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Fatty acid and amino acid composition of eggs, muscle and midgut glands of freshwater prawns, *Macrobrachium rosenbergii* (de Man), raised in fertilized ponds, unfertilized ponds or fed prepared diets

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Abstract

Fatty acid and amino acid profiles were determined in three tissues (eggs, muscle and midgut glands) from freshwater prawns, *Macrobrachium rosenbergii* (De Man), raised unfed in ponds with no organic fertilization, unfed in ponds with organic fertilization or fed a formulated, pelleted diet. Amino acid profiles of tail muscle and eggs were not treatment-dependent. Comparison of amino acid profiles of the diet with those of tissues of animals fed that diet suggest that dietary levels of arginine, histidine, methionine, and especially, lysine may be suboptimal. Fatty acid profiles of the midgut gland, tail muscle, and to a lesser extent, eggs reflected the fatty acid composition of the diet in the fed treatment, the organic fertilizer material in the fertilized treatment and natural food organisms in the unfed treatment. Direct consumption of organic fertilizer particles by the prawns is supported by these data. Selective retention of essential fatty acids appears likely in all three treatments. Comparison of the fatty acid composition of prawn eggs and muscle tissue with that of the pelleted diet indicates that 18 : 3(n-3), 20 : 5(n-3), and especially 20 : 4(n-6) are probably below optimal levels. However, under practical culture conditions, prawn growth may not be significantly reduced because of relatively low dietary requirements and contributions from natural foods in the ponds.

Introduction

Few studies have addressed fatty acid and amino acid requirements and metabolism in the freshwater prawn, *Macrobrachium rosenbergii* (De Man) (D'Abramo & Sheen 1993; Reed & D'Abramo 1989). As a result, prawn diets have principally been adapted from penaeid shrimp diets. However, recent studies have strongly suggested that distinct differences in polyunsaturated fatty acid nutrition exist between freshwater prawns and species of marine shrimp (D'Abramo & Sheen 1993).

Little or no information exists concerning dietary nutrient requirements of prawns under practical, semi-intensive pond production conditions (Tacon 1995). Several methods exist to investigate or estimate the nutritional requirements of aquatic animals, even under practical farming conditions. Information on fatty acid requirements can be generated by analysing tissues of animals subjected to different nutritional regimes (D'Abramo & Sheen 1993). High levels of fatty acids in tissues relative

to dietary levels may indicate relative importance (Reigh & Stickney 1989). Fatty acid profiles from tissues of prawns exhibiting superior weight gains may indicate when dietary requirements are met (D'Abramo & Sheen 1993). Amino acid requirements for some species have also been predicted through analyses of body tissues (Wilson & Poe 1985).

The midgut gland is the primary site of fatty acid metabolism in the prawn (D'Abramo & Sheen 1993) and levels of particular fatty acids may reflect consumption, or even preference for, certain food items (Tidwell & Robinette 1990). Conservation of particular fatty acids during periods of starvation or nutritional stress may reflect relative importance to the respective tissue (Reigh & Stickney 1989). Also, the biochemical composition of eggs is largely fixed and may serve as a good reference to evaluate the potential quality of a diet (Soivivo, Niemisto & Backstrom 1989).

This study was conducted to evaluate the amino acid composition of eggs and tail muscle, and fatty acid composition of eggs, muscle, and midgut gland in prawns solely subsisting on natural foods in unfertilized ponds, prawns raised in organically fertilized ponds, and prawns raised in unfertilized ponds and fed a formulated, pelleted diet. These data may be useful in evaluating relative suitability of types of feeding strategies based on comparisons of nutrient composition between tissue and food items and serve as indicators of nutritional importance and essentiallity of specific nutrients.

Materials and methods

Description, preparation and stocking of ponds

Less than 1 week prior to the anticipated stocking date, the ponds located at the Aquaculture Research Centre, Kentucky State University (KSU), Frankfort, KY were filled with water from a reservoir that receives, and is filled by, runoff from the surrounding watershed. The water surface area of all the experimental ponds was 0.04 ha and the average water depth was approximately 1.3 m. A 0.5 hp vertical lift pump operated continuously in the deepest area of each pond to prevent thermal stratification and provide aeration.

Size-graded juvenile prawns were transported by truck from the Aquaculture Research Unit of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University on 1 June 1994. Juveniles were held in three 3000 l flow-through tanks, containing plastic netting to provide substrate, and then stocked into nine ponds on 8 June 1994 at a density of 39 250/ha. Average weight (± SD) was 0.50 g (± 0.15 g) at stocking. Three replicate ponds were randomly assigned to each of the three experimental treatments: fed (FD; prawns fed a pelleted feed with no pond fertilization), fertilized (FRT; ponds organically fertilized, but prawns not fed) and unfed (UNFD; stocked with prawns but no feed or fertilizer).

Table 1 Ingredient composition (%) and proximate analysis of the diet and fed to pond cultured freshwater prawns in the fed (FD) treatment

Ingredient	Percentage composition
Menhaden fish meal (67% protein)	7.50
Soybean meal (44% protein)	13.75
DDGS	40.00
Wheat flour	12.50
Meat and bone meal (54% protein)	7.50
Ground corn meal	5.25
Mineral mix[1]	1.25
Vitamin mix[2]	1.25
Choline chloride	0.05
Cod liver oil	0.50
Dicalcium phosphate	0.50
Lignosulfonate binder	10.00
Analysed composition (%)[3]:	
protein	31.89 ± 1.87
lipid	6.54 ± 2.54
moisture	10.07 ± 0.40

[1]Mineral mix contained: Mn, 10.0% (as $MnSO_4$); Zn, 10.0% (as $ZnSO_4$); Fe, 7.0% (as $FeSO_4$); Cu, 0.7% (as $CuSO_4$); I, 0.24% (as $CaIO_3$); Co, 0.10% (as $CoSO_4$).

[2]Vitamin mix contained: thiamine (B_1), 1.01%; riboflavin (B_2), 1.32%; pyridoxine (B_6), 0.9%; nicotinic acid, 8.8%; folic acid, 0.22%, cyanocobalamine (B_{12}) 0.001%; pantothenic acid, 3.53%; menadione (K), 0.2%; ascorbic acid (c), 22.1%; retinolpalmitate (A), 4409 IU kg^{-1}; cholecalciferol (D_3), 2204 600 IU kg^{-1}; α-tocopherol (E), 66.2 IU kg^{-1}; ethoxyquin, 0.66%.

[3]Protein and lipid on a dry weight basis.

Feed and feeding rates

The diet was formulated to contain 32% protein. Ingredient composition of the diet (Table 1) was similar to that of the diet utilized by D'Abramo,

Table 2 Composition (dry weight) of distillers dried grains with solubles (DDGS) used as an organic fertilizer in the FRT treatment

Component	% of total
Protein[1]	28.1 ± 0.14
Lipid[1]	14.45 ± 0.21
Moisture[1]	10.48 ± 0.01
Phosphorous[2]	7.05 ± 0.07
Potassium[2]	0.71
Magnesium[2]	0.44
Calcium[2]	0.15

[1]Replicate analyses.
[2]NRC (1983).

Heinen, Robinette & Collins (1989) and Tidwell, Webster, Clark & D'Abramo (1993), and contained 7.5% menhaden fish meal. Dietary ingredients were processed into 5 mm sinking pellets by a commercial feed mill (Farmers Feed Mill, Lexington, KY, USA).

One-half of the daily ration of diet was distributed over the entire surface of each pond in the FD treatment, twice daily, between 0900 and 1000 h and between 1500 and 1600 h. The prawns were fed a percentage of the estimated mean individual body weight using a computer generated feeding schedule (Daniels & D'Abramo 1994). Feeding rates were adjusted weekly based on an assumed 2.5:1 feed to gain conversion (D'Abramo *et al.* 1989). Every 3 weeks, biomass estimates for each pond were adjusted according to sample weights. Mortality was assumed to be 1% per week. In ponds receiving organic fertilization, distillers dried grains with solubles (DDGS) was added at a rate isonitrogenous with that of the FD treatment according to the same schedule. The DDGS used in the study was a homogeneous composite from several distilleries as provided by the Distillers Feed Research Council, Ft. Wright, KY, USA. A partial nutrient profile of the DDGS used as organic fertilizer is provided in Table 2.

Tissue sampling and analyses

At the conclusion of the study (September 27 1994), the tail muscle tissue from five individuals from each pond was removed, chopped into small pieces, immediately frozen in liquid nitrogen (–196 °C), pooled, and stored in one screw cap vial at –40 °C until amino acid analysis. Tail muscle and midgut gland tissue from five additional prawns per pond were similarly prepared and stored for future fatty acid analyses. Eggs in late stages of development were also removed from five mature female prawns per pond, frozen, pooled, and stored, as described previously for muscle, until used for amino acid and fatty acid analyses.

Essential amino acid profiles of diets were compared to those of prawn muscle and eggs using the following index: A/E = the content of the individual essential amino acid/total essential amino acid content × 1000.

Extractions for amino acid analyses were performed according the method of the AOAC (1984). Acid digests (24 h HCl) were analysed by high-performance liquid chromatography (HPLC) using a Hewlett-Packard Model 1050 with post-column derivitization with ninhydrin (Pickering Trione Ninhydrin reagent; Pickering Chemical Co., Mountain View, CA, USA). Detection was at 570 or 440 nm as appropriate. Samples analysed for methionine and cystine were treated for 18 h with performic acid and then neutralized with sodium meta-bisulfite prior to the 24 h digestion with HCl. Performic acid-treated samples were analysed by post-column derivitization with ortho-phthalic aldehyde (OPA: Fluoraldehyde; Pierce Chemical Co., Rockford, IL, USA) using fluorescence detection. Chromatography for either of these digests was accomplished with Interaction AA 511 cation exchange columns (4.6 × 120 mm, 5 µm) using the Pierce BuffElute gradient system (Pierce Chemical Co.). All samples had norieucine added as internal standard with quantification performed by comparison to a standard amino solution (Pierce Standard H; Pierce Chemical Co.) supplemented with norieucine and cysteic acid (Sigma Chemical Co., St Louis, MO, USA) For tryptophan the alkaline digests were analysed by chromatography on a 4.6 × 150 mm 5 µm C18 reverse phase column (Interaction Chemicals Inc., Mountain View, CA, USA) with UV detection at 281 nm. These samples had 5-methyl tryptophan added as internal standard and they were quantified by comparison to a standard containing 0.05 µM 5-methyl tryptophan and 0.05 µM tryptophan.

Lipid extraction was according to Bligh and Dyer (1959). Fatty acid methyl esters were obtained according to the method of the AOAC (1984) and analysed using a Hewlett-Packard 5890 II gas chromatograph equipped with an Omegawax 320 30 m fused-silica capillary column (Supelco, Inc.,

Bellefonte, PA, USA) and a flame-ionization detector. The carrier gas was helium. The oven temperature was programmed from 160 to 220 °C at 2 °C min^{-1} and then from 200 to 270 °C at 10 °C min^{-1}. The detector response was recorded and quantified with an electronic integrator–recorder. An internal standard was added and fatty acid methyl esters were identified by comparison and their retention times with those of authentic standards (Supelco, Bellefonte, PA, USA).

Statistical analysis

Data were analysed by ANOVA using the SAS ANOVA procedure (SAS 1988). Fisher's least significant difference test was used to compare treatment means. Fatty acid and amino (percentage) acid data were transformed to arcsine values prior to analysis (Zar 1984).

Results and discussion

Growth data for prawns in the fertilized system was greater than expected, producing > 1000 kg/ha (Tidwell, Coyle, Webster, Sedlacek, Western, Knight, Hill, D'Abramo, Daniels & Fuller 1997) when carrying capacity of a fertilized system for prawns is normally considered to be about 600 kg/ha (Moore 1986; Tidwell, Webster, Sedlacek, Weston, Knight, Hill, D'Abramo, Daniels, Fuller & Montanez 1995). Sampling of prawn gut contents indicated that the material provided as fertilizer (DDGS) was being consumed directly. Accordingly, amino acid and fatty acid profiles of the DDGS were determined. Therefore, effects on the profiles of prawn tissue may be considered to be at least partially due to direct consumption.

Amino acids

The prepared diet contained significantly higher ($P \leq 0.05$) levels of arginine and lysine than the DDGS (Table 3). The amino acid composition of tail muscle and eggs from prawns in the three treatments are presented in Tables 4 and 5, respectively. Although there were some statistically significant differences ($P < 0.05$), the actual magnitude of those differences was quite small, and they were probably not of biological significance.

Muscle tissue and prawn eggs *A/E* ratios did not appear to be influenced by dietary treatment (Table 6). A comparison of *A/E* ratios in muscle and eggs with those of the diet indicates that the relative abundance of arginine, histidine, methionine, and

Table 3 Essential amino acid composition (% of total amino acids) plus cystine and tyrosine of a complete diet and organic pond fertilizer (Distillers Dried Grains with Solids:DDGS)*

	Treatment	
Amino acid	**Diet**	**DDGS**
Thr	4.21 ± 0.01[a]	4.07 ± 0.05[a]
Cys	1.65 ± 0.13[a]	1.85 ± 0.02[a]
Val	4.53 ± 0.07[a]	4.85 ± 0.02[a]
Met	2.17 ± 0.07[a]	2.32 ± 0.03[a]
Ile	3.57 ± 0.05[a]	3.58 ± 0.05[a]
Leu	9.57 ± 0.01[b]	12.15 ± 0.24[a]
Tyr	2.90 ± 0.03[a]	2.72 ± 0.02[a]
Phe	4.97 ± 0.06[a]	5.08 ± 0.04[a]
His	3.05 ± 0.02[a]	3.68 ± 0.15[a]
Lys	4.18 ± 0.03[a]	2.29 ± 0.05[b]
Arg	5.66 ± 0.09[a]	3.70 ± 0.04[b]

*Means within a row are significantly different when not followed by the same letter ($P \leq 0.05$).

Table 4 Essential amino acid composition (% of total amino acids) plus cystine and tyrosine of tail muscle of pond raised prawns either unfed (UNFD), fed a complete diet (FD), or raised in ponds organically fertilized (FRT) with Distillers Dried Grains with Solubles (DDGS)*

		Treatment	
Amino acid	**UNFED**	**FD**	**FRT**
Thr	3.98 ± 0.09[a]	4.06 ± 0.07[a]	4.19 ± 0.03[a]
Cys	1.02 ± 0.10[a]	1.03 ± 0.10[a]	1.01 ± 0.01[a]
Val	4.58 ± 0.19[b]	4.90 ± 0.04[a]	4.81 ± 0.07[ab]
Met	2.66 ± 0.04[a]	2.63 ± 0.06[a]	2.60 ± 0.30[a]
Ile	4.32 ± 0.05b	4.53 ± 0.03[a]	4.38 ± 0.03[a]b
Leu	7.77 ± 0.07[a]	7.82 ± 0.07[a]	7.74 ± 0.05[a]
Tyr	3.46 ± 0.02[a]	3.39 ± 0.09[a]	3.37 ± 0.10[a]
Phe	4.32 ± 0.03[a]	4.30 ± 0.19[a]	4.15 ± 0.30[a]
His	3.76 ± 0.05[a]	3.99 ± 0.04[a]	3.98 ± 0.11[a]
Lys	7.82 ± 0.16[a]	8.05 ± 0.15[a]	7.76 ± 0.02[a]
Arg	7.89 ± 0.12[a]	7.79 ± 0.05[a]	7.74 ± 0.01[a]
Trp	0.93 ± 0.07[a]	1.09 ± 0.03[a]	0.93 ± 0.21[a]

*Means within a row are significantly different when not followed by the same letter ($P \leq 0.05$).

Table 5 Essential amino acid composition (% of total amino acids) plus cystine and tyrosine of eggs from pond raised either unfed (UNFD), fed a complete diet (FD), or raised in ponds organically fertilized (FRT) with Distillers Dried Grains with Solubles (DDGS)*

	Treatment		
Amino acid	**UNFED**	**FD**	**FRT**
Thr	5.35 ± 0.16[a]	5.19 ± 0.11[a]	5.69 ± 0.29[a]
Cys	1.41 ± 0.11[a]	1.19 ± 0.05[b]	1.51 ± 0.03[a]
Val	5.69 ± 0.01[a]	5.53 ± 0.14[a]	6.15 ± 0.26[a]
Met	3.05 ± 0.01[a]	2.84 ± 0.16[a]	3.53 ± 0.25[a]
Ile	4.65 ± 0.03[a]	4.64 ± 0.11[a]	5.03 ± 0.17[a]
Leu	8.08 ± 0.02[a]	8.10 ± 0.14[a]	8.59 ± 0.21[a]
Tyr	3.78 ± 0.17[a]	3.79 ± 0.07[a]	3.76 ± 0.23[a]
Phe	4.52 ± 0.16[a]	4.65 ± 0.14[a]	4.47 ± 0.27[a]
His	4.18 ± 0.06[a]	4.29 ± 0.16[a]	4.07 ± 0.18[a]
Lys	7.79 ± 0.05[a]	7.95 ± 0.19[a]	7.81 ± 0.15[a]
Arg	7.32 ± 0.11[a]	7.38 ± 0.07[a]	7.58 ± 0.28[a]
Trp	1.57 ± 0.07[a]	1.73 ± 0.07[a]	1.75 ± 0.01[a]

*Means within a row are significantly different when not followed by the same letter ($P \leq 0.05$).

lysine was lower in the diet than in prawn tissues, with lysine being lowest. These data agree with other results reported by Tidwell *et al.* (1993) and may indicate the prepared diet is somewhat deficient in these essential amino acids. The *A/E* ratios of DDGS indicate that it may contain suboptimal levels of isoleucine as well as arginine and lysine. However, DDGS may well play a dual role being consumed directly, but also acting as an organic fertilizer (Tidwell *et al.* 1997). The resulting increased availability of high quality natural foods could possibly compensate for low dietary levels of essential nutrients, such as lysine.

Fatty acids

There were several statistically significant ($P \leq 0.05$) differences in individual fatty acids between the prepared diet and DDGS, although the actual magnitude of some differences was quite small (Table 7). Primary differences were that DDGS had a significantly higher level ($P \leq 0.05$) of linoleic acid, reflecting its high corn content, while the prepared diet had significantly higher concentrations ($P < 0.05$) of arachidonic acid, eicosapentaenoic acid (EPA), and DHA, probably reflecting the contribution of the marine oil and fish meal ingredients. The prepared diet had significantly higher levels ($P \leq 0.05$) of saturated, monoene, and (n-3) fatty acids, and a lower (n-6)/(n-3) ratio than DDGS.

Saturated fatty acids

Myristic acid (14 : 0) was found in eggs (Table 8) and muscle tissue (Table 9) of prawns in the FD treatment at levels similar to those in the diet. Levels of 14 : 0 were significantly higher ($P \leq 0.05$) in the eggs of UNFD prawns than in those of prawns fed the formulated diet (FD). Levels of myristic acid were significantly higher ($P \leq 0.05$) in the midgut gland of UNFD prawns than in that of prawns raised in fertilized ponds (FRT).

Levels of palmitic acid (16 : 0) were very similar in eggs and tail muscle and were not significantly different ($P > 0.05$) among prawns in the three treatments. In the midgut gland, levels of 16 : 0 were significantly higher ($P \leq 0.05$) in prawns from the UNFD treatment in those of the other treatments (Table 10).

In prawns in the FD treatment, levels of stearic acid (18 : 0) were higher in eggs, muscle, and midgut gland than levels found in the feed. Levels of stearic acid were significantly lower ($P \leq 0.05$) in the midgut gland and eggs of prawns in FRT treatment than in those of prawns fed the formulated diet (FD). Levels of 18 : 0 in the eggs of UNFD prawns were significantly lower ($P < 0.05$) than those of eggs from prawns in other treatments.

Monoenes

Palmitoleic acid (16 : 1(n-7)) was found in eggs, muscle, and midgut glands of FD prawns at higher levels than those found in the diet. These data are in agreement with Tidwell *et al.* (1993) who reported concentrations of palmitoleic acid in the tail muscle of prawns to be much higher than that found in the experimental diet. Also, levels of 16 : 1(n-7) in all three tissues were significantly higher ($P \leq 0.05$) in UNFD prawns than in prawns in the FD or FRT treatments. These data may indicate the relative importance for this fatty acid. Increased production of 16 : 1(n-7) may be stimulated by high dietary levels of 16 : 0 (Guary, Kayama, Murakami & Ceccaldi 1976) or low dietary levels of polyenoic fatty acids (Reigh & Stickney 1989). The latter

Table 6 *A/E* ratios for essential of tail muscle and eggs from prawns with either unfed (UNFD), fed a prepared diet (FD), or raised in organically fertilized ponds (FRT)

	Tail muscle			Prawn eggs			Nutrient material	
	UNFD	FD	FRT	UNFD	FD	FRT	Diet	DDGS
Arg	164.5 ± 2.1[a]	158.3 ± 1.8[a]	160.8 ± 1.4[a]	140.3 ± 1.6[a]	141.1 ± 1.8[a]	138.5 ± 2.0[a]	135.1 ± 2.1[a]	88.7 ± 0.31[b]
His	78.3 ± 0.8[b]	81.1 ± 0.2[ab]	82.6 ± 2.6[a]	80.1 ± 0.9[a]	82.1 ± 3.2[a]	74.3 ± 0.9[a]	72.8 ± 0.5[a]	88.1 ± 2.5[a]
Ile	88.9 ± 1.2[a]	92.2 ± 0.8[a]	90.9 ± 0.3[a]	89.1 ± 0.9[a]	88.7 ± 1.9[a]	91.8 ± 0.5[a]	85.1 ± 1.2[a]	85.9 ± 0.2[a]
Leu	161.9 ± 0.9[a]	159.1 ± 0.7[a]	160.8 ± 0.3[a]	154.7 ± 1.0[a]	154.9 ± 2.4[a]	157.1 ± 1.6[a]	228.4 ± 0.3[b]	291.3 ± 2.1[a]
Lys	162.8 ± 2.9[a]	163.8 ± 2.4[a]	160.2 ± 0.3[a]	149.3 ± 1.5[a]	152.0 ± 4.1[a]	142.9 ± 2.3[a]	99.8 ± 0.7[a]	55.0 ± 0.5[b]
Met	55.2 ± 1.0[a]	53.6 ± 1.3[a]	53.9 ± 5.8[a]	58.4 ± 0.0[a]	54.2 ± 2.8[a]	64.5 ± 4.6[a]	51.7 ± 1.5[a]	55.6 ± 0.1[a]
Phe	89.9 ± 0.7[a]	87.4 ± 3.3[a]	85.3 ± 6.4[a]	86.7 ± 2.7[a]	89.0 ± 2.9[a]	81.6 ± 1.9[a]	118.6 ± 1.6[a]	121.8 ± 0.6[a]
Thr	82.9 ± 2.2[a]	82.6 ± 0.9[a]	86.5 ± 0.9[a]	102.3 ± 2.6[a]	99.3 ± 1.7[a]	103.9 ± 1.8[a]	100.4 ± 0.1[a]	97.6 ± 2.5[a]
Val	95.3 ± 2.8[a]	99.6 ± 0.9[a]	99.8 ± 1.8[a]	109.0 ± 0.5[a]	105.8 ± 2.6[a]	112.4 ± 2.0[a]	108.0 ± 1.7[a]	116.2 ± 1.8[a]
Try	19.3 ± 1.4[a]	22.2 ± 0.5[a]	19.5 ± 4.2[a]	30.1 ± 1.5[a]	33.0 ± 1.3[a]	33.1 ± 0.3[a]	–	–

*Means within a row, within a tissue, are significantly different when not followed by the same letter ($P \leq 0.05$).

Table 7 Fatty acid composition (% of total fatty acids) of the diet and organic fertilizer material (Distillers Dried Grains with Solubles:DDGS)*

	Treatment	
Fatty acid	Diet	DDGS
14 : 0	1.18 ± 0.00[a]	0.14 ± 0.01[b]
16 : 0	17.21 ± 0.09[a]	15.07 ± 0.02[b]
16 : 1(n-7)	1.62 ± 0.00[a]	0.19 ± 0.01[b]
18 : 0	3.74 ± 0.03[a]	2.43 ± 0.01[b]
18 : 1(n-9)	22.42 ± 0.08[a]	22.16 ± 0.01[a]
18 : 2(n-6)	45.19 ± 0.03[b]	55.91 ± 0.00[a]
18 : 3(n-3)	2.38 ± 0.01[a]	3.10 ± 0.00[b]
20 : 4(n-6)	0.21 ± 0.01[a]	0.00 ± 0.00[b]
20 : 5(n-3)	1.81 ± 0.02[a]	0.00 ± 0.00[b]
22 : 5(n-3)	0.29 ± 0.00[a]	0.00 ± 0.00[b]
22 : 6(n-3)	1.29 ± 0.01[a]	0.00 ± 0.00[b]
Other		
Total	99.89 ± 0.09	100.00 ± 0.00
Saturates	23.03 ± 0.03[a]	18.79 ± 0.06[a]
Monoenes	25.05 ± 0.07[a]	23.16 ± 0.07[b]
Diene	45.29 ± 0.08[b]	55.51 ± 0.08[a]
Triene	2.45 ± 0.08[a]	2.20 ± 0.00[a]
PUFA	51.81 ± 0.19[b]	58.11 ± 0.08[a]
(n-3)	4.25 ± 0.02[a]	2.09 ± 0.07[b]
(n-6)	43.35 ± 0.03[b]	56.02 ± 0.08[a]
(n-6)/(n-3)	10.68 ± 0.03[b]	26.81 ± 0.04[a]

*Means within a row are significantly different when not followed by the same letter ($P \leq 0.05$).

condition may be true for UNFD prawns in this study. Also, prawns in the UNFD treatment were consuming natural food organisms, such as benthic macroinvertebrates. Chironomids are important natural food items for prawns (Tidwell *et al.* 1997) and contain high levels of 16 : 1(n-7) (Bell, Ghioni & Sargent 1994).

Oleic acid, 18 : 1(n-9) was the second most abundant fatty acid in the diet (22%) and DDGS fertilizer (22%). Reigh & Stickney (1989) found that levels of 18 : 1(n-9) respond to different dietary treatments. However, in this study 18 : 1(n-9) showed less response than 16 : 1(n–7). Levels of 18 : 1(n-9) in the midgut gland even showed a significant decrease ($P \leq 0.05$) in the unfed prawns while 16 : 1(n-7) showed an increase.

(n-6) fatty acids

Within the (n-6) family of fatty acids linoleic acid (18 : 2(n-6)) was the most abundant individual fatty acid in both the diet and DDGS. Tissue levels of 18 : 2(n-6) in eggs, muscle, and midgut gland were lower than levels in the diet or DDGS. Treatment responses relative to tissue concentrations varied widely. Levels of 18 : 2(n-6) were significantly higher ($P \leq 0.05$) in all three tissues of prawns raised in FRT ponds than those of prawns in other treatments. This condition possibly reflects direct consumption of DDGS because the level of 18 : 2(n-6) in DDGS was significantly higher ($P \leq 0.05$) than in the

Table 8 Fatty acid composition (% of total) fatty acids in eggs from pond raised freshwater prawns either unfed (UNFD), fed a complete diet (FD), or raised in ponds organically fertilized (FRT) with Distillers Dried Grains with Solubles (DDGS)*

	Treatment		
Amino acid	**UNFED**	**FD**	**FRT**
14 : 0	2.21 ± 0.18^{a}	1.09 ± 0.06^{b}	1.06 ± 0.03^{b}
16 : 0	17.99 ± 0.04^{a}	17.56 ± 0.42^{a}	17.63 ± 0.48^{a}
16 : 1(n-7)	7.29 ± 0.61^{a}	3.10 ± 0.61^{a}	1.85 ± 0.31^{b}
18 : 0	5.45 ± 0.23^{c}	7.21 ± 0.17^{a}	6.22 ± 0.17^{b}
18 : 1(n-9)	26.00 ± 1.62^{a}	25.70 ± 0.99^{a}	20.34 ± 0.47^{b}
18 : 2(n-6)	8.00 ± 0.64^{c}	27.56 ± 1.32^{b}	35.70 ± 2.04^{a}
18 : 3(n-3)	7.11 ± 0.66^{a}	2.40 ± 0.14^{b}	2.40 ± 0.33^{b}
20 : 4(n-6)	3.91 ± 0.18^{a}	1.51 ± 0.13^{b}	1.92 ± 0.05
20 : 5(n-3)	4.24 ± 0.76^{a}	3.17 ± 0.15^{ab}	1.33 ± 0.30^{b}
22 : 6(n-3)	0.98 ± 0.38^{ab}	1.54 ± 0.04^{a}	0.21 ± 0.07^{b}
Other			
PUFA	30.21 ± 0.81^{c}	39.57 ± 1.50^{b}	46.01 ± 1.87^{a}
(n-3)	14.26 ± 1.91^{a}	8.16 ± 0.28^{b}	4.68 ± 0.14^{b}
(n-6)	15.09 ± 1.17^{c}	31.12 ± 1.29^{c}	40.54 ± 1.98^{a}
(n-6)/(n-3)	1.11 ± 0.21^{c}	3.81 ± 0.06^{b}	8.69 ± 0.55^{a}

*Means within a row are significantly different when not followed by the same letter ($P \leq 0.05$).

Table 9 Fatty acid composition (% of total fatty acids) in tail muscle from pond raised freshwater prawns either unfed(UNFD), fed complete diet (FD), or raised in ponds organically fertilized (FRT) with distillers dried grains with solubles (DDGS)*

	Treatment		
Amino acid	**UNFED**	**FD**	**FRT**
14 : 0	1.11 ± 0.15^{a}	0.83 ± 0.03^{ab}	0.64 ± 0.01^{b}
16 : 0	17.80 ± 0.10^{a}	17.23 ± 0.29^{a}	17.15 ± 0.42^{a}
16 : 1(n-7)	3.12 ± 0.25^{a}	2.32 ± 0.22^{b}	1.29 ± 0.17^{c}
18 : 0	8.48 ± 0.53^{a}	10.00 ± 0.59^{a}	9.57 ± 0.26^{a}
18 : 1(n-9)	17.56 ± 0.92^{b}	19.71 ± 0.24^{a}	15.74 ± 0.28^{b}
18 : 2(n-6)	7.69 ± 0.59c	22.87 ± 0.77^{b}	30.75 ± 0.84^{a}
18 : 3(n-3)	6.19 ± 0.72^{a}	2.45 ± 0.21^{b}	2.43 ± 0.02^{b}
20 : 4(n-6)	11.57 ± 0.39^{a}	5.81 ± 0.25^{b}	6.56 ± 0.25^{c}
20 : 5(n-3)	10.85 ± 0.18^{a}	6.91 ± 0.44^{b}	4.71 ± 0.75^{c}
22 : 6(n-3)	2.19 ± 0.36^{a}	1.51 ± 0.11^{a}	0.55 ± 0.16^{b}
Other			
PUFA	42.16 ± 1.44^{b}	43.10 ± 1.18^{b}	48.70 ± 0.13^{a}
(n-3)	20.25 ± 0.35^{a}	11.44 ± 0.76^{b}	7.73 ± 1.08^{c}
(n-6)	21.48 ± 1.26^{c}	31.26 ± 0.72^{b}	40.61 ± 1.23^{c}
(n-6)/(n-3)	1.06 ± 0.06^{b}	2.75 ± 0.18^{b}	5.49 ± 0.87^{a}

*Means within a row are significantly different when not followed by the same letter ($P \leq 0.05$).

prepared diet. Further, levels of 18 : 2(n-6) are not very abundant (2–10%) in the freshwater invertebrates that are the primary natural food for prawns (Bell *et al.* 1994). Levels of 18 : 2(n-6) were significantly lower ($P < 0.05$) in egg and muscle tissue in prawns in the UNFD treatment compared to prawns in the FRT or FD treatments. Levels of 18 : 2(n-6) in the midgut gland showed the same relationship (UNFD < FD < FRT), but the difference between UNFD and FD was not statistically significant ($P > 0.05$).

Arachidonic acid (20 : 4(n-6)) is an elongation and desaturation product of 18 : 2(n-6) and is important as a structural component of muscle cell membranes (Lilly & Bottino 1981). Reigh & Stickney (1989) found that in fasted freshwater prawns 20 : 4(n-6) concentrations doubled in all sampled tissues. In the present study 20 : 4(n-6) levels in UNFD prawns were 159% greater in muscle and 450% greater in the midgut gland than 20 : 4(n-6) concentrations in the corresponding tissues of the FD prawns. These differences were statistically significant ($P \leq 0.05$). Levels of 20 : 4(n-6) in tissues of animals in the FD and FRT treatments were higher than levels in the diet and DDGS, respectively. Although neither diet nor DDGS had high levels of 20 : 4(n-6), eggs and especially tail muscle tissue had characteristically high levels of 20 : 4(n–6). This predominant occurrence of arachidonic acid probably reflects the important role of this fatty acid in muscle cell membranes and may be due to selective retention (Reigh & Stickney 1989). Tissue arachondonic acid may also be being secured from forage organisms as levels of this fatty acid are relatively abundant in oligochaetes (Bell *et al.* 1994), which are an important natural food item for prawns (Tidwell *et al.* 1995).

(n-3) family

Linolenic acid (18 : 3(n-3)) was the most abundant (n-3) fatty acid in both the prepared diet and DDGS (Table 7). Levels in eggs of prawns fed the diet (FD) were very similar to dietary levels, while muscle and midgut concentrations were slightly lower. Levels of 18 : 3(n-3) were significantly higher ($P \leq 0.05$) in all three tissues of UNFD prawns compared to those of prawns in other treatments. This is probably due to selective retention and/or dietary input from natural

Table 10 Fatty acid composition (% of total fatty acids) in midgut glands from pond raised freshwater prawns either unfed (UNFD), fed a complete diet (FD), or raised in ponds organically fertilized (FRT) with Distillers Dried Grains with Solubles (DDGS)*

	Treatment		
Amino acid	**UNFED**	**FD**	**FRT**
14 : 0	3.00 ± 0.49^{a}	2.24 ± 0.01^{ab}	1.27 ± 0.11^{b}
16 : 0	19.34 ± 0.79^{a}	17.29 ± 0.23^{b}	15.59 ± 0.43^{b}
16 : 1(n-7)	6.40 ± 1.37^{a}	3.20 ± 0.06^{b}	1.53 ± 0.11^{b}
18 : 0	6.30 ± 0.95^{a}	7.18 ± 0.44^{a}	3.69 ± 0.44^{b}
18 : 1(n-9)	22.29 ± 3.79^{b}	31.98 ± 0.97^{a}	26.52 ± 0.58^{ab}
18 : 2(n-6)	14.01 ± 4.86^{b}	21.91 ± 1.64^{b}	39.78 ± 1.02^{a}
18 : 3(n-3)	5.97 ± 2.33^{a}	1.29 ± 0.44^{a}	1.61 ± 0.11^{a}
20 : 4(n-6)	2.38 ± 1.09^{a}	0.43 ± 0.06^{a}	0.33 ± 0.02^{a}
20 : 5(n-3)	3.38 ± 1.33^{a}	0.64 ± 0.12^{b}	0.34 ± 0.01^{b}
22 : 6(n-3)	0.93 ± 0.22^{a}	0.34 ± 0.07^{b}	0.03 ± 0.03^{b}
Other			
PUFA	22.91 ± 2.48^{b}	27.12 ± 1.99^{b}	44.04 ± 1.59^{a}
(n-3)	11.73 ± 4.23^{a}	2.76 ± 0.74^{b}	2.30 ± 0.06^{b}
(n-6)	18.29 ± 3.73^{b}	25.15 ± 1.74	42.38 ± 0.92
(n-6)/(n-3)	3.18 ± 2.27^{c}	10.16 ± 1.97^{b}	18.44 ± 0.46^{a}

*Means within a row are significantly different when not followed by the same letter ($P \leq 0.05$).

foods. All 10 freshwater invertebrates analysed by Bell *et al.* (1993) contained levels of 18 : 3(n-3) higher than those in the diet used in this study.

EPA, 20 : 5(n-3), is important in membrane maintenance and prostaglandin synthesis. There were proportionately greater levels of this fatty acid in eggs and muscle of FD prawns than in the diet. Levels in the midgut gland were lower than levels in the diet. Levels in muscle and midgut tissue of UNFD prawns were significantly higher ($P \leq 0.05$) than those found in tissues of prawns in the FD or FRT treatments. Reigh and Stickney (1989) found that in prawns 20 : 5(n-3) could be synthesized from other tissue (n-3) sources. However, D'Abramo & Sheen (1993) stated that the ability to convert C18 to C20 fatty acids in the (n-3) linolenic family was extremely limited. Selective retention of EPA is likely and chironomids and oligochaetes are rich sources of EPA (Bell *et al.* 1994).

Summary categories

The total levels of (n-3) fatty acids in the feed (4.3%) was significantly higher ($P \leq 0.05$) than in DDGS (2.1%). This difference was reflected in the tail muscle tissue where levels in FD prawns were significantly greater ($P \leq 0.05$) than in those prawns raised in FRT ponds. UNFD prawns had significantly greater ($P \leq 0.05$) levels of total *n*-3 fatty acids in all three tissues (egg, muscle, and midgut gland) than in those tissues of prawns in the FRT or FD treatments.

Total levels of (n-6) fatty acids were significantly higher ($P \leq 0.05$) in the DDGS (56.0%) than in the diet (43.4%). This difference was reflected in levels of (n-6) fatty acids being significantly higher ($P \leq 0.05$) in prawns in the FRT treatment than in the FD or UNFD treatments. These data further support the direct consumption of DDGS by prawns. In tail muscle and eggs from UNFD prawns, (n-6) levels were significantly lower ($P < 0.05$) than in those tissues of prawns from other treatments. This is probably due to the fact that (n-6) fatty acids are less abundant than (n-3) fatty acids in freshwater invertebrates (Bell *et al.* 1994) which are natural forage items. Also, (n-6) fatty acids probably serve as excellent sources of energy. Selective retention was indicated for only one fatty acid in the (n-6) family, (20 : 4(n-6)).

The ratio of (n-6) to (n-3) fatty acids was very low in the prepared diet and DDGS. Treatment relationships of (n-6)/(n-3) ratios in ascending order were UNFD < FD < FRT in all three tissues. These relationships principally reflect differences in dietary availability, with (n-6) fatty acids being most abundant in DDGS (FRT treatment) and (n-3)s being abundant in natural forage items (UNFD). The (n-6)/(n-3) ratio was significantly lower in all sampled tissues of UNFD prawns with an approximate 1 : 1 ratio in both eggs and muscle. These tissues may be the best indicators of dietary requirements.

Data on body composition of UNFD prawns are probably the best indicators of dietary requirements. These prawns would reflect the fatty acid profile of their natural food organisms and/or preferential retention of important fatty acids during the period of nutritional stress. These data would indicate that the fatty acids of primary importance are 16 : 1, 18 : 3(n-3), 20 : 4(n-6), and 20 : 5(n-3), but not 22 : 6(n–3). These data are in agreement with those reported by D'Abramo & Sheen (1993).

The present study indicates large differences between the fatty acid composition of body tissue of prawns and the fatty acid profiles of the formulated diet. The ratio of (n-6)/(n-3) fatty acids in the diet

(10.7) was approximately 10 times the (n-6)/(n-3) ratio in the tail muscle or eggs of unfed prawns (1.1). Individual fatty acids that appear limiting in the diet formulation include 18 : 3(n-3), 20 : 5(n-3), and especially 20 : 4(n–6). Feed ingredients such as menhaden oil which contains high levels of 20 : 5(n-3) and canola oil which contains significant levels of 18 : 3(n-3) and 20 : 4(n-6) should be evaluated. However, essential fatty acid requirements of freshwater prawns appear to be quite low when compared to the estimated requirements of marine shrimp (D'Abramo & Sheen 1993). Low levels in prepared diets may be adequately compensated for by consumption of even small amounts of natural food organisms from the ponds. This possible condition emphasizes the need to understand and quantify the contribution of natural food organisms to the overall nutritional budget of pond raised crustaceans (Tacon 1995).

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