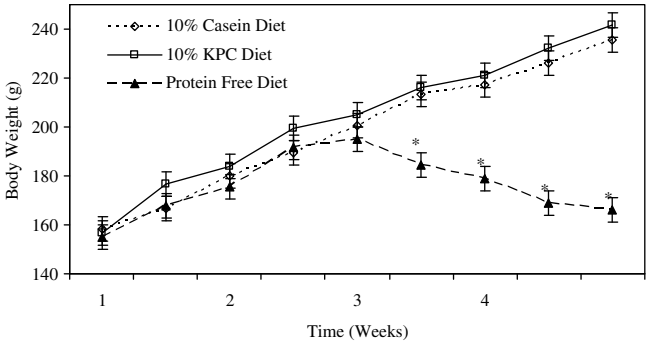

Fig. 1. Body weights of female rats fed 10% protein diets consisting of casein, KPC, or 2 weeks of 10% casein followed by 2 weeks of a protein-free diet. Symbol (*) indicates significant difference at $P < 0.05$ by one-way ANOVA followed by Tukey's test. KPC = krill protein concentrate.

Table 3
Protein quality measurements, body weight, fat pads and food intake measurements[a]

Protein quality measurement	Casein	KPC
Digestibility (%)	93.3 ± 2.0	93.2 ± 1.0
Biological value	0.69 ± 0.01	0.58 ± 0.02*
Net protein utilization (%)	64.7 ± 1.0	54.1 ± 2.0*
PDCAAS	1	1
Protein efficiency ratio (g body wt/g protein)	1.57 ± 0.05	1.44 ± 0.15
Final body weight (g)	235.4 ± 5.8	241.8 ± 3.4
Total body weight gain (g/4 wk)	73.3 ± 3.0	80.0 ± 3.5
Total fat pad weights (g)	7.9 ± 0.4	9.6 ± 0.4*
Retroperitoneal fat pad (g)	2.5 ± 0.2	3.3 ± 0.3*
Gonadal fat pads (g)	5.3 ± 0.3	6.3 ± 0.2*
Total food intake (g)	451.1 ± 11.5	462.7 ± 11.6
Feed efficiency (g body wt/g food intake)	0.16 ± 0.005	0.17 ± 0.009

KPC, krill protein concentrate; PDCAAS, protein digestibility corrected for amino acid score.

[a] Values are mean ± SEM with $n = 10$.

* Significant difference ($P < 0.05$) by t-test.

for the PER (Gropper et al., 2005). In the current study, there was no difference in the PER between rats fed KPC and casein diets (Table 3).

Table 3 shows that there were no differences in final body weights and total weight gain between rats fed casein or KPC. However, rats consuming KPC had greater total ($P = 0.01$), retroperitoneal ($P = 0.02$), and gonadal ($P = 0.03$) fat pad weights than rats fed casein. The diets were isocaloric and no significant differences in food intake or feed efficiency was observed when rats fed KPC were compared to rats fed casein (Table 3). Greater total nitrogen excretion ($P < 0.001$) in rats fed KPC (1.8 ml ± 0.07) compared to rats fed the casein diet (1.4 ml ± 0.07) suggests that more amino acid deamination may have occurred in rats fed KPC. It is possible that the excess carbon skeleton were converted to triglycerides for energy storage. However, further studies are needed to address the mechanism responsible for the greater fat mass associated with KPC consumption.

Based on the current study, KPC appears to be a high quality protein that supports growth and is comparable to casein for digestibility, PER and PDCAAS scores. In addition, the 8.1% lipid content of KPC may provide the advantage of having a healthier lipid composition than other animal proteins. Therefore, we evaluated the fatty acid profile of the lipid component of KPC.

3.4. KPC fatty acid composition

The fatty acid composition showed that KPC lipid contained approximately 37% saturated fatty acids, 21% monounsaturated fatty acids, and 27% ω-3 PUFAs (Table 4). Of the ω-3 PUFAs, eicosapentaenoic acid (EPA, 20:5, ω-3) accounted for 12.7% and docosahexaenoic acid (DHA, 20:6, ω-3) for 12.3%. KPC contained higher amounts of EPA and DHA (25%) compared to Coho Salmon (18%), a fatty fish high in ω-3 PUFAs. Based on fatty acid composition, KPC appears to be rich in the biologically active ω-3 PUFAs, EPA, and DHA. This is important because ingestion of EPA and DHA has been linked to various health benefits (Ruxton, Reed, Simpson, & Millington, 2004). However, ω-3 PUFAs are susceptible to oxidation. Oxidized oils are associated with a short shelf-life and when ingested have adverse health effects such as increased risk of CVD (Khan-Merchant, Penumetcha, Meilhac, & Parthasarathy, 2002). Lipid oxidation was determined by measuring TBARS and lipid hydroperoxide concentrations in KPC after 3 d storage at room temperature (22 °C). TBARS concentrations of 0.11 mmol MDA equivalents/kg (Table 4) were within the 0.01-2.3 mmol MDA equivalents/kg range reported for fresh dietary fats (Eder, Keller, Hirche, & Brandsch, 2003). In the literature, lipid hydroperoxide concentrations of 4 mmol /kg were reported for fresh dietary fats (Brandsch, Nass, & Eder, 2003). In our study, KPC lipid hydroperoxide concentration was 0.015 mmol/kg after 3 d storage. Therefore, measurement of TBARS and hydroperoxide concentrations indicated that KPC remained stable against oxidation for up to 3 d at 22 °C.

Table 4
Fatty acid composition of krill protein concentrate, whole Antarctic krill, and Coho salmon

Fatty Acid (%)	KPC	Whole Antarctic krill[a]	Coho salmon[b]
SFAs			
14:0	10.0	4.9	4.5
16:0	25.2	18.8	12.7
18:0	2.1	1.0	3.5
MUFAs			
16:1	7.51	4.9	8.5
18:1	13.5	16.4	20.3
PUFAs			
ALA 18:3 (n−3)	1.5	1.1	2.6
EPA 20:5 (n−3)	12.7	17.4	7.2
DHA 22:6 (n−3)	12.3	12.4	11.1
18:2 (n−6)	3.1	3.3	6.5
20:4 (n−6)	1.2	0.5	2.2
TBARs (3 d) mmol MDA equivalents/kg KPC	0.11		
Lipid hydroperoxides (3d) mmol/kg KPC	0.015		

KPC, krill protein concentrate; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.
[a] Values from Suzuki and Shibata (1990).
[b] Values from USDA (2004).

3.5. Serum lipid and lipoprotein profile

Serum total cholesterol was lower ($P = 0.04$) in rats fed KPC compared to casein-fed rats (Table 5). The sterols associated with shellfish such as krill, have been reported to interfere with cholesterol absorption (Vahouny, Connor, Roy, Lin, & Gallo, 1981) and this in turn may explain the decrease in blood cholesterol. Lower serum cholesterol in rats fed KPC was accompanied by decreased HDL ($P = 0.003$). In a human study, Childs, Kind, and Knopp (1990) found that male subjects consuming fish oils rich in EPA had decreased HDL cholesterol. In contrast, Bunea, El Farrah, and Deutsch (2004) found that hyperlipidemia subjects consuming krill oil had increased HDL and decreased LDL compared to subject consuming fish oil. The authors suggested that the greater lipogenic effects of krill oil was due to the ω-3 PUFAs in krill being associated with phospholipids; whereas, the ω-PUFAs in fish are mainly associated with triglycerides. More studies are needed in order to clarify the effect of the ω-3 PUFAs provided by krill oil on lipoproteins.

3.6. Organ weights and clinical blood measurements

No difference in adrenal weights indicated that rats were not differentially stressed due to the treatments (data not shown). Major organ weights were measured as indicators of toxicity. Of the major organs, only the kidney weights differed between the treatment groups (Table 5). Kidney weights were lower ($P = 0.003$) in the rats fed KPC compared to casein. Studies have shown that feeding different protein sources influences mineral deposition in the kidneys.

Table 5
Kidney weights, serum measurements of kidney function, and serum lipid profile of rats fed either 10% krill protein concentrate or casein[a]

Measurement	Casein	KPC
Kidney weights		
Absolute kidneys weight (mg)	2.1 ± 0.05	1.9 ± 0.04*
Relative kidneys weight (mg/100g body weight)	897.2 ± 18.2	780.7 ± 20.2*
Kidney function		
Serum total protein (U/L)	6.0 ± 0.7	6.3 ± 1.0
Serum albumin (U/L)	4.2 ± 0.5	4.1 ± 0.9
Serum BUN (U/L)	12.1 ± 1.3	10.1 ± 3.2
Serum creatinine (U/L)	0.5 ± 0.15	0.5 ± 0.1
Serum calcium (U/L)	11.5 ± 1.3	11.4 ± 2.7
Serum phosphorus (U/L)	11.3 ± 0.4	10.7 ± 0.5
Serum lipid profile		
Triglycerides (mg/dl)	218.6 ± 24.6	235.1 ± 19.3
Total cholesterol (mg/dl)	97.9 ± 6.5	67.9 ± 11.0*
VLDL (mg/dl)	43.7 ± 4.9	47.1 ± 3.9
LDL (mg/dl)	38.1 ± 8.7	31.8 ± 5.2
HDL (mg/dl)	95.2 ± 7.3	66.3 ± 3.7*

KPC, krill protein concentrate; BUN, blood urea nitrogen; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.
* Indicates significant difference with $P < 0.05$ by t-test.
[a] Values are given as mean ± SEM of $n = 10$.

Zhang and Beynen (1992) provided rats with casein, soybean, or cod meal and observed that the female rats fed ~18% casein had the highest kidney Ca concentration. In our study, total mineral content of the kidney was higher ($P < 0.001$) in rats fed the casein (0.3 g/g kidney ± 0.04) compared to rats fed KPC (0.07 g/g kidney ± 0.01). Kidney Ca content was higher ($P = 0.002$) in rats fed casein (7.0 mg/g kidney ± 0.9) than rats fed KPC (0.2 mg/g kidney ± 0.05). Similarly, kidney P content was also higher ($P < 0.001$) in rats fed casein (5.8 mg/g kidney ± 0.6) compared to KPC fed rats (1.0 mg/g kidney ± 0.2). Our study results suggest that KPC may protect against kidney calcification and mineralization.

Higher ($P = 0.03$) urinary output was observed in rats fed KPC (14.5 ml/d ± 3.0) compared to rats fed casein (6.0 ml/d ± 2.2). However, no difference was observed in kidney function as indicated by the absence of statistical differences in serum creatinine, blood urea nitrogen, total protein, Ca, and P in the rats fed KPC compared to the rats fed casein (Table 5). Despite differences in kidney mineralization and urinary output, there were no differences in kidney function between rats fed KPC and casein. Studies of longer duration may be needed to confirm whether the changes in kidney weight accompanying KPC consumption were protective.

In conclusion, the large biomass of krill makes it an economical and sustainable alternative to current commercially available protein sources. The isoelectric solubilzation/precipitation method enabled protein from krill to be successfully recovered. Nutritional evaluation of the recovered KPC indicates it is a high quality protein that is suitable as a protein source in foods designated for adult and infant consumption based on the ability to support growth, and a PDCAAS and PER score equal to the milk protein, casein. In addition, KPC also had the advantage of being high in ω-3 PUFAs and was shown to be safe to consume. Based on the current study, KPC appears to be a nutritious and safe food source for human consumption. Further studies are needed to determine the health benefits associated with consuming KPC.

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