Growth response and tail-muscle fatty acid quality of Pacific white shrimp, *Litopenaeus vannamei* (Boone) fed with diets containing different levels of rice protein concentrate

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Received: December 2013 Accepted: July 2014

Abstract

The effect of five isonitrogenous diets (36.6% protein), formulated by replacing 0, 25, 50, 75, and 100% of fish meal (FM) with rice protein concentrate (RPC), was investigated on the growth and tail-muscle fatty acid (FA) quality of juvenile Pacific white shrimp, *Litopenaeus vannamei*. The feeds were fed to shrimp (initial weight of 6.99 ± 0.08 g) five times daily to apparent satiation for 60 days. Final weight of shrimp fed with FM, 25 and 50% RPC was higher than that of shrimp fed with 75 and 100% RPC. Survival in shrimp was not significantly affected by dietary protein source and level ($p>0.05$). Regarding FAs, $\sum n6$ and PUFA increased significantly as the RPC levels increased, but the n-3/n-6 ratio, EPA+DHA, MUFA, SFA and $\sum n3$ were significantly declined ($p<0.05$). However, tail-muscle FA composition reflected the inclusion of plant protein. Lipid quality indices (AI and TI) showed significant variation, but were very favourable for the consumers' health. The present study suggests that RPC can replace FM up to 50% in diets for *L. vannamei* with no significant effect on the growth, but a decreasing trend in quality of tail-muscle FAs was observed when the RPC level increased.

Keywords: Fish meal, Fatty acids, Growth, Rice protein concentrate, Tail-muscle

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Introduction

Application of feeds with low levels of FM but high levels of less expensive plant protein sources is considered as a major factor to reduce production costs and increase profitability in shrimp farming. Although soybean meal is considered as one of the most promising alternatives for many reasons (Floreto *et al*., 2000; Lemos *et al*., 2000), its increased acceptability and utilization in the feed industry and its limited availability worldwide have resulted in high feed costs. Thus, there is a need to investigate the possibility of using other more economical plant protein sources with a nutritive value comparable to that of soybean meal. RPC as an ingredient for aquafeed has been evaluated in several works (Palmegiano *et al*., 2006, 2007; Sanchez-Lozano *et al*., 2009; Oujifard *et al*., 2012 a, b). Its amino acid profile is appropriate for aquaculture feeds and its digestibility is similar to most other plant meals (Palmegiano *et al*., 2006, 2007). However, when compared to FM, RPC is characterized by a lower composition of essential amino acids (NRC, 1993), and by its lack of the n-3 marine FAs, EPA and DHA, which are essential for the growth and survival of marine shrimp (Fox *et al*., 2004).

Information on the role of dietary protein sources on lipid metabolism is limited. In higher vertebrates, protein level and source is known to affect lipid metabolism and FA bioconversion potential (Lindholm and Eklund, 1991; Terasawa *et al*., 1994; Potter, 1995; Aoyama *et al*., 2000). Furthermore, the influence of dietary fat level on feed intake, growth and fat deposition on whitefish, *Coregonus lavaretus*, is demonstrated (Koskela *et al*., 1998). However, little information on the role of dietary protein sources on lipid metabolism on shrimp is available. Although the effect of RPC on amino acids composition and apparent digestibility coefficient of nutrients is demonstrated in shrimp (Oujifard *et al*., 2012a, b), no work has been done on its influence on FA composition. In the current work the effects of its dietary levels on tail-muscle FA have been investigated.

Materials and methods

Shrimp and experimental conditions

Juvenile shrimp were obtained from a semi-intensive farm in Bushehr (Iran). Shrimp were acclimated to laboratory conditions for 4 days before being randomly distributed into concrete tanks of 10 m^3 capacity. Shrimp of 6.99 g initial body weight were distributed into 15 experimental tanks in triplicate groups of 80 shrimp each. Continuous aeration was provided by an electric blower and air-stones. Water quality showed that temperature averaged 27ºC ±0.47, salinity ranged between 44 and 46 g L^{-1}, and dissolved oxygen did not fall below 5.7 mg L^{-1}. Light cycle was 12 h light and 12h dark (artificial photoperiod). Mortalities were registered daily, and dead shrimp were removed and weighed.

Diet preparation

The commercial RPC used in this study was purchased from BGMP Company Limited (South Korea). Five experimental diets were formulated to be isoproteic, (CP 36.6% dry weight) and isolipidic, (LP 8.7% dry weight) with an increasing level of RPC in: 0% (RPC0), 25% (RPC 25), 50% (RPC 50), 75% RPC 75 and 100% (RPC 100) respectively.

The experimental diets were fed to the shrimp, *L. vannamei*, over 60 days to investigate the effect on its growth and tail-muscle FAs. Nutrition compositions of the diets were done according to the procedures of the Association of official analytical chemists, (AOAC, 2005). Ingredients and nutrition composition of the experimental diets are shown in Table 1; the initial FA profile of the shrimp, fish oil, soybean oil, FM and RPC are shown in Table 2. Respective diets were hand-fed five times a day at 8, 12, 16, 20 and 24 h to visual satiety. At the end of the feeding trials, shrimp were analyzed for FA profile on the base of dietary treatment.

Table 1: Ingredient composition of the experimental diets.

	% Replacement				
Ingredient (%)	**0**	**25**	**50**	**75**	**100**
Fish meal (*Clupeonella* sp.)	45.7	34.3	22.85	11.42	0
Rice protein concentrate[1]	0	11.4	22.85	34.27	45.7
Shrimp meal	15	15	15	15	15
Binder (Amet)	1.5	1.5	1.5	1.5	1.5
Lecithin	1	1	1	1	1
Cholesterol[2]	0.5	0.5	0.5	0.5	0.5
Wheat flour	25.73	25.73	25.73	25.73	25.73
Fish oil (*Clupeonella* sp.)	0.65	0.99	1.34	1.68	2.02
Soybean oil	0.65	0.99	1.34	1.68	2.02
Mineral premix	2	2	2	2	2
Vitamin premix	2	2	2	2	2
Antioxidant (Ethoxyquin)	0.02	0.02	0.02	0.02	0.02
Anti fungus[3]	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	1.5	1.5	1.5	1.5	1.5
Cellulose	3	2.32	1.62	0.93	0.25
Chromic oxide[2]	0.5	0.5	0.5	0.5	0.5
Analysed compositions					
Dry matter	94.4	94.5	93.8	92.8	92.6
Crude protein	36.7	36.6	36.6	36.5	36.7
Crude lipid	8.2	8.7	8.5	9.1	9.3
Fiber	3	3.5	3.8	4.2	4.4
Gross energy (kJ g^{-1} diet)	17.5	17.7	17.4	17.6	17.9

[1]Rice protein concentrate (supplied by EUNJIN International Co., Ltd, South Korea), [2]Merck, Germany,
[3]Anti fungi: ToxiBan premix (Component: Aluminosilicate, zeolite, bentonate, propionate ammonium).

Table 2: Main fatty acids composition in initial tail-muscle of *Litopenaeus vannamei*, fish oil, soybean oil, FM and RPC (as percentage of the identified fatty acids).

Fatty acid (%)	Initial tail-muscle	Fish oil	Soybean oil	FM	RPC
C14:0 (Myristic acid)	0.39 ± 0.04	2.88	0.75	3.05	0.79
C16:0 (Palmitic acid)	21.96 ± 0.59	20.70	7.81	22.01	27.88
C17:0 (Margaric acid)	0.98 ± 0.03	0.83	-	0.8	0.7
C18:0 (Stearic acid)	11.29 ± 0.21	4.64	2.68	4.47	2.51
C16:1*n*7 (Palmitoleic acid)	0.99 ± 0.01	4.17	3.76	4.25	0.15
C18:1*n*9 (Oleic acid)	15.80 ± 0.24	28.24	22.4	28.25	29.67
C20:1n9 (Eicosenoic acid)	0.43 ± 0.01	1.68	-	0.2	0.36
C18:2*n*6 (Linoleic acid)	17.49 ± 0.17	1.76	51.1	1.76	35.21
C18:3*n*3 (α-Linolenic acid)	1.14 ± 0.00	1.20	4.56	1.18	1.16
C20:4*n*6 (Arachidonic acid)	3.22 ± 0.04	0.49	-	0.62	[4] nd
C20:5*n*3 (Eicosapentaenoic acid, EPA)	7.92 ± 0.05	5.37	-	5.12	0.07
C22:6*n*3 (Docosahexanoic acid, DHA)	8.59 ± 0.06	16.96	-	17.01	nd
∑ SFA[1]	34.64 ± 0.38	29.05	11.24	31.61	32.8
∑ MUFA[2]	19.76 ± 0.13	34.09	26.16	34.7	30.23
∑ PUFA[3]	38.37 ± 0.33	25.78	55.66	26.28	36.47
∑*n*3	17.65 ± 0.11	23.53	4.56	23.41	1.23
∑*n*6	20.72.4 ± 0.22	2.25	51.1	2.87	35.24
n-3/*n*-6	0.85 ± 0.00	10.45	0.08	8.15	0.03
DHA+EPA	16.51 ± 0.12	22.33	-	22.13	0.07

[1]SFA: Saturated fatty acids, [2]MUFA: Monounsaturated fatty acids, [3]PUFA: Polyunsaturated fatty acids, [4]"nd"= not detected,

Growth study

Weight of the shrimp was recorded at 15-day intervals for 60 days. At the end of the experiment, the growth and survival rates (Goytortua-Bores *et al*., 2006) of the shrimp were calculated. Final body weights were calculated based on total shrimp biomass.

Survival % = (final number of shrimp/ initial number of shrimp) ×100

Water stability

Water stability of each diet was determined at 1, 2, 4 and 8 h, following the method of Maguire *et al*. (1988). Water stability was measured as the retained percentage of dry matter of feed immersed in seawater for a certain period of time.

Sample preparation

An initial sample of ten shrimp was taken at the start of the experiment to

determine baseline levels of FA profile in tail-muscle. Prior to sampling, shrimp were fasted for 24 h. The shrimp were killed by immersion in ice-cold water (hypothermia) and then transported to the laboratory within ~2 h in foamed polystyrene, self-draining boxes with a suitable quantity of flaked ice (the ice/shrimp ratio was 3:1, w/w). The homogenized samples were kept at -40ºC prior to FA analysis. The same procedures were used for the samples collected at the end of the feeding trials.

FA composition

Total lipid of tail-muscle and diets was extracted by homogenization in chloroform/methanol (2:1, v/v) according to the method of Folch *et al*. (1957). Fatty acid methyl esters (FAME) samples were analyzed using a Philips PU 4400 gas chromatograph (GC) (Phillips Scientific, Cambridge, United Kingdom), equipped with a fused silica capillary column BPX-70 (25 m × 0.32 mm, film thickness 0.25 µm) and a FID detector. The carrier gas was helium. Temperature program included a gradient from 160 up to 230ºC with an increase rate of 1.5ºC min^{-1}. FAMEs were identified by known purified standards and quantified using a response factor to an internal and external FA standard. Final values were averages of the three replicate injections. Lipid quality indices, i. e., atherogenic index (AI) and thrombogenic index (TI), were calculated according to Ulbricht and Southgate (1991).

Statistical analyses

Descriptive statistics for analysis of the results were calculated for each treatment. One-way ANOVA was performed. Duncan's multiple range test was used to identify significant differences among digestibility coefficients; $p<0.05$ was considered to be statistically significant. Excel, Microsoft Office, 2007, and SPSS version 16 (SPSS Inc., Chicago, IL, USA) were used for data manipulations and statistical analyses.

Results

All of the experimental diets were readily accepted and no mortality was recorded in the different treatments. FA composition of the RPC (Table 2) showed high percentages of C16:0, C18:1n9 and C18:2n6 (27.88%, 29.67% and 35.21%, respectively). These FAs in the diets increased with increasing RPC inclusion level, while C14:0, C16:1n7, C17:0, C17:1n9, C18:0, C20:4n6, C20:5n-3 (EPA), and C22:6n-3 (DHA) decreased. The PUFA n-3 content in RPC diets was lower than in the control diet (FM). Due to higher concentration of linoleic acid (C18:2n6) in RPC diet, the PUFA n-6 content increased and the n-3/n-6 ratio decreased with increasing RPC inclusion level (Table 3).

Table 3: Fatty acid composition of experimental diets.

	% Replacement				
FA (%)	0	25	50	75	100
C14:0	2.32	2.01	1.67	1.31	1.14
C16:0	20.83	20.98	21.28	21.89	22.9
C16:1*n*-7	3.63	3.04	2.41	1.82	1.39
C17:0	0.74	0.62	0.51	0.26	0.28
C17:1*n*-7	0.49	0.57	0.4	0.24	0.24
C18:0	4.85	4.57	4.24	4.18	3.63
C18:1*n*-9	25.97	26.15	26.58	27.51	28.05
C18:2*n*-6	10.92	15.99	21.59	25.5	29.39
C18:3*n*-3	1.88	2.03	2.2	2.15	2.32
C20:1*n*-9	0.26	0.26	0.29	0.33	0.33
C20:4*n*-6	0.53	0.46	0.35	0.26	0.16
EPA	4.29	3.76	2.99	2.07	1.38
DHA	12.48	10.6	7.93	5.4	2.82
Others	3.23	2.77	2.34	2.12	1.8
SFA	30.24	29.56	28.97	29.05	29.31
MUFA	31.55	30.99	30.4	30.42	30.31
PUFA	30.63	33.26	35.41	35.57	36.21
HUFA[1]	17.30	14.82	11.27	7.73	4.36
∑*n*3	18.72	16.45	13.17	9.62	6.54
∑*n*6	11.91	16.81	22.24	25.95	29.67
*n*3/*n*6	1.57	0.97	0.59	0.37	0.22
EPA+DHA	16.77	14.36	10.92	7.47	4.2

FA values (percentages of total FAME) were adjusted to express as percent of total area identified in the chromatograms, unidentified peaks were not considered in the computations. [1]HUFA: C20:4*n*-6, EPA, DHA.

Figs. 1 and 2 illustrate the growth curve and survival of shrimp fed with different dietary proteins for 60 days. Final weight of shrimp fed with control, 25 and 50% RPC was significantly higher ($p<0.05$) than that of shrimp fed with 75 and 100% RPC. No significantly different results were observed concerning the survival.

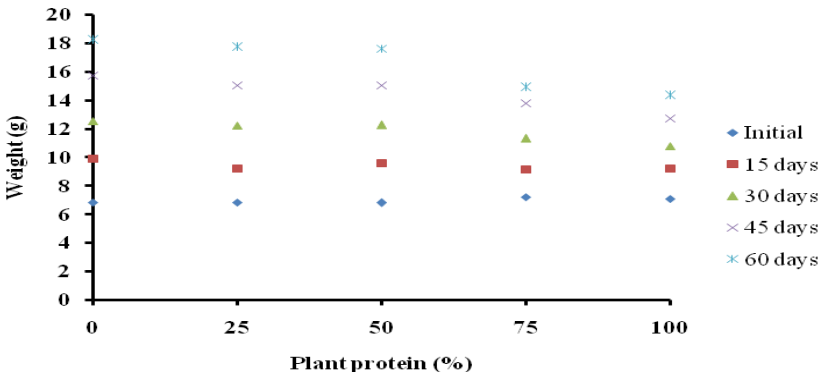


Figure 1: Weight (g) of *Litopenaeus vannamei* given diets with an increasing level of RPC (0-100%) in concrete tanks from the start of the trial and after 15, 30, 45 and 60 days.

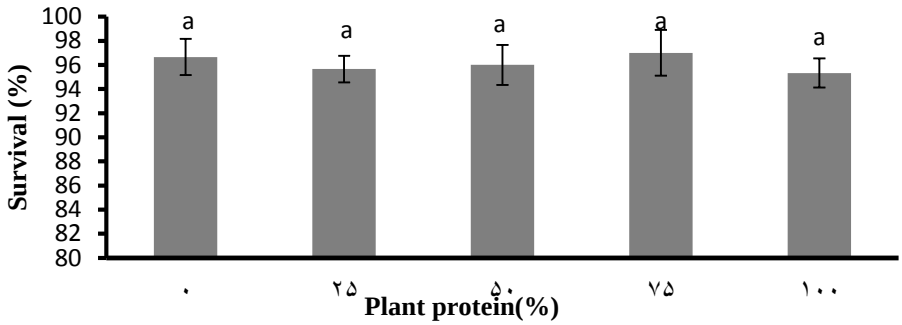


Figure 2: Survival (%) of *Litopenaeus vannamei* fed with increasing levels of RPC (0-100%) in concrete tanks.

The difference in level of RPC influenced water stability of the diets significantly (Table 4). Data of the percentage of dry retained matter after immersion showed a decreasing trend in water stability for the diets at higher RPC inclusion rates. After 8 h of immersion in seawater, retained dry matter for FM was the highest.

Table 4: Water stability (% Dry matter retained) of experimental diets in seawater observed at different immersion periods.

	% Replacement				
Period (h)	**0**	**25**	**50**	**75**	**100**
1	94.23 ± 0.08^{a}	86.18 ± 0.04^{b}	85.40 ± 0.02^{c}	78.01 ± 0.04^{d}	75.95 ± 0.05^{e}
2	93.42 ± 0.06^{a}	85.21 ± 0.02^{b}	83.62 ± 0.01^{c}	75.80 ± 0.01^{d}	74.00 ± 0.02^{e}
4	91.19 ± 0.01^{ab}	83.89 ± 0.05^{b}	82.80 ± 0.02^{bc}	75.01 ± 0.05^{d}	72.40 ± 0.02^{e}
8	87.66 ± 0.22^{a}	81.53 ± 0.11^{b}	81.26 ± 0.10^{b}	73.03 ± 0.09^{c}	71.60 ± 0.26^{d}

Data are mean of triplicate samples (SE). Value in the same row having different superscripts are significantly different ($p<0.05$).

FA analysis of the tail-muscle FAs reflected those of the supplied diet. Replacement of FM with RPC reduced diet's content of EPA, DHA, C20:4*n*-6, ∑*n*3, MUFA and EPA+DHA, but increased the ∑*n*6 (Table 3). Furthermore, high content of linoleic acid (C18:2*n*-6) and low content of DHA and EPA in the RPC raw material (Table 2) determined higher percentage of

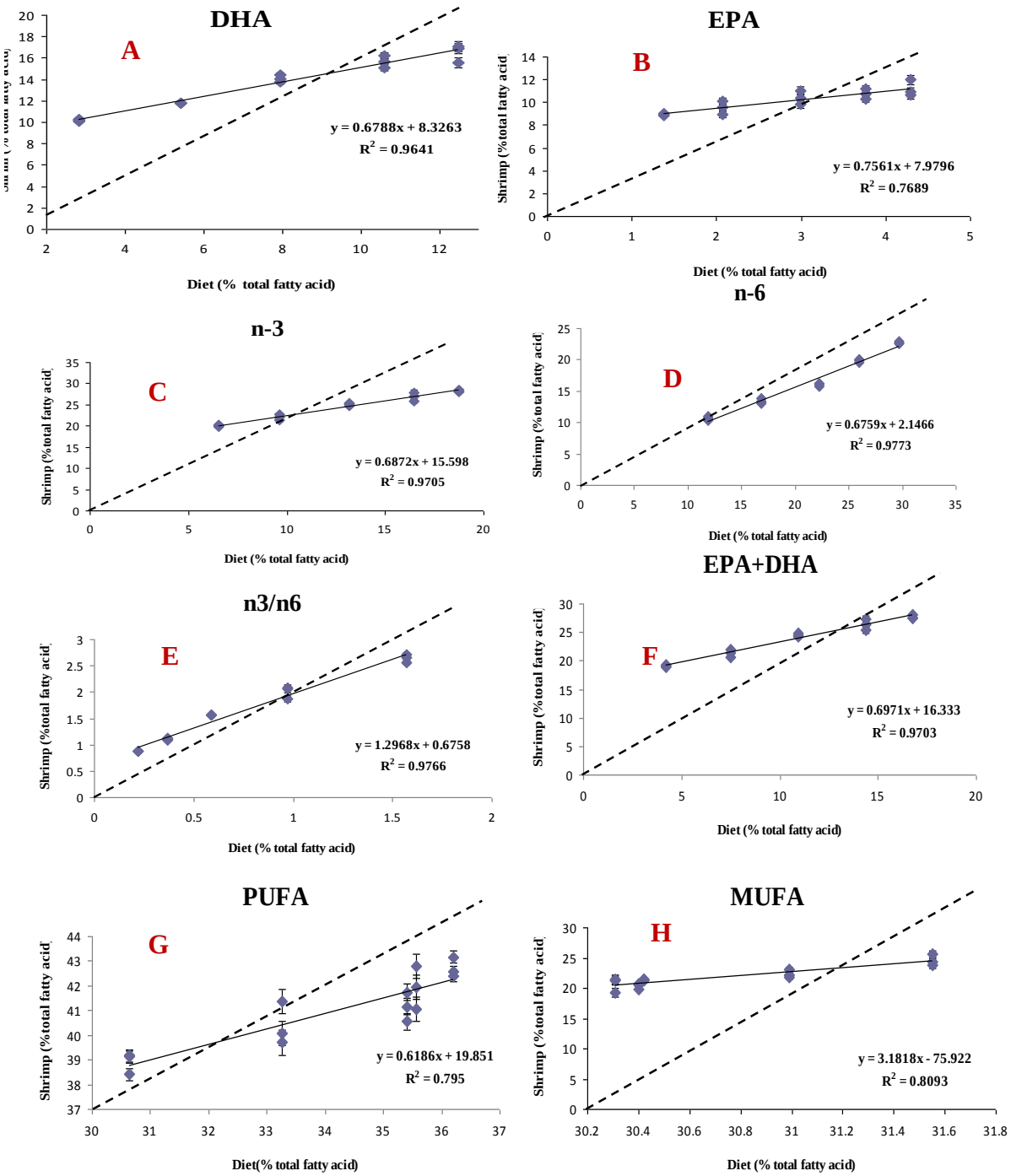
C18:2*n*-6 and lower percentage of DHA and EPA in tail-muscle of shrimp fed with RPC diets than those fed with FM. The C18:2*n*-6 content rose from 6.99% in the FM to 18.87% in the RPC100% (Table 5). Conversely, a decreasing trend from 11.18% to 8.95% and 16.53% to 10.18% was found in C20:5*n*-3 (EPA) and C22:6*n*-3 (DHA) contents, respectively. The ratio of *n*-3 to *n*-6 FAs was highest (2.64%) in tail-muscle of shrimp fed with FM and lowest (0.87%) in shrimp fed with 100% RPC. In order to emphasize these trends, a regression approach was used, plotting these FAs in the tail-muscle and the FAs diets (Figs 3, A-H). The high correlation coefficients confirm our findings that some FAs in the tail-muscle reflected those of the diets. The atherogenicity and trombogenicity indexes showed significant differences between the experimental diets, and TI increased with an increasing RPC inclusion level. The lowest values of the AI, and the most desirable level from the health point of view, was 0.344 and 0.342 % for tail-muscle of shrimp fed with FM and 75% RPC, respectively.

Table 5: Tail-muscle fatty acid composition of juvenile *Litopenaeus vannamei* reared on different diets (% of total fatty acid expressed as mean ± SEM).

FA (%)	% Replacement				
	0	**25**	**50**	**75**	**100**
C14:0	0.33 ± 0.00^{a}	0.34 ± 0.00^{a}	0.29 ± 0.00^{b}	0.35 ± 0.35^{a}	0.27 ± 0.00^{c}
C16:0	20.51 ± 0.12^{ab}	21.23 ± 0.30^{a}	20.31 ± 0.21^{b}	20.29 ± 0.08^{b}	21.26 ± 0.35^{a}
C16:1*n*-7	1.12 ± 0.04^{a}	0.77 ± 0.00^{b}	0.56 ± 0.06^{c}	0.21 ± 0.00^{d}	0.51 ± 0.02^{c}
C17:0	1.30 ± 0.01^{a}	1.19 ± 0.02^{b}	0.98 ± 0.00^{c}	0.78 ± 0.01^{d}	0.70 ± 0.00^{e}
C17:1*n*-7	0.31 ± 0.00^{a}	0.32 ± 0.00^{a}	0.32 ± 0.03^{a}	0.18 ± 0.00^{b}	nd
C18:0	11.01 ± 0.09^{a}	11.12 ± 0.05^{a}	11.00 ± 0.08^{a}	10.43 ± 0.00^{b}	10.12 ± 0.15^{c}
C18:1*n*-7	2.88 ± 0.06^{a}	2.69 ± 0.02^{b}	2.27 ± 0.02^{c}	1.91 ± 0.03^{d}	1.70 ± 0.02^{c}
C18:1*n*-9	19.77 ± 0.56^{a}	18.01 ± 0.38^{bc}	16.63 ± 0.31^{c}	18.49 ± 0.26^{ab}	17.92 ± 0.65^{bc}
C18:2*n*-6	6.99 ± 0.21^{e}	9.82 ± 0.29^{d}	12.21 ± 0.17^{c}	15.93 ± 0.19^{b}	18.87 ± 0.15^{a}
C18:3*n*-3	0.50 ± 0.03^{c}	0.52 ± 0.00^{c}	0.58 ± 0.01^{c}	0.74 ± 0.02^{b}	0.84 ± 0.03^{a}
C20:1*n*-9	0.54 ± 0.01^{ab}	0.53 ± 0.00^{abc}	0.51 ± 0.00^{bc}	0.55 ± 0.00^{a}	0.50 ± 0.00^{c}
C20:2*n*-6	1.02 ± 0.02^{d}	1.31 ± 0.01^{c}	1.42 ± 0.04^{bc}	1.49 ± 0.03^{ab}	1.60 ± 0.05^{a}
C20:4*n*-6	2.66 ± 0.03^{a}	2.30 ± 0.12^{b}	2.39 ± 0.06^{b}	2.45 ± 0.03^{ab}	2.24 ± 0.09^{b}
EPA	11.18 ± 0.39^{a}	10.73 ± 0.26^{a}	10.43 ± 0.35^{ab}	9.53 ± 0.32^{bc}	8.95 ± 0.04^{c}
DHA	16.53 ± 0.47^{a}	15.66 ± 0.31^{b}	14.10 ± 0.18^{c}	11.77 ± 0.01^{d}	10.18 ± 0.04^{e}
SFA	33.17 ± 0.21^{a}	33.90 ± 0.34^{c}	32.59 ± 0.13^{b}	31.86 ± 0.10^{d}	32.37 ± 0.23^{e}
MUFA	24.63 ± 0.54^{a}	22.34 ± 0.36^{b}	20.59 ± 0.06^{c}	21.37 ± 0.09^{bc}	20.65 ± 0.69^{c}
PUFA	38.90 ± 0.24^{d}	40.37 ± 0.50^{c}	41.15 ± 0.34^{bc}	41.93 ± 0.50^{ab}	42.71 ± 0.23^{a}
∑*n*3	28.21 ± 0.12^{a}	26.93 ± 0.58^{b}	25.11 ± 0.18^{c}	22.05 ± 0.37^{d}	19.98 ± 0.11^{e}
∑*n*6	10.68 ± 0.18^{e}	13.43 ± 0.19^{d}	16.03 ± 0.15^{c}	19.87 ± 0.13^{b}	22.73 ± 0.12^{a}
*n*3/*n*6	2.64 ± 0.04^{a}	2.00 ± 0.06^{b}	1.56 ± 0.00^{c}	1.10 ± 0.01^{d}	0.87 ± 0.00^{e}
EPA+DHA	27.71 ± 0.14^{a}	26.40 ± 0.58^{b}	24.53 ± 0.16^{c}	21.31 ± 0.34^{d}	19.14 ± 0.08^{e}
[1]AI	0.344^{b}	0.360^{a}	0.349^{ab}	0.342^{b}	0.352^{ab}
[2]TI	0.303^{d}	0.325^{c}	0.332^{c}	0.353^{b}	0.383^{a}

[1]AI = [C12:0 + 4(C14:0) + C16:0] / [MUFA + *n*-3 PUFA + *n*-6 PUFA]; [2]TI = [C14:0 + C16:0 + C18:0] / [0.5MUFA + 0.5(*n*-6PUFA) + 3(*n*-3PUFA) + (*n*-3PUFA/*n*-6PUFA)]

Value in the same row having different superscripts are significantly different ($p<0.05$).



Figures 3: Relationships (means ± SEM) between dietary and tail-muscle fatty acid concentrations of A: DHA, B: EPA, C: *n*-3, D: *n*-6, E: *n*-3/*n*-6, F: EPA+DHA, G: PUFA and H: MUFA in total lipids of juvenile *Litopenaeus vannamei* reared on different diets. The spotted line indicates the line of a 1:1 correlation between % in diet and % in flesh.

Discussion

It seems that replacement of FM by plant proteins can alter the nutritional quality of shrimp muscle. This alteration from the human's nutritional and health points of view is important. Chemical composition of the experimental diets was equalled in protein, lipid and energy

and at levels supposed to be optimal for the Pacific white shrimp, but the FAs of the diets were not balanced. A consistent finding in feeding trials of all fish and shrimp species so far implies that FM is a much more digestible and useful feed ingredient than plant proteins. Better growth- promoting effect of FM diet obtained in the present study may be due to higher HUFA content in FM diet, especially EPA and DHA that have better nutritional value than 18:2*n*-6. No difference in growth was evident by increasing the dietary levels of RPC up to 50%, but further inclusions to 75 and 100% brought about a growth reduction (Fig. 1). In general, there are several reasons for total substitution of FM by plant proteins, such as problems related to amino acid imbalances and complex carbohydrates (NRC, 1993), anti-nutritional factors (Francis *et al*., 2001), and low digestibility of nutrient (Palmegiano *et al*. 2006, 2007).

Water stability of feed pellets is significantly affected by the type of plant protein in the diets, which are most likely to be caused by different fibre contents of the diets (Akiyama *et al*., 1992). The lowest water stability was exhibited by the 100% RPC diet that also possessed significantly higher fibre content (4.4%) than other diets. In contrast, FM with the lowest fibre content (3%) was the most stable of all diets tested.

FA composition of the aquatic animals may be altered by feeding (Bell *et al*., 2002; Lane and Kohler, 2006), so controlled diets can be used to manipulate FA profile of tissue lipids. This was confirmed in this study, which clearly established linear correlations between percentage of individual FAs in the dietary lipids and total lipids in muscle (Fig. 3 A-H). The main difference between experimental diets was in their C18:2*n*-6 and HUFA (EPA and DHA) levels. The percentage of C18:2*n*-6 was higher in RPC (35.21%), whereas that of EPA and DHA was higher in FM (5.12 and 17.1%, respectively). Certain FAs (C20:4*n*-6, EPA and DHA) have reportedly been selectively retained and/or synthesized in muscle tissue of shrimp (Gonzalez-Felix *et al*., 2002). In this regard, it is of interest that complete replacement of FM in *L. vannamei* diet with RPC reduced the percentage of DHA in dietary lipids around six folds but in the muscle lipid only by around one and half fold. The relatively high levels of HUFA in body lipids of shrimp, when fed essential FA free diets, is suggested to be, probably the result of preferential utilization of short- and medium chained FA as energy source for metabolism rather than an increase in the absolute content of the HUFA (Xu *et al*., 1994).

Since penaeid shrimps have a limited capacity to synthesize highly unsaturated fatty acids (HUFA) from linoleic and linolenic acids (Kanazawa *et al*., 1979; Read, 1981), some of these FAs are therefore needed.

In addition, Ulbricht and Southgate (1991) reported that lipid nutritional quality and the values of the AI or TI are inversely related. Higher values of AI and TI (>1.0) are detrimental to human health (Bobe *et al*., 2004). In this study

the AI and TI showed significant variation, but were very favourable for the consumers' health. Those values were also lower than those registered in terrestrial products (Palmegiano *et al*., 2000).

RPC can be used in *L. vannamei* diet up to 50% inclusion without any reduction in growth performance, but resulted in an almost linear reduction in the quality of tail-muscle FA profile. FAs levels in shrimp muscle were strongly influenced by the dietary levels of FAs. Replacement of FM with RPC resulted in reduced levels of PUFA n-3 in shrimp muscle tissue. Decreasing the levels of n-3 PUFA could be a potential drawback for plant protein substitution from a human nutritional point of view.

Acknowledgements

The authors would like to thank the Jonob Feridis Aquaculture Center (Bushehr Province, IRAN), particularly Mr. M. Mousavi and E. Zariffard for providing the facilities. This study has been financed by Tarbiat Modares University. We are also grateful to BGMP Co., Ltd. of South Korea for supplying RPC.

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