DOI:10.1021/jf9036408

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Determination of Digestibility, Tissue Deposition, and Metabolism of the Omega-3 Fatty Acid Content of Krill Protein Concentrate in Growing Rats

Kayla M. Bridges, Joseph C. Gigliotti, Stephanie Altman, Jacek Jaczynski, and Janet C. Tou*

Human Nutrition and Foods, Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, West Virginia 26506

Krill protein concentrate (KPC) consists of high-quality protein (77.7% dry basis) and lipids (8.1% dry basis) that are rich (27% of total fatty acids) in omega-3 polyunsaturated fatty acids (ω-3 PUFAs). The objective of the study was to determine digestibility, tissue deposition, metabolism, and tissue oxidative stability of the ω-3 PUFAs provided by KPC. Young female Sprague−Dawley rats (n = 10/group) were fed ad libitum isocaloric diets for 4 weeks with either 10% freeze-dried KPC or 10% casein. The casein diet contained 5.3% added corn oil (CO), whereas the KPC contained 5.3% total lipids from 0.9% krill oil (KO) provided by KPC and 4.4% added corn oil (KO + CO). Fatty acid compositions of various tissues were analyzed by gas chromatography. Lipid peroxidation was determined by thiobarbituric acid reactive substances (TBARS). Total antioxidant capacity and urinary eicosanoid metabolites were determined by enzyme immunoassay. The ω-3 PUFAs provided in KO from KPC increased (P = 0.003) docosahexaenoic acid (DHA) concentration in the brain. DHA and eicosapentaenoic acid (EPA) content in fat pads and liver were increased (P < 0.01), whereas the ω-6 PUFA, arachidonic acid (AA), was decreased (P < 0.01) in rats fed the KPC diet containing the KO + CO mixture compared to rats fed the casein diet containing pure CO. Feeding the KPC diet decreased pro-inflammatory 2-series prostaglandin and thromboxane metabolites. There was no significant difference in TBARS or total antioxidant capacity in the tissues of rats fed the different diets. On the basis of the study results, the low amount of ω-3 PUFAs provided by the KO content of KPC provides beneficial effects of increasing tissue EPA and DHA deposition and reduced AA-derived 2-series eicosanoid metabolites without increasing lipid peroxidation. Therefore, consumption of KPC has the potential to provide a healthy and sustainable source of ω-3 PUFAs.

KEYWORDS: Krill protein concentrate; ω-3 PUFAs; docosahexaenoic acid; eicosapentaenoic acid; arachidonic acid

INTRODUCTION

Krill is a marine crustacean with the most abundant animal biomass on Earth (*1*). On the basis of body size, krill has the highest content of protein (> 65% dry weight) among all organisms (*2*). Lipid analysis showed that ~19% of the fatty acids in Antarctic krill (*Euphausia superba*) consisted of omega-3 polyunsaturated fatty acids (ω-3 PUFAs), with eicosapentaenoic acid (EPA, 20:5 ω-3) and docosahexaenoic acid (DHA, 22:6 ω-3) being particularly abundant (*2*). Furthermore, mercury concentrations in Antarctic krill are reportedly low (*3*). Yet, krill has remained virtually untapped as a food source for human consumption.

Commercial development of krill as a food has been hindered by the lack of an effective technology for meat recovery from krill. Using isoelectric solubilization/precipitation, krill protein concentrate (KPC) was recovered (*4*). KPC consisted of 77.7% crude protein (dry basis) with protein quality determined to be similar to the milk protein, casein (*5*). Jacques et al. (*6*) reported reduced circulating plasma cholesterol concentration in laboratory animals fed protein derived from seafood compared to the milk protein, casein. The dietary combination of seafood protein and lipid has been suggested to be synergistic (*7*). KPC also contains a small amount of lipid (8.1% dry weight). Analysis of the lipids associated with KPC showed that 27% of the fatty acids were ω-3 PUFAs. Of the ω-3 PUFAs, EPA accounted for 12.7% and DHA for accounted for 12.3% (*5*).

The ω-3 PUFAs in krill have been reported to be in both phospholipid (PL) and triacylglycerol (TAG) form (*8*). The structural form of fatty acids may affect bioavailability because PLs are digested differently from TAGs. Rats fed DHA as egg PLs showed better apparent absorption indicated by lower fecal excretion of DHA and higher feed efficiency ratios than rats fed DHA in TAG form (*9*). In turn, this may affect ω-3 PUFA incorporation into tissues. Valenzuela et al. (*10*) reported that feeding female rats DHA supplemented in the form of egg PLs resulted in higher accretion of DHA in the liver compared to

*Address correspondence to this author at the Division of Animal and Nutritional Sciences, West Virginia University, P.O. Box 6108, Morgantown, WV 26506 [telephone (304) 293-2631, ext. 4437; fax (304) 293-2232; e-mail janet.tou@mail.wvu.edu].

 Published on Web 02/04/2010

supplementation in TAG form. In contrast, Song and Miyazawa (*11*) found that rats fed DHA in PL form resulted in lower incorporation in the liver than rats fed DHA in TAG form. Therefore, it is important to determine the structural form of fatty acids provided by KPC and their bioavailability.

Tissue incorporation of ω-3 PUFAs from KPC is important because EPA and DHA exert various health benefits. The ω-3 PUFA, EPA, competes with arachidonic acid (AA) for enzymes involved in the synthesis of eicosanoids. The long-chain omega-6 polyunsaturated fatty acid (ω-6 PUFA) AA (20:4 ω-6) is the precursor of the 2-series eicosanoids (i.e., prostanoids and thromboxanes). The 2-series prostanoids are pro-inflammatory, and 2-series thromboxanes facilitate platelet aggregation and vasoconstriction. At high concentrations, EPA competitively inhibits the 2-series eicosanoids, resulting in reduced inflammation, platelet aggregation, and vasodilation (*12*). On the other hand, at high concentrations the ω-3 PUFAs, EPA and DHA, are highly susceptible to lipid peroxidation due to their high degree of unsaturation (*13*). Song and Miyazawa (*11*) demonstrated that increased levels of tissue DHA increased thiobarbituric acid reactive substances (TBARS), but not when provided in PL form. Therefore, the objective of this study was to determine digestibility, tissue deposition, metabolism, and oxidative stability of the ω-3 PUFAs provided by KPC.

MATERIALS AND METHODS

Diets. Whole frozen Antarctic krill (*Euphausia superba*) was purchased from Krill Canada (Langley, BC, Canada). KPC was recovered from whole krill using an isoelectric solubilization/precipitation method (*4*). The proximate composition of recovered KPC after freeze-drying was 77.7% crude protein and 8.1% total lipid on a dry basis (*5*). Diets were based on a standard purified AIN-93G diet (*14*). **Table 1** shows the diet ingredients and modifications of the AIN-93G diet. Dietary modifications consisted of providing 10% protein as KPC or casein and replacing soybean oil with corn oil. Diets were adjusted to be isocaloric and isonitrogenous with both diets containing 5.3% total lipid. The casein diet contained 5.3% corn oil (CO), whereas the KPC contained 5.3% total lipids from the 0.9% KO provided by KPC and 4.4% added corn oil (KO + CO). Diets containing KPC were prepared weekly and stored at 4 °C.

Animal Feeding Study. All animal procedures were approved by the Animal Care and Use Committee at West Virginia University and were conducted in accordance with the guidelines set forth by the Institute of Laboratory Animal for the Care and Use of Laboratory Animals (*15*). Young (28 day) growing female Sprague−Dawley rats were purchased from Taconic Farms (Rockville, MD). Upon arrival at the animal care facility, rats were individually housed in metabolic cages throughout the study duration (4 weeks) to determine food intake and to collect urine and feces. Rats were kept caged in rooms maintained 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, Indianapolis, IN). Following the 14 day acclimation period, rats (n = 10/group) were randomly assigned to be fed ad libitum either 10% casein with 5.3% CO or KPC with 0.9% KO and 4.4% added CO (KO + CO). Food intake was measured biweekly. At the end of the 4 week study, rats were euthanized by CO_2 inhalation. Brain, liver, and retroperitoneal and gonadal fat pads were dissected and weighed.

Digestibility. The apparent digestibility of dietary lipid was determined using fecal samples collected during the final week of the 4 week study. Fecal samples were freeze-dried for 48 h. Total fecal lipid content was determined by Soxhlet extraction. Apparent digestibility (%) was determined according to the method of Deuchi et al. (*16*) using the formula [(lipid intake − fecal lipid)/(lipid intake)] × 100. Similarly, apparent digestibility (%) of individual fatty acids was determined using the formula [(fatty acid intake − fecal fatty acids)/(fatty acid intake)] × 100.

Fatty Acid Analysis of Oils and Lipid Classes. Lipids were extracted from the diet, various tissues, and feces according to the method by Bligh and Dyer (*17*). All samples were conducted in duplicates. Samples were weighed, and 48 μL of heptadecenoic acid (17:1) was added as an internal standard. Weighed samples were added to Tris/EDTA buffer, pH 7.4. A chloroform/methanol/acetic acid (2:1:0.15 v/v/v) solution was added to the samples. Samples were centrifuged at 900*g* for 10 min at 10 °C, and the bottom chloroform layer was collected. The collected chloroform was then filtered through one-phase separation filters to remove any remaining water and precipitated material. The remaining layer was then mixed with chloroform/methanol (4:1 v/v) and centrifuged at 900*g* for 10 min at 10 °C. The chloroform layer was collected and filtered. The extracted lipid was dried under nitrogen gas.

Table 1. Composition of Diets

ingredient	casein, corn oil[a] (g/kg of diet)	KPC, krill oil + corn oil[a] (g/kg of diet)
casein	115	0
KPC[b]	0	128
DL-methionine	1.5	0
sucrose	531.8	609.8
corn starch	200	229.4
corn oil	53.5	49.9
cellulose	52	59.6
vitamin mix[c]	10	11.5
ethoxyquin	0.01	0.01
mineral mix[c]	13.4	15.3
calcium phosphate	20.2	20.9
calcium carbonate	2.6	3.6
gross energy (kcal/g)	4.3	4.3

[a] The KPC diet contains a mixture of 0.9% krill oil and 4.4% corn oil. The casein diet contains 5.3% corn oil. [b] KPC, krill protein concentrate. Diet formulated for 872g of diet + 128g addition of KPC. [c] Based on the AIN-93G diet vitamin and mineral mixes Reeves et al. (*14*).

The extracted lipid samples were transmethylated following the procedure described by Fritsche and Johnston (*18*). Briefly, extracted fatty acids were methylated by adding 4% H_2SO_4 in anhydrous methanol to the dried lipid samples followed by incubation in a 90 °C water bath for 60 min. Samples were cooled to room temperature, and 3 mL of ddH_2O was added to stop the reaction. Chloroform was added to the methylated sample. Samples were dried under nitrogen gas, and iso-octane (3 mL) was used as a diluent.

The methylated lipid samples were analyzed by gas chromatography (CP-3800, Varian Inc., Walnut Creek, CA) using an initial temperature of 140 °C held for 5 min and then increased at 1 °C/min to a final temperature of 220 °C. A wall-coated open tubular fused silica capillary column (Varian Inc.) was used to separate fatty acid methyl esters with CP-Sil 88 as the stationary phase. Nitrogen was used as the carrier gas, and total separation time was 110 min. Quantitative 37 component fatty acid methyl ester Sigma Mix (Supelco, Bellefonte, PA) was used to identify fatty acid composition. Fatty acids were identified using retention time and peak area counts.

Thin-layer chromatography (TLC) was performed to separate the lipid classes. The oil sample (10 mg) was dissolved in chloroform/methanol (1:1 v/v) and spotted onto Whatman K6F silica plates with 60 Å pore size (PJ Cobert Associates, St. Louis, MO). Plates were developed using a hexane/ether/acetic acid solution (80:20:1.5 v/v/v) as the mobile phase. Plates were air-dried for 5 min and observed by Fluorchem 8000 densitometer (Alpha Innotech Corp., San Leandro, CA) using transluminating white light. Plate images were captured using a camera interfaced to a PC, and images were analyzed using the spot densitometer tab in the Fluorchem (version 1.0) computer program. PL and TAG were identified using R_f values obtained from triolein (Sigma-Aldrich, St. Louis, MO) and soybean lecithin (Fisher Scientific, Pittsburgh, PA) standards. PL and TAG bands were scraped from the plates and suspended in chloroform/methanol (1:1 v/v). To determine the fatty acid composition of the TAG and PL, samples were methylated and analyzed by gas chromatography as described above for dietary oil fatty acid composition.

Thiobarbituric Acid Reactive Substances (TBARS). Tissue TBARS were determined in homogenates of brain, liver, and retroperitoneal and gonadal fat pads. To determine urinary TBARS, rats were individually housed in metabolic cages to allow for urine collection. Urine samples were collected during the final week of the 4 week feeding study.

Table 2. Fatty Acid Composition of Oil and Lipid Classes of Diets Fed to Young Female Rats[a]

	KPC with 0.9% KO + 4.4% CO			casein with 5.3% CO	
fatty acid	FA content (mg/mL)	FA-TAG[b] (%)	FA-PL[b] (%)	FA content (mg/mL)	FA-TAG[c] (%)
ω-6 PUFAs					
18:2 (ω-6), LA	2.92 ± 0.24	0.31 ± 0.24	47.4 ± 0.24	4.5 ± 0.20	56.1 ± 0.86
20:4 (ω-6), AA	0.016 ± 0.002	0.23 ± 0.24	0.06 ± 0.24	ND	ND
ω-3 PUFAs					
18:3 (ω-3), ALA	0.40 ± 0.03	0.85 ± 0.24	0.81 ± 0.24	0.09 ± 0.04	0.90 ± 0.02
20:5 (ω-3), EPA	0.40 ± 0.03	ND	0.27 ± 0.24	ND	ND
22:6 (ω-3), DHA	0.27 ± 0.02	12.7 ± 0.24	0.03 ± 0.24	ND	ND

[a] Values are given as mean ± SEM of triplicate samples. Abbreviations: ND, nondetectable; KO, krill oil; CO, corn oil; PUFAs, polyunsaturated fatty acids; FA, fatty acid; TAG, triacylglycerol; PL, phospholipid; LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. [b] 0.9% KO + 4.4% CO consists of 22 ±0.2% phospholipids and 61 ± 1.2% triacylglycerol. [c] 5.3% CO consists of 67.1 ±1.9% triacylglycerol and non-detectable phospholipids.

Collected urine samples were centrifuged at 1500*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 for lipid peroxidation. TBARS were measured using a commercially available enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). Briefly, tissue homogenate or urine samples were mixed with sodium dodecyl sulfate solution and a color reagent containing thiobarbituric acid, acetic acid, and sodium hydroxide. Samples were incubated for 60 min in a 90 °C water bath and then incubated in an ice bath for 10 min to stop the reaction. Following centrifugation for 10 min at 1600*g* at 4 °C, absorbance was read at 540 nm using a Spectramax Plus microplate reader (Molecular Devices, Sunnyvale, CA). Values were expressed as micromolar malondialdehyde (μM MDA).

Total Antioxidant Capacity. Urine samples were collected during the final week of the 4 week feeding study. Collected urine samples were centrifuged at 1500*g* for 10 min at 4 °C. Urine samples were aliquoted into fresh tubes and stored at −80 °C until assayed for total antioxidant capacity. Total antioxidant capacity in urine samples was measured according to a commercially available Antioxidant Assay EIA kit (Cayman Chemical). Briefly, urine samples were diluted 1:20 v/v with 5 mM potassium phosphate buffer containing 0.9% sodium chloride and 0.1% glucose at pH 7.4. In a 96-well microplate, duplicate diluted samples were mixed with the water-soluble tocopherol analogue, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and metmyoglobin and 2,2′-azinobis(3-ethylbenzthiazoline sulfonate). To initiate the reaction, hydrogen peroxide was added to each well. Samples were then incubated at room temperature on a shaker for 5 min. Absorbance was read at 750 nm using a Spectramax Plus microplate reader. Values were expressed as micromolar Trolox.

Eicosanoid Metabolite Measurements. The unstable eicosanoid PGE_2 is converted to the stable metabolite 13,14-dihydro-15-keto PGA_2, which can be easily quantified by EIA. Similarly, unstable TXA_2 is converted to the stable metabolite 11-dehydro TXB_2, which can be easily quantified by EIA. These prostaglandin and thromboxane metabolites were determined by commercially available EIA kits (Cayman Chemical). Briefly, urine samples were diluted 1:2 and in a 96-well microplate mixed with acetylcholinesterase conjugate tracer (50 μL) and rabbit antiserum (50 μL) specific to the metabolite being measured. The microplate was incubated on an orbital shaker for 18 h for the PGE_2 metabolite and for 2 h for the 11-dehydro TXB_2 at room temperature. Following incubation, the wells were washed (five times). Ellman's reagent (200 μL) was added to each well and the microplate covered and allowed to develop in the dark for 90 min. Absorbance was read at 412 nm using a Spectramax Plus microplate reader.

Statistical Analysis. Student's *t* test was used to compare differences between diet groups. The Mann−Whitney rank sum test was performed on data not normally distributed. Differences were considered to be significant at $P < 0.05$. Results are expressed as mean ± standard error of the mean (SEM). Results were analyzed using SigmaStat 3.1 statistical software program (Systat Software Inc., San Jose, CA).

RESULTS

Lipid Composition of Diets. Diets were based on a modification of the standard purified AIN-93G diet (*14*). Modification of diet ingredients consisted of reducing protein content to 10% and replacing casein with KPC as the crude protein source. Another dietary modification was the replacement of soybean oil in the AIN-93G diet with corn oil. Diet ingredients were adjusted so that the diets were isocaloric and isonitrogenous and contained 5.3% total lipid (**Table 1**). The casein diet contained 5.3% corn oil (CO), whereas the KPC contained a mixture of 0.9% krill oil (KO) provided by KPC with 4.4% added corn oil (KO + CO). Shown in **Table 2**, pure CO is rich in the essential ω-6 fatty acid and precursor of long-chain ω-6 PUFAs linoleic acid (LA, 18:2 ω-6). CO also contains the essential ω-3 fatty acid and precursor of long-chain ω-3 PUFAs α-linolenic acid (ALA, 18:3 ω-3). The contents of the long-chain ω-6 PUFA AA and long-chain ω-3 PUFAs EPA and DHA were negligible in CO. However, adding 0.9% KO from KPC to 4.4% CO resulted in measurable amounts of AA, EPA, and DHA.

Separation of the lipid classes by TLC showed that CO was composed of 67.1 ± 1.9% TAGs and no detectable PLs. The KO + CO mixture was composed of 22 ± 0.2% PLs and 61 ± 1.2% TAGs. **Table 2** shows the fatty acids of these different lipid classes. LA was associated with TAG in the casein diet with CO. In contrast, LA was predominantly in PL in the KPC diet containing KO + CO. However, the long-chain ω-6 PUFA AA was predominantly in TAG in the KO + CO mixture. ALA was associated with TAG in the casein diet containing pure CO and equally distributed between PL and TAG in the KPC diet containing KO + CO. The long-chain ω-3 PUFA EPA was associated with PL, whereas DHA was predominantly in TAG in the KPC diet.

Digestibility of Dietary Fatty Acids. There was no significant difference in food intake for rats fed the KPC diet containing the KO + CO mixture (16.1 ± 0.4 g/day) compared to rats fed the casein diet containing pure CO (16.5 ± 0.4 g/day). There were no significant differences in total lipid intake, total lipid content of feces, or apparent digestibility of total dietary lipids between the diet groups. However, there were differences in digestibility of the individual fatty acids. In **Table 3**, apparent digestibility of the ω-6 fatty acid LA was significantly decreased ($P = 0.02$) in rats fed the KPC diet containing the KO + CO mixture compared to rats fed the casein diet containing pure CO. Rats fed the KPC diet had a lower ($P = 0.001$) intake of LA, but no difference in fecal excretion of LA compared to rats fed casein containing CO. In rats fed the casein diet containing CO only, the content of the long-chain ω-6 PUFA AA was negligible. Rats fed the KPC diet showed low (22.75 ± 6.45%) apparent digestibility of AA.

Of the ω-3 fatty acids, the apparent digestibility of ALA was not statistically different between the diet groups despite rats fed the KPC diet containing the KO + CO mixture having higher ($P < 0.05$) intake of ALA than rats fed casein containing pure CO. Fecal excretion of ALA by rats fed the KPC diet containing the KO + CO mixture was higher, although not statistically, compared

Table 3. Apparent Digestibility of Lipids and Fatty Acids in Young Female Rats Fed Casein Diet Consisting of Corn Oil or Krill Protein Concentrate Diet Consisting of Krill Oil and Corn Oil Mix[a]

	intake (mg)		fecal excretion (mg)		apparent digestibility (%)	
fatty acid	KPC, KO + CO	casein, CO	KPC, KO + CO	casein, CO	KPC, KO + CO	casein, CO
ω-6 PUFAs						
LA (18:2 ω-6)	303.13 ± 6.17*	346.50 ± 13.79	17.34 ± 3.66	10.68 ± 2.54	93.76 ± 1.06*	96.93 ± 1.06
AA (20:4 ω-6)	1.27 ± 0.03	ND	0.97 ± 0.20	ND	22.75 ± 6.45	ND
ω-3 PUFAs						
ALA (18:3 ω-3)	6.32 ± 0.13*	5.49 ± 0.22	2.88 ± 0.65	2.07 ± 0.41	49.02 ± 10.58	62.86 ± 6.26
EPA (20:5 ω-3)	13.28 ± 0.27	ND	3.60 ± 0.57	ND	70.12 ± 3.52	ND
DHA (22:6 ω-3)	12.89 ± 0.29	ND	0.77 ± 0.21	ND	93.42 ± 1.64	ND
total lipids (g)	6.16 ± 0.13	5.91 ± 0.24	0.78 ± 0.11	0.76 ± 0.10	86.10 ± 1.36	87.17 ± 1.36

[a] Values are given as mean ± SEM of $n = 10$ rats/group. * indicates significant difference at $P < 0.05$ by Student's t test. Abbreviations: ND, nondetectable; KPC, krill protein concentrate; KO, krill oil; CO, corn oil; PUFAs, polyunsaturated fatty acids; LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 4. Fatty Acid Analysis of Tissues of Young Female Rats Fed Casein Diet Consisting of Corn Oil or Krill Protein Concentrate Diet Consisting of Krill Oil and Corn Oil Mix[a]

	brain (mg/g of tissue)		liver (mg/g of tissue)		retroperitoneal fat pad (mg/g of tissue)		gonadal fat pad (mg/g of tissue)	
fatty acid	KPC, KO + CO	casein, CO	KPC, KO + CO	casein, CO	KPC, KO + CO	casein, CO	KPC, KO + CO	casein, CO
ω-6 PUFAs								
LA (18:2 ω-6)	0.20 ± 0.06	0.18 ± 0.041	3.38 ± 0.29	3.24 ± 0.25	38.25 ± 3.25	43.51 ± 4.12	58.06 ± 6.25	60.79 ± 3.74
AA (20:4 ω-6)	1.99 ± 0.16	2.09 ± 0.28	1.94 ± 0.10*	3.94 ± 0.15	0.32 ± 0.12*	0.90 ± 0.09	0.69 ± 0.11	1.04 ± 0.12
ω-3 PUFAs								
ALA (18:3 ω-3)	0.15 ± 0.06	0.21 ± 0.07	ND	ND	1.38 ± 0.12	0.99 ± 0.15	1.54 ± 0.15	1.67 ± 0.13
EPA (20:5 ω-3)	ND	ND	0.76 ± 0.04*	ND	0.74 ± 0.19*	ND	0.87 ± 0.26*	ND
DHA (22:6 ω-3)	2.85 ± 0.24*	2.46 ± 0.37	1.67 ± 0.10*	0.72 ± 0.026	0.78 ± 0.19*	ND	0.92 ± 0.36*	ND

[a] Values are given as mean ± SEM of $n = 10$ rats/group. * indicates significant difference at $P < 0.05$ by Student's t test. Abbreviations: ND, nondetectable; KPC, krill protein concentrate; KO, krill oil; CO, corn oil; PUFAs, polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

to rats fed the casein diet containing pure CO. In rats fed the casein diet containing CO, the content of the long-chain ω-3 PUFAs EPA and DHA was negligible. Rats fed the KPC diet showed moderate (70.12 ± 3.52%) apparent digestibility of EPA and high (93.42 ± 1.64%) apparent digestibility of DHA.

Body Weight, Organ Weights, and Fatty Acid Analysis of Tissues. At the end of the 4 week study, there was no significant difference in the final body weight of rats fed the KPC diet (242 ± 3 g) compared to rats fed the casein diet containing CO (235 ± 6 g). There was no significant difference in brain weight between rats fed the KPC diet (1.63 ± 0.03 g) compared to rats fed the casein containing CO (1.69 ± 0.03 g). There was no significant difference in liver weight between rats fed the KPC diet (10.1 ± 0.2 g) and rats fed the casein diet containing CO (9.3 ± 0.3 g). Rats fed the KPC diet had higher ($P = 0.02$) retroperitoneal fat pad weight (3.33 ± 0.26 g) than rats fed the casein diet containing CO (2.54 ± 0.16 g). Rats fed the KPC diet had higher ($P = 0.03$) gonadal fat pad weight (6.28 ± 0.24 g) than rats fed the casein diet containing CO (5.34 ± 0.32 g).

According to **Table 4**, there were no significant differences in brain, liver, or fat pad LA content among the diets despite different fatty acid compositions of the dietary oils. Although AA content was higher in the KO + CO provided by the KPC diet, AA deposition was decreased in the liver ($P < 0.001$) and retroperitoneal fat pads ($P = 0.002$), and there was a tendency ($P = 0.056$) for lower AA deposition in the gonadal fat pads compared to rats fed the casein diet containing CO. No difference in AA brain deposition was found between the diet groups.

Of the ω-3 PUFAs, ALA showed no significant differences in brain, liver, or fat pad content among the diet groups. Rats fed the KPC diet providing 0.9% KO containing EPA resulted in increased tissue deposition of EPA in liver ($P < 0.001$) and retroperitoneal and gonadal fat pads ($P = 0.004$) compared to rats fed the casein diet with CO containing no EPA. EPA was not present in detectable amounts in brain tissue of either diet group. Rats fed the KPC diet with 0.9% KO containing DHA increased DHA in brain ($P = 0.003$), liver ($P < 0.001$), and retroperitoneal ($P = 0.003$) and gonadal ($P = 0.021$) fat pads compared to rats fed the casein diet with CO containing no DHA.

Oxidative Stability. In **Table 5**, rats fed the KPC diet containing the KO + CO mixture had decreased ($P = 0.03$) TBARS in the gonadal fat pads compared to rats fed the casein diet containing pure CO. There were no significant differences in retroperitoneal fat pads, liver, or brain TBARS between the diet groups. There were no significant differences in urinary TBARS or total antioxidant capacity concentrations between the diet groups. Correcting for creatinine, to limit variability due to changes in renal excretory function, resulted in no significant differences in urinary TBARS or total antioxidant capacity among the diet groups.

Eicosanoid Measurements. Efficacy in reducing the AA products, prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2), was determined. Unstable PGE_2 was converted to the stable metabolite 13,14-dihydro-15-keto PGA_2, which was measured in urine samples. There was no statistically significant difference in urinary 13,14-dihydro-15-keto PGA_2 concentration in rats fed the KPC diet containing the KO + CO mixture compared to rats fed the casein diet containing pure CO (**Figure 1A**). Correcting for creatinine resulted in reduced ($P = 0.009$) 13,14-dihydro-15-keto PGA_2 concentration in rats fed the KPC diet compared to rats fed the casein diet containing CO (**Figure 1B**).

Unstable TXA_2 was converted to the stable metabolite 11-dehydro TXB_2, which was measured in urine samples. There was no statistically significant difference in urinary 11-dehydro TXB_2

Table 5. TBARS and Total Antioxidant Capacity in Young Female Rats Fed Casein Diet Consisting of Corn Oil or Krill Protein Concentrate Diet Consisting of Krill Oil and Corn Oil Mix[a]

	KPC, KO + CO	casein, CO
TBARS		
gonadal fat pad TBARS (μM MDA/g of tissue)	1.14 ± 0.25*	2.00 ± 0.15
retroperitoneal fat pad TBARS (μM MDA/g of tissue)	2.30 ± 0.21	2.20 ± 0.23
liver TBARS (μM MDA/g of tissue)	13.40 ± 0.48	13.07 ± 0.76
brain TBARS (μM MDA/g of tissue)	5.57 ± 0.28	5.93 ± 0.38
urinary TBARS (μM MDA/day)	53.5 ± 11.8	56.7 ± 11
urine TBARS/creatinine (μM MDA/mg of creatinine)	11.7 ± 2.0	6.6 ± 1.5
total antioxidant capacity		
urinary total antioxidant capacity (μM Trolox/day)	3.5 ± 0.8	7.8 ± 2.3
urinary total antioxidants/creatinine (μM Trolox/mg of creatinine)	42.3 ± 11.9	30.2 ± 16.1

[a] Values are given as mean ± SEM of $n = 6-10$ samples. * indicates significant difference at $P < 0.05$ by Student's t test. Abbreviations: KPC, krill protein concentrate; CO, corn oil; KO, krill oil; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

concentration in rats fed the KPC diet containing the KO + CO mixture compared to rats fed the casein diet containing pure CO (**Figure 2A**). Correcting for creatinine resulted in reduced ($P = 0.009$) urinary 11-dehydro TXB_2 concentration in rats fed the KPC diet compared to rats fed the casein diet containing CO (**Figure 2B**).

DISCUSSION

Fatty acid bioavailability influences tissue incorporation and conversion to other fatty acids. The KPC diet provided EPA in PL form, which showed moderate (70.12 ± 3.52%) digestibility. DHA was predominantly in TAG form, which showed high (93.42 ± 1.64%) digestibility. This difference in digestibility may be due to intestinal hydrolysis of TAGs yielding 2-monoacylglycerols using enzymes other than PLs, which yields 1-lysophospholipids (*19*). Feeding growing rats KPC containing the KO + CO mixture resulted in higher ($P < 0.05$) DHA content in the brain compared to rats fed casein containing pure CO. Innis et al. (*20*) reported that feeding rats a diet composed of a 10% fish oil and 2% safflower oil blend (15.1% EPA, 7.3% DHA, 0.9% AA) for 4 weeks increased brain EPA and DHA and decreased AA content compared to rats fed 12% pure safflower oil. In our study, rats fed the KPC diet containing 0.9% KO + 4.4% added CO (12.5% EPA, 12.7% DHA, 1.5% AA) for 4 weeks did not lead to detectable EPA content in the brain. EPA is typically found in low to nondetectable amounts in brain tissue (*21*). However, rats fed the KPC diet had increased DHA without decreasing AA content in the brain. This is important because DHA in conjunction with AA is considered necessary to support proper brain development (*20*, *22*). In our study, DHA and AA were also detectable in the brains of rats fed the CO, despite the negligible content in the oil source. According to Rapoport et al. (*23*), DHA content in the brain can be maintained in the absence of dietary DHA by liver conversion of ALA to circulating DHA. In the present study, the fatty acid composition of the liver was also determined because it is the main site of fatty acid synthesis and conversion (*21*).

The body can synthesize EPA and DHA provided sufficient ALA is consumed in the diet. In our study, detectable DHA in the liver tissue of rats fed the casein diet with DHA-deficient CO as the oil source suggests conversion from ALA. In rats fed KPC, liver ALA content was negligible despite higher ALA content in the KPC diet containing KO + CO mixture compared to the casein diet containing CO only. ALA digestibility was 49.02 ± 10.58% in rats fed the KPC diet compared to 62.86 ± 6.26% digestibility in rats fed casein diet with pure CO. Lower digestibility in the KPC diet may be due to some ALA being distributed in PL form. Also, decreased ALA may be due to conversion to EPA and DHA. Werner et al. (*24*) found that feeding ALA in the PL form compared to the TAG form increased DHA content in the liver. In our study, DHA and EPA contents were increased in the liver of rats fed KPC. Therefore, reduced liver ALA in rats fed the KPC diet may be due to the poor digestibility of ALA in PL form as well as efficient conversion of ALA in PL form to the long-chain ω-3 PUFAs EPA and DHA.

The casein diet containing CO was rich in LA but negligible in AA. Detectable AA in the liver tissue of rats fed the casein diet with AA-deficient CO as the oil source suggests conversion from LA. In rats fed KPC diet, liver AA concentration was lower ($P < 0.05$) despite higher AA content in the KO + CO mixture compared to the casein diet containing pure CO. The AA provided by the KPC diet was predominantly distributed in TAGs and had low (22.75 ± 6.45%) digestibility. However, the body can synthesize AA provided sufficient LA is consumed in the diet. Rats fed the KPC diet had lower ($P = 0.001$) intake and reduced ($P = 0.02$) digestibility of LA compared to the rats fed the casein diet containing CO. In the KPC diet, LA was predominantly in PL form, whereas LA was in TAG form in the casein diet containing CO. Additionally, tissue AA content is affected by EPA and DHA due to competition for the sn-2 position of PLs in cell membranes (*25*). Liver EPA and DHA contents were increased in rats fed the KPC diet. Therefore, reduced liver AA in rats fed the KPC diet may be due to the poor digestibility of LA and AA and possibly competitive inhibition associated with higher EPA and DHA tissue incorporation.

Froyland et al. (*26*) found that feeding male rats a diet containing 94% of total fatty acids as pure EPA in the form of ethyl esters increased liver EPA 17-fold compared to CO. The authors also found that feeding 91% of total fatty acids as pure DHA in the form of ethyl esters increased liver DHA 3-fold compared to CO. In the current study, the EPA and DHA from 0.9% KO individually contributed to < 0.01% of the total fatty acids. Despite this low amount, the long-chain ω-3 PUFAs provided from KPC were capable of increasing EPA and DHA and lowering liver AA concentrations in the liver. Lower AA content in the liver, but not the brain, may have resulted from the liver being the major site for fatty acid synthesis, whereas fatty acid uptake occurs in peripheral tissues. Studies have reported that biosynthesis of fatty acids resulted in preferential accretion in some tissues (i.e., brain, retina, and skeletal muscles) and efflux from tissues known to process and/or store fatty acids (*27*).

In the present study, the adipose tissue was also analyzed because dietary fatty acids not incorporated into membranes or metabolized are stored in adipose tissue as TAG. Therefore, adipose tissue reflects the dietary intake of fatty acids. In human adipose tissue, LA is the most abundant ω-6 PUFA at ~12−16% of fatty acids and ALA is the most abundant ω-3 PUFA at

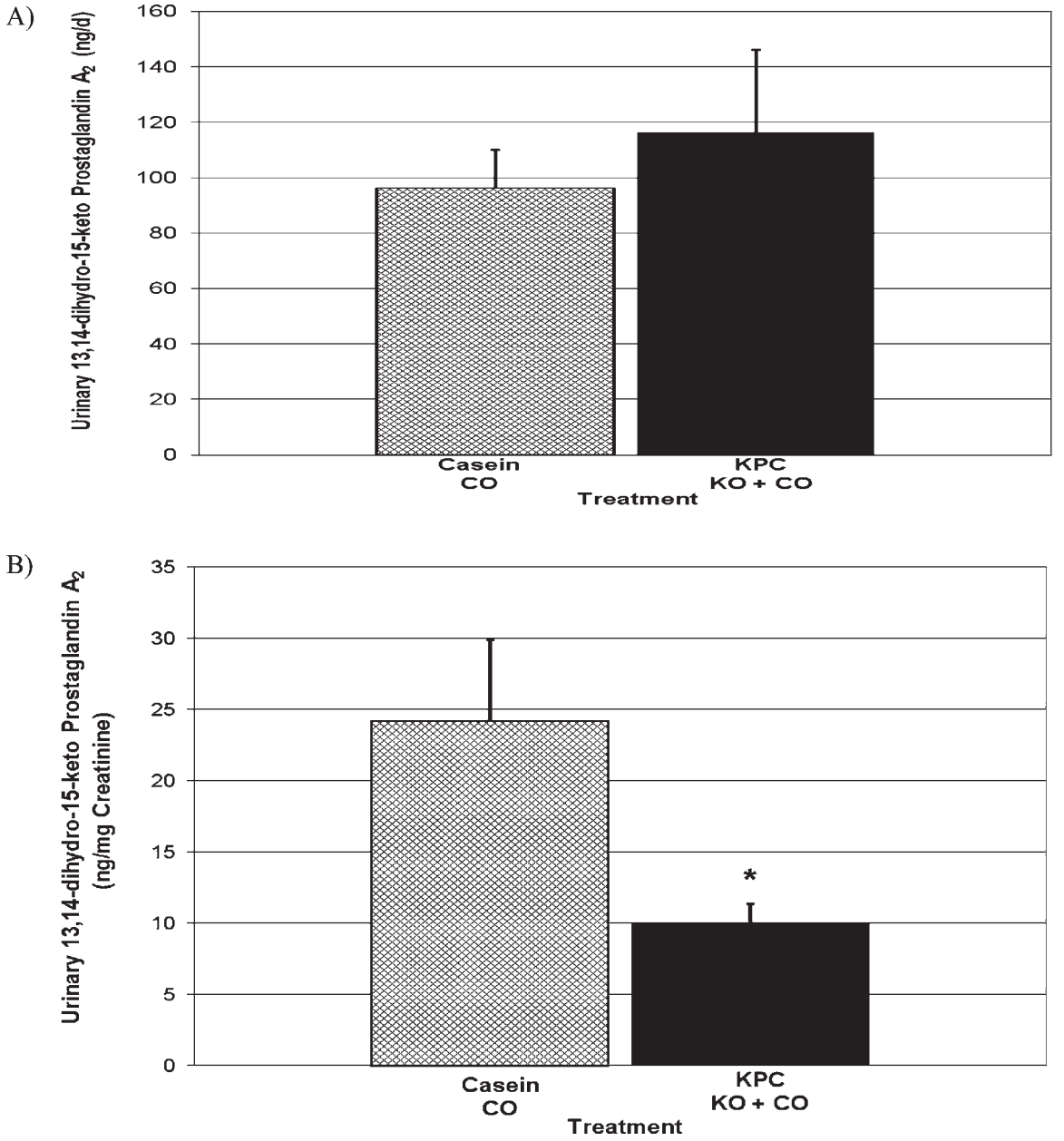

Figure 1. Effect of feeding young female rats diets consisting of casein containing 5.3% corn oil (CO) or krill protein concentrate (KPC) containing 0.9% krill oil and 4.4% added corn oil (KO + CO) on PGE_2 metabolite: (**A**) urinary 13,14-dihydro-15-keto PGA_2; (**B**) urinary 13,14-dihydro-15-keto PGA_2 corrected for creatinine to limit variability due to changes in renal excretory function. Values are given as mean ± SEM of $n = 6$ rats/group. * indicates significant difference at $P < 0.05$ by Student's t test.

~1% (*21*). In our animal study, LA and ALA contents of fat pads were similar in rats fed both diets. EPA and DHA were increased in the fat pads of rats fed the KPC diet containing the KO + CO mixture compared to rats fed the casein diet containing pure CO. Detectable AA content in the fat pads of rats fed AA-deficient CO provided by the casein diet indicated conversion from LA. Lower AA incorporation in the fat pads of rats fed the KPC diet likely resulted from the poor digestibility of AA. Froyland et al. (*26*) found that feeding male rats a diet with 94% of total fatty acids as EPA ethyl esters increased epididymal fat pad EPA content 41-fold compared to CO. The authors also found that feeding 91% of total fatty acids as DHA ethyl esters increased epididymal fat pad DHA 11-fold compared to CO. In our study, the long-chain ω-3 PUFAs provided by KPC were capable of increasing EPA and DHA in the adipose tissue. The results suggested that even the small lipid content provided by KPC improves ω-3 PUFA status. This is important because large doses of pure oil or isolated fatty acids are often used in studies to increase EPA and DHA tissue incorporation (*28*). However, small doses of EPA and DHA and oil mixes rather than pure oils are more representative of the human diet.

The present study also determined whether the increased tissue ω-3 PUFA content observed with KPC consumption altered lipid metabolism. At high concentrations, ω-3 PUFAs competitively inhibit AA, the precursor of 2-series eicosanoids such as prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2) (*12*). The 2-series PGE_2 is pro-inflammatory, and TXA_2 promotes platelet aggregation and vasodilation. Therefore, reduction in tissue content of the long-chain ω-6 PUFA AA has been suggested for health benefits. On the basis of the current study, KPC providing EPA or DHA at the level of 0.01% of the total fatty acids in the diet decreased PGE_2 and TXA_2 metabolites

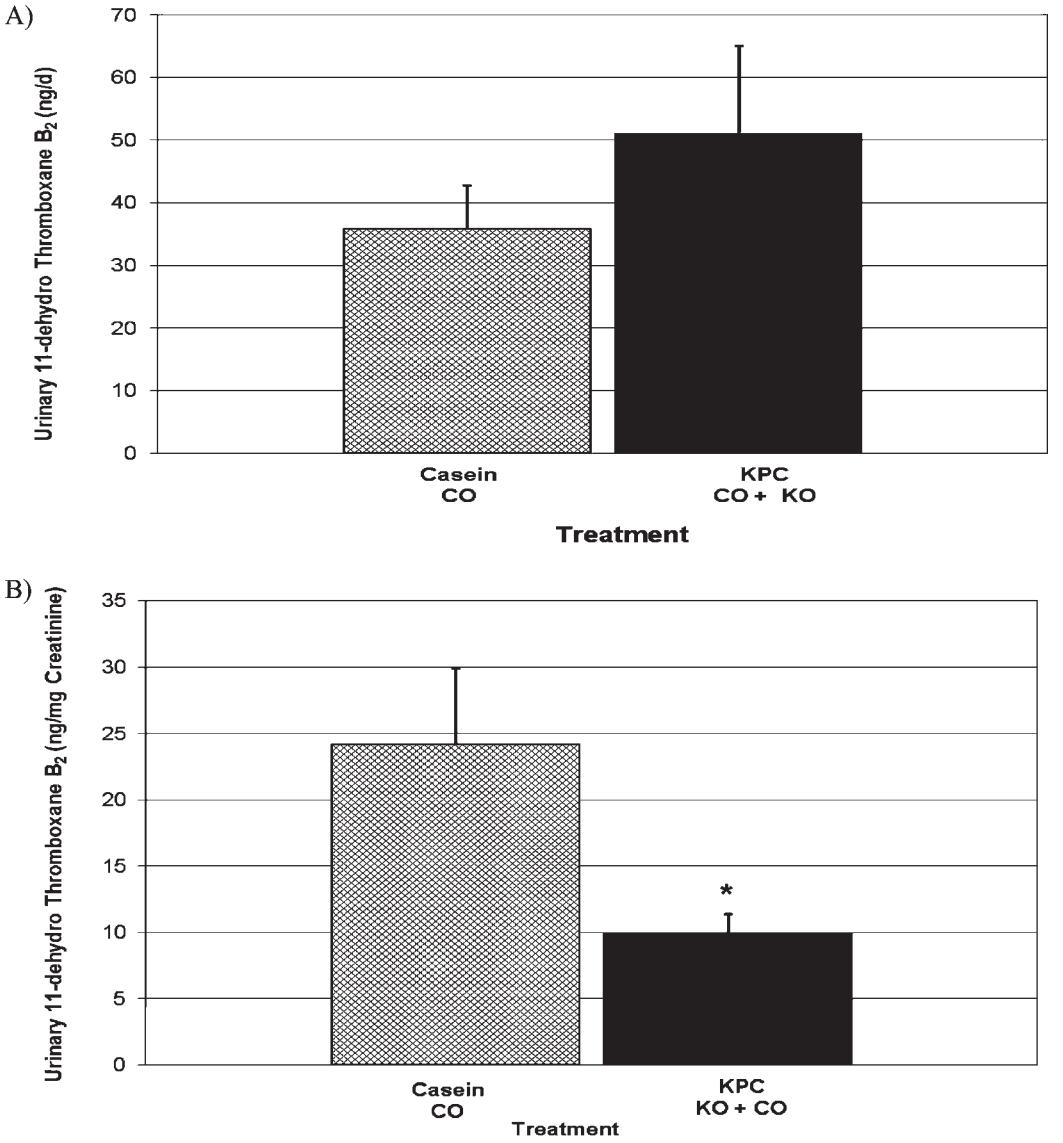

Figure 2. Effect of feeding young female rats diets consisting of casein containing 5.3% corn oil (CO) or krill protein concentrate (KPC) containing 0.9% krill oil and 4.4% added corn oil (KO + CO) on TXA_2 metabolite: (**A**) urinary 11-dehydro TXB_2; (**B**) urinary 11-dehydro TXB_2 corrected for creatinine to limit variability due to changes in renal excretory function. Values are given as mean ± SEM of $n = 6$ rats/group. ∗ indicates significant difference at $P < 0.05$ by Student's *t* test.

compared to the casein diet with CO. This is consistent with reports that supplementation with either EPA and/or DHA decreases pro-inflammatory eicosanoid production (*25*).

Providing health benefits at low doses is important to consider because EPA and DHA are highly susceptible to lipid peroxidation due to their high degree of unsaturation. Saito and Kubo (*29*) found that feeding rats 8.4% total energy as purified DHA for 30 days significantly increased lipid peroxidation indicated by increased TBARS. In our study, tissue incorporation of EPA and DHA in rats fed the KPC diet containing the KO + CO mixture did not increase tissue and urinary TBARS or decrease total antioxidant capacity compared to rats fed the casein diet containing pure CO. Song and Miyazawa (*11*) observed that increased levels of DHA in membrane PLs did not increase lipid peroxidation when DHA was provided in the PL form. In our study, DHA was predominantly in the TAG form. Regardless of the structural form, the ω-3 PUFA content of KPC did not contribute significantly to oxidative stress.

In summary, KPC containing 0.9% KO provides some fatty acids in PL form. Generally, fatty acid digestibility was greater in TAG than PL form. The EPA and DHA associated with the KO provided by KPC increased tissue concentrations despite individually contributing to < 0.01% of the total fatty acids in the diet. The lipid content (KO) provided by KPC increased long-chain ω-3 fatty acids and decreased AA tissue accretion and pro-inflammatory eicosanoid metabolites without changing oxidative stability. A source of ω-3 PUFAs that provides high tissue accretion with the least lipid oxidation favors maximal health benefits. Therefore, the study results indicated that consumption of KPC provides a healthy source of ω-3 PUFAs with the advantage of offering a sustainable source of ω-3 PUFAs due to its large biomass and underutilization as a food source for humans.

ABBREVIATIONS USED

ω-3 PUFAs, omega-3 polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ALA, α-linolenic acid; ω-6 PUFAs, omega-6 polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; KPC, krill protein concentrate; KO, krill oil; CO, corn oil; PL, phospholipid; TAG, triacylglycerol; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TXA_2, thromboxane A_2;

TXB_2, 11-dehydrothromboxane B_2; PGE_2, prostaglandin E_2; PGA_2, 13,14-dihydro-15-keto prostaglandin A_2.

ACKNOWLEDGMENT

We thank Kylash Konaur for assisting in the preparation of the fatty acid samples.

LITERATURE CITED

(1) Nicol, S.; James, A.; Pitcher, G. A first record of daytime surface swarming by *Euphausia lucens* in the Southern Benguela region. *Mar. Biol.* **1987**, *94*, 7–10.

(2) Kolakowska, A.; Kolakowska, E.; Szcygielski, M. Winter season krill (*Euphausia superba* Dana) as a source of n-3 polyunsaturated fatty acids. *Nahrung* **1994**, *38*, 128–134.

(3) Nygard, T.; Lie, E.; Rov, N.; Steinnes, E. Metal dynamics in the Antarctic food chain. *Mar. Pollut. Bull.* **2001**, *42*, 598–602.

(4) Chen, Y. C.; Tou, J. C.; Jaczynski, J. Amino acid and mineral composition of protein and other components and their recovery yields from whole Antarctic krill (*Euphausia superba*) using isoelectric solubilization/precipitation. *J. Food Sci.* **2009**, *74*, 31–39.

(5) Gigliotti, J.; Jaczynski, J.; Tou, J. C. Determination of the nutritional value, protein quality and safety of krill protein concentrate isolated using an isoelectric solubilization/precipitation technique. *Food Chem.* **2008**, *111*, 209–214.

(6) Jacques, H.; Gascon, A.; Bergeron, N.; Lavigne, C.; Hurley, C.; Deshaies, Y.; Moorjani, S.; Julien, P. Role of dietary fish protein in the regulation of plasma lipids. *Can. J. Cardiol.* **1995**, *11*, 63–71.

(7) Wergedahl, H.; Gudbrandsen, O. A.; Rost, T. H.; Berge, R. K. Combination of fish oil and fish protein hydrolysate reduces plasma cholesterol level in high-fat-fed Wistar rats. *Nutrition* **2009**, *25*, 98–104.

(8) Bottino, N. R. Lipid composition of two species of Antarctic krill: *Euphausia superba* and *E. crystalorophias*. *Comp. Biochem. Physiol. B* **1975**, *50*, 479–484.

(9) Amate, L.; Gil, A.; Ramírez, M. Dietary long-chain polyunsaturated fatty acids from different sources affect fat and fatty acid excretions in rats. *J. Nutr.* **2001**, *131*, 3216–3321.

(10) Valenzuela, A.; Nieto, S.; Sanhueza, J.; Nunez, M. J.; Ferrer, C. Tissue accretion and milk content of docosahexaenoic acid in female rats after supplementation with different docosahexaenoic acid sources. *Ann. Nutr. Metab.* **2005**, *49*, 325–332.

(11) Song, J. H.; Miyazawa, T. Enhanced level of n-3 fatty acid in membrane phospholipids induces lipid peroxidation in rats fed dietary docosahexaenoic acid oil. *Atherosclerosis* **2001**, *155*, 9–18.

(12) Calder, P. C. Polyunsaturated fatty acids and inflammation. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2006**, *75*, 197–202.

(13) Gardner, H. W. Oxygen radical chemistry of polyunsaturated fatty acids. *Free Radical Biol. Med.* **1989**, *7*, 65–86.

(14) Reeves, P. G.; Nielson, F.; Fahey, G. C.Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939–1951.

(15) Institute of Laboratory Animal Resources Commission on Life Sciences. *Guide For The Care And Use of Laboratory Animals*; National Academy Press: Washington, DC, 1996.

(16) Deuchi, K.; Kanauchi, O.; Imasato, Y.; Kobayashi, E. Decreasing effect of chitosan on the apparent fat digestibility by rats fed on a high-fat diet. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1617–1620.

(17) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917.

(18) Fritsche, K. L.; Johnston, P. V. Effect of dietary α-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two murine mammary adenocarcinomas. *J. Nutr.* **1990**, *189*, 52–60.

(19) Brenna, J. T.; Salem, N.Jr.; Sinclair, A. J.; Cunnane, S. C. International Society for the Study of Fatty Acids and Lipids, ISSFAL. α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2009**, *80*, 85–91.

(20) Innis, S. M.; Rioux, F. M.; Auestad, N.; Ackman, R. G. Marine and freshwater fish oil varying in arachidonic, eicosapentaenoic and docosahexaenoic acids differ in their effects on organ lipids and fatty acids in growing rats. *J. Nutr.* **1995**, *125*, 2286–2293.

(21) Arterburn, L. M.; Hall, E. B.; Oken, H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am. J. Clin. Nutr.* **2006**, *83*, 1467–1476.

(22) Van Elswyk, M. E.; Kuratko, C. Achieving adequate DHA in maternal and infant diets. *J. Am. Diet. Assoc.* **2009**, *109*, 403–404.

(23) Rapoport, S. I.; Rao, J. S.; Igarashi, M. Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2007**, *77*, 251–261.

(24) Werner, A.; Havinga, R.; Kuipers, F.; Verkade, H. J. Treatment of EFA deficiency with dietary triglycerides or phospholipids in a murine model of extrahepatic cholestasis. *Am. Physiol. Gastrointest. Liver Physiol.* **2004**, *286*, 822–832.

(25) Schmitz, G.; Ecker, J. The opposing effects of n-3 and n-6 fatty acids. *Prog. Lipid Res.* **2008**, *2*, 147–155.

(26) Froyland, L.; Vaagenes, H.; Asiedu, D. K.; Garras, A.; Lie, O.; Berge, R. K. Chronic administration of eicosapentaenoic acid and docosahexaenoic acid as ethyl esters reduced plasma cholesterol and changed the fatty acid composition in rat blood and organs. *Lipids* **1996**, *31*, 169–178.

(27) DeMar, J. C.Jr.; DiMartino, C.; Baca, A. W.; Lefkowitz, W.; Salem, N.Jr. Effect of dietary docosahexaenoic acid on biosynthesis of docosahexaenoic acid from α-linolenic acid in young rats. *J. Lipid Res.* **2008**, *49*, 1963–1980.

(28) Fritsche, K. Important differences exist in the dose−response relationship between diet and immune cell fatty acids in humans and rodents. *Lipids* **2007**, *42*, 961–979.

(29) Saito, M.; Kubo, K. An assessment of docosahexaenoic acid intake from the viewpoint of safety and physiological efficacy in matured rats. *Ann. Nutr. Metab.* **2002**, *46*, 176–181.

Received for review October 16, 2009. Revised manuscript received January 11, 2010. Accepted January 20, 2010. Funding for this study was provided by the West Virginia University Agriculture and Forestry Experimental Station Hatch Grant H45.