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Optimal protein replacement of fish meal by mackerel condensate in diet for giant freshwater prawn (*Macrobrachium rosenbergii*)

Wattana Wattanakul[1], Uraiwan Wattanakul[2], Karun Thongprajukaew[3,4] & Chutchawan Muenpo[5]

[1]Department of Fisheries Technology, Faculty of Sciences and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang, Thailand

[2]Department of Food Industry and Fisheries Products, Faculty of Sciences and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang, Thailand

[3]Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

[4]Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science, Kasetsart University, Bangkok, Thailand

[5]Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand

Correspondence: K Thongprajukaew, Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand. E-mails: karun.t@psu.ac.th (or) karun114@hotmail.com

Abstract

The optimal protein replacement of fish meal (FM) by mackerel condensate (MC) was investigated in giant freshwater prawn, *Macrobrachium rosenbergii* (0.90 ± 0.01 g initial weight) over a 12-week trial. The experimental diets replaced the FM partially in a baseline FM-based diet (0MC), by substituting MC for 10% (10MC), 20% (20MC), 30% (30MC), 40% (40MC), 50% (50MC) and 60% (60MC) of the FM, and a commercial diet (CD) was included for comparison. The prawn fed with 40MC had superior growth performance and feed utilization. Significant increases in amylase and cellulase-specific activities indicated improved carbohydrate utilization in this dietary group ($P < 0.05$). The proteolytic activity was maintained through the key gastrointestinal enzymes (pepsin, trypsin and chymotrypsin). A slight improvement in carcass compositions was also observed in the prawns fed 40MC, and the total haemocyte count was significantly increased by this diet, without negative effects on the hepatopancreatic histology. These findings indicate that a 40% protein replacement of FM by MC is optimal for giant freshwater prawn, and similar use of it in the aquafeed of other species appears worth further studies.

Keywords: by-product, carcass composition, digestive enzyme, feed utilization, haemocyte

Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is a commercially important crustacean species native to the Indo-Pacific region, northern Australia and Southeast Asia. The global annual production of this species has progressively increased to over 200 000 tonnes within the past 10 years (FAO 2014). The success of intensive aquaculture depends on efficient use of the available food sources. In culturing giant freshwater prawns, the feed constitutes 40–60% of the operational costs (D'Abramo & Sheen 1991; Mitra, Mukhopadhyay & Chattopadhyay 2005). Therefore, cost savings in aquaculture are often possible by replacing expensive animal proteins, mainly fish meal (FM), with lower cost protein sources. These include plant proteins (Du & Niu 2003; Gupta, Sehgal & Sehgal 2007; Hasanuzzaman, Siddiqui & Chisty 2009), low cost animal proteins such as meat and bone meal (Hossain & Islam 2007), and special opportunistic options such as golden apple snail meal (Jintasataporn, Tabthipwon & Yenmark 2004) and *Artemia* (Anh, Hien, Mathieu, Hoa & Sorgeloos 2009).

The export of canned mackerel from Thailand has grown rapidly in recent years. The recovered solid by-products are often used to prepare FM. This feedstuff is an effective source of proteins in animal feed, and its high demand has significantly increased its price (Anh *et al.* 2009). However, the condensate from steam cooking of fish is rarely used, and the wastewaters from seafood processing factories have high levels of organic loading (Prasertsan, Jaturapornpipat & Siripatana 1997; Uttamangkabovorn, Prasertsan & Kittikun 2005). This condensate has high protein and lipid contents (Wattanakul, Wattanakul & Is-Haak 2011) and can act as a feed attractant in aquafeed (Somboon & Semachai 2004). Using the condensate as feedstuff would have benefits in both reducing effluents and providing cost-effective nutrition to aquaculture.

The giant freshwater prawn accepts a pelleted diet and has omnivorous feeding habits. The replacement of a feed ingredient might negatively affect the utilization of proteins and carbohydrates, and such responses can be assessed through the activities of various digestive enzymes (Anand, Kohli, Kumar, Sundaray, Roy, Venkateshwarlu, Sinha & Pailan 2014). Pepsin, trypsin and chymotrypsin are the major enzymes contributing to protein digestion in the stomach and the intestine (Thongprajukaew & Kovitvadhi 2013; Kanghae, Thongprajukaew, Madlee & Kittiwattanawong 2014). The ratio of the intestinal enzymes trypsin and chymotrypsin (the T/C ratio) appears to correlate with growth (Thongprajukaew, Kovitvadhi, Kovitvadhi, Somsueb & Rungruangsak-Torrissen 2011) and with feed utilization efficiency (Sunde, Eiane, Rustad, Jensen, Opstvedt, Nygard, Venturini & Rungruangsak-Torrissen 2004) in aquatic animals. On the other hand, the bioavailability of carbohydrates can be observed through the activities of amylase and cellulase, in relation to digestible and indigestible elements in the diet (Anand *et al.* 2014; Kanghae *et al.* 2014). A close connection exists between the nutritional status assessed from digestive enzymes, and the feed utilization of animals.

The feed utilization of mackerel condensate (MC), a by-product from the steam cooking of mackerels, in aquafeed by the giant freshwater prawn is not known, and this can depend on the partial replacement level used. This study was motivated by the potential of finding a low cost MC-containing diet formulation, and by the desire to convert an industrial waste stream to a useful value-added by-product.

Materials and methods

Preparation of the experimental diets

All feedstuffs were purchased from Phatthalung Livestock, Phatthalung, Thailand. The raw MC ingredient was obtained from a canned seafood factory (Kuang Pei San Food Products Public, Trang, Thailand). This industrial by-product is a condensate from the steam conditioning (at 100°C for 30 min) of mackerels (including Indian mackerel, short-bodies mackerel, island mackerel, mackerel scad, and blue mackerel) and then boiled (at 100°C) until constant total soluble solids (57–60° Bx and ≤400 g kg^{-1} moisture content). This MC contained 667 g kg^{-1} crude proteins (CP), 214 g kg^{-1} crude lipids and 119 g kg^{-1} ash, on dry weight basis. The six diets were produced by partly replacing FM with MC, the fraction replaced being 10% (10MC), 20% (20MC), 30% (30MC), 40% (40MC), 50% (50MC) or 60% (60MC). The baseline FM-based diet (0MC) and a commercial diet (CD) for giant freshwater prawn (Charoen Pokphand PCL., Samut Sakhon, Thailand) were included as controls. All the experimental diets were produced by mixing the main feed ingredients with additives and vitamin-mineral premixes, and water was added to reach an appropriate moisture content. The glutinous mixture was passed through a meat mincer (2-mm-die diameter) and then dried at 60°C for 24 h. The dried pellets were cut, sieved and stored in black polyethylene bags at 4°C, until used in feeding.

Proximate chemical compositions

The feedstuffs, experimental diets and CD were analysed for moisture, CP, crude lipid, ash and crude fibre, according to standard methods of AOAC (1990). All the analyses were performed in triplicates and are reported on g kg^{-1} wet weight basis. Nitrogen free extract (NFE) and gross energy (GE) were calculated from the chemical constituents. For both MC and FM, their amino acid profiles were also determined according to the method proposed by White, Hart and Fry (1986). Samples were preprocessed by hydrolysis in 6 mol L^{-1} HCl at 110°C for 24 h to obtain free amino acids. These free amino acids were

derivatized with phenylisothiocyanate, as proposed by Hagen, Frost and Augustin (1989).

Feeding trial of giant freshwater prawns

The 40-day-old giant freshwater prawns were obtained from Satun Inland Fisheries Research and Development Center, Satun, Thailand, and then transported to the Department of Fisheries Technology, Faculty of Sciences and Fisheries Technology, Rajamangala University of Technology Srivijaya. The prawns were acclimatized in a cement pond (1.5 m × 4 m × 1 m) containing 6000 L water for 10 days. They were fed 0MC diet *ad libitum* twice daily at 08:00 and 17:00 hours. Subsequently, the prawns (0.90 ± 0.01 g initial weight) were randomly distributed into 24 cement ponds (1 m × 3 m × 0.5 m) with 30 cm water levels, at an initial density of 25 prawns per pond, with three ponds sharing each assigned dietary treatment. The experiment was conducted for 12 weeks with 12:12 light/dark, the water was partly replaced (20%) daily, and aeration was continuously supplied by air pumps throughout the experiment. The prawns were initially fed with an experimental diet at 10% of body weight per day, and the feed amount was adjusted weekly according to the actual feeding performance. The sinking diet was placed on a small dip net (75 cm × 75 cm) that was kept submerged for 2 h during the meal times (08:00 and 17:00 hours), after which the food scraps were siphoned off. Dead prawns were removed at feedings, and their counts recorded daily. The food scraps were dried and used to estimate feed intake (FI), feed conversion ratio (FCR) and the protein efficiency ratio (PER). Survival, growth and feed utilization parameters were summarized every other week for eventual analysis. At the end of the experiment, all the prawns were starved for 24 h prior to measuring body weight and length. In each treatment, three pooled samples (three prawns per pond) were sampled for their digestive tracts in order to study digestive enzymes; another three pooled samples (three prawns per pond) were used for carcass composition; and similarly three pooled samples (three prawns per pond) were used for haemolymph and hepatopancreatic histopathology studies. The digestive tracts and carcasses were kept in test tubes at −20°C until analysis. The haemolymph and hepatopancreatic studies were performed immediately after necropsy.

Water quality analysis

Water quality was measured every other week. The parameters monitored included temperature (Hg thermometer, pp. 2–61), pH (pH meter, pp. 4–87 to 4–91), dissolved oxygen (Multiparameter Display System; YSI 650MDS, YSI Incorporated, USA), total alkalinity (titration method, pp. 2–27 to 2–29), ammonia (phenate method, pp. 4–108 to 4–109) and nitrite (colorimetric method, pp. 4–112 to 4–114), determined according to standard methods of APHA, AWWA and WPCF (1998).

Determination of digestive enzymes

Enzyme extraction

The frozen digestive tract was extracted using a micro-homogenizer (THP-220; Omni International, Kennesaw, GA, USA) in the presence of 0.2 M phosphate buffer pH 8 (1: 3 *w*/*v*). The homogenate was centrifuged at 15 000 × *g*, at 4°C for 30 min, and then the supernatant was collected and kept at −20°C for the digestive enzyme assays.

Quantification of protein in enzyme extracts

The protein concentration of a crude enzyme extract was determined using the method of Lowry, Rosenbrough, Farr and Randall (1951), and bovine serum albumin (BSA) was used as the protein standard. The concentration of protein (mg mL^{-1}) was used to quantify the enzyme-specific activities (U mg $protein^{-1}$).

Digestive enzyme assays

Pepsin (EC 3.4.23.1) activity was assayed according to the method of Rungruangsak and Utne (1981), using casein as the substrate. The quantity of digested product was measured spectrophotometrically at 720 nm against *L*-tyrosine standard. The activities of trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) were assayed according to Rungruangsak-Torrissen, Moss, Andresen, Berg and Waagbo (2006), using *N*-benzoyl-*L*-arginine-*p*-nitroanilide (BAPNA) and *N*-Succinyl-ala-ala-prophe-*p*-nitroanilide (SAPNA) as the substrates respectively. The amount of liberated *p*-nitroanilide was measured at 410 nm to quantify the enzyme activities. The T/C ratio was calculated for each

sample separately from the activities of trypsin and chymotrypsin (Rungruangsak-Torrissen *et al.* 2006). Activities of the carbohydrate-digesting enzymes α-amylase (EC 3.2.1.1) and cellulase (EC 3.2.1.4) were determined based on Areekijseree, Engkagul, Kovitvadhi, Thongpan, Mingmuang, Pakkong and Rungruangsak-Torrissen (2004) and Mendels and Weber (1969), using soluble starch and carboxymethylcellulose (CMC) as the substrates respectively. These enzyme activities were determined spectrophotometrically at 540 nm, by comparisons to standard maltose and glucose respectively.

Analysis of carcass (whole body) composition

Carcass compositions in terms of moisture, CP, crude lipid, and ash, were analysed according to standard methods of AOAC (1990). All these analyses were performed in triplicates per dietary treatment, and are reported on % wet weight basis.

Haemolymph collection and count

Haemolymph samples were collected from the third walking leg pairs using 1-mL syringes (needle size 20–25G). The 0.2 mL samples were diluted with 0.15% trypan blue dissolved in 2.5% normal saline. Measurement of total haemocytes was conducted using a haemocytometer (Precicolor; HBG, Giessen-Luetzellinden, Germany) under a light microscope.

Hepatopancreatic histopathology

The preparation of tissue samples was performed according to the method described in Humason (1979). A 3–4 μm hepatopancreas tissue slice was obtained using a rotary microtome (Shandon AS325 Retraction; Life Sciences International, Malmesbury, Wiltshire, UK), and stained with Haematoxylin and Eosin (H&E). Histopathology of the hepatopancreas was assessed, based on medium location, under a light microscope set at 400× magnification.

Statistical analysis

The experimental design was completely randomized with three replicates each across eight treatments. The data were analysed using SPSS Version 14 (SPSS, Chicago, USA), and the results are summarized as mean ± SEM. One-Way ANOVA was used for evaluating the observed variables and the differences between means were tested with Tukey's multiple range test for significance ($P < 0.05$). Relationships between the specific activities of digestive enzymes were examined from Pearson correlation coefficients (r).

Results

Chemical compositions of FM, MC and the experimental diets

The proximate chemical compositions of FM and MC are given in Table 1, on dry matter basis. The MC was higher in CP, crude lipid and GE than the FM, and *vice versa* for ash content. No detectable amounts of crude fibre and NFE were found in MC, while their contents were 1 and 44 g kg^{-1} in FM. In contents of essential amino acids (EAA) the MC was inferior to the FM, except for histidine, the total being 109.3 and 279.0 g kg^{-1} ΣEAA respectively. Non-essential amino acids (NEAA) were also higher in FM than in MC, except for asparagine, cysteine and hydroxyproline. The total NEAA contents and the ratios EAA/NEAA were 331.8 g kg^{-1} and 0.84, and 207.2 g kg^{-1} and 0.53, in the same order.

Chemical compositions of the experimental diets are shown in Table 2. The control and MC-containing diets were generally similar in their proximate compositions. The prepared experimental diets were higher in CP, crude lipid, ash and GE, and lower in NFE, relative to CD.

Survival, growth and feed utilization

There were no significant differences between the treatments in survival at the end of experiment ($P > 0.05$), and the prawns across all treatments had an average 84.5% survival (Table 3). The growth performance in terms of final weight, specific growth rate (SGR) or average daily gain (ADG), was similar in the prawns fed with 20MC, 30MC and 40MC, but weight gain (WG) was significantly lower in 20MC than in 30MC or 40MC ($P < 0.05$). The feed utilization, as observed in the FI and FCR, was lower in the prawns fed with 20% to 40% replacement levels. These three dietary treatments were also superior in the PER. The hepatosomatic index (HSI) was significantly higher in the prawns fed with 20MC or 50MC compared

Table 1 Proximate chemical compositions (g kg^{-1} dry weight) of FM and MC. The values were averaged from triplicate determinations

Chemical composition	FM	MC
Dry matter	935	598
CP	611	667
EAA*		
Arginine	53.1	18.1
Histidine	17.2	27.8
Isoleucine	22.0	9.0
Leucine	45.3	3.7
Lysine	46.3	24.6
Methionine	19.3	1.8
Phenylalanine	20.9	1.3
Threonine	27.6	6.9
Valine	27.3	16.1
ΣEAA	279.0	109.3
NEAA†		
Alanine	41.5	32.4
Aspartic acid	56.6	25.8
Asparagine	16.0	25.8
Cysteine	6.4	11.4
Glutamic acid	84.5	36.3
Glycine	44.6	32.4
Hydroxyproline	5.5	8.5
Proline	29.9	18.2
Serine	26.6	6.0
Tyrosine	20.2	10.4
ΣNEAA	331.8	207.2
Ration EAA/NEAA	0.84	0.53
Crude lipid	78	214
Ash	266	119
Crude fibre	1	nd
NFE‡	44	nd
GE (kcal kg^{-1})§	4342	5755

*EAA, essential amino acids; does not include tryptophan which was destroyed by acid-hydrolysis of protein.
†NEAA, non-essential amino acids.
‡Nitrogen free extract (NFE) = 1000 − (CP + crude lipid + ash + crude fibre).
§Gross energy (GE) = (CP × 5.6) + (crude lipid × 9.44) + (crude fiber × 4.1) + (NFE × 4.1) (Anand *et al.* 2014).
FM, fish meal; MC, mackerel condensate; nd, not detected; GE, gross energy.

to the 0MC treatment, and the similar levels in the other MC-containing diets and CD were next in rank order. Based on the measurements overall, the CD and 0MC treatments gave generally inferior growth and feed utilization relative to the other treatments (diets containing MC).

Water quality

The water quality remained similar across the dietary treatment groups during the 12-week feeding trial, and all water quality parameters measured were in their standard ranges for aquaculture (Hossain & Islam 2007; Anh *et al.* 2009; Hasanuzzaman *et al.* 2009). The average quality parameters were: temperature 28.65 ± 0.13°C (min–max = 27.90–29.72°C), pH 7.40 ± 0.11 (7.01–7.67), dissolved oxygen 6.79 ± 0.10 mg L^{-1} (6.15–7.40 mg L^{-1}), alkalinity 105.01 ± 0.53 mg L^{-1} (101.86–109.08 mg L^{-1}), ammonia 0.41 ± 0.02 mg L^{-1} (0.31–0.59 mg L^{-1}) and nitrite 0.23 ± 0.02 mg L^{-1} (0.14–0.33 mg L^{-1}).

Digestive enzyme specific activity

There were no significant differences among the treatments in the specific activities of pepsin (Fig. 1a), trypsin (Fig. 1b) and chymotrypsin (Fig. 1c). The highest specific activity of amylase was in the prawns fed 40MC, followed by 60MC and 50MC in rank order (Fig. 1d). The prawns in the 10MC and the 40MC dietary groups had similar specific activities of cellulase ($P > 0.05$, Fig. 1e) that were significantly higher than in the other groups ($P < 0.05$). The T/C ratio was highest with the 40MC and the 60MC diets, while it was lowest with 20MC that was similar to CD (Fig. 1f).

Carcass composition

The carcass moisture and lipid contents were similar across the eight dietary treatments (Table 4). The protein content was highest in the prawns fed 40MC, but this group differed significantly only from the 60MC group. The ash contents were highest with the CD treatments that differed significantly only from the 10MC group. Overall, the carcass compositions show no clear consistent trends with the fraction of FM replaced by MC.

Total haemocyte count

The haemocyte population was highest in the prawns fed 30MC and 40MC diets, followed by CD (Fig. 2). The highest levels of MC (50–60% replacement) resulted in a dramatic decrease in the total haemocyte count.

Hepatopancreatic microanatomy

Hepatopancreas tissue samples were assessed from all the treatment groups, but these samples exhibited similar and normal histoarchitecture, and showed no structure differences in the various cell

Table 2 Formulations (g kg^{-1} wet weight) and proximate chemical compositions (g kg^{-1} wet weight) of the experimental diets

Item	0MC	10MC	20MC	30MC	40MC	50MC	60MC	CD*
Ingredient								
FM	438	394	350	306	263	219	175	–
MC	0	55	111	166	222	277	332	–
Soybean meal	154	158	161	165	167	171	172	–
Rice bran	103	98	93	88	83	78	74	–
Corn meal	103	98	93	88	83	78	74	–
Broken rice	103	98	93	88	83	78	74	–
Alpha starch	40	40	40	40	40	40	40	–
Soybean oil	10	10	10	10	10	10	10	–
Fish oil	10	10	10	10	10	10	10	–
Vitamin premix†	19	19	19	19	19	19	19	–
Mineral premix‡	20	20	20	20	20	20	20	–
Chemical composition								
Moisture	44	43	49	44	55	64	43	87
CP	350	351	355	360	360	358	355	323
Crude lipid	109	108	111	118	112	101	100	58
Ash	125	127	126	130	127	129	128	106
Crude fibre	15	13	18	18	11	16	19	18
NFE§	357	358	341	330	335	332	355	408
GE (kcal kg^{-1}) ‖	4514	4506	4508	4557	4492	4385	4465	4103

*CD, commercial diet; not examined in certain feedstuff ingredients.
†Vitamin premix, 1 kg contained 12 000 U vitamin A, 2000 U vitamin D, 3000 mg vitamin E, 20 mg vitamin K, 100 mg thiamine HCl, 150 mg riboflavin, 500 mg niacin, 500 mg *D*-pantothenic acid, 25 mg pyridoxine HCl, 1 mg biotin, 5 mg folic acid, 125 mg vitamin B_{12}, 1000 mg ascorbic acid, 1500 mg choline and 1 mg inositol.
‡Mineral premix, 1 kg contained 3 g sodium chloride, 1 g potassium chloride, 1.4 g magnesium sulphate, 0.2 g ferric citrate, 0.25 g manganese sulphate, 0.01 g potassium iodide, 0.13 g zinc carbonate, 0.01 g copper sulphate and 6 g dicalcium phosphate.
§Nitrogen free extract (NFE) = 1000 − (moisture + CP + crude lipid + ash + crude fibre).
‖Gross energy (GE) = (CP × 5.6) + (crude lipid × 9.44) + (crude fibre × 4.1) + (NFE × 4.1) (Anand *et al.* 2014).
MC, mackerel condensate; FM, fish meal; CP, crude protein; GE, gross energy.

types, tubular structures, or their arrangement (Fig. 3).

Discussion

The giant freshwater prawn is an omnivorous species, and is generally fed a diet with varying 50–250 g kg^{-1} FM (Tacon & Metian 2008). In attempts to reduce the feed costs, plant proteins, particularly soybean meal, have been used to replace costly FM (Du & Niu 2003; Gupta *et al.* 2007; Hasanuzzaman *et al.* 2009). The fraction that can be replaced is limited by an unbalance of the EAA, and by low palatability that manifests as reduced food consumption, poor growth and low feed efficiency (Floreto, Bayer & Brown 2000; Du & Niu 2003). Animal protein ingredients, including golden apple snail meal (Jintasataporn *et al.* 2004) and *Artemia* (Anh *et al.* 2009), have successfully replaced 50% or even 100% of the FM in the diet of giant freshwater prawn. Only limited nutritional information is available on the MC, while studies on bioremediation and waste treatment have reported its chemical oxygen demand (66 222 mg L^{-1}), oil and grease (1727 mg L^{-1}), total solids (57 192 mg L^{-1}) and suspended solids (7000 mg L^{-1}) (Uttamangkabovorn *et al.* 2005). During processing, the fish is precooked by steaming at 95–100°C for 30–75 min (Uttamangkabovorn *et al.* 2005; current study). The condensate from this precooking mainly contains soluble proteins, fats and ash released from the fish, but very little crude fibre and NFE (Table 1).

In this study, the 40MC diet improved growth performance (WG, SGR and ADG) and feed utilization (FI, FCR and PER) of giant freshwater prawn, relative to the control groups. Although the condensate can improve the palatability of feed due to the presence of peptides and free amino acids, the prawn fed with 40MC diet had dramatically reduced FI. Apparently the 40MC diet provided sufficient nutritional constituents in order to

Table 3 Survival, growth performance and feed utilization of giant freshwater prawns subjected to various dietary treatments for 12 weeks

Parameter	0MC	10MC	20MC	30MC	40MC	50MC	60MC	CD
Survival (%)	83.67 ± 1.18	84.81 ± 1.85	86.83 ± 1.21	82.33 ± 1.61	84.67 ± 1.02	85.24 ± 1.57	83.78 ± 1.16	84.45 ± 1.51
Final body weight (g)	3.91 ± 0.17^{abc}	3.66 ± 0.24^{cd}	4.16 ± 0.52^{ab}	4.32 ± 0.41^{a}	4.43 ± 0.12^{a}	3.45 ± 0.45^{cd}	3.41 ± 0.16^{d}	3.71 ± 0.33^{bcd}
WG (g week^{-1})*	0.251 ± 0.002^{bc}	0.230 ± 0.001^{cd}	0.273 ± 0.002^{ab}	0.290 ± 0.003^{a}	0.301 ± 0.002^{a}	0.218 ± 0.004^{d}	0.211 ± 0.002^{d}	0.231 ± 0.003^{cd}
SGR (% day^{-1})†	1.73 ± 0.04^{bc}	1.66 ± 0.02^{c}	1.82 ± 0.03^{ab}	1.87 ± 0.05^{a}	1.90 ± 0.04^{a}	1.61 ± 0.06^{cd}	1.56 ± 0.02^{d}	1.67 ± 0.04^{bc}
ADG (g day^{-1})‡	0.036 ± 0.001^{bc}	0.033 ± 0.001^{cd}	0.038 ± 0.002^{ab}	0.039 ± 0.002^{a}	0.042 ± 0.001^{a}	0.030 ± 0.002^{d}	0.029 ± 0.001^{d}	0.033 ± 0.001^{cd}
FI (g day^{-1})§	0.059 ± 0.001^{c}	0.061 ± 0.001^{b}	0.057 ± 0.001^{cd}	0.056 ± 0.001^{d}	0.055 ± 0.001^{d}	0.062 ± 0.001^{b}	0.064 ± 0.001^{a}	0.061 ± 0.001^{b}
FCR (g feed g gain^{-1})‖	2.46 ± 0.06^{cd}	2.54 ± 0.08^{c}	2.39 ± 0.05^{d}	2.35 ± 0.03^{d}	2.33 ± 0.05^{d}	2.59 ± 0.04^{bc}	2.68 ± 0.01^{b}	2.56 ± 0.06^{c}
PER (g gain g protein^{-1})**	1.16 ± 0.03^{bc}	1.12 ± 0.05^{c}	1.19 ± 0.08^{ab}	1.21 ± 0.05^{a}	1.23 ± 0.04^{a}	1.10 ± 0.06^{cd}	1.07 ± 0.02^{d}	1.11 ± 0.04^{c}
HSI (%)††	4.16 ± 0.02^{c}	5.01 ± 0.09^{b}	5.73 ± 0.07^{a}	4.89 ± 0.11^{b}	4.86 ± 0.13^{b}	5.53 ± 0.06^{a}	5.14 ± 0.21^{b}	4.85 ± 0.16^{b}

Data were expressed as mean ± SEM (n = 3). Different superscripts in the same row indicate statistical significances ($P < 0.05$).

*Weight gain (WG, g week^{-1}) = Final weight (g) − initial weight (g)/rearing period (week).

†Specific growth rate (SGR, % day^{-1}) = 100 (ln W_t − ln W_0)/(t − t_0) Where W_t = mean weight (g) at day t, W_0 = mean weight (g) at day t_0,

‡Average daily gain (ADG, g day^{-1}) = Net weight gain (g)/rearing period (days).

§Feed intake (FI, g day^{-1}) = F/(W_0 + W_1/2)(N_0 + N_1/2)t Where F = dry feed fed (g), W_0 = average initial body weight (g), W_1 = average final body weight (g), N_0 = initial fish number, N_1 = final fish number, t = rearing period (day).

‖Feed conversion ratio (FCR, g feed g gain^{-1}) = Dry feed consumed (g)/wet weight gain (g).

**Protein efficiency ratio (PER, g gain g protein^{-1}) = Wet weight gain (g)/protein intake (g).

††Hepatosomatic index (HSI, %) = 100 (Wet weight of hepatopancreas/wet body weight).

MC, mackerel condensate; CD, commercial diet; FI, feed intake.

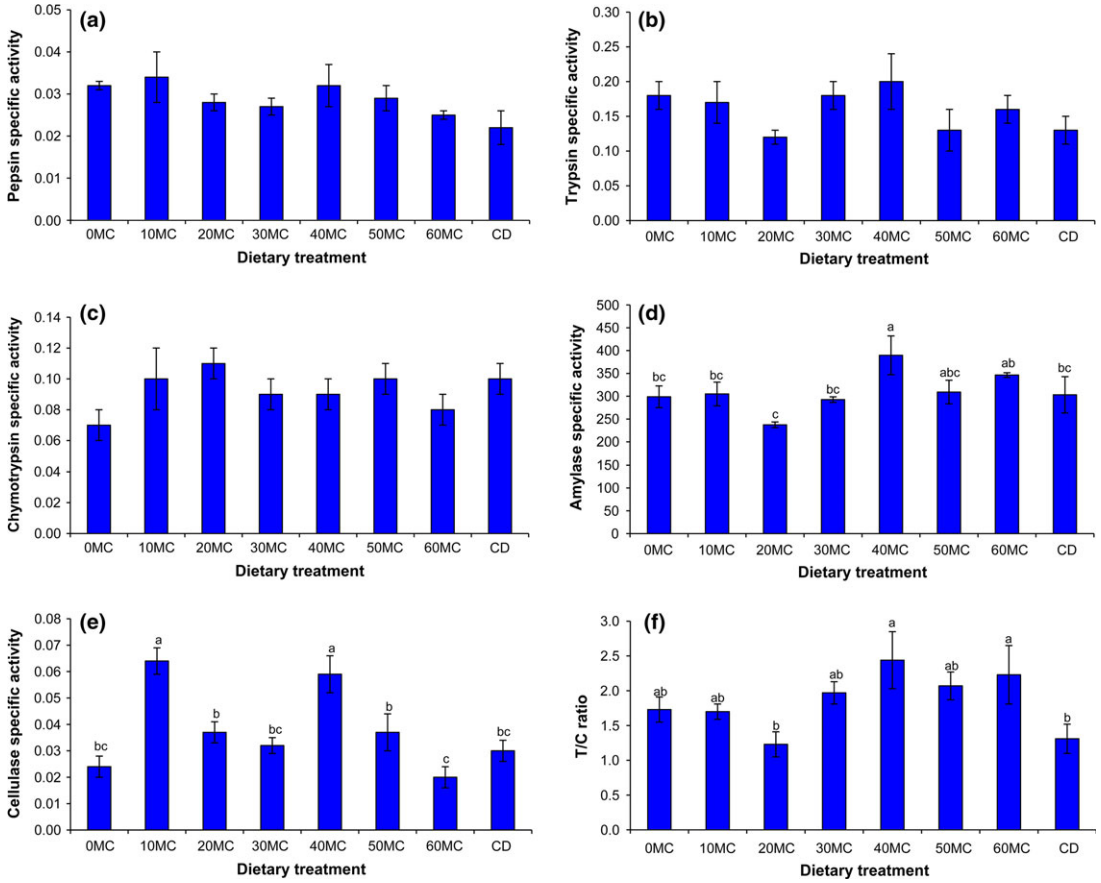

Figure 1 The specific activities (U mg protein^{-1}) of pepsin (a), trypsin (b), chymotrypsin (c), amylase (d) and cellulase (e), and the specific activity ratio of trypsin to chymotrypsin (f), in giant freshwater prawns subjected to various dietary treatments. The data are expressed as mean ± SEM (n = 3). Different superscripts indicate significant differences between groups ($P < 0.05$).

induce satiation, as well as a positive response in growth performance. This hypothesis agrees well with the decreased FCR and increased PER along with enhanced growth performance. From available data, the fish condensates from canned tuna or mackerel seafood factories can contain 512–831 g kg^{-1} CP, 38–274 g kg^{-1} crude lipids and 119–289 g kg^{-1} ash, on dry weight basis (Somboon & Semachai 2004; Wattanakul *et al.* 2011, current study). Variations in chemical composition of the condensate can be due to the processing conditions or the types of processed fish. In prior studies, the fish condensate has replaced 25% of the FM in a 40%-protein-diet for the climbing perch, *Anabas testudineus* (Wattanakul & Wattanakul 2013), and 80% of FM in a 27%-protein-diet for the Vietnamese Tra fish, *Pangasius hypophthalmus* (Is-Haak & Koydon 2010). The optimal replacement level in the current study is in agreement with the findings in white shrimp (*Litopenaeus vannamei*) fed a FM-based 35%-protein-diet in which MC replaced 40% of the FM (Wattanakul *et al.* 2011). Generally, the maximal inclusion levels of fish condensate in aquafeed are in the range 100–200 g kg^{-1} of feed, while higher levels or total replacement have negative effects on both growth and feed utilization (Is-Haak & Koydon 2010; Wattanakul *et al.* 2011; Wattanakul & Wattanakul 2013). The near optimal 40% protein replacement level and the feed formulation in the current study appear profitable, as its current purchase cost is 0.64 USD kg^{-1}, which compares favourably against both the 0MC (0.73 USD kg^{-1}) and especially the CD (1.05 USD kg^{-1}). Although the higher 50–60% replacement levels gave slightly lower feed costs (0.61–0.64 USD kg^{-1}) they impacted growth and feed utilization negatively. For the MC alone (0.45 USD kg^{-1}), current

Table 4 Carcass compositions (% wet weight) of the giant freshwater prawns subjected to various dietary treatments for 12 weeks

Carcass composition	0MC	10MC	20MC	30MC	40MC	50MC	60MC	CD
Moisture	76.96 ± 0.25	77.27 ± 0.64	77.49 ± 0.23	76.23 ± 0.57	76.22 ± 0.51	76.44 ± 0.40	77.75 ± 0.71	76.44 ± 0.40
CP	15.43 ± 0.21^{ab}	14.97 ± 0.50^{ab}	14.78 ± 0.27^{ab}	15.80 ± 0.39^{ab}	16.26 ± 0.32^{a}	15.36 ± 0.19^{ab}	14.15 ± 0.48^{b}	15.58 ± 0.30^{ab}
Crude lipid	0.22 ± 0.01	0.19 ± 0.01	0.20 ± 0.02	0.21 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.20 ± 0.01	0.22 ± 0.01
Ash	4.32 ± 0.10^{ab}	3.74 ± 0.10^{b}	3.81 ± 0.23^{ab}	4.04 ± 0.16^{ab}	4.31 ± 0.06^{ab}	4.12 ± 0.03^{ab}	3.86 ± 0.17^{ab}	4.42 ± 0.10^{a}

Data were expressed as mean ± SEM (n = 3). Different superscripts in the same row indicate statistical significances ($P < 0.05$).
MC, mackerel condensate; CD, commercial diet; CP, crude protein.

purchasing prices are very inexpensive compared to the FM (0.84 USD kg^{-1}). In addition, based on our feeding management, water quality was unaffected by the MC replacement level, and it also remained within appropriate range for the normal growth of prawn (Hossain & Islam 2007; Anh *et al.* 2009; Hasanuzzaman *et al.* 2009).

The growth of an aquatic animal is positively correlated with its gastrointestinal weight (Thongprajukaew *et al.* 2011). The stomach and intestine perform practically all of the digestion and absorption of nutrients. In the stomach, protein digestion depends on the pepsin activity (Kageyama 2002). This enzyme cleaves peptide bonds between hydrophobic and preferably aromatic amino acids, such as phenylalanine, tryptophan and tyrosine (Dunn 2001). In the intestinal digestion, 40–50% of the dietary proteins are digested by trypsin activity (Eshel, Lindner, Smirnoff, Newton & Harpaz 1993). This enzyme cleaves the carboxyl side of charged polar R groups (including lysine and arginine) in polypeptide chains. Trypsin can activate itself along with other zymogens, producing chymotrypsin, elastase and carboxypeptidase. Both of the serine proteases, trypsin and chymotrypsin, respond to feed protein stressors in white shrimps (CÓrdova-Murueta, García-Carreño & Navarrete-del-Toro 2003).

We observed no significant differences in the activities of the three proteolytic enzymes, or in the sum of proteolytic activities (0.284 ± 0.018 U mg protein^{-1} on average), between the treatments, indicating stable ability to digest proteins. This indicates that the quality and quantity of protein in the diets had little effect on the response of protein catabolism in the prawns. Under laboratory conditions with ample feed available, ingestion rates tended to be high, and a high enzymatic activity allowed crustaceans to extract most of the digestible compounds, retrieving nutritional entities (Le Vay, Jones, Puello-Cruz, Sangha & Ngamphonsai 2001). That no effects were observed on proteolytic activity in the current study is similar to a prior observation, where the activities of total protease and chymotrypsin were observed in mysis sub-stages and post-larvae stage (PL1) of white shrimp fed by basal microcapsules, microcapsules containing krill hydrolysate or *Artemia franciscana* Nauplii (Gallardo, Martínez, Palomino, Paredes, Gaxiola, Cuzon & Pedroza-Islas 2013). In the current study, the elevated PER in the prawns fed by 20MC, 30MC or 40MC diets was due to the better

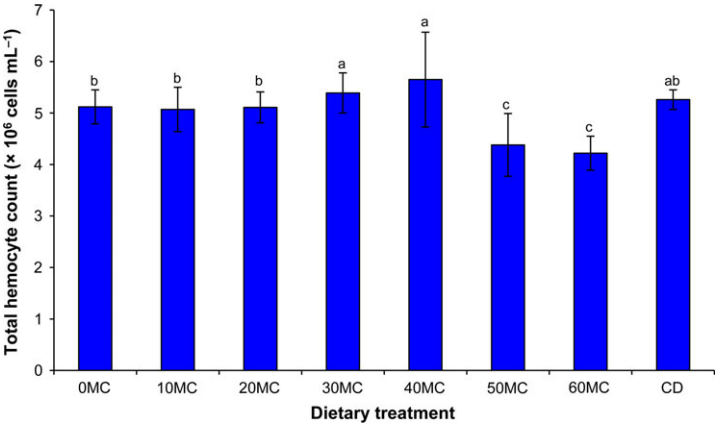

Figure 2 The total haemocyte counts ($\times 10^6$ cells mL^{-1}) for giant freshwater prawns subjected to various dietary treatments. The data are expressed as mean ± SEM ($n = 3$). Different superscripts indicate significant differences between groups ($P < 0.05$).

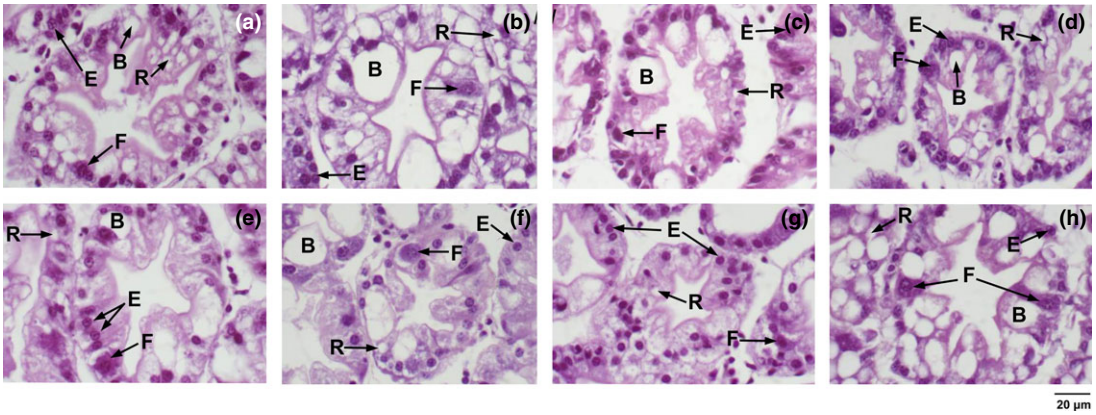

Figure 3 The hepatopancreatic microanatomy for giant freshwater prawns fed for 12 weeks with 0MC (a), 10MC (b), 20MC (c), 30MC (d), 40MC (e), 50MC (f), 60MC (g) or CD (h). All of the images have 400× magnification. The labels indicate cell types: Blasenzellen (B), embryonic cell (E), Fibrillenyellen (F) and Restzellen (R).

amino acid profile, not due to better protein digestibility. The growth and feed efficiencies have been positively associated with the T/C ratio in Atlantic salmon, *Salmo salar* (Sunde *et al.* 2004; Rungruangsak-Torrissen *et al.* 2006) and in blue swimming crab, *Portunus pelagicus* (Chamchuen, Pratoomchat, Engkakul, Kovitvadhi & Rungruangsak-Torrissen 2014). However, no correlation between this ratio and growth was observed in Siamese fighting fish (*Betta splendens*) fed various modified diets (Thongprajukaew *et al.* 2011). In the giant freshwater prawn the T/C ratio appears not predictive of growth, based on the current study, which might indicate effects of the species, the metamorphosis stage or the regulation of protein metabolism.

A close association between the protein- (total protease and trypsin) and carbohydrate-digesting enzymes (amylase) has been reported in fish (Thongprajukaew *et al.* 2011). This is in agreement with a positive correlation ($r = 0.315$–0.663, $n = 24$, $P < 0.05$) between the protein-digesting enzymes (either pepsin, trypsin, chymotrypsin or T/C ratio) and the carbohydrate-digesting enzymes (either amylase or cellulase) in the current study. Amylase and cellulase are mainly produced in the hepatopancreas of the giant freshwater prawn, and are then secreted into the foregut to cleave glycosidic bonds (González-Peña, Anderson, Smith & Moreira 2002). The increased amylase and cellulase-specific activities with 40MC diet indicate improved capacity for digesting the NFE and the structural carbohydrates respectively. Similar observations have also been reported in giant tiger prawn (*Penaeus monodon*) fed by a diet containing 4% biofloc, as compared to the control and the other inclusion levels (Anand *et al.* 2014). However, there are studies in which an increase in the

activity of these enzymes may also decrease the digestibility of dry matter and protein (Potty 1996; González-Peña *et al.* 2002). To replace FM they not only included MC but also soybean meal, which has many non-starch polysaccharides that have low digestibility; these can require increased enzyme activity.

Rapid growth with low feed consumption had no negative effects on the carcass composition in the prawns fed 40MC, as compared to other dietary treatments. The carcass composition in terms of moisture, protein, lipid and ash were similar with the 0MC diet ($P > 0.05$). Fish meal replacement exceeding 50% tended to decrease the protein deposition in the carcass, as compared to the 40MC. Floreto *et al.* (2000) reported the replacement of FM with soybean meal at no more than 50% of dietary protein appears feasible, with multiple amino acid supplementation (arginine, methionine and tryptophan) significantly enhancing growth performance of American lobster (*Homarus americanus*). Such high replacement levels of FM by MC in practical diets for giant freshwater prawn might also be feasible with EAA supplementation (except histidine).

The safety of this by-product in aquafeed is supported by the current study, in that no negative effects were detected on the total haemocyte count or the hepatopancreatic microanatomy, or on survival. Haemocytes are involved in defence processes of shrimp such as phagocytosis, encapsulation, melanization and coagulation (Johansson, Keyser, Sritunyalucksana & Söderhäll 2000). The haemocyte count exhibits great variation in shrimps exposed to stressful test conditions, and it is used as a sensitive indicator of health status (Perazzolo, Gargioni, Ogliari & Barracco 2002). In the current study, the increase in total haemocytes and growth with 30MC and 40MC diets could be due to mitogenic stimulation, as earlier observed in Kuruma prawn, *Penaeus japonicus* (Sequeira, Tavares & Arala-Chaves 1996). On the other hand, decreased total haemocytes in the prawns fed 50MC or 60MC may be associated with the lower growth rate. An increase in haemocyte count may also be due to immunostimulation, induced by contamination with microorganisms, or by the presence of some polysaccharides in MC, like lipopolysaccharide, β-glucan, etc. (Sequeira *et al.* 1996). Moreover, the amount of histidine in 40MC may be suitable for triggering the haemocytes through production of histamines that take part in allergic and inflammatory reactions (Ahmad & Khan 2005), while lower (0MC, 10MC and 20MC) or higher (50MC and 60MC) amounts did not boost immunity. Further microbiological and nutritional analyses of the MC-containing diets or the MC might be necessary before large-scale applications in aquaculture. The stable histoarchitecture of prawn hepatopancreas indicates that the use of MC had no toxic effects. The embryonic cells (E-cell) are involved in mitotic activity and can differentiate into Blasenzellen (B-cell), Fibrillenyellen (F-cell) and Restzellen (R-cell) (Ramadevi, Shyamasundari & Rao 1990). The B-cells are filled with digestive enzymes and are responsible for intracellular digestion, concentrating the absorbed materials (Al-Mohanna & Nott 1986) and secreting the vacuolar content at the end of digestive process (Sousa & Petriella 2000). The F-cells synthesize the digestive enzymes and have a high rate of protein synthesis (Al-Mohanna & Nott 1989). The R-cells absorb nutrients from the lumen and function in the storage of lipid droplets and glycogen (Sousa & Petriella 2000). Based on observed histoarchitectures, cell types and acini, the hepatopancreases in all prawn groups appeared to function normally. Also, the different diets with FM protein replacement or with increased soybean inclusion had no evident negative effects. An increased HSI in the current study correlated well with growth, and the correlation between hepatopancreatic and body weights was strongly positive ($r = 0.767$, $n = 24$, $P < 0.01$). The values were less sensitive in trend, but may indicate an improvement in the health status of shrimp, based on comparison with activity of aspartate aminotransferase and alanine aminotransferase (Xie, Tian, Li, Zhou, Zeng, Yang & Liu 2015). In the current study, although HSI fluctuated and exhibited varying trends, a moderate ratio along with the highest haemocytes and normal hepatopancreatic histoarchitecture supports the 40% protein replacement of FM by MC, in the diet for giant freshwater prawn.

Conclusions

Mackerel condensate (MC) is a nutrient-rich waste from the cooking and canning of fish. The nutritional use of this industrial by-product is attractive both for reducing effluents and for cost savings in aquafeed. We experimentally investigated its use in the diet for giant freshwater prawn. A 40%

protein replacement of FM by MC was optimal, and gave the prawns both superior growth and feed utilization, significantly improved carbohydrate digestion and maintained protein digestion. No effects of diet on water quality were observed during the 12-week experiment. The optimal diet had no negative effects on carcass composition, total haemocyte count, hepatopancreatic microanatomy or survival. Moreover, this feedstuff, similarly as the MC-containing diets, is very inexpensive compared to conventional FM. Overall, these findings suggest that the diet of giant freshwater prawn is well-suited for the value-added use of the industrial by-product, mackerel condensate.

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