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Effects of TwoDietary Lipid Sources at Two Levels on the Survival, Growth and Fatty Acid Composition of the Giant Freshwater Prawn, *Macrobrachium rosenbergii*

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Abstract

A 10 week feeding trial was conducted to investigate dietary crude palm oil (CPO) or squid liver oil (SLO), at 3.5% (low)or 9.5% (high) as a 2 × 2 factorial design on survival, growth and whole body fatty acid composition of the giant freshwater prawn, *Macrobrachium rosenbergii*. Growth performance waslowest and highest for prawns fed the CPO-lowand SLO-highdiets, respectively. While prawns fed the CPO-high diet had lower growth than those fed the SLO-low diet, no significant differences were detected.Significant interactions were found for oleic acid, arachidonic acid, n-6 polyunsaturated fatty acids (PUFA) and n-3/n-6 PUFA, while long chain PUFA, eicosapentaenoic acid and docosahexaenoic acid of the prawns were significantly lower when fed the CPO-based diets. Lower growth of *M. rosenbergii* fed the CPO-based diets may be related to less favorable fatty acid ratios and/ or lowered digestibility but higher dietary CPO can help mitigate this.

Keywords: lipid sources; fatty acid; macrobrachium rosenbergii

1. INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii*, is becoming an increasingly important aquaculture species throughout the tropics and the annual production has grown from 38,000 tonnes in 1995 to over 220,000 tonnes in 2010 [1].This species is especially popular throughout Asia for its good taste and ability to be integrated with farms such rice or fish production, as well as being an alternative to marine shrimp production that have been affected by white spot syndrome virus [2,3]. It has been stated that for this industry to continue expanding, it will become necessary to adopt more sustainable practices [4]. In particular, identifying alternative dietary sources to the increasingly costly and less available marine oilsrepresents an essential part in achieving this goal.

Among the various proposed alternatives to marine oil, crude palm oil (CPO) has a number of beneficial characteristics conducive for the aquaculture industry that includes being relatively cheap, contains a high carotenoid content that can improve thecolor/appearance of prawns [5] a wide spectrum of vitamin E isomers that protects against lipid peroxidation [6] and is one of the most highly produced and cheapest plant oils in the world [7,8,9]. However, CPO has relatively high levels of saturated fatty acids (SFA),while being deficient in long chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). As such, since many aquatic animals have a limited ability for LC-PUFA synthesis*de novo*, this will likely substantially alter their fatty acid composition as well as potentially reducing aquaculture productivity.

Our previous investigation on *M. rosenbergii*showed that dietary CPO led to significantly lower growth compared to dietarysquid liver oil (SLO), but not fish oil, which may have been related to unfavorable fatty acid ratios and/or lowered lipid digestibility [5]. However, it is unclear whether higher dietary inclusion rates of CPO can mitigate this and whether any potential interactions exist. Indeed, to the best of our knowledge, research data exploring interactions between vegetable and marine oils at different levelson the growth performance, feeding efficiency or fatty acid composition are currently available for only one species of fish [9].In this study, Kenari et al. (2011) demonstrated that the growth ofcaspian brown trout, *Salmo trutta caspius* fed a vegetable oil blend, consisting of soybean and canola oil, at a low (10%) and high (20%) level wereboth superior to the same dietary levels of fish oil. However, to the best of our knowledge, investigations on such interactions on dietary lipid and type have not been performed for crustaceans.

The purpose of the present study was to investigate the effects of two dietary lipid sources of CPO or SLO added at two different levels of 3.5% (low) or 9.5% (high), respectively on thesurvival, growth, whole body moisture/lipid content and fatty acid composition of the giant freshwater prawn, *M. rosenbergii*after 10 weeks.

2. MATERIALS AND METHODS

A total of four isonitrogenous and isoenergeticdiets were formulated to contain crude palm oil (CPO; rich in SFA) at 3.5 or 9.5%, or squid liver oil (SLO; rich in n-3 LC-PUFA) at 3.5 or 9.5%, respectively (Table 1). Diets containing 3.5% of added CPO or SLO will hereafter be referred to as CPO-low or SLO-low, respectively, while diets containing 9.5% of added CPO or SLO will hereafter be referred to as CPO-high or SLO-high, respectively. The diets were formulated to be isoenergetic by adjusting dextrin levels while soybean meal (59.2%) and fish meal (12.0%) were included as the main protein sources in all diets.

Table 1: Ingredient and Proximate composition (% Dry Matter) of Experimental Diets with Two Lipid Sources and Levels for the Giant Freshwater Prawn, *Macrobrachium rosenbergii*.

Ingredients	Diets			
	CPO-low	SLO-low	CPO-high	SLO-high
Danish fish meal	12.0	12.0	12.0	12.0
Soybean meal [1]	59.2	59.2	59.2	59.2
Dextrin	13.6	13.6	0.6	0.6
CPO [2]	3.5	-	9.5	-
SLO [2]	-	3.5	-	9.5
Vitamin premix [3]	3.0	3.0	3.0	3.0
Mineral premix [3]	4.0	4.0	4.0	4.0
CMC [4]	1.5	1.5	1.5	1.5
α-cellulose	3.2	3.2	10.7	10.7
Proximate composition				
Dry matter	86.4	86.7	88.8	90.0
Crude protein	38.5	37.3	37.8	38.8
Crude lipid	5.4	4.5	12.3	11.5
Crude ash	8.8	8.8	8.6	8.6
Crude fiber	3.2	2.2	5.2	5.6
NFE[5]	44.1	47.2	36.1	35.5
Lipid to carbohydrate ratio[6]	1:8.2	1:10.5	1:2.9	1:3.1

[1] Solvent-extracted
[2] Squid liver oil (SLO) was obtained from HS AQUA FEED Co. Ltd. (Milyang, South Korea) and crude palm oil (CPO)was obtained from Wilmar Ltd. (Penang, Malaysia).
[3] Vitamin and mineral premix according to Ng and Wang (2011).
[4] CMC = carboxymethyl cellulose.
[5] Nitrogen-free extract = 100 - (% protein+ % lipid+ % ash+ % fiber).
[6] Lipid to carbohydrate ratio = NFE/ crude lipid.

Each experimental diet was prepared and stored as previously described by Kim et al. (in press). The proximate composition of the experimental diets is shown in Table 1 and values obtained were relatively similar for all diets as expected. Dietary crude protein remained relatively constant at 36.1-38.8%. The crude lipid approximated the intended dietary lipid level of 5.4 and 4.5% for the CPO-low and SLO-low diets, respectively and 12.3 and 11.5% for the CPO-high and SLO-high diets, respectively, taking into account residual oils from the feed ingredients used.

The fatty acid composition of the experimental diets generally reflected the fatty acid composition of the added oils (Table 2). The CPO-based diets contained the highest SFA and monounsaturated fatty acids (MUFA) content, with palmitic acid (PA, 16:0) and oleic (OA, 18:1n-9) acid contributing the most to these groups, respectively. The LC-PUFA content of the CPO-based diets were low with EPA and DHA in a range of 0.4 - 0.9 and 0.6 - 1.1%, respectively while arachidonic acid (ARA, 20:4n-6) were at undetectable levels. In contrast, the SLO-based diets contained the highest n-3/n-6 PUFA ratio as well as highest PUFA, n-3 PUFA and LC-PUFA content, of which, EPA, DHA and ARA were in a range of 8.8 - 10.9%, 10.5 - 12.6%, and 0.5 - 0.7%, respectively. Moreover, while the total PUFA content was higher in the SLO-based diets, the α-linoleic acid (ALA), linolenic acid (LA) and n-6 PUFA concentrations were somewhat similar between diets of the same lipid levels, although ALA tended to be higher in the SLO-based diets and LA and n-6 PUFA tended to be higher in the CPO-based diets.

The prawns were obtained from a commercial hatchery (Perak, Malaysia). Upon arrival, they were acclimated for three weeks and fed twice daily with a commercial prawn diet (Gold Coin Ltd., Malaysia) to satiation in our laboratory prior to commencing the experiment.A total of 20 prawns, with an initial weight of 0.07 ± 0.01 g (mean ± SD), were randomly selected and distributed into each of the 12 glass aquaria [90-L capacity; 30cm (W) × 70cm (L) × 46cm (H)]. Each aquarium was then randomly assigned to one of three replicates for the four dietary treatments. A flow-though freshwater system supplied all aquaria after prior sand and carbon filtration.The set-up and conditions of the culture system used in the present study were previously described by Ng and Andin (2011) [10]. At the bottom of each aquarium were twenty PVC pipes (diameter 25 mm; length 45 mm) to provide refuge for prawns during their molting process. Throughout the feeding trial, the water temperature ranged between 28 to 31ºC.

Initially, the prawns were fed the experimental diets three times daily (0900, 1300 and 1700 h) to apparent satiation (about 15% of wet body weight per day). By week 4, the prawns were then fed twice daily (09:00 and 17:00 h) to apparent satiation (about 8% of wet body weight). Each day the tanks were siphoned for feces and any uneaten food. The feeding trial was conducted for 10 weeks.

At the beginning of the experiment, the prawns in the acclimation tanks were starved for 24-h and then 20 prawns were randomly selected to determine the initial fatty acid content. At the end of the experiment, all prawns were removed to measure the final total body weight and length and then euthanized and stored in a freezer (-20ºC) for subsequent determination of whole body moisture, lipid and fatty acid composition.

Proximate analysis of diet ingredients, experimental diets and prawn whole-body samples was conducted using standard AOAC methods [11].All samples were freeze dried to constant weight and finely ground before lipid extraction. Lipids for fatty acid analysis were extracted from the samples with chloroform and methanol according to Bligh and Dyer (1959) and methylated and transesterified with boron trifluoride in methanol [12,11]. The fatty acid analysis was performed by gas chromatography and using procedures previously described in Ng and Wang (2011) [13].

All data were subjected to a one-way and two-way ANOVA using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). When a significant treatment effect was observed, a Duncan's Multiple Range test was used to compare means. Treatment effects were considered at $P<0.05$ level of significance.

Table 2: The Fatty Acid Composition (% Total Fatty Acids) of the Experimental Diets with Two Lipid Sources and Levelsfor the Giant Freshwater Prawn, *Macrobrachium rosenbergii*.

Fatty acids	Diets[1]			
	CPO-low	SLO-low	CPO-high	SLO-high
12:0	0.3	ND[2]	0.4	0.1
14:0	1.4	3.3	1.2	3.9
16:0	34.6	18.1	39.2	19.0
16:1	0.7	3.0	0.4	3.5
16:3n4	0.1	0.2	ND	0.3
16:4n1	ND	0.1	ND	0.1
17:0	0.1	0.3	0.1	0.3
17:1	ND	0.1	ND	0.2
18:0	4.0	2.6	4.2	2.2
18:1n7	0.9	2.8	0.1	3.1
18:1n9	30.7	14.1	35.9	14.3
18:2n6	19.9	17.7	14.2	10.1
18:3n3	2.3	3.6	1.2	2.5
18:3n4	0.1	0.4	ND	0.5
18:4n3	0.2	1.8	0.1	2.2
20:0	0.3	0.1	0.3	0.1
20:1	0.9	3.6	0.6	3.9
20:2n6	ND	0.3	ND	0.4
20:3n3	ND	0.2	ND	ND
20:3n6	ND	ND	ND	ND
20:4n3	ND	0.6	ND	0.7
20:4n6	ND	0.5	ND	0.7
20:5n3	0.9	8.8	0.4	10.9
22:0	0.1	0.1	0.1	0.1
22:1n9	ND	0.5	0.1	2.0
22:5n3	ND	0.4	ND	0.5
22:6n3	1.1	10.5	0.6	12.6
24:0	ND	ND	0.1	ND
24:1n9	0.1	0.4	0.1	0.4
Total SFA[3]	41.0	24.9	45.5	26.1
Total MUFA[4]	33.3	24.6	37.1	27.5
Total PUFA[5]	24.5	45.3	16.7	41.6
Total LC-PUFA[6]	2.0	21.1	1.0	25.4
Total n-3 PUFA	4.5	26.0	2.4	29.5
Total n-6 PUFA	19.9	18.3	14.2	10.8
n-3/n-6	0.2	1.4	0.2	2.7

[1] CPO, crude palm oil; SLO, squid liver oil.
[2]ND = not detectable.
[3]SFA = saturated fatty acid.
[4]MUFA = monounsaturated fatty acid.
[5]PUFA = polyunsaturated fatty acid.
[6]LC-PUF = long chain polyunsaturated fatty acid

3. RESULTS

The survival of the prawns ranged between 76.7 - 88.3%, and no significant differences among the treatments were detected ($P > 0.05$). However, for the final weight and weight gain of the prawns, both the dietary lipidsource and level had a significant effect ($P < 0.05$) (Table 3).

Table 3: Growth Performance and Survival of the Giant Freshwater Prawn, *Macrobrachium rosenbergii*, Fed Experimental Diets with Two Lipid Sources and Levels for 10 Weeks.[1]

	Diets [2]				Two-way ANOVA		
	CPO-low	SLO-low	CPO-high	SLO-high	Level	Source	Interaction
Initial weight (g)	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.00	-	-	-
Final weight (g)	0.65±0.08^{c}	0.97±0.09^{b}	0.74±0.07^{bc}	1.34±0.07^{a}	$P<0.05$	$P<0.05$	NS[3]
Weight gain (%)[4]	830±99^{c}	1,306±130^{b}	965±108^{bc}	1,813±128^{a}	$P<0.05$	$P<0.05$	NS
Total length (cm)	5.20±0.27^{ab}	5.58±0.08^{ab}	4.95±0.26^{b}	5.78±0.05^{a}	NS	$P<0.05$	NS
Survival (%)[5]	88.3±9.3	83.3±8.3	81.7±3.3	76.7±4.4	NS	NS	NS

[1]Values are means of triplicate groups, and values in the same row with different superscripts are significantly different ($P<0.05$).
[2] CPO, crude palm oil; SLO, squid liver oil.
[3]NS = not significant.
[4]Weight gain (%) = [(final wt.(g) - initial wt.(g))/ initial wt.(g)] × 100.
[5]Survival = (final fish number/initial fish number) × 100.

The highest final weight and weight gain of the prawns were for those fed the SLO-highdiet and were significantly different than all other dietary treatments, while prawns fed the CPO-lowdiet had the lowest final weight and weight gain, which was significantly lower than the SLO-based diets. However, for prawns fed the SLO-loworCPO-highdiets, no significant differences for the final weight or weight gain were detected. For the final length, prawns fed the SLO-high diet were significantly longer than prawns fed the CPO-high diet, but not for those fed the CPO-low or SLO-lowdiets (Table 3). No significant interaction was detected between the lipid source orlevel on growth performance.

A significant dietary lipid level effect was detected on the whole body crude lipid content of the prawns with those fed the CPO-highor SLO-highdiets being significantly higher than the prawns fed the CPO-lowor SLO-lowdiets (Table 4). No significant dietary lipid source or interactive effect was detected on the whole body crude lipid content. For the whole body moisture content of the prawns, there was no significant dietary lipid source, level or interactive effect of these two detected (Table 4).

Table 4: The Whole Body Moisture and Crude Lipid Content (%; Dry Matter Basis) of the Giant Freshwater Prawn, *Macrobrachium rosenbergii*, Fed Experimental Diets with Two Lipid Sources and Levels for 10 Weeks.[1]

	Diets [2]				Two-way ANOVA		
	CPO-low	SLO-low	CPO-high	SLO-high	Level	Source	Interaction
Moisture	74.1±0.7	74.4±0.5	74.8±0.6	73.4±0.4	NS[3]	NS	NS
Crude lipid	6.1±0.6[b]	5.5±0.4[b]	11.3±0.2[a]	11.3±0.5[a]	$P<0.05$	NS	NS

[1]Values are means of triplicate groups, and values in the same row with different superscripts are significantly different ($P<0.05$).
[2] CPO, crude palm oil; SLO, squid liver oil
[3]NS = not significant.

The fatty acid composition of the prawns at the end of the feeding trial generally reflected the fatty composition of the added dietary lipids. Table 5 presents these results,as a percentage of total fatty acids, and the major findingsare as follows:

- Total SFA of the prawns was not significantly different among all the dietary treatments.
- Total MUFA was highest and lowest for prawns fed the CPO-high and SLO-low diets, respectively, which were significantly different from each other. Both a significant dietary lipid source and level were detected on the total MUFA level.
- Total PUFA was highest and lowest for prawns fed the SLO-low and CPO-high diets, respectively, which were significantly different from each other. A significant dietary lipid level effect was detected, although no significant dietary lipid source or interactive effect was detected.
- The n-6 PUFA was significantly lower for prawns fed higher levels of both dietary lipids, while the n-3 PUFA was significantly lower for prawns fed the CPO-based diets than the SLO-based diets.
- Total LC-PUFA was significantly higher for prawns fed the SLO-based diets than those fed the CPO-based diets. No significant differences in the LC-PUFA levels of the prawns were detected between prawns fed the same lipid sources.

- The n-3/n-6 PUFA were significantly higher for prawns fed the SLO-high, followed by prawns fed the SLO-low diet which were significantly different from each other. The lowest n-3/n-6 PUFA ratio was for the prawns fed both CPO-based diets. A significant dietary lipid source, level and interactive effect of these two were detected on the n-3/n-6 PUFA ratios of the prawns.

The major fatty acids that include ALA (18:3n-3), LA (18:2n-6), EPA (20:5n-3), DHA (22:6n-3) and ARA (20:4n-6) are reported as follows:

- ALA levels were significantly different at each treatment with prawns fed the SLO-low and CPO-high diets having the highest and lowest ALA levels, respectively. Both a significant dietary lipid source and level effect was detected on the ALA levels on the prawns but no significant interaction between these two was found.
- LA levels were highest and lowest for prawns fed the CPO-low and SLO-high diets, respectively. A significant dietary lipid source and level were detected on the LA levels of the prawns but no significant interaction between these two was found.
- EPA and DHA levels were both significantly higher for prawns fed the SLO-based diets than those fed the CPO-based diets. A significant dietary lipid source effect was detected on both the EPA and DHA levels of the prawns, but no significant dietary lipid level or interactive effect was found.
- Both a significant dietary level and interaction effect was detected on the ARA levels of the prawns, but not a significant dietary lipid source. The highest and lowest ARA level of the prawns were for those fed the SLO-low and SLO-highdiets, respectively.

Table 5: Whole Body Fatty Acid Composition (% Total Fatty Acid) of the Giant Freshwater Prawn, *Macrobrachium rosenbergii*, Fed Experimental Diets with Two Lipid Sources and Levels for 10 Weeks.[1]

Fatty acids	Initial	Diets[2]				Two way ANOVA		
		CPO-low	SLO-low	CPO-high	SLO-high	Level	Source	Interaction
14:0	0.9	1.1±0.1^{c}	2.3±0.1^{b}	1.4±0.1^{c}	3.6±0.3^{a}	*P*<0.05	*P*<0.05	*P*<0.05
15:0	0.2	0.2±0.0^{c}	0.4±0.0^{b}	0.2±0.0^{c}	0.6±0.0^{a}	*P*<0.05	NS[4]	*P*<0.05
16:0	23.8	23.2±0.3^{bc}	22.0±0.3^{c}	26.0±0.7^{a}	24.7±1.4^{ab}	*P*<0.05	*P*<0.05	NS
16:1	1.2	1.5±0.2^{b}	1.9±0.1^{ab}	1.5±0.2^{b}	2.3±0.1^{a}	NS	*P*<0.05	NS
16:3n4	6.8	5.9±0.5^{a}	3.7±0.2^{bc}	4.1±0.5^{ab}	1.9±0.9^{c}	*P*<0.05	*P*<0.05	NS
17:0	0.4	0.3±0.0^{c}	0.5±0.0^{b}	0.2±0.0^{d}	0.7±0.0^{a}	NS	*P*<0.05	*P*<0.05
17:1	ND[3]	0.1±0.0^{c}	0.2±0.0^{b}	0.1±0.0^{c}	0.2±0.0^{a}	NS	*P*<0.05	*P*<0.05
18:0	8.2	9.9±0.1^{a}	10.5±0.2^{a}	7.7±0.2^{c}	8.5±0.3^{b}	*P*<0.05	*P*<0.05	*P*<0.05
18:1n7	3.6	3.9±0.2^{c}	4.8±0.1^{b}	3.2±0.1^{d}	5.4±0.1^{a}	NS	*P*<0.05	*P*<0.05
18:1n9	20.4	25.8±0.3^{b}	18.2±0.3^{d}	32.7±0.9^{a}	20.8±0.4^{c}	*P*<0.05	*P*<0.05	*P*<0.05
18:2n6	14.2	14.5±0.2^{a}	12.5±0.3^{b}	11.8±0.5^{b}	8.2±0.5^{c}	*P*<0.05	*P*<0.05	NS
18:3n3	1.0	0.9±0.1^{c}	1.4±0.1^{a}	0.6±0.0^{d}	1.1±0.1^{b}	*P*<0.05	*P*<0.05	NS

18:3n4	ND	0.0±0.0^{c}	0.3±0.0^{b}	0.0±0.0^{c}	0.4±0.0^{a}	*P*<0.05	*P*<0.05	*P*<0.05
18:3n6	ND	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.02±0.01^{a}	NS	NS	NS
18:4n3	ND	0.0±0.0^{b}	0.3±0.0^{a}	0.0±0.0^{b}	0.3±0.1^{a}	NS	*P*<0.05	NS
20:0	0.2	0.2±0.0	0.3±0.0	0.2±0.0	0.2±0.0	NS	NS	NS
20:1n9	0.6	0.8±0.1^{c}	2.4±0.1^{b}	0.6±0.0^{c}	3.3±0.1^{a}	*P*<0.05	*P*<0.05	*P*<0.05
20:2n6	0.5	0.8±0.0^{b}	1.2±0.1^{a}	0.5±0.0^{c}	0.8±0.1^{b}	*P*<0.05	*P*<0.05	NS
20:3n3	ND	0.1±0.0^{c}	0.9±0.0^{a}	0.0±0.0^{c}	0.3±0.1^{b}	*P*<0.05	*P*<0.05	*P*<0.05
20:3n6	ND	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.04±0.01^{a}	*P*<0.05	*P*<0.05	*P*<0.05
20:4n3	0.1	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.3±0.1^{a}	*P*<0.05	*P*<0.05	*P*<0.05
20:4n6	2.4	1.9±0.1^{b}	2.5±0.2^{a}	1.5±0.2^{bc}	1.0±0.0^{c}	*P*<0.05	NS	*P*<0.05
20:5n3	9.2	5.5±0.3^{b}	7.7±0.4^{a}	4.8±0.7^{b}	8.7±0.2^{a}	NS	*P*<0.05	NS
21:0	ND	0.0±0.0^{c}	0.1±0.1^{a}	0.0±0.0^{c}	0.1±0.0^{b}	*P*<0.05	*P*<0.05	*P*<0.05
22:0	ND	0.1±0.0^{ab}	0.2±0.0^{a}	0.1±0.0^{b}	0.1±0.0^{ab}	NS	*P*<0.05	NS
22:1n9	0.2	0.0±0.0^{c}	0.4±0.0^{a}	0.2±0.0^{b}	0.2±0.0^{b}	NS	*P*<0.05	*P*<0.05
22:4n6	ND	0.0±0.0^{b}	0.1±0.0^{a}	0.0±0.0^{b}	0.05±0.03^{a}	NS	*P*<0.05	NS
22:5n3	ND	0.1±0.1^{ab}	0.2±0.0^{a}	0.0±0.0^{c}	0.1±0.1^{ab}	NS	*P*<0.05	NS
22:6n3	ND	3.1±0.2^{b}	5.0±0.0^{a}	2.6±0.2^{b}	5.7±0.5^{a}	NS	*P*<0.05	NS
24:0	6.1	ND	ND	ND	ND	NS	NS	NS
24:1n9	ND	0.1±0.0^{b}	0.2±0.0^{a}	0.1±0.0^{b}	0.3±0.0^{a}	NS	P<0.05	NS
Total SFA[5]	39.7	35.0±0.4	36.2±0.4	35.8±0.6	38.5±2.1	NS	NS	NS
Total MUFA[6]	26.1	32.2±0.7^{b}	28.1±0.5^{c}	38.3±1.2^{a}	32.6±0.6^{b}	*P*<0.05	*P*<0.05	NS
Total PUFA[7]	34.2	32.9±0.9^{ab}	35.7±0.7^{a}	25.9±1.8^{c}	29.0±2.6^{bc}	*P*<0.05	NS	NS
Total LC-PUFA[8]	11.8	10.7±0.6^{b}	16.4±0.6^{a}	8.9±1.1^{b}	16.2±0.9^{a}	NS	*P*<0.05	NS
Total n-3 PUFA	10.2	9.7±0.4^{b}	15.4±0.4^{a}	8.1±0.9^{b}	16.2±1.0^{a}	NS	*P*<0.05	NS
Total n-6 PUFA	17.1	17.2±0.2^{a}	16.3±0.3^{a}	13.7±0.6^{b}	10.1±0.7^{c}	*P*<0.05	*P*<0.05	*P*<0.05
n-3/n-6	0.6	0.6±0.0^{c}	0.9±0.0^{b}	0.6±0.1^{c}	1.6±0.0^{a}	*P*<0.05	*P*<0.05	*P*<0.05

[1]Values are means of triplicate groups, and values in the same row with different superscripts are significantly different (*P*<0.05).
[2]CPO, crude palm oil; SLO, squid liver oil.
[3]ND = not detectable.
[4]NS = not significant.
[5]SFA = saturated fatty acid.
[6]MUFA = monounsaturated fatty acid.
[7]PUFA = polyunsaturated fatty acid.
[8]LC-PUFA = long chain polyunsaturated fatty acid.

4. DISCUSSION

The results of the current study show that SLO was the better lipid source for the growth of *M. rosenbergii*. Nevertheless, no significant growth differences were detected between the CPO-highand the SLO-low diet, which may indicate a compensatory effect, while dietary lipid source or level had no effect on survival. As such, thismay thus may provide an avenue for substantially reducing feeding costs by replacing the more expensive SLO with themuch cheaper and more readily available CPO, albeit at higher dietary levels.

Regardless of the dietary lipid source, higher dietary lipid levels led to improved growth of *M. rosenbergii*,which may indicate a protein sparing effect, whereby lipids and carbohydrates are utilized as energy sources while protein is utilized more for growth. Based on various metabolic indicators, it has been shown that protein sparing for *M. rosenbergii* is maximized at a 1:4 dietary lipid to digestible carbohydrateratio [14]. Sheen and D'Abramo (1991) made a similar suggestion to explain no significant growth improvement to *M. rosenbergii* when fed diets containing lipid levels of 2 - 10% [15]. On the other hand, A-S Goda (2008) demonstrated that among various tested protein to energy (P:E) ratios in a range from 16 to 21 mg CP kJ g^{-1}, a P:E ratio of 17 mg CP kJ g^{-1} resulted in optimal growth performance and feeding efficiency [16]. However, it is interesting to note that the measured optimal P:E ratio also corresponded to a lipid to carbohydrate ratio of 1:4 [16]. Since all the diets used in the current study had similar energy levels but with different lipid and carbohydrate inclusion levels, the higher growth of *M. rosenbergii* fed the CPO-high and SLO-high diets may be due to a more favorablelipid to carbohydrate ratio of approximately 1:3. In contrast, the CPO-low and SLO-low diets had a substantially higher lipid to carbohydrate ratio of 1:12 and 1:9.5, respectively.

Our previous experiment showed that, among various dietary lipid sources tested with*M. rosenbergii*, LC-PUFA deficient canola oil (CO) actually outperformed the more LC-PUFA rich SLO [5], indicating LC-PUFA deficiencies are unlikely the cause for lower growth in the current study. Indeed, D'Abramo and Sheen (1993) have shown that the LC-PUFA requirement of *M. rosenbergii* is relatively low at around 0.08%, and these fatty acids derived from residual fish oil (in the fish meal) in the current study, along with our previous study [17], may have met their requirements. The suitability of CO for *M. rosenbergii* may be due to the relatively high amounts of SFA and MUFA, which tend to better utilized as energy sources than PUFA or LC-PUFA [18,19], particularly when oleic acids (OA; 18:1n9) are well balanced to LA or ALA ratios [20]. However, despite CPO being high in both SFA and MUFA, there are indications that the fatty acid digestibility and/ or their utilization as an energy source are less than other lipid sources. It has been demonstrated that, among various marine oil sources as well as palm oil and CO, the black tiger prawn, *Penaeus monodon*, digested SFA in palm oil much more poorly

(62 - 72%) than SFA in all the other tested oil sources (70 - 95%) [18]. Moreover, the OA to LA or ALA ratios are much higher in CPO than other lipid sources (*e.g.* SLO, CO, fish oil) which may have inhibitedβ-oxidation [21]. However more research is required.

In the current study, the fatty acid composition of the prawns generally reflected those of the diets. After 10 weeks, the whole body ALA levels of the prawnswere lower than those provided in all the tested diets and, conversely, the whole body EPA and DHA of the prawns fed the CPO-based diets were substantially higher than those of the diets. On the other hand, prawns fed the SLO-based diets had a lower LC-PUFA content compared to the levels found in their respective diets. This may potentially be the result of LC-PUFA synthesis in *M. rosenbergii*when these fatty acids are deficient in the diets (*i.e.* CPO-based diets), while elevated levels of dietary LC-PUFA (*i.e.*in the SLO-based diets) can inhibit their synthesis [22,20,24,25,26]. However, since there were no significant differences in these whole body fatty acid composition between the CPO-lowand CPO-high dietary treatments,this may indicate that if LC-PUFA synthesis exists for *M. rosenbergii*, than such conversions may not be substrate dependent.

There were several significant interactions between the dietary lipid source and level on the composition of various fatty acid levels in the whole body of the prawns. In particular, there were significant interactions on theOA, ARA and total n-6 PUFA content as well as the total n-3/n-6 PUFAratio. Prawns fed the CPO diets had significantly higher OA than those fed the SLO diets, while higher inclusions of both lipid sources significantly increased the whole body OA content compared to their respective lipid sources.Accumulation of OA in*M. rosenbergii*, particularly for those in the CPO-high dietary treatment, appears to support the suggestion that this fatty acid is not utilized at sufficient rates for energy compared to those being consumed.

In contrast, higher dietary inclusions of either CPO or SLO tended to reduce the ARA and total n-6 PUFA whole body content of the prawns, but this was less pronounced for those fed the CPO-based diets. Furthermore, while increasing dietary CPO had no effect on the n-3/n-6 PUFA ratio, increasing dietary SLO increased the n-3/n-6 PUFA ratio. Since LA, which is a precursor to ARA, substantially reduced in the prawns compared to what was provided in the diets while the whole body ARA increased substantially above the dietary levels (in the case of the CPO-based diets, ARA was at a non-detectable level) may indicate that*M. rosenbergii* synthesized this fatty acid to a certain degree regardless of the dietary lipid content. The exact cause for lower dietary inclusions of either CPO or SLO increasing the ARA and n-6 PUFA in the whole bodymay also indicate these are being conserved but more research is required.

The dietary lipid source or level had no significant effect on the moisture content of the prawns in the current study, although higher dietary inclusion of either CPO or SLO significantly increased the crude lipid content to a similar level. This finding of a significant dietary lipid level, but not source, on the crude body content is in agreement with those of *S. trutta caspius* when fed diets containing different levels of fish oil or vegetable oil blends [9].

In conclusion, an over two fold increase to the dietary inclusion of CPO from 3.5% to 9.5% can replace the more expensive SLO at 3.5% without significantly affecting the growth of the prawns after 10 weeks. However, since growth was slightly lower for the prawns fed the CPO-high and SLO-low diet, significant differences may become apparent on a longer time frame. As such, further research into the effects of fatty acid ratios, such as OA to LA or ALA, may be worthwhile to substantially reduce feeding costs without compromising productivity during the grow-out culture of *M. rosenbergii*.

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