Available online at www.sciencedirect.com

SCIENCE DIRECT®

Aquaculture 250 (2005) 317–327

Aquaculture

www.elsevier.com/locate/aqua-online

Dietary value of different vegetable oil in black tiger shrimp *Penaeus monodon* in the presence and absence of soy lecithin supplementation: Effect on growth, nutrient digestibility and body composition

K.P. Kumaraguru vasagam*, S. Ramesh, T. Balasubramanian

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608502, Tamilnadu, India

Received 1 December 2004; received in revised form 11 February 2005; accepted 11 February 2005

Abstract

Sunflower oil, peanut oil, palm oil and sardine oil were evaluated for their dietary value in juvenile *Penaeus monodon* in the presence or absence of soy lecithin (SL). Eight isonitrogenous and isocaloric diets were formulated to contain each of the oils as the lipid source. Evaluation was based on the growth performance of shrimp (for 42 days) and apparent digestibility coefficients (ADC). The ADC of the diets was determined by comparing concentrations of the digestibility marker, chromic oxide in the feed and faeces of the shrimp. Shrimps fed diets containing vegetable oil with SL had significantly higher values ($P<0.05$) than those fed diets containing the same oil without SL in all the performance parameters tested. No significant differences ($P>0.05$) were observed among SL supplemented dietary treatments, although weight gain and feed efficiency were higher in shrimp fed diets containing peanut oil (PNL) and sardine oil (FOL) respectively. Among the dietary treatments without SL, shrimp fed sardine oil diet (FO) had significantly ($P>0.05$) higher values than other diets in all the performance parameters tested which did not differ from those fed SL supplemented diets. There was no significant difference ($P>0.05$) in survival of shrimp among the dietary treatments. Though apparent dry matter digestibility (ADMD) was not significantly differed among dietary oils, there exists a significant difference in ADC for crude protein, crude lipid and energy. All SL supplemented diets showed a marked increase in apparent crude lipid digestibility (ACLD) than the diets without SL. A high correlation was found between dietary phospholipid (PL) level and ACLD ($r=0.95$; $P<0.05$) of the experimental diets. Carcass composition of shrimp fed the different vegetable oil sources was similar. However, the lipid content was higher in the shrimp fed diets containing FO and FOL. The fatty acid composition of the test diets was reflected to a certain extent in the fatty acid composition of whole shrimp. The findings of the present work have shown that, shrimp fed vegetable oil supplemented with SL had significantly higher growth and nutrient digestibility comparable to that of sardine oil without SL.

Keywords: Shrimp diets; *Penaeus monodon*; Vegetable oil; Soy lecithin; Digestibility; Fatty acids

* Corresponding author. Tel.: +95 4144 243223; fax: +95 4144 243555.
E-mail address: vasagamguru@rediffmail.com (K.P. Kumaraguru vasagam).

0044-8486/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.aquaculture.2005.02.035

1. Introduction

Crustaceans require dietary lipid as a source of essential fatty acids (EFA) and other lipid classes like phospholipids (PL), sterols and carotenoids. The unique aspect of lipid nutrition in penaeid shrimp is the requirement of EFA. Various researchers consider that there are four fatty acids that are essential for *Penaeus monodon*: linoleic (18:2n-6, LOA), linolenic (18:3n-3, LNA), eicosapentaenoic (20:5n-3, EPA), and docosahexaenoic (22:6n-3, DHA) acids with latter two n-3 highly unsaturated fatty acids (HUFA) being the most indispensable (Catacutan, 1991; Merican and Shim, 1996; Glencross and Smith, 2001; Glencross et al., 2002).

IFOMA (2000) estimated that, given continuation of the current rate of expansion of aquaculture, global demand for fish oil for aquafeeds will equal the total global supply of fish oil by circa 2009. Therefore, in order to sustain the rapid growth of global aquaculture industry, it is imperative for the aquafeed industry to evaluate alternatives to fish oil (New and Wijkstrom, 2002; Tacon, 2002). Studies on the use of vegetable oil like palm oil (Ng et al., 2000; Tortensen et al., 2000) and sunflower oil (Hoffman and Prinsloo, 1995; Ng et al., 2003) have shown encouraging results in fish diets compared to fish fed equivalent levels of fish oil. However in shrimp, studies comparing lipid sources have shown contradictory results. Colvin (1976) observed that the nutritional value of sunflower oil, linseed oil, soybean oil and peanut oil were similar, although peanut oil gave the best performance in *P. indicus*. Vegetable oil high in linolenic acid promoted better growth of *P. japonicus* than those high in linoleic acid, while sardine and short-necked clam oil provided better growth and survival than any of the vegetable oils (Guary et al., 1976). Work done elsewhere in penaeid shrimp by Kanazawa et al. (1977), Catacutan (1991), and González-Félix and Perez-Velazquez (2002) found that fish oil rich in n-6 and n-3 HUFA proved more efficient in terms of growth than vegetable oils poor in these fatty acids. However in many studies, supplementing PL to the diet of shrimp showed increased growth regardless of the lipid source tested (Kanazawa et al., 1985; Pascual, 1986; Coutteau et al., 1996; Gong et al., 2001; González-Félix and Perez-Velazquez, 2002).

Kontara et al. (1997) proposed that PL may possibly improve the utilization efficacy of EFA supplied in the diet as neutral lipid, mostly triacylglycerol, and thus reduce the quantitative requirements for n-3 HUFA in shrimp diets. Glencross (1998) demonstrated the digestibility enhancing potential of PL in *P. monodon* through both in vivo and in vitro experiments. Meyers (1993) observed that PL also plays a functional role in improving the physical properties of manufactured feeds. Soybean lecithin has been widely used for dietary supplementation of PL in shrimp diet. Considering the diverse functional roles of PL and limited nutritive value of vegetable oil, the present investigation was designed to evaluate the dietary value of vegetable oil in the presence and absence of soy lecithin (SL) supplementation in juvenile *P. monodon*. Evaluation was made based on growth parameters, nutrient digestibility and final carcass composition including fatty acids.

2. Materials and methods

2.1. Diet formulation

Eight isonitrogenous (37.6 ± 0.27 g kg^{-1} crude protein) and isocaloric (18.74 ± 0.02 MJ kg^{-1} dry matter) diets were formulated for each of the oils (sardine oil—FO; sunflower oil—SO; peanut oil—PN; palm oil—PO) or their blend with SL (sardine oil + SL—FOL; sunflower oil + SL—SOL; peanut oil + SL—PNL; palm oil + SL—POL) as the lipid source. The lipid sources were included at a uniform level of 7 g kg^{-1}. Composition of experimental diets and their lipid combinations are presented in Table 1. All the ingredients except lipid sources were finely ground, sieved (100 μm), mixed in a Hobart mixer and made into dough like consistency by adding water. The dough was steamed in an autoclave at 100 °C for 10 min. The cooled dough was hand mixed thoroughly after adding the lipid source and vitamin mix. The dough was pelletized through a 2 mm die and dried overnight at 55 °C using a hot air oven. After drying, the pellets were stored at −5 °C until used.

Table 1
Ingredient composition (g kg $^{-1}$) and proximate composition of the test diets

Ingredients	FO	SO	PN	PO	FOL	SOL	PNL	POL
Common ingredients[a]	920	920	920	920	920	920	920	920
Fish oil[b]	70	–	–	–	50	–	–	–
Soy lecithin[c]	–	–	–	–	20	20	20	20
Coconut oil[d]	–	70	–	–	–	50	–	–
Peanut oil[d]	–	–	70	–	–	–	50	–
Palm oil[d]	–	–	–	70	–	–	–	50
Chemical composition[e] *(Percentage dry matter basis)*								
Dry matter	91.55	91.32	91.48	91.75	91.77	91.05	91.85	91.65
Crude protein	37.44	37.55	37.95	37.86	37.65	37.12	37.45	37.75
Crude lipid	8.68	8.64	8.62	8.64	8.63	8.63	8.64	8.63
Crude fibre	4.40	4.38	4.41	4.39	4.38	4.39	4.35	4.32
Ash	13.63	13.85	13.86	13.65	13.69	13.78	13.90	13.85
NFE[f]	35.83	35.62	35.08	35.5	35.68	36.01	35.70	35.48
Phospholipid	1.52	0.220	0.315	0.362	2.414	1.567	1.710	1.741
Total cholesterol	0.857	0.782	0.816	0.882	0.842	0.746	0.793	0.835
Gross energy (MJ/kg DM)	18.75	18.73	18.73	18.76	18.71	18.73	18.77	18.73

FO—sardine oil; SO—sunflower oil; PN—peanut oil; PO—palm oil; FOL—sardine oil+SL; SOL—sunflower oil+SL; PNL—peanut oil+SL; POL—palm oil+SL.

[a] All ingredients as g kg^{-1}, unless stated otherwise: fish meal (defatted), 252; squid meal, 50; shrimp meal, 40; soybean meal (defatted), 205; wheat flour, 285; CMC, 5; cholesterol, 5; spirulina, 5; di-calcium phosphate, 50; mould inhibitor, 3; vitamin mix, 10 (provided the following levels of nutrients—vitamin A acetate, 87,912 IU; cholecalciferol (D3), 2200 IU; tocopheryl acetate, (E), 550 IU; menadione, 22 mg; D—calcium pantothenate, 189 mg; pyridoxine HCl, 77.6 mg; riboflavin, 66 mg; niacin, 330 mg; folic acid, 22 mg; thiamin mononitrate, 73.9 mg; biotin, 2.2 mg; cyanocobalamin (B12), 0.1 mg; inositol, 220 mg; butylated hydroxytoluene, 22 mg) and mineral mix, 10 (provided the following levels of nutrients Cu, 2.8 mg; Fe, 39.1 mg; Zn, 107.3 mg; Mn, 41.1 mg; K, 1674 mg; I, 10 mg; Co, 0.5 mg; and Se, 0.4 mg).

[b] Body oil of sardine obtained from Coastal Aquatic Proteins, Mangalore, India.

[c] Standard fluid soy lecithin (63% phospholipid), obtained from—Central Soya, Fort Wayne, Indiana, United States of America.

[d] Food grade oil obtained from local grocery shop.

[e] Value is mean of triplicate samples.

[f] Nitrogen free extract calculated by difference.

2.2. Growth trial

Growth trials were performed in triplicate for 42 days in substrate free, circular plastic troughs (100-l capacity) provided with continuous aeration. Juvenile *P. monodon* (1.34 ± 0.07 g) were randomly stocked in each aquarium at a rate of eight-shrimp/trough. Previously, hatchery produced (Best India shrimp hatchery, Marakkanam, Chennai, India) post larvae were grown in concrete tanks with a commercial shrimp feed (Waterbase Private Limited, India) labeled to have 35% crude protein. Filtered seawater was used with a daily water exchange of 80%. Temperature was maintained at 28 ± 2 °C. Photo exposure was provided as alternating 12 h light and dark periods. Salinity, pH, dissolved-oxygen and ammonia–nitrogen concentrations in the water were measured once a week following the method of Strickland and Parsons (1972). Shrimp were fed three times per day, at approximately 09:00, 15:00 and 19:00 hours following a fixed feeding regime which was adjusted every week based on the growth and observed mortalities. One hour after feeding uneaten feed was siphoned from the bottom of tank and sieved to segregate the uneaten feed from faeces. Collected feed was gently rinsed with distilled water to eliminate excess salts and dried in a hot air oven to calculate feed intake. At the end of the growth trial, mean weight gain and survival for each dietary treatment were determined. Based on the feed input and comparative carcass analyses, feed efficiency (FE = wet weight gain × 100/dry weight feed offered), protein efficiency ratio (PER = wet weight gain × 100/dry

weight protein offered), energy retention efficiency (ERE = 100 × energy deposited / energy consumed) and daily growth rate (DGR = wet weight gain / experimental days × 100) were evaluated. Finally all shrimp were chill-killed and stored frozen at −20 °C for subsequent determination of whole body composition. Earlier samples of shrimp from the initial population were collected and stored frozen for the conduct of a similar evaluation.

2.3. Digestibility trial

The apparent digestibility coefficients (ADC) of dry matter, nutrients (protein and lipid) and energy in feeds were measured in vivo using chromic oxide (Cr_2O_3) an inert marker. Feed formulations were the same as for the growth trial except the addition of Cr_2O_3 (5 g kg^{-1}) at the expense of wheat flour. The feeds were prepared following the regrinding and re-extruding technique proposed by Smith and Tabrett (2004). Initially the feed dough was pelletized and air dried. Subsequently dried pellets were reground and pelletized again as explained in Section 2.1. Juvenile *P. monodon* (3.25 ± 0.7 g) grown in concrete tanks were randomly stocked in 27 circular plastic troughs (100 l). Each trough housed 6 shrimp with three replicate troughs per treatment. Shrimp were acclimated to the digestibility trough for one week before the initiation of trial and adapted to consume each experimental diet containing Cr_2O_3. Shrimp were fed four rations per day (05:00, 11:00, 17:00 and 23:00 hours) and allowed to feed 45 min after each feeding. The uneaten feed including a few faecal strands were siphoned from the bottom of the tanks after feeding time and discarded. Thereafter, the faecal matter was collected twice from the tank bottom before the next feeding (3 h after earlier feeding and 0.5 h before next feeding) for 20 days. The collected faeces was gently rinsed with distilled water to remove excess salt and dried in a hot air oven at 55 °C. The dried faecal material from each tank was pooled and stored frozen at −20 °C for analysis. The ADC of dry matter, nutrients and energy were determined according to Smith and Tabrett (2004) by following the equation: Digestibility % = 100 − (100 × % Cr_2O_3 in diet / % Cr_2O_3 in faeces × % nutrient in faeces / % nutrient in diet).

2.4. Biochemical analysis

Experimental feed and faecal samples were finely ground, sieved before analysis. Dry matter was calculated by gravimetric analysis following oven drying at 100 °C for 24 h. Ash content was determined gravimetrically by burning in Muffle furnace at 550 °C for 6 h. Standard methods of AOAC (1990) was followed for estimation of crude protein content (AOAC 955.04). Crude fibre was analysed according to Van Soest et al. (1991). Estimation of crude lipid, total cholesterol and total PL were carried out following the methods of Folch et al. (1957), Zlatkis et al. (1953) and Rouser (1970), respectively. Gross energy (GE) was determined with an adiabatic bomb calorimeter (Parr Model 124), using benzoic acid as standard. Fatty acids were saponified and methylated using 2% NaOH in methanol, 14% BF_3/methanol and heptane. The fatty acid methyl esters (FAME) were determined on a Hewlett Packard HP 5890 gas chromatograph equipped with a flame ionisation detector. The sample was injected at 190 °C onto a J and W Scientific DB23 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with hydrogen as the carrier gas. The column was operated isothermally at an oven temperature of 180 °C and a detector temperature of 210 °C. Fatty acids were identified by comparing with authentic standards. The chromic oxide content of diets and faecal samples was analysed by the method of Furukawa and Tsukahara (1966).

2.5. Statistical analysis

To determine the effect of SL on oil type, dietary treatments were analysed by 4 × 2 factorial ANOVA in which the oil source with and without SL were treated as two blocks of variables. Treatments with in each block were compared by one way ANOVA. Duncan's multiple range test (Duncan, 1955) was applied to ascertain any significant differences between treatment means. Correlation analysis was done on the PL content of feeds against the ADC obtained and performance parameters tested to delineate the relation between the variables. All the above mentioned statistical analyses were performed using SPSS statistical software (Ver.10 for Windows, SPSS,

Chicago, IL, USA). Limits of significance for all critical ranges were set at $P<0.05$.

3. Results

All growth trials were conducted without interruption or disease problems. The water quality parameters across all experiments were: salinity, 28–30.1‰; temperature, 25.5–28.5 °C; dissolved oxygen, >5.67 mg l^{-1}; total ammonia–nitrogen, 0.04–0.07 mg l^{-1}; nitrite–nitrogen, 0.09–0.11 mg l^{-1}; and pH, 7.9–8.2.

Composition of the feeds on a dry weight basis is presented in Table 1. Crude protein and energy content of the test diets were relatively constant; therefore the diets were considered isonitrogenous and isocaloric, respectively. All test diets contained the same level of lipid (8.64 ± 0.02 g kg^{-1}) regardless of the sources and combination of lipid sources used. The fatty acid content varied significantly among experimental diets with respect to the lipid source (Table 2). Fatty acid contribution of SL seemed to be particularly important in the case of LOA, because SL is rich in this fatty acid.

3.1. Growth performance

Data on growth performance and survival of *P. monodon* are presented in Table 3. Shrimp fed diets containing vegetable oil with SL (SOL, PNL and POL) had significantly higher values ($P<0.05$) than those fed diets containing the same oil without SL (SO, PN and PO) for all the performance parameters tested (weight gain, FE, PER, ERE and DGR). No significant differences ($P>0.05$) were observed among the SL supplemented dietary treatments, although weight gain and FE were higher in shrimp fed diets PNL and FOL respectively. Among the dietary treatments without SL, shrimp fed FO had significantly ($P>0.05$) higher values than diets SO, PN and PO in all the performance parameters tested. On the other hand, shrimp fed FO did not differ from those fed SL supplemented diets (FOL, SOL, PNL and POL), indicating a better utilization of those vegetable oils comparable to sardine oil in the presence of SL. Survival of shrimp was not significantly ($P>0.05$) affected by dietary treatments. Despite the considerable enhancement of PL content in SL supplemented diets, a weak

Table 2
Fatty acid composition (percentage of total) of experimental diets[a]

Fatty acid	Experimental diets							
	FO	SO	PN	PO	FOL	SOL	PNL	POL
8:0	–[b]	–	–	–	–	–	–	–
10:0	–	–	–	–	–	–	–	–
12:0	0.2	–	–	0.2	0.1	–	–	0.1
14:0	0.4	0.2	0.5	1.1	0.3	0.2	0.4	0.8
14:1	1	0.8	1.1	0.8	0.9	0.8	1	0.8
16:0	14.2	7.4	17.7	37.7	15	10.3	17.5	31.3
16:1	5.1	0.1	0.3	0.1	3.6	0.1	0.2	0.1
16:2	1	0.2	–	–	0.9	0.3	0.1	0.1
16:3	1.2	0.3	0.6	0.3	0.9	0.3	0.5	0.3
16:4	0.5	0.2	0.9	0.2	0.4	0.2	0.7	0.2
18:0	3.3	3.4	8.4	3.3	3.3	3.4	6.8	3.3
18:1	10.9	22.9	18.4	35.6	9.3	17.5	14.5	26.5
18:2n-6	20.8	54.2	27.4	10	31.1	54.1	35.6	23.5
18:3n-6	0.4	0.1	0.1	0.1	0.3	0.1	0.1	0.1
18:3n-3	3.3	0.5	2	0.6	4.6	2.7	3.7	2.7
18:4n-3	1.3	1.1	1.2	1.1	1.2	1.1	1.2	1.1
20:0	3.3	2	2	2.3	2.9	2	2	2.2
20:1	1.4	0.4	1	0.4	1.1	0.4	0.8	0.4
20:2n-6	0.5	0.1	2.6	0.1	0.4	0.1	1.8	0.1
20:3n-3	1.3	1	1	1	1.2	1	1	1
20:4n-6	0.8	0.2	0.9	0.2	0.7	0.2	0.7	0.2
20:3n-6	0.8	0.1	0.4	0.1	0.6	0.1	0.3	0.1
22:0	1.9	0.3	0.3	0.3	1.4	0.3	0.3	0.3
20:5n-3	10.1	1.2	5.5	1.1	7.5	1.3	4.3	1.3
22:1	2	0.6	1.6	0.6	1.6	0.7	1.4	0.7
22:2	0.7	0.3	0.4	0.3	0.6	0.3	0.4	0.3
22:3	0.6	–	–	–	0.4	–	–	–
22:4	0.5	0.2	0.3	0.2	0.4	0.2	0.3	0.2
22:5n-3	2.4	0.7	1.1	0.8	1.9	0.8	1	0.8
22:6n-3	10.1	1.5	4.3	1.5	7.4	1.5	3.4	1.5
Saturates[c]	23.3	13.3	28.9	44.9	23	16.2	27	38
Monounsaturates[d]	20.4	24.8	22.4	37.5	16.5	19.5	17.9	28.5
PUFA and HUFA[e]	56.3	61.9	48.7	17.6	60.5	64.3	55.1	33.5
Total n-3[f]	28.5	6	15.1	6.1	23.8	8.4	14.6	8.4
Total n-6[g]	23.3	54.7	31.4	10.5	33.1	54.6	38.5	24

FO—sardine oil; SO—sunflower oil; PN—peanut oil; PO—palm oil; FOL—sardine oil+SL; SOL—sunflower oil+SL; PNL—peanut oil+SL; POL—palm oil+SL.

[a] Fatty acid values (percentage of total fatty acid methyl esters) were adjusted to express a percent of the total area identified in the chromatograms, unidentified peaks were not considered in the computations.

[b] –=0.0, not detected.

[c] Saturates: 12:0, 14:0, 16:0, 18:0, 20:0, 22:0.

[d] Monounsaturates: 14:1, 16:1, 18:1, 20:1, 22:1.

[e] PUFA and HUFA: 16:2, 16:3, 16:4, 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2, 22:3, 22:4, 22:5n-3, 22:6n-3.

[f] Total n-3: 18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.

[g] Total n-6: 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6.

Table 3
Growth performances of *Penaeus monodon* fed diets containing different lipid sources and their blend with soy lecithin

Parameters	FO	SO	PN	PO	FOL	SOL	PNL	POL
Initial weight (g)	1.25 ± 0.15	1.32 ± 0.12	1.35 ± 0.18	1.4 ± 0.07	1.45 ± 0.21	1.28 ± 0.19	1.25 ± 0.13	1.42 ± 0.13
Weight gain (g)	2.95 ± 0.51^{b}	2.18 ± 0.57^{a}	2.12 ± 0.45^{a}	2.15 ± 0.41^{a}	3.06 ± 0.23^{b}	3.05 ± 0.37^{b}	3.1 ± 0.18^{b}	3.07 ± 0.37^{b}
FE (%)	45.88 ± 1.2^{b}	34.60 ± 1.6^{a}	33.65 ± 2.1^{a}	34.4 ± 2.3^{a}	46.36 ± 1.1^{b}	46.0 ± 1.3^{b}	44.73 ± 2.4^{b}	45.96 ± 1.3^{b}
PER	1.23 ± 0.05^{b}	0.92 ± 0.08^{a}	0.89 ± 0.12^{a}	0.91 ± 0.09^{a}	1.23 ± 0.05^{b}	1.24 ± 0.15^{b}	1.19 ± 0.28^{b}	1.22 ± 0.14^{b}
ERE (%)	10.89 ± 1.5^{b}	8.24 ± 0.89^{a}	8.04 ± 0.92^{a}	8.18 ± 1.24^{a}	11.03 ± 1.23^{b}	10.92 ± 1.34^{b}	10.58 ± 0.94^{b}	10.9 ± 1.47^{b}
DGR[a]	70.24 ± 4.12^{b}	51.9 ± 3.45^{a}	50.48 ± 2.65^{a}	51.19 ± 3.54^{a}	72.86 ± 3.12^{b}	72.62 ± 2.57^{b}	73.81 ± 2.14^{b}	73.1 ± 1.56^{b}
Survival (%)	98.6 ± 1.4	97.7 ± 2.9	97.7 ± 2.9	98.6 ± 1.4	99.5 ± 0.8	99.1 ± 0.8	99.5 ± 0.8	99.1 ± 0.8

FE—feed efficiency; PER—protein efficiency ratio; ERE—energy retention efficiency; DGR—daily growth rate, FO—sardine oil; SO—sunflower oil; PN—peanut oil; PO—palm oil; FOL—sardine oil+SL; SOL—sunflower oil+SL; PNL—peanut oil+SL; POL—palm oil+SL. Means in the same row sharing different superscripts are significantly different ($P<0.05$).
[a] mg/day/shrimp.

correlation was observed between dietary PL versus weight gain, FE and ERE ($r=0.76$, $r=0.76$ and $r=0.76$ respectively, $P<0.05$).

3.2. Nutrient digestibility

Results of the digestibility study are shown in Table 4. The apparent dry matter digestibility (ADMD) did not differ significantly ($P>0.05$) among dietary treatments and ranged from 70.7% to 73.2%. However, significant differences in apparent digestibility for crude protein (ACPD), crude lipid (ACLD) and energy (AED) were observed (range: 86.7–91.2%, 81.6–92.8% and 67.5–72.5%, respectively). Shrimp fed SL supplemented diets showed significantly higher ADC for crude protein, energy and a striking increase in crude lipid than the diets without SL. While a significant correlation was observed between dietary PL level and ACLD ($r=0.95$; $P<0.05$), the correlation between dietary PL level versus ACPD and AED ($r=0.53$ and $r=0.63$; $P>0.05$) were not significant. On the other hand, ACLD was positively correlated with weight gain, FE and ERE ($r=0.95$, $r=0.94$ and $r=0.93$, respectively; $P<0.001$).

Table 4
Apparent digestibility coefficients for dry matter (ADMD), crude protein (ACPD), crude lipid (ACLD) and energy (AED) in experimental diets containing different lipid sources and their blend with soy lecithin consumed by *Penaeus monodon* (mean ± SE)

Test diets	ADMD (%)	ACPD (%)	ACLD (%)	AED (%)
FO	70.7 ± 0.008	88.8 ± 0.011^{b}	88.6 ± 0.006^{b}	68.3 ± 0.013^{a}
SO	71.4 ± 0.007	89.3 ± 0.013^{b}	83.5 ± 0.007^{a}	69 ± 0.006^{ab}
PN	70.8 ± 0.008	89 ± 0.007^{b}	81.6 ± 0.012^{a}	68.6 ± 0.009^{a}
PO	72.3 ± 0.009	86.7 ± 0.014^{a}	82.6 ± 0.017^{a}	67.5 ± 0.002^{a}
FOL	71.9 ± 0.009	90 ± 0.003^{cb}	91.2 ± 0.017^{c}	70.2 ± 0.004^{b}
SOL	73.2 ± 0.011	90.8 ± 0.007^{c}	91.8 ± 0.013^{c}	72.3 ± 0.016^{c}
PNL	72.7 ± 0.009	91.2 ± 0.004^{c}	92.8 ± 0.013^{c}	72.5 ± 0.030^{c}
POL	73.2 ± 0.013	90.9 ± 0.009^{c}	90.7 ± 0.015^{cb}	71.7 ± 0.007^{c}

FO—sardine oil; SO—sunflower oil; PN—peanut oil; PO—palm oil; FOL—sardine oil+SL; SOL—sunflower oil+SL; PNL—peanut oil+SL; POL—palm oil+SL.
Means in the same column sharing different superscripts are significantly different ($P<0.05$).

3.3. Biochemical composition

Whole body carcass composition of shrimp fed each diet is presented in Table 5. There were no significant differences among dietary groups ($P>0.05$) for dry matter, crude protein, crude lipid, ash and gross energy, although shrimp fed diets FO and FOL had higher lipid content than the rest of the dietary treatments. PL and cholesterol content differed significantly ($P<0.05$) among shrimp receiving various dietary oils and their combination with SL, ranging between 0.68–0.86% and 0.15–0.20%, respectively. The fatty acid composition of the dietary treatments was reflected to a certain extent in the fatty acid composition of whole shrimp (Table 6). For instance, arachidonic acid (20:4n-6, AA), EPA and DHA were always significantly higher in shrimp fed sardine oil diets (FO and FOL).

4. Discussion

In the present study, the lipid level of experimental diets averaged 86.4 g kg^{-1} and that too was

Table 5
Final carcass composition of *Penaeus monodon* fed with diets containing different lipid sources and their blend with soy lecithin

Composition (% dry matter basis)	FO	SO	PN	PO	FOL	SOL	PNL	POL
Dry matter	26.23 ± 0.95	25.65 ± 1.03	25.72 ± 0.35	25.71 ± 0.47	26.68 ± 0.24	25.65 ± 0.64	25.63 ± 0.25	25.72 ± 0.54
Crude protein	73.26 ± 0.31	73.45 ± 0.51	73.65 ± 0.57	73.72 ± 0.67	73.85 ± 0.14	73.43 ± 0.21	73.44 ± 0.45	73.86 ± 0.23
Crude lipid	3.85 ± 0.9[a]	3.38 ± 0.8[a]	3.42 ± 0.65[a]	3.55 ± 0.57[a]	4.31 ± 1.07[c]	3.26 ± 0.55[a]	3.23 ± 0.69 [a]	3.24 ± 0.97[a]
Crude ash	13.80 ± 1.25	13.48 ± 0.67	13.39 ± 0.66	13.48 ± 0.57	14.37 ± 0.67	13.50 ± 0.57	13.46 ± 0.29	13.47 ± 1.31
Phospholipid	0.856 ± 0.009[b]	0.716 ± 0.006[a]	0.678 ± 0.004[a]	0.747 ± 0.005[a]	0.815 ± 0.005[b]	0.777 ± 0.005[b]	0.759 ± 0.002[b]	0.760 ± 0.006[b]
Total cholesterol	0.196 ± 0.17	0.152 ± 0.10	0.148 ± 0.14	0.176 ± 0.17	0.198 ± 0.19	0.200 ± 0.21	0.198 ± 0.15	0.185 ± 0.12
Gross energy (kcal/kg)	4150 ± 1.5	4156 ± 0.98	4148 ± 0.57	4149 ± 0.76	4135 ± 0.67	4138 ± 0.57	4139 ± 1.57	4139 ± 1.36

FO—sardine oil; SO—sunflower oil; PN—peanut oil; PO—palm oil; FOL—sardine oil + SL; SOL—sunflower oil + SL; PNL—peanut oil + SL; POL—palm oil + SL.
Means in the same row sharing different superscripts are significantly different ($P < 0.05$).

constituted not only by the test oils but also by other basal ingredients. Glencross et al. (2002) also suggested a dietary lipid level of 75 g kg^{-1} of which 30 g kg^{-1} was essential fatty acids as an optimum for *P. monodon*. The variation observed in growth response, nutrient digestibility and carcass composition of shrimp will mainly be due to the differences in the oil components.

Diets containing vegetable oil with SL (SOL, PNL and POL) had significantly higher growth performance and feed utilization compared to the diets having respective oil without SL (SO, PN and PO) and this suggests that growth of juvenile *P. monodon* increased significantly when SL was included in the diet regardless of the vegetable oils tested. An obvious increase in dietary PL content in all SL supplemented diets might lead to enhanced growth promotion in shrimp; a beneficial effect documented for many shrimp species (Kanazawa et al., 1979; Teshima et al., 1982; Kanazawa, 1993; Coutteau et al., 1996; Lim et al., 1997; González-Félix et al., 2002), including *P. monodon* (Pascual, 1986). González-Félix et al. (2002) obtained higher final weight and lower feed conversion ratio (FCR) in shrimp fed vegetable oil plus lecithin than those fed with diets containing same oil without PL also support the present observation. Results of the present study also showed that the presence of SL with sardine oil had no significant effect on shrimp growth performance than its absence; thus SL supplementation is superfluous with sardine oil containing HUFA, suggesting that either one is sufficient to promote better growth. According to this, a possible explanation for this shared role in shrimp growth is that the better availability of HUFA in that fish oil provided the elements for synthesis of PL required by the shrimp (D'Abramo and Sheen, 1993). Survival, however, was not significantly affected in the present study, indicating the juvenile shrimps are less sensitive to dietary PL level.

Shrimp fed diet FOL had higher FE, followed by other SL supplemented diets. A similar observation was made by Lim et al. (1997) who found that menhaden oil was better utilized in the presence of 1% SL. The present study also showed that shrimp fed FO had higher growth performance and feed utilization than dietary treatments without SL. The poor performance of vegetable oil fed shrimp might be due to the inadequate level of HUFA in those oils and it corroborates the observation made by Lim et al. (1997) with *P. vannamei* fed vegetable oils poor in n-3 fatty acids. Glencross et al. (2002) demonstrated an undesirable effect in weight gain when balance of fatty acids was inappropriate. In the present study, an improved growth performance was obtained in sardine oil fed shrimp might be due to higher HUFA content in fish oil, especially 20:5n-3 and 22:6n-3 which has better nutritional value than that of 18:3n-3 (Glencross and Smith, 2001). Guary et al. (1976) obtained a higher growth rate and lower FCR in *P. japonicus* fed diet containing sardine oil as lipid source. HUFA are also preferentially incorporated and

Table 6
Fatty acid composition (percentage of total) of final carcass[a]

Fatty acid	Dietary treatments							
	FO	SO	PN	PO	FOL	SOL	PNL	POL
8:0	–[b]	–	–	–	–	–	–	–
10:0	–	–	–	–	–	–	–	–
12:0	0.3	0.45	0.32	0.48	0.2	0.43	0.28	0.45
14:0	0.85	0.43	0.26	1.18	0.95	0.38	0.25	
14:1	0.14	0.1	0.13	0.23	0.24	0.1	0.11	0.19
16:0	20.8	18.6	23.07	28.52	19.8	17.6	20.02	27.8
16:1	1.95	0.32	0.28	0.34	1.98	0.66	0.75	0.62
16:2	0.28	–	–	–	0.15	–	–	–
16:3	0.35	0.3	0.28	0.22	0.25	0.33	0.32	0.3
16:4	1.85	0.78	0.75	0.94	2.21	0.85	0.87	0.78
18:0	9.45	10.92	8.74	8.26	9.86	10.25	8.76	8.45
18:1	12.11	14.24	18.26	13.27	12.15	12.91	19.54	11.95
18:2n-6	14.25	38.2	29.15	23.65	16.88	41.14	32.3	21.85
18:3n-6	0.08	–	–	–	0.11	–	–	–
18:3n-3	1.85	2.56	2.85	1.92	1.77	2.36	1.65	3.12
18:4n-3	1.27	–	0.11	–	–	–	0.17	–
20:0	0.15	–	–	–	0.18	–	–	–
20:1	0.78	0.68	0.61	0.75	0.79	0.33	0.34	0.29
20:2n-6	2.62	3.61	3.28	2.72	2.69	2.65	3.25	2.75
20:3n-3	0.88	0.31	0.27	0.27	0.29	0.33	0.32	0.33
20:4n-6	1.38	0.81	0.76	0.85	1.35	0.82	0.79	0.84
20:3n-6	0.14	–	–	–	0.12	–	–	–
22:0	0.74	0.06	–	0.08	0.91	–	–	–
20:5n-3	17.15	3.65	5.94	12.13	16.82	4.75	6.16	13.72
22:1	0.58	1.25	0.65	1.36	0.65	0.68	0.52	0.78
22:2	0.18	0.17	0.15	0.15	0.14	0.14	0.15	0.14
22:3	–	–	–	–	0.06	–	–	–
22:4	0.16	0.12	0.11	0.1	0.11	0.06	0.06	0.11
22:5n-3	0.86	0.33	0.38	0.3	0.82	0.28	0.28	0.29
22:6n-3	8.85	2.11	3.65	2.28	8.52	2.95	3.11	3.08
Saturates[c]	32.29	30.46	32.39	38.52	31.9	28.66	29.31	38.86
Monounsaturates[d]	15.56	16.59	19.93	15.95	15.81	14.68	21.26	13.83
PUFA and HUFA[e]	52.15	52.95	47.68	45.53	52.29	56.66	49.43	47.31
Total n-3[f]	30.86	8.96	13.2	16.9	28.22	10.67	11.69	20.54
Total n-6[g]	18.47	42.62	33.19	27.22	21.15	44.65	36.34	25.46

FO—sardine oil; SO—sunflower oil; PN—peanut oil; PO—palm oil; FOL—sardine oil+SL; SOL—sunflower oil+SL; PNL—peanut oil+SL; POL—palm oil+SL.

[a] Fatty acid values (percentage of total fatty acid methyl esters) were adjusted to express a percent of the total area identified in the chromatograms, unidentified peaks were not considered in the computations.

[b] –=0.0, not detected.

[c] Saturates: 12:0, 14:0, 16:0, 18:0, 20:0, 22:0.

[d] Monounsaturates: 14:1, 16:1, 18:1, 20:1, 22:1.

[e] PUFA and HUFA: 16:2, 16:3, 16:4, 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2, 22:3, 22:4, 22:5n-3, 22:6n-3.

[f] Total n-3: 18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.

[g] Total n-6: 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6.

conserved in the polar lipid of crustacean tissue (D'Abramo and Sheen, 1993).

Result of the digestibility trial indicates the optimistic influence of SL on ACLD of the experimental diets. All SL supplemented diets were rich in PL content and there was a significant correlation between ACLD and PL content of diets, suggesting higher lipid digestibility due to emulsification. In

support of this, Glencross (1998) reported that PL can significantly increase the lipid digestibility in *P. monodon* by way of enhanced emulsification of the neutral lipid to an extent of 85 g kg^{-1} in the diet. However, PL content of SL supplemented diets were lower than the recommended level and even with its inferior dietary PL content the lipid digestibility was influenced significantly in the present study. Lester et al. (1975) observed that the increased solubilization of sterols by the crustacean's intestinal detergent, acylsarcosyltaurine, when low concentration of lecithin was included in the emulsion. It has been found that SL had a minor influence on ACPD and those values obtained in the present study are generally found good. This is the first report of such kind on the effect of lecithin on nutrient digestibility other than lipid.

The fatty acid composition of the test diets was reflected to a certain extent in the fatty acid composition of whole shrimp as earlier reported (Colvin, 1976; Catacutan, 1991; Deering et al., 1997; González-Félix et al., 2002). However, certain fatty acids appeared to be actively synthesized and/or retained, because they were present in small amounts in some diets, but in relatively higher amounts in tissue of whole shrimp, such as the case of AA, EPA and DHA. Sparing or preferential retention of specific HUFA at the expense of saturated and monounsaturated fatty acid (SFA and MUFA) has also been demonstrated in other shrimp (Xu et al., 1994; Deering et al., 1997; González-Félix et al., 2002). A study conducted by Xu et al. (1994) in *Fenneropenaeus chinensis*, suggested that the relatively high levels of HUFA such as AA, EPA and DHA in the body lipids of shrimp fed EFA-free diets were probably the result of preferential utilization of short and medium chained fatty acid as energy sources for metabolism rather than an increase in the absolute content of the HUFA.

Proximate composition of the whole body except crude lipid did not seem to be related to the types of dietary lipid tested. Shrimp fed diet containing sardine oil (FO and FOL) had the highest fat content. In a similar study, Catacutan (1991) also noticed an increased level of carcass lipid in *P. monodon* fed a diet containing cod liver oil. On other hand, Glencross et al. (2002) found that the increase in lipid deposition occurred because the prawns could not effectively utilise the entire available lipid for growth, due to an imbalance in the proportions of dietary EFA. But it cannot be the case with fish oil, which is proven for its fatty acid balance and growth promoting potential in shrimp (Guary et al., 1976; Kanazawa et al., 1977; Catacutan, 1991; Glencross and Smith, 2001; González-Félix and Perez-Velazquez, 2002). The present study also demonstrated a statistical difference in the total cholesterol and PL content of shrimp receiving different dietary oil showing a strong relationship between the dietary PL level and final PL content of the shrimp. The constant accumulation of PL in the body tissue clearly indicated their dietary essentiality in shrimp nutrition. However the reason for variation in cholesterol content is unpredictable and has no connection with its dietary level.

The results of the present investigation are encouraging and confirmed the dietary essentiality of PL in shrimp nutrition that reported in earlier literature (Meyers, 1993; Kontara et al., 1997; Glencross, 1998; Gong et al., 2001; González-Félix and Perez-Velazquez, 2002). In the present study, dietary SL at 20 g kg^{-1} has significantly enhanced the lipid digestibility and growth performance of juvenile *P. monodon* on vegetable oils as their lipid source. However with sardine oil, SL supplementation showed only a marginal improvement in growth of shrimp.

Acknowledgements

The authors are grateful to the authorities of Annamalai University for the facilities. This research was supported in part by a grant from Ocean Science and Technology Cell (OSTC), Department of Ocean Development (DOD), Government of India. They are thankful to Dr. T. Rajakumar, Business Manager, Kemin Nutritional Technologies (India) Private Limited, Tamilnadu, India for the encouragement and technical assistance all through the study. They are also indebted to Dr. R.P. Wilson, Editor of this journal for amending the English version and suggesting improvements to an earlier version of this manuscript.

References

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Catacutan, M.R., 1991. Growth and fatty acid composition of *Penaeus monodon* juveniles fed various lipids. Isr. J. Aquac.-Bamidgeh 43, 47–56.

Colvin, P.M., 1976. The effect of selected seed oils on the fatty acid composition of *Penaeus indicus*. Aquaculture 8, 81–89.

Coutteau, P., Camara, M.R., Sorgeloos, P., 1996. The effect of different levels and sources of dietary phosphatidylcholine on the growth, survival, stress resistance, and fatty acid composition of postlarval *Penaeus vannamei*. Aquaculture 147, 261–273.

D'Abramo, L.R., Sheen, S.S., 1993. Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquaculture 115, 63–86.

Deering, M.J., Fielder, D.R., Hewitt, D.R., 1997. Growth and fatty acid composition of juvenile leader prawns, *Penaeus monodon*, fed different lipids. Aquaculture 151, 131–141.

Duncan, D.B., 1955. Multiple range and multiple *F*-tests. Biometrics 11, 1–42.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Furukawa, H., Tsukahara, H., 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish fed. Bull. Jpn. Soc. Sci. Fish. 32, 502–508.

Glencross, B.D., 1998. Effect of dietary phospholipids on digestion of neutral lipid by prawn *Penaeus monodon*. J. World Aquacult. Soc. 29, 365–369.

Glencross, B.D., Smith, D.M., 2001. Optimising the essential fatty acids, eicosapentaenoic and docosahexaenoic acids in the diet of the prawn, *Penaeus monodon*. Aquacult. Nutr. 7, 59–69.

Glencross, B.D., Smith, D.M., Thomas, M.R., Williams, K.C., 2002. Optimising the essential fatty acids in the diet for weight gain of the prawn, *Penaeus monodon*. Aquaculture 204, 85–99.

Gong, H., Lawrence, A.L., Gatlin, D.M., Jiang, D.H., Zhang, F., 2001. Comparison of different types and levels of commercial soybean lecithin supplemented in semipurified diets for juvenile *Litopenaeus vannamei* Boone. Aquacul. Nutr. 7, 11–17.

González-Félix, M.L., Perez-Velazquez, M., 2002. Current status of lipid nutrition of Pacific white shrimp *Litopenaeus vannamei*. In: Cruz-Surez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Corte's, M.G., Simoes, N. (Eds.), Advances on Nutricion Acuicola VI. Memorias del VI Simposium International de Nutriocion Acuicola, Cancun, Quitana Roo, Mexico.

González-Félix, M.L., Lawrence, A.L., Gatlin, D.M., Perez-Velazquez, M., 2002. Growth, survival and fatty acid composition of juvenile *Litopenaeus vannamei* fed different oils in the presence and absence of phospholipids. Aquaculture 205, 325–343.

Guary, J.V., Kayama, M., Murakami, Y., Ceccaldi, H.J., 1976. The effect of a fat-free diet and compounded diets supplemented with various oils on moults, growth and fatty acid composition of prawn *Penaeus japonicus*. Aquaculture 7, 245–254.

Hoffman, L.C., Prinsloo, J.F., 1995. The influence of different dietary lipids on the growth and body composition of the African sharptooth catfish, *Clarias gariepinus* (Burchell). S. Afr. J. Sci. 91, 315–320.

IFOMA, 2000. International fishmeal and fish oil manufacturers association. Predicted use of fishmeal and fish oil in aquaculture—revised estimate, IFOMA Update, vol. 98. St. Albans, UK.

Kanazawa, A., 1993. Essential phospholipids of fish and crustaceans. In: Kaushik, S.J., Luquet, P. (Eds.), Fish Nutr.-Pract., 24–27 June, Biarritz, France. Les Colloques, vol. 61. INRA, Paris, pp. 519–530.

Kanazawa, A., Tokiwa, S., Kayama, M., Hirata, M., 1977. Essential fatty acids in the diet of prawn: I. Effects of linoleic and linolenic acids on growth. Bull. Jpn. Soc. Sci. Fish. 43, 1111–1114.

Kanazawa, A., Teshima, A.S., Tokiwa, S., Endo, M., Abdel Razek, F.A., 1979. Effects of short-necked clam phospholipids on the growth of prawn. Bull. Jpn. Soc. Sci. Fish. 45, 1151–1153.

Kanazawa, A., Teshima, S., Sakamsto, M., 1985. Effects of dietary lipids, fatty acids and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture 50, 39–49.

Kontara, E.K.M., Coutteau, P., Sorgeloos, P., 1997. Effect of dietary pohospholipid on requirements for and incorporation of n-3 highly unsaturated fatty acids in postlarval *Penaeus japonicus* Bate. Aquaculture 158, 305–320.

Lester, R., Carey, M.C., Little, J.M., Dowd, S.R., 1975. Crustacean intestinal detergent promotes sterol solubilisation. Science 189, 1098–1100.

Lim, C., Ako, H., Brown, C.L., Hahn, K., 1997. Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. Aquaculture 151, 143–153.

Merican, Z.O., Shim, K.F., 1996. Qualitative requirements of essential fatty acids for juvenile *Penaeus monodon*. Aquaculture 147, 275–291.

Meyers, S.P., 1993. Soy lecithin and phospholipids in aquatic diets. In: Lim, C.E., Sessa, D.J. (Eds.), Nutrition and Utilization Technology in Aquaculture. AOCS press, Champaign, Illinosis, pp. 105–113.

New, M.B., Wijkstrom, U.N., 2002. Use of fishmeal and fish oil in aquafeeds: further thoughts on the fishmeal trap. FAO Fish. Circ., vol. 975. FAO, Rome, Italy.

Ng, W.K., Tee, M.C., Boey, P.L., 2000. Evaluation of crude palm oil and refined palm oil as dietary lipids in pelleted feeds for a tropical bagrid catfish, *Mystus nemurus* (Cuvier and Valenciennes). Aquac. Res. 31, 337–347.

Ng, W.K., Lim, P.K., Boey, P.L., 2003. Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, *Clarias gariepinus*. Aquaculture 215, 229–243.

Pascual, F.P., 1986. Effect of supplemental lecithin and lipid sources on the growth and survival of *Penaeus monodon* juveniles. In: Maclean, J.L., Dizon, L.B. (Eds.), Proceedings of the First Asian Fisheries Forum. Asian Fisheries Society, Philippines, pp. 615–618.

Rouser, L., 1970. Estimation of phospholipids by phosphorus assay. Lipids 5, 494–496.

Smith, D.M., Tabrett, S.J., 2004. Accurate measurement of in vivo digestibility of shrimp feeds. Aquaculture 232, 563–580.

Strickland, J.D.H., Parsons, T.R., 1972. Practical handbook of seawater analysis. Bull. Fish. Res. Board Can., 167–311.

Tacon, A.G.J., 2002. Global review of feeds and feed management practices in shrimp aquaculture. Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment. Work in Progress for Public Discussion, Published by the Consortium. 2002, p. 68.

Teshima, S., Kanazawa, A., Sasada, H., Kawasaki, M., 1982. Requirements of larval prawn, *Penaeus japonicus* for cholesterol and soybean phospholipids. Mem. Fat. Fish. Kagoshima Univ. 31, 193–199.

Tortensen, B.E., Lie, O., Froyland, L., 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)—effects of capelin oil, palm oil and oleic-enriched sunflower oil as dietary lipid sources. Lipids 35, 653–664.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Xu, X., Wenjuan, J., Castell, J.D., O'Dor, R., 1994. Essential fatty acid requirement of the Chinese prawn, *Penaeus chinensis*. Aquaculture 127, 29–40.

Zlatkis, A., Zak, B., Boyle, H.J., Mich, D., 1953. A new method for direct determination of serum cholesterol. J. Lab. Clin. Med. 41, 486–492.