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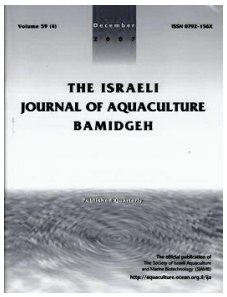

The Effect of Replacement of Fish Oil by Soybean Oil in Practical Diets, on Tissue Fatty Acid and Expression of Related Genes in Pacific White Shrimp *Litopenaeus vannamei*

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Key words: Replacement; fish oil; sobean oil; gene-expression; white shrimp

Abstract

High prices and unsustainable supply have rendered the use of high levels of fish oil in aquafeeds problematic. In the present study, an eight-week feeding trial was conducted to evaluate the replacement of fish oil with less expensive and more sustainable soybean oil in a practical diet containing 17% fish meal, which is widely used in China for Pacific white shrimp *Litopenaeus vannamei*. Five diets with five levels of fish oil replacement (0%, 25%, 50%, 75%, and 100%) by soybean oil were fed to shrimp for 56 days. At the end of the trial results obtained after analysis of the shrimp showed that shrimp fed diets containing 50% fish oil and 50% soybean oil displayed no significant differences in weight gain, specific growth rate, survival, and feed conversion ratio. Quantitative polymerase chain reactions revealed that the expression levels of fatty acid binding protein and fatty acid synthase, two critical genes in fat metabolism, gradually decreased with increased levels of soybean oil in diets. Fatty acid profiling showed that complete replacement of fish oil with soybean oil affected fatty acid content in shrimp muscles, including monounsaturated fatty acid, polyunsaturated fatty acid, and highly-unsaturated fatty acid, as well as the ratio of n-3/n-6 polyunsaturated fatty acids. The expression of two genes decreased with increased soybean oil level in diets. The growth results indicate that soybean oil can be used to replace 50% fish oil in this practical diet for *Litopenaeus vannamei* without adverse effects on shrimp growth performance.

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Introduction

The demand for seafood is expected to keep rising as the world population continues to grow and people become more affluent and conscious of healthy food choices. However capture fisheries have reached maximum sustainable level, therefore aquaculture has become the best way to meet the demand for sea food (FAO 2006). Pacific white shrimp *Litopenaeus vannamei*, which is native to the Pacific coast of central and south America and was introduced to the Eastern hemisphere in 1985, is the leading cultured shrimp species worldwide (Lin et al. 1990; FAO 2009). In just 8 years from 1999 to 2007, the total aquaculture production of *L. vannamei* increased dramatically from 186,113 tons to 2,296,630 tons (FAO 2009). The growth of the shrimp industry has been accompanied by an increase in feed production, leading to increased cost and lower availability of some ingredients. One of these ingredient is fish oil, which is widely used in formulated aquatic feeds as a major source of nutrient lipids (Cuzon et al. 2004; Tacon and Metian 2008). In 2006, ~835,000 tons of fish oil was used in aquatic feeds, constituting ~88.5% of the total reported fish oil production for that year (Tacon and Metian 2008). The uncertainty of availability and increasing costs has elevated the price of fish oil. The cost of feeds can be 40-60% of production costs, thus the cost of shrimp production would be substantially decreased by reducing or completely replacing expensive ingredients like fish oil in formulated feeds (Hertrampf et al. 2000). In addition, increasing social and environmental concerns regarding sustainability of marine products further discourage the use of fish oil. A considerable reduction in the use of fish oil or even complete replacement by alternative oils represents an important strategy for further development of shrimp feeds.

Fish oil has long been used in formulated practical shrimp feeds as the main source of lipids (Cuzon et al. 2004; Tacon and Metian 2008). Lipids provide energy and are essential for shrimp growth, health, welfare, and reproduction (Lim et al. 1997; Gonzalez-Felix et al. 2010). In addition to cost and sustainability, there are other considerations to take into account when planning the replacement of fish oil with alternative oils. Essential fatty acid (EFA) requirements of shrimp must be met to avoid deficiencies; shrimp possess limited ability to synthesize *de novo* the n-6 (including linoleic acids 18:2n-6, LOA) and n-3 (including linolenic acids 18:3n-3, LNA) families of polyunsaturated fatty acids (PUFA). They also have very limited capability to elongate and de-saturate these PUFA to highly unsaturated fatty acids (HUFA), including arachidonic (20:4n-6, AA), eicosapentaenoic (20:5n-3, EPA), and decosahexaenoic (22:6n-3, DHA) acids (Kanazawa et al. 1979; Kayama et al. 1980). 5–10g/kg of lipid content and the associated C18 PUFA, as well as n-3 and n-6 HUFA are required in feeds for shrimp and other crustaceans (Akiyama et al. 1992; Gonzalez-Felix et al. 2002a). In addition the quality of the final shrimp product should not be compromised by alterations in feeds. Marine animals including shrimp are a good dietary source of the n-3 FAs which are considered healthier than n-6 FAs. n-6 FAs are pro-inflammatory and thus promote pathogenesis of diseases, while n-3 FAs are anti-inflammatory and able to prevent sudden cardiac death caused by arrhythmias (Russo 2009). Several studies have shown that the fatty acid profile of shrimp tissue reflects that of the diets (Deering et al. 1997; Glencross et al. 2001; Gonzalez-Felix et al. 2002b; Gonzalez-Felix et al. 2003). Thus it is critical to ensure that changes in formulated shrimp diets do not lead to decreased n-3 FAs levels and n-3/n-6 FAs ratio.

Several studies have been conducted to test replacement of fish oil with alternative lipid sources in feeds for *Litopenaeus vannamei,* and have demonstrated mixed success (Patnaik et al. 1996; Lim et al. 1997; Zhou et al. 2007; Gonzalez-Felix et al. 2010; Samocha et al 2010; Samocha et al. 2011). More studies are needed to further investigate the development of shrimp feeds using alternative oils, because failure on an industry wide level will inevitably have a negative impact on shrimp production. There are no studies on the effect of soybean oil on the expression levels of genes involved in fat metabolism in shrimp muscle. In this study, our aim was to determine a practical diet for for *L. vannamei* with partial replacement of fish oil with soybean oil which is more cost-effective, and to evaluate the relationship between the experimental diets and gene expression on fat metabolism in their muscle. We chose soybean oil because of its low cost, global availability, and because it has been tested in other formulated shrimp feeds (Sookying et al. 2013).

Materials and Methods

Experimental Diets. Five iso-nintrogenous (36% crude protein) and iso-energetic (14.13 kJ/g diet) experimental diets were formulated to contain 7.5% lipid with different combinations of fish and soy oil. Five levels of soybean oil substitution for fish oil were tested; 3:1, 1:1, 1:3, fish oil alone and

soybean oil alone. The nutritional content and the preparation and storage of diets were based on previous reports (Shiau 1998; Kureshy and Davis 2002; Cuzon et al. 2004). The proximate composition of the experimental diets are shown in Table 1 and the dietary fatty acid composition are shown in Table 2. Water and lipids were added to the premixed dry ingredients and thoroughly mixed in a Hobart-type mixer. The 1.6 mm diameter sinking pellets were wet-extruded, air-dried to about 11% moisture, sealed in plastic bags, and stored frozen until feeding.

Table 1. Ingredient composition (g/kg diet) and proximate analysis (g/kg dry weight) of practical diets containing increasing levels of soybean oil to replace fish oil on an equal oil basis.

Ingredients	*Diets*				
	FO	*FO3:SO1*	*FO1:SO1*	*FO1:SO3*	*SO*
Fish meal (anchovy by-product)[1]	170	170	170	170	170
Soybean meal	200	200	200	200	200
Wheat meal	300	300	300	300	300
Peanut meal	150	150	150	150	150
Rapeseed meal	100	100	100	100	100
Soya lecithin	20	20	20	20	20
Calcium phosphate monobasi	20	20	20	20	20
Mineral mixture[2]	10	10	10	10	10
Vitamin mixture[3]	10	10	10	10	10
Fish oil	20	15	10	5	0
Soybean oil	0	5	10	15	20
Total	1000	1000	1000	1000	1000
Proximate analysis					
Moisture			105		
Crude protein (N×6.25)			375		
Ether extract			75.4		
Calcium			13.5		
Phosphate			13		
Gross energy (kj/g)			18.6		

[1] Playa Seca, Punta Puntillas s/n ,Distritoy Provincia de Paita, Departamento de PIURA. Others feed ingredients were purchased from Guangdong Evergreen Feed Industry Co., Ltd (Zhanjiang, China).

[2] Mineral premix consisted of (g/kg premix) the following: KCl, 55; $MgSO_4 \cdot 7H_2O$, 70; $NaH_2PO_4 \cdot 2H_2O$, 310; Ca–lactate, 200; Ferric citrate, 20; $AlCl_3 \cdot 6H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$, 1.8; $CuCl_2$, 1.6; $MnSO_4 \cdot H_2O$, 0.3; KI, 0.1; $CoCl_2 \cdot 6H_2O$, 0.5; and a-cellulose, 368.

[3] Vitamin premix supplied the diet with (mg/kg premix) the following: retinyl acetate, 0.1; all-rac-atocopherol, 450; menadione, 45; thiamin hydrochloride, 65; riboflavin, 200; pyridoxine hydrochloride, 40; nicotinic acid,740; D-Ca pantothenate, 230; inositol,450; biotin, 6.0; folic acid, 15; PABA, 400; choline chloride, 8000; cyanocobalamin, 0.1; h-carotene, 12.

Table 2. Fatty acid compositions (percentage of weight to total fatty acids) of the five experimental diets.

Fatty acid	*Diets*				
	FO	*FO3:SO1*	*FO1:SO1*	*FO1:SO3*	*SO*
C14:0	3.48	3.02	2.54	2.10	1.59
C15:0	0.38	0.34	0.29	0.24	0.18
C16:0	17.23	17.15	17.09	17.05	17.06
C16:1	3.64	3.19	2.73	2.29	1.78
C17:0	0.90	0.79	0.68	0.56	0.43
C18:0	3.82	3.94	4.04	4.15	4.18
C18:1n-9	18.72	20.17	21.53	23.10	24.61
C18:2n-6	21.39	23.57	26.03	28.45	31.52
C18:3n-3	2.10	2.24	2.44	2.61	2.85
C20:0	0.56	0.55	0.53	0.52	0.50
C20:1n-9	1.37	1.33	1.19	1.08	0.92
C21:0	0.09	0.09	0.07	0.08	0.00
C20:4n-6	0.52	0.47	0.42	0.36	0.28
C22:0	0.62	0.63	0.63	0.64	0.64
C20:5n-3	6.19	5.25	4.35	3.36	2.28
C22:6n-3	7.15	6.40	5.71	4.80	3.72
Saturated fatty acid (SFA)	27.08	26.50	25.88	25.34	24.58
Monounsaturated fatty acid (MUFA)	23.72	24.69	25.46	26.47	27.31
Polyunsaturated fatty acid (PUFA)	37.35	37.92	38.94	39.57	40.64
Highly unsaturated fatty acid (HUFA)	13.86	12.12	10.47	8.51	6.28
n3/n6	0.70	0.58	0.47	0.37	0.28

Shrimp, Tank System, and Feeding. Juvenile Pacific white shrimp were obtained from Guangdong Evergreen Group (Zhanjiang, Guangdong province, China). Prior to the present study, the shrimp were acclimated to commercial diets (diets for white shrimp, Guangdong Evergreen Feed Industry Co., Ltd, Zhanjiang, Guangdong Province, China) for two weeks. Twenty tanks (0.3 m^3) were stocked with 30 shrimp (initial weight 0.3±0.01 g) per tank, with four replicates per diet. Each experimental diet was randomly assigned to four tanks. Shrimp were hand fed four times per day at approximately 0700, 1100, 1600, and 2000 hours. The amount of feed consumed by the shrimp in each tank was recorded daily and the quantity adjusted according to feed consumption every day. The culture tanks were cleaned weekly. During the 8-week experimental period, temperature range was 26-32 ℃, salinity was 22-25 g/l, pH was 7.5-8.2, ammonia nitrogen was lower than 0.05 mg/l, and dissolved oxygen was no less than 5.0 mg/l.

Growth Parameters. At the end of the growth trial, mean weight gain, and survival rate for each dietary treatment were calculated. Finally all shrimp were chilled and stored frozen at -20 ℃ for subsequent determination of whole body nutrient composition. Parameters calculated were:

Weight gain (WG) =100× (Wt-Wi)/Wi

Specific growth rate (SGR %/day) = [(lnWt−lnWi/T)]*100

Feed conversion ratio (FCR) = Feeds consumed (g, dry weight)/body weight gain (g)

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

where Wt= final weight, Wi= initial weight, and T= total experimental days

Proximate Analysis, Extraction and Fatty Acid Analysis. Crude protein, crude lipid, moisture, crude ash, calcium, and total phosphorus in diets, muscle, and whole body were determined by standard methods (AOAC 1995). Moisture was determined by oven-drying at 105 ℃ for 24 h. Crude protein (N×6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (Xianjian, Shanghai, China). Crude lipid was determined by the ether-extraction method using a Soxtec System HT (Xianjian, Shanghai, China). Ash content was determined by muffle furnace (Rongfeng, Shanghai, China) at 550 ℃ for 24 h. At end of the feeding trial fatty acid analysis was carried out on dissected tail muscle tissue of 15 shrimp for each of the experimental diets (Table 2).

Lipid sources and experimental diets were analyzed for fatty acid composition at the beginning of the feeding trial. Lipids for fatty acid analysis were extracted from diets with chloroform and methanol (Bligh and Dyer 1959), methylated, and transesterified with boron trifluoride in methanol (AOAC 1995). Fatty acid methyl esters were identified and analyzed by an Agilent 7890A gas-liquid chromatography (California, USA) equipped with a flame ionization detector and an EZChrom Elite Compact. The esters were separated on a DB-23 fused in silica capillary column (60 m×0.32 mm, California, USA). Column temperature was set at 50 ℃ for the first 2 min, then increased to 220 ℃ at 4 ℃/min and held at this temperature for 20 min. An injector (split ratio 1/30) was used. Injector port and detector temperatures were 250 and 260 ℃, respectively. Helium was used as the carrier gas. Fatty acids were identified by comparing retention time.

Total RNA Isolation and Quantitative Polymerase Chain Reaction (qPCR) Analysis of Shrimp Fatty Acid Binding Protein (FABP) and Fatty Acid Synthase (FAS). Total RNA was isolated from the muscle of freshly ice-killed shrimp using TRI Reagent (Omega Bio-Tek, Norcross, USA) according to the manufacturer's description. The concentration and quality of RNA were determined by A260/A280 measurement and 2 μg of total RNA was pretreated with RQ1 RNase-free DNase (Omega Bio-Tek, Norcross, USA). Subsequently, treated RNA was reverse transcribed using M-MLV Reverse Transcriptase (Omega Bio-Tek, Norcross, USA) with random primer for synthesis of the first-strand cDNA.

Primers for amplification of shrimp fatty acid binding protein (FABP) (Accession DQ398572), FAS (Accession HM595630), and β-actin (Accession JF288784, used as an internal control) were designed based on nucleotide sequences deposited in the GenBank. Primers for FABP were 5′ - GGTTCACCCTCGACCCTTC-3′ (forward) and 5′ - ACATTGCCATTCTGGGACA-3′ (reverse). For FAS, forward and reverse primers were 5′ - TACGGAGAACCTAGTGGAAC-3′ and 5′- CTACCGACGACGAAAAGTGA -3′, respectively. For β-actin, the forward and reverse primers were 5′ - CAAGGAGAAACTGTGCTA -3′ and 5′- AGGAATGAGGGCTGGAAC -3′, respectively. Expected PCR product sizes for FABP, FAS and β-actin were 163, 115 and 165 bp, respectively. Real-time qPCR was performed using TaKaRa SYBR® Premix Ex Taq™ II (Dalian, China) on a Bio-Rad CFX96 Real-Time PCR System (California, USA).

Statistical Analysis. The results were expressed as mean ± standard deviation (SD). All data were subjected to one-way ANOVA analysis. When there were significant differences, the group means were further compared with Duncan's multiple range test. Effects with a probability of $P < 0.05$ were considered significant. All statistical analyses were performed using the SPSS 11.5 (SPSS, Chicago, USA)

Results

Growth Performance and Muscle Biochemical Composition of Shrimp. Shrimp fed diets containing 100% fish oil had slightly higher WG and SGR, and significant differences were observed when the fish oil content was less than 50% ($P<0.05$). Results showed that no significant differences in survival rate were observed among the different diets and no notable interaction was detected between the two oil sources ($P>0.05$). Shrimp fed diets containing more fish oil had lower FCR, but there were no statistically significant differences (Table 3).

Table 3. Growth performance and feed utilization efficiency of shrimp fed with different dietary lipids for 8 weeks*

Diet	BW	WG	SGR	Survival	FCR
FO	12.88^{a}±0.38	1949.67^{a}±15.38	4.96^{a}±0.01	95.56±2.22	1.43±0.01
FO3:SO1	12.52^{ab}±0.21	1943.24^{a}±36.16	4.95^{a}±0.03	98.89±1.11	1.43±0.03
FO1:SO1	12.22^{bc}±0.28	1845.41^{ab}±20.57	4.90^{ab}±0.01	95.56±2.22	1.51±0.03
FO1:SO3	11.96^{c}±0.22	1783.39^{b}±40.28	4.81^{b}±0.06	94.44±1.11	1.55±0.07
SO	12.01^{bc}±0.28	1808.24^{b}±56.56	4.82^{b}±0.05	96.67±1.92	1.50±0.04

*Different symbols denote significant differences ($p < 0.05$) ;Values are means ± SD.

Muscle proximate biochemical composition of the shrimp was not significantly affected by the dietary lipid source ($P>0.05$) (Table 4). Moisture, protein, lipid, ash, and total phosphorus contents of muscle did not differ among different dietary treatments. Muscle lipid and calcium levels decreased in relation to the inclusion of soybean oil from an initial mean value of 2.94% and 0.12% to a mean value of 2.8% and 0.07% respectively. There was no statistical significance between the diets.

Table 4. Muscle proximate composition (g/kg wet weight) of shrimp fed with different dietary lipids for 8 weeks*

Diet	Moisture	Protein	Lipid	Ash	Calcium	Total phosphorus
FO	728.83±13.72	751.22±25.08	29.39±0.52	45.70±3.54	1.23±0.14	8.24±0.22
FO3:SO1	735.63±7.91	788.64±14.61	29.20±1.49	45.88±2.17	0.88±0.14	9.38±0.79
FO1:SO1	735.47±11.66	803.21±16.09	28.77±0.49	45.82±2.40	0.76±0.10	9.52±0.81
FO1:SO3	714.60±15.29	788.37±18.66	28.04±0.33	45.86±2.31	0.70±0.12	8.65±0.43
SO	728.67±12.72	803.61±12.03	28.15±0.77	45.77±0.44	0.74±0.09	9.46±0.14

*Values are means ± SD

Muscle Fatty Acid Composition of Shrimp. Fatty acid content in the tail muscles of the shrimp were strongly influenced by dietary treatments (Table 5). In general, there was higher concentration of fatty acids in the muscles, and the inverse was also true. Increased amounts of C18:1, C18:2 and C18:3 were deposited in the muscle of shrimp fed with the diets which had higher levels of soybean oil. The concentration of C20:5n-3 (EPA), 22:6n-3 (DHA) and the ratio of n-3/n-6 decreased gradually with the increase of soybean oil inclusion. 20:4n-6 (ARA) was generally low in the muscle irrespective of the diet. In short, shrimps fed diets containing more soybean oil had higher monounsaturated (MUFA) but lower poly-unsaturated fatty acid and highly-unsaturated fatty acid (PUFA and HUFA) ($P<0.05$), and there were no significant differences between saturated fatty acid(SFA) ($P > 0.05$).

Table 5. Fatty acid compositions (% of total fatty acid) in the muscle lipids of shrimp fed with different dietary lipids for eight weeks*

Fatty acids	*Diets*				
	FO	*FO3:SO1*	*FO1:SO1*	*FO1:SO3*	*SO*
C14:0	0.29^{a}±0.01	0.30^{a}±0.01	0.26^{b}±0.02	0.22^{c}±0.01	0.20^{c}±0.01
C15:0	0.22^{a}±0.01	0.22^{a}±0.01	0.20^{a}±0.01	0.18^{b}±0.01	0.17^{c}±0.01
C16:0	18.82±0.07	18.70±0.06	18.65±0.11	18.64±0.15	18.60±0.09
C16:1	0.98^{a}±0.02	1.00^{a}±0.01	0.85^{b}±0.02	0.71^{c}±0.01	0.66^{c}±0.03
C17:0	1.08^{a}±0.01	1.06^{a}±0.01	1.02^{b}±0.02	0.96^{c}±0.01	0.88^{d}±0.01
C18:0	10.92^{a}±0.24	11.17^{ab}±0.08	11.18^{ab}±0.11	11.72^{b}±0.10	11.53^{b}±0.23
C18:1n-9	18.51^{a}±0.17	18.91^{a}±0.12	18.93^{b}±0.10	19.42^{b}±0.09	19.41^{c}±0.01
C18:2n-6	13.04^{a}±0.18	12.86^{a}±0.27	13.53^{a}±0.23	15.16^{b}±0.25	18.89^{c}±0.38
C18:3n-3	0.64^{a}±0.01	0.60^{a}±0.03	0.65^{ab}±0.03	0.72^{b}±0.02	0.85^{c}±0.03
C20:0	0.49±0.02	0.52±0.01	0.49±0.01	0.49±0.02	0.48±0.01
C20:1n-9	1.03^{a}±0.01	0.99^{a}±0.03	1.02^{a}±0.02	1.02^{a}±0.04	0.84^{b}±0.03
C21:0	0.13±0.01	0.12±0.01	0.12±0.01	0.12±0.01	0.11±0.01
C20:4n-6	1.88^{a}±0.03	2.23^{b}±0.04	1.98^{b}±0.02	1.99^{b}±0.09	1.66^{c}±0.03
C22:0	0.36±0.02	0.38±0.01	0.35±0.01	0.38±0.01	0.34±0.02
C20:5n-3	13.67^{a}±0.06	11.76^{b}±0.09	11.83^{b}±0.23	10.40^{c}±0.13	8.81^{d}±0.10
C22:6n-3	12.71^{a}±0.05	12.57^{a}±0.10	12.64^{a}±0.05	11.94^{b}±0.08	10.39^{c}±0.09
Saturated fatty acid (SFA)	32.31±0.30	32.47±0.10	32.28±0.04	32.71±0.15	32.31±0.17
Monounsaturated fatty acid (MUFA)	20.51^{a}±0.14	20.90^{b}±0.29	20.80^{ab}±0.12	21.15^{b}±0.11	20.92^{b}±0.06
Polyunsaturated fatty acid (PUFA)	41.94^{a}±0.28	40.01^{b}±0.43	40.62^{b}±0.49	40.21^{b}±0.26	40.60^{b}±0.34
Highly unsaturated fatty acid (HUFA)	28.26^{a}±0.19	26.55^{b}±0.25	26.44^{b}±0.48	24.33^{c}±0.20	20.86^{d}±0.10
n3/n6	1.81^{a}±0.02	1.65^{b}±0.03	1.62^{b}±0.03	1.34^{c}±0.02	0.98^{d}±0.03

*Different symbols denote significant differences ($p < 0.05$) ;Values are means ± SD.

Expression of FABP and FAS Gene in Muscle of Shrimp. The expression of FABP and FAS were strongly influenced by soybean oil levels in the diets ($P<0.05$) see Table 6.The results showed that the expression levels of FABP and FAS decreased with increased soybean oil levels in diets. The relative expression level of FABP and FAS in shrimp was 1.28 and 1.25 respectively when fed FO diet, but dropped to 0.03 and 0.12 respectively when fed SO diet.

Table 6. Expression of FABP and FAS gene in muscle of shrimp*

Diets	*FABP*	*FAS*
FO	1.28^{a}±0.33	1.25^{a}±0.10
FO3:SO1	0.86^{b}±0.11	1.03^{a}±0.06
FO1:SO1	0.43^{c}±0.02	0.68^{b}±0.03
FO1:SO3	0.13^{c}±0.02	0.32^{c}±0.03
SO	0.03^{c}±0.01	0.12^{c}±0.01

Discussion

Good lipid sources are vital to shrimp growth as they have limited ability to synthesize PUFA and desaturate PUFA to HUFA (Kanazawa et al. 1979; Kayama et al. 1980). Fish oils have been historically included in formulated aquafeeds as excellent sources of lipids (Tacon and Metian 2008). However, given the growing demand for fish oils by feed industries and the limited supply, prices are likely to increase. Growing economic, environmental, and market pressures, have discouraged the use of fish oil in aquafeeds (Tacon and Metian 2008). In light of these considerations, various studies have focused on the reduction of fish oil in commercial diets; however, complete replacement of fish oil with non-marine lipid sources has not produced optimal results. Menhaden oil rich in n-3 HUFA was better utilized by *P. vannamei* than all six tested vegetable oils (Lim et al. 1997). Supplementation of plant oils with DHA- and ArA-rich oils from fermented products was found to be a viable option to replace marine fish oil for *L. vannamei* (Samocha et al. 2010, 2011).

Growth performance and protein efficiency ratio were significantly higher for shrimp fed diets containing Pollack fish oil or a mixture of fish oil and soy oil than vegetable oil or pork lard (Zhou et al. 2007). These observations show that shrimp have very limited ability to synthesize PUFA and desaturate PUFA to HUFA (Kanazawa et al. 1979; Kayama et al. 1980). In addition to the EFA requirement of shrimp, it is important that the content of n-3 fatty acid is not compromised by alternative lipid sources. In a plant-based diet, replacement of up to 90% fish oil with soy oil had no adverse effects on shrimp growth performance, but dramatically reduced n-3/n-6 ratio, suggesting that 10% fish oil can meet the EFA requirement of shrimp but is not sufficient to maintain a good n-3/n-6 ratio (Gonzalez-Felix et al. 2010). Results of our study showed that 50% replacement of fish oil with soy oil, at least in the commercial diet tested here, had no adverse effects on shrimp growth performance, indicating that EFA requirement was met. Moreover, our results also showed that fish oil replacement by soy oil markedly alters the fatty acid content as well as n-3/n-6 ration in shrimp muscle. We noticed that the other feeding trials were carried out on totally plant-based diets checking protein in shrimp muscle, (Lim et al. 1997; Samocha et al. 2010 and 2011; Zhou et al. 2007; Gonzalez-Felix et al. 2010), but the diets in our study included 17% soy oil in fish meal. One explanation is that 17% soy oil in fish meal can meet the EFA requirement of shrimp.

Consistent with previous observations, our results also showed that the fatty acid content of shrimp reflects that of the diets, as the more FA there is in the diets the higher the content in the tail muscle (Deering et al. 1997; Glencross et al. 2001; Gonzalez-Felix et al. 2002b and 2003). Changes in FA content of shrimp induced by different soy oil inclusion was statistically significant and markedly affected shrimp growth and the n-3/n-6 FA ration of meat.

FABP and FAS are two critical genes in fatty acid metabolism and their expression is affected by the level of in-vivo fatty acid levels (Kaikaus et al. 1993; Sul et al. 1998; Smith et al. 2003). FABP is a family of carrier proteins for fatty acids and other lipophilic substances such as eicosanoids and retinoids (Chmurzynska 2006; Smathers and Petersen 2011). FABP bind to the intracellular fatty acids, thus maintaining a low level of unbound free fatty acids that could otherwise be detrimental to cells. FABP expression is modulated by developmental, hormonal, fatty acids, and pharmacological factors (Kaikaus et al. 1993). At present, there are few studies on the relationships between FABP and lipid sources. It was reported that fish oil replacement by vegetable oil led to decreased FABP expression in Atlantic salmon *Salmo salar* (Torstensen et al. 2009). Consistently, our results clearly showed that the expression of FABP in shrimp muscle was also down-regulated by soy oil in feeds.

Animal FAS is a multi-enzyme protein that catalyzes the synthesis of long-chain fatty acids, primarily palmitate, using acetyl-CoA and malonyl-CoA as substrates and NADPH as the reducing equivalent. It is not a single enzyme but a whole enzymatic system composed of two identical 272 kDa multifunctional polypeptides, in which substrates are handed from one functional domain to the next (Sul and Wang 1998; Smith et al. 2003). The transcription of FAS is regulated by nutritional and hormonal cues, including dietary fatty acids (Sul and Wang 1998). FAS was cloned from *L. vannamei* and its expression in both muscle and hepatopancreas was induced by white spot syndrome virus infection (Yang et al., 2011). We found that muscle expression level of FAS decreased gradually with the increase in dietary soy oil inclusion. Dietary PUFA suppresses FAS expression (Sul and Wang 1998). Our results showed that increasing soy oil inclusion in shrimp feeds led to increased PUFA levels in both diets and shrimp muscles. But whether this elevation in PUFA levels resulted in down-regulation in FAS expression needs further investigation.

In summary, results of the present study indicate that 50% fish oil could be replaced by soybean oil in practical diets for *L. vannamei* without any significant negative effect on the growth performance of shrimp. The expression of FABP and FAS decreased when soybean oil levels were increased in diets. Further investigation is needed to see whether similar results could be obtained from shrimp raised in ponds.

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