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Effects of Low Dietary Fishmeal on Growth, Nitrogen Loading and Some Physical and Chemical Indices of Asian Sea Bass *Lates calcarifer* (Bloch, 1790)

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ABSTRACT

A 60-day trial was conducted to determine the effects of a low-fishmeal diet on Asian sea bass, *Lates calcarifer*. A 50% fishmeal diet was used as a control and in other experimental diets, fishmeal was replaced by soybean and canola meals to give diets containing 40, 30, 20 and 10% fishmeal. The effect of supplementing the 10% fishmeal diet with 1% betaine was also tested. Fish fed the 10–30% fishmeal diets showed significantly higher specific growth rate than did fish fed the control diet. Reducing the amount of dietary fishmeal did not affect the fish survival rate, feed intake or feed conversion ratio, but decreased the nitrogen loading. Reduced dietary fishmeal increased the amount of flesh protein and most essential amino acids contents, but decreased the flesh lipid, ash and bone protein contents. The hepato-somatic and viscero-somatic indices and the condition factor were significantly increased with reducing dietary fishmeal. Supplementing the 10% fishmeal diet with 1% betaine reduced the hepato-somatic index and condition factor, but increased the feed intake, feed conversion ratio and nitrogen loading; however, this did not improve the specific growth rate and survival rate, and adversely affected fish quality by decreasing the flesh protein and increasing the lipid content.

Keywords: growth, feed intake, soybean meal, canola meal, betaine

INTRODUCTION

Culture of Asian sea bass, a tropical fish of economic importance, has been well established in Southeast Asian countries, Australia and Taiwan as the fish can be cultured in both outdoor and indoor systems with fresh, brackish and salt water (Glencross, 2006a). This species requires approximately 45–55% dietary protein (Glencross, 2006a). Fishmeal provides a high protein content and has a good balance of essential amino acids (National Research Council, 1993), and has generally been preferred as the main dietary protein source for Asian sea bass, typically at inclusion levels of 20–50% (Tacon and Metian, 2008). Unfortunately, global fishmeal production is currently tending to decrease, resulting in an increased price (Tacon *et al*., 2012), which directly affects the price of feed, particularly for carnivorous fish species which require a high-protein diet (Glencross, 2006b). Hence, it is necessary to reduce the amount of dietary fishmeal

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used in any fish feed formula to reduce the feed cost. This would also reduce concerns about destroying fishery stocks (Naylor *et al*., 2000), because under-sized economic fish are caught with trash fish during the large-scale fishing practices, leading to depletion of stocks. Furthermore, reducing dietary fishmeal will lower the amount of nitrogen that is loaded into the environment (Abou *et al*., 2012), which will help to make fish farming sustainable (World Bank, 2006).

To reduce the dependency on fishmeal, alternative protein sources have been investigated and tested in several farmed aquatic species (Gatlin *et al*., 2007). Some potential sources are plant meals from soybean, canola, or cottonseed, or meals from rendered animal products such as blood, meat and bone, feathers and shrimp heads. Among these alternatives, soybean and canola meals rank first and second in global production, respectively (Suárez *et al*., 2009; Tacon *et al*., 2012) and are available in large quantities.

This study determined the effects on fish growth, feed utilization, nitrogen loading and the physical and chemical qualities of reducing dietary fishmeal from 50 to 10% by replacement with soybean and canola meals. However, high inclusion rates of these plant meals may affect the high condition factor making fish fatter. Using a high rate of plant meal reduces dietary methionine and lysine and leads to a lack of carnitine; these amino acids are the precursors of the production of carnitine, which plays a key role in lipid metabolism (Kidd *et al*., 1997). Lacking this nutrient leads to increases in fish hepato-somatic and viscero-somatic indices, which in turn affect the high condition factor (Sang *et al*., 2012) and these fatter fish are not preferred by Thai consumers (Plaipetch *et al*., 2008). Furthermore, high levels of dietary plant meal can affect feed intake leading to slow growth (Boonyaratpalin *et al*., 1998). A solution is to supplement the diet with betaine, a feed attractant, which helps to increase feed intake (Tiril *et al*., 2008). Betaine also possibly helps to improve the condition factor by compensating for deficiencies of methionine and lysine for the production of carnitine (Kidd *et al*., 1997).

MATERIALS AND METHODS

Preparation of the test diets

Six isonitrogenous and isolipidic test diets were prepared. A 50% fishmeal diet was used as a control. In diets 1–4, fishmeal was replaced by soybean and canola meals to give diets containing 40, 30, 20 and 10% fishmeal. Diet 5 was the 10% fishmeal diet supplemented with 1% betaine. Different levels of fish oil and soybean oil were used to provide dietary docosahexaenoic acid (22:6n-3) and an optimum n-3/n-6 fatty acids ratio of 1.50, as recommended by Williams and Barlow (1999). In batches of 1 kg, each diet was mixed with 30% water and formed into pellets by forcing through a feed mincing machine with a 2 mm pore size for first month feeding and then with a 3 mm pore size for the second month. All the test diets were dried at 60 °C overnight, then broken into an optimum length of 3–5 mm and kept in a freezer at -20 °C until use. The ingredients of the test diets and their proximate and essential amino acid compositions are shown in Tables 1 and 2, respectively.

Fish husbandry

Groups of 15 Asian sea bass fingerlings with an average body weight of 8 g were randomly stocked into each of 18 100 L aquaria. All the aquaria were connected with a recirculation system to clean the water and maintain the same water quality in all aquaria. This system comprised a sedimentation unit, a protein skimmer, a biofilter unit and a cleaned water unit. Water from all the fish aquaria flowed through the sedimentation unit before being pumped through the protein skimmer and then passed through the biofilter and the cleaned water units under gravity. Then, the cleaned water was pumped back into the fish aquaria. The flow rates of both the outlet and inlet water were fixed

Table 1 Ingredients of the test diets.

Ingredient	Dietary fishmeal (%)					
	50	40	30	20	10	10 + betaine
Thai fishmeal[1]	50.00	40.00	30.00	20.00	10.00	10.00
Soybean meal[2]	–	10.00	20.00	32.25	45.00	44.00
Canola meal[3]	–	10.00	15.00	15.00	15.00	5.00
Squid liver meal	5.00	5.00	5.00	5.00	5.00	5.00
Shrimp head meal	8.00	8.00	8.00	8.00	8.00	8.00
Wheat gluten	8.60	5.00	5.00	5.00	5.00	5.00
Cassava flour	15.78	10.03	5.28	2.85	–	–
Soybean oil	3.70	3.45	3.25	3.00	2.75	2.75
Tuna oil	3.20	4.10	5.10	6.13	7.03	7.03
Vitamin premix[4]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[5]	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin C 35 %	0.20	0.20	0.20	0.20	0.20	0.20
BHT	0.02	0.02	0.02	0.02	0.02	0.02
Cellulose	3.50	2.20	1.15	0.55	–	–
Betaine	–	–	–	–	–	1.00

BHT = Butylated hydroxytoluene.

[1] Tuna byproduct contains 60.05% crude protein, 9.51% crude lipid, 18.47% ash.

[2] Contains 45.50% crude protein, 1.47% crude lipid, 5.82% ash.

[3] Contains 38.67% crude protein, 1.65% crude lipid, 7.12% ash.

[4] Contains (g.kg^{-1}): A 0.138; D 0.002; E 10; K 5; B_1 6; B_2 10; B_3 40; B_5 10; B_6 4; B_{12} 0.01; p-amino benzoic acid 5; folic acid 1.5; biotin 0.6; inositol 200; choline chloride 500 and vitamin C, 50 g.

[5] Contains (g.kg^{-1}): KCl 131.7; $NaH_2PO_4 .2H_2O$ 394.7; $CaHPO_4$ 210.5 and KH_2PO_4 263.1.

Table 2 Proximate composition and essential amino acids of the test diets.

	Dietary fishmeal (%)					
	50	40	30	20	10	10 + betaine
Moisture	7.00	6.72	6.48	6.22	6.61	6.17
Crude protein	42.43	42.96	43.52	42.66	42.52	43.51
Crude lipid	12.79	13.16	12.93	12.65	12.86	13.14
Ash	14.32	13.59	12.79	11.82	11.16	10.95
Crude fiber	5.81	6.03	5.85	6.01	5.96	6.11
NFE	17.65	17.54	18.43	20.64	20.89	20.12
Essential amino acids[1] (% of protein)						
Phenylalanine	4.70	4.49	4.40	4.28	4.06	4.00
Valine	4.18	3.96	3.84	3.74	3.50	3.54
Threonine	4.92	4.83	4.68	4.60	4.39	4.35
Isoleucine	4.93	4.70	4.53	4.41	4.27	4.20
Methionine	2.37	2.21	2.14	2.05	1.89	1.85
Histidine	2.06	1.97	1.82	1.71	1.65	1.61
Arginine	6.09	5.86	5.70	5.48	5.20	5.18
Lysine	6.16	5.69	5.30	5.11	4.97	4.94
Leucine	8.64	7.86	7.62	7.50	7.43	7.41
Calculated n-3/n-6	1.50	1.51	1.52	1.54	1.56	1.56
Calculated % DHA	10.44	10.44	10.47	10.62	10.70	10.72

NFE = Nitrogen free extract, DHA = Docosahexaenoic acid shown as % of total fatty acid.

[1] Tryptophan is destroyed during sample preparation.

at 2.50 $L.min^{-1}$. The water was exchanged by 50% every 4 d in both the recirculation system and fish aquaria to maintain good water quality. During the experiment, some water quality parameters in all the fish aquaria were measured and shown to be within suitable values—namely pH, alkalinity, total ammonia nitrogen, nitrite, temperature and dissolved oxygen, with ranges of 6.9–7.1, 120 $mg.L^{-1}$, 0.5–0.7 $mg.L^{-1}$ 0.01 $mg.L^{-1}$, 27–30 ˚C and 5.0–6.0 $mg.L^{-1}$, respectively.

For each diet, three groups of fish were fed twice a day (at 0900 and 1500 hours) to apparent satiation. The fish from each aquarium were weighed as a whole batch every 15 d over the 60 d experimental period; the diet consumed and the remaining numbers of live fish were also recorded. At the end of the trial, six fish from each aquarium were sacrificed to determine physical and chemical qualities. The test fish were individually weighed and the standard length measured, before separation and individual weighing of the liver, visceral mass, flesh and bone. The total fish flesh from each aquarium was dried to a constant weight at 60 °C before chemical analysis.

Chemical analysis

Analysis of moisture, ash and crude fiber contents of the test diets and fish samples followed the methods of Association of Official Analytical Chemists (1995). Crude protein content was analyzed using a Truspec CN determination machine (Leco; St. Joseph, MI, USA). A fat extractor (TFE 2000; Leco; St. Joseph, MI, USA) was used to analyze the crude lipid content. Nitrogen free extract was calculated by deduction of the sums of other nutrients from 100%. The amino acids profile of the sample after acid hydrolysis with 6M HCl was determined by high performance liquid chromatography (HPLC), using an Agilent 1100 series instrument (Agilent Technologies; Santa Clara, CA, USA). The HPLC was performed on an ion exchange column type Na^+, (8µm particle size, 3.0 × 250 mm) fitted with a guard column type Na^+, 8 µm (2.0 × 20 mm). The sample solution was injected and the flow rate was 0.3 $mL.min^{-1}$. The column temperature and the pressure were set at 48 °C and 2×10^8 Pa, respectively.

Calculations and statistical analysis

Growth, survival rate, feed utilization, and some physical qualities were calculated according to the following equations:

MW = [sum of body weight / number of fish] (1)

where MW is the mean weight measured in grams;

ML = [sum of standard length / number of fish] (2)

where ML is the mean length measured in centimeters;

SGR = [100 × [(ln(FW) – ln(IW)] / day] (3)

where SGR is the specific growth rate measured in percent per day and IW is the initial weight and FW the final weight both measured in grams;

SR = [100 × (remaining number of fish) / (initial number of fish)] (4)

where SR is the survival rate measured in percent;

FI = [100 × (sum of dried diet consumed / mean of initial and final body weight) / day] (5)

where FI is the feed intake measured in percent;

FCR = [sum of dried diet consumed / weight gain] (6)

where FCR is the feed conversion ratio;

PER = [weight gain / protein consumed] (7)

where PER is the protein efficiency ratio;

PR = [100 × (final − initial body protein) / protein consumed] (8)

where PR is the protein retention measured in percent;

NL = [(nitrogen consumed − nitrogen deposited) / weight gain] (9)

where NL is the nitrogen loading measured in grams per kilogram of fish (Vielma *et al*., 2002);

CF = [100 × (W / L^3)] (10)

where CF is the condition factor, W is the weight in grams and L is length in centimeters (Nash *et al.*, 2006);

FR = [100 × flesh weight / total body weight] (11)

where FR is the flesh ratio measured in percent;

HSI = [100 × liver weight / total body weight] (12)

where HIS is the hepato-somatic index measured in percent;

VSI = [100 × visceral mass weight / total body weight] (13)

where VSI is the viscero-somatic index measured in percent.

All data were calculated as mean ± SD and were subjected to one-way analysis of variance. Duncan's new multiple range test was used to test for significant differences at the (P < 0.05) level and for highly significant differences at the (P < 0.01) level.

RESULTS

Growth, feed utilization, nitrogen loading and survival rate

Table 3 presents the results on the growth, feed utilization, nitrogen loading and survival rate of fish fed with the test diets. Overall, the final mean weight and length were not significantly affected by the treatment, but specific growth rates of fish fed the 10, 20 or 30% fishmeal diets were highly significantly greater than those of fish fed with the control. This parameter also showed an increasing trend with reducing dietary fishmeal, as did the feed intake, protein efficiency ratio and protein retention. The survival rates of the test fish were high and no significant differences were observed among all the diets. In contrast, the fish showed highly significant decreases in nitrogen loading. Supplementing 10% fishmeal diet with 1% betaine led to increases in the feed intake, feed conversion ratio and nitrogen loading, and decreases in the specific growth rate, protein efficiency ratio and protein retention compared to those fish without this supplementation.

Physical qualities, proximate composition, and essential amino acids

Some physical indices and the proximate composition of the fish fed the test diets are presented in Table 4. Reducing dietary fishmeal from 50% to 10% by partial replacement with

Table 3 Growth, feed utilization, nitrogen loading and survival rate of fish fed the test diets.

	Dietary fishmeal (%)					
	50	40	30	20	10	10 + betaine
Initial MW (g)	8.20±0.07	8.17±0.19	8.19±0.15	8.09±0.09	8.22±0.18	8.29±0.21
Final MW (g)	86.44±2.75	88.57±3.32	89.87±2.76	88.96±1.66	91.95±1.16	87.45±2.24
Initial ML (cm)	8.35±0.15	8.20±0.09	8.17±0.21	8.41±0.18	8.27±0.10	8.16±0.14
Final ML (cm)	17.85±0.34	17.60±0.81	17.21±0.45	17.08±0.59	17.98±0.36	17.63±0.72
SGR (% d^{-1})^2	3.92±0.02^b	3.97±0.04^{ab}	3.99±0.02^a	4.00±0.03^a	4.03±0.04^a	3.93±0.02^b
FI (% BW d^{-1})^1	3.48±0.21^{bc}	3.70±0.02^{abc}	3.44±0.04^c	3.72±0.15^{ab}	3.66±0.14^{abc}	3.85±0.18^a
FCR	1.04±0.08	1.06±0.01	1.02±0.03	1.04±0.05	1.11±0.07	1.12±0.04
PER	2.23±0.17	2.32±0.04	2.27±0.06	2.40±0.12	2.36±0.15	2.15±0.13
PR (%)^2	27.05±0.75^b	27.70±0.84^b	30.42±0.72^a	31.89±0.81^a	31.64±0.50^a	26.71±0.88^b
NL (g Kg^{-1})^2	51.73±1.21^a	48.22±1.76^{ab}	46.53±1.88^{bc}	42.97±2.48^c	43.94±2.57^{bc}	51.97±3.39^a
SR (%)	91.11±3.84	88.89±3.84	95.55±3.85	91.11±3.84	93.33±6.66	93.33±6.66

Values are shown as mean±SD.

Different lowercase superscript letters in a row show significant differences, where 1 = (P < 0.05) and 2 = (P < 0.01).

MW = Mean weight; ML = Mean length; SGR = Specific growth rate; FI = Feed intake; FCR = Feed conversion ratio; PER = Protein efficiency ratio; PR = Protein retention; NL = Nitrogen loading; SR = Survival rate.

plant protein sources (soybean and canola meals) significantly increased the condition factor and the hepato-somatic index and highly significantly increased the viscera-somatic index. The flesh ratios showed an increasing trend but this was not significant. Supplementing the 10% fishmeal diet with 1% betaine decreased these parameters compared to those of fish without supplementation, except for the viscera-somatic index, which still increased. The results also indicated that the flesh protein of fish fed the test diets highly significantly increased as the dietary fishmeal was reduced. However, the flesh lipid and ash contents, including bone protein were highly significantly decreased. Supplementing the 10% fishmeal diet with 1% betaine led to decreases in the flesh protein and ash contents, whereas the flesh lipid content increased.

Table 5 presents the essential amino acid contents in the flesh of fish fed the test diets. Some essential amino acid contents showed significant increases as dietary fishmeal was reduced, except

Table 4 Physical indices and proximate composition (% DM) of fish fed the test diets.

	Dietary fishmeal (%)					
	50	40	30	20	10	10 + betaine
CF [1]	$1.47±0.05^{c}$	$1.51±0.08^{bc}$	$1.54±0.13^{abc}$	$1.59±0.07^{ab}$	$1.61±0.09^{ab}$	$1.63±0.03^{a}$
Flesh (%)	42.08±2.31	43.91±2.00	45.24±2.68	45.10±4.39	45.80±4.71	44.66±3.57
HSI (%)[1]	$2.07±0.20^{b}$	$2.43±0.23^{a}$	$2.53±0.24^{a}$	$2.51±0.45^{a}$	$2.67±0.29^{a}$	$2.55±0.11^{a}$
VSI (%)[2]	$5.53±0.59^{c}$	$5.58±0.86^{c}$	$5.73±0.32^{bc}$	$5.83±0.65^{bc}$	$6.72±0.99^{ab}$	$7.14±1.20^{a}$
Flesh						
Moisture	68.65±2.87	70.20±1.23	69.94±1.50	72.69±2.02	72.55±2.58	71.33±1.43
Crude protein[2]	$58.56±0.45^{c}$	$58.93±0.85^{c}$	$60.67±0.25^{b}$	$63.23±0.23^{a}$	$62.26±0.70^{a}$	$58.24±0.60^{c}$
Crude lipid[2]	$20.84±0.54^{a}$	$17.93±0.06^{c}$	$19.56±0.49^{b}$	$16.19±0.21^{e}$	$16.86±0.34^{d}$	$20.56±0.19^{a}$
Ash[2]	$13.79±0.17^{b}$	$14.32±0.17^{a}$	$11.47±0.14^{e}$	$11.94±0.08^{d}$	$12.27±0.03^{c}$	$11.02±0.06^{f}$
Bone						
Moisture	56.21±2.28	58.14±1.79	56.68±2.11	59.68±1.80	58.13±1.69	58.23±1.05
Crude protein[2]	$26.86±0.18^{a}$	$26.93±0.14^{a}$	$25.89±0.08^{b}$	$24.68±0.41^{c}$	$24.81±0.17^{c}$	$26.23±0.09^{b}$

DM = Dry matter; CF = Condition factor; HSI = Hepato-somatic index; VSI = Viscero-somatic index.
Values are shown as mean±SD.
Different lowercase superscript letters in a row show significant differences, where 1 = ($P < 0.05$) and 2 = ($P < 0.01$).

Table 5 Essential amino acid contents in flesh of fish fed the test diets (% protein, DM).

	Dietary fishmeal (%)					
	50	40	30	20	10	10 + betaine
Phe[1]	$3.83±0.03^{c}$	$3.87±0.03^{bc}$	$3.93±0.07^{abc}$	$3.96±0.09^{ab}$	$3.94±0.02^{ab}$	$4.03±0.04^{a}$
Val[1]	$4.00±0.06^{b}$	$4.11±0.10^{ab}$	$4.02±0.03^{b}$	$4.21±0.04^{a}$	$4.20±0.11^{a}$	$4.21±0.11^{a}$
Thr	4.47±0.12	4.56±0.04	4.22±0.17	4.43±0.16	4.41±0.07	4.42±0.15
Ile[2]	$3.48±0.02^{c}$	$3.60±0.07^{bc}$	$3.54±0.04^{bc}$	$3.75±0.04^{a}$	$3.66±0.10^{ab}$	$3.75±0.09^{a}$
Met[1]	$2.69±0.01^{c}$	$2.76±0.03^{a}$	$2.74±0.03^{ab}$	$2.71±0.01^{bc}$	$2.74±0.02^{ab}$	$2.77±0.03^{a}$
His	1.87±0.07	1.91±0.09	1.97±0.08	1.90±0.06	1.89±0.10	1.85±0.05
Arg	5.91±0.23	6.06±0.77	6.39±0.77	5.72±0.48	5.51±0.38	6.05±0.83
Lys[2]	$7.17±0.11^{b}$	$7.43±0.12^{ab}$	$7.67±0.20^{a}$	$7.67±0.19^{a}$	$7.72±0.17^{a}$	$7.68±0.06^{a}$
Leu[2]	$6.70±0.03^{c}$	$7.04±0.04^{b}$	$7.01±0.09^{b}$	$7.32±0.10^{a}$	$7.25±0.12^{a}$	$7.33±0.08^{a}$

Phe = Phenylalanine; Val = Valine; Thr = Threonine; Ile = Isoleucine; Met = Methionine; His = Histidine; Arg = Arginine; Lys = Lysine; Leu = Leucine; Tryptophan is destroyed during sample preparation.Values are shown as mean±SD.
Different lowercase superscript letters in a row show significant differences, where 1 = ($P < 0.05$) and 2 = ($P < 0.01$).

threonine, histidine and arginine. Supplementing the 10% fishmeal diet with 1% betaine seemed to increase the essential amino acid contents in the fish flesh slightly compared to those of fish without supplementation.

DISCUSSION

This study indicated that reducing the content of dietary fishmeal for Asian sea bass from the typically used inclusion rates of 20–50% down to 10% had no adverse effects on the specific growth rate, survival rate or feed conversion ratio; these are key economic parameters for the fish farmer. This result was similar to that reported by Glencross *et al*. (2011) who showed that a reduction to 15% dietary fishmeal for this species had no adverse impact on growth. The satisfactory result with the low fishmeal content confirmed that it is possible to use alternative protein sources with higher inclusion rates than those currently recommended. For example, soybean meal could be used at a level of 45%, which is higher than the 25% recommended for marine fish by Tacon *et al*. (2012). However, the high level of dietary fiber introduced by using plant protein sources with high ratios, such as 60% by the combination of soybean and canola meals used in this study, can potentially affect digestibility. Hence, the plant protein sources have to be partially replaced with low fiber plant meal or animal by-products to control the dietary fiber content, which should not be above 2.54% (Food and Agriculture Organization, 1987).

Soybean meal is a key alternative protein source for use in aquatic animal feeds, but its price has tended to increase for several reasons such as competing uses for terrestrial animal feed and biodiesel production (Tacon *et al*., 2012). This has driven the aquatic feed companies to seek alternatives to soybean meal. Canola meal is now becoming popular because it has a comparable essential amino acids profile to soybean meal and is cheaper (Glencross, 2003). The present study showed that canola meal could be used at 15% without adverse impact on growth. This inclusion rate is within the recommended levels of 7–20% for marine fish (Newkirk, 2009; Tacon *et al*., 2012). However, it is possible that canola meal could be the main protein source for Asian sea bass, since dietary levels of 60% have been used for red sea bream (Glencross, 2003) and 28% for European sea bass (Lanari and D' Agaro, 2005). Further research is needed to evaluate the possibilities of using canola meal as the main protein source and replacing soybean meal at a high ratio.

The reduction to 10% dietary fishmeal without an adverse impact on the specific growth rate in the present study may have been achieved because it provides the main essential amino acids in relative amounts similar to those in the whole body amino acids of this species, as reported by Glencross (2006), and this might be higher than the actual requirements. Another factor could be the increase in fish oil levels to balance the n-3/n-6 fatty acids ratio to the value of 1.50 (Williams and Barlow, 1999). This ratio has been recommended to ensure that Asian sea bass will obtain sufficient essential fatty acids, especially eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) for normal growth. As with European sea bass, the satisfactory results with 5% dietary fishmeal seemed to be related to the use of a higher fish oil level (Kaushik *et al*., 2004).

In the current study, reducing the level of dietary fishmeal did not decrease the feed intake of the test fish, as reported by Williams (1998), possibly because of the use of higher levels of fish oil that contains high arachidonic acid (20:4n-6) and linolenic acid (18:3n-3) that have been suggested to be feed attractants (Teruya *et al*., 2001). It has been shown clearly that fish oil can be a feed attractant for spotted Babylon, *Babylonia areolata* (Kun-Art *et al*., 2010).

The use of soybean and canola meals to replace fishmeal increased the feed intake and feed conversion ratio, possibly because of effects on the digestibility of anti-nutritional factors such as phytic acid and trypsin inhibitor contained in these plant meals (Francis *et al*., 2001). Furthermore, proteins of both soybean and canola meal contain higher nitrogen contents compared with fishmeal protein (Tacon *et al*., 2009). However, nitrogen loading tended to decrease with a reduction in the level of dietary fishmeal, possibly because the high fishmeal diets (30–50% fishmeal) contained higher amounts of essential amino acids than the requirements of the fish, for example methionine, lysine and arginine (Millamena, 1996). Other essential amino acids were possibly also higher than the values assumed from their contents in the whole body (Glencross, 2006). The fish might have to excrete these excess amino acids in higher amounts than was required with the 10% and 20% fishmeal diets.

Generally, some physical qualities such as fatty fish (high condition factor), and high lipid flesh are not preferred by Thai consumers (Plaipetch *et al*., 2008). This study showed that a low fishmeal diet, especially the 10% fishmeal diet, clearly decreased flesh lipid, but the hepato-somatic and viscero-somatic indices and the condition factor were higher than for the control diet. These seemed to be related to lower dietary methionine and lysine when fishmeal was replaced with soybean and canola meals. Both these essential amino acids are precursors of carnitine, which promotes lipid metabolism by carrying fatty acids through the outer mitochondrial membrane. A lack of carnitine has been shown to cause disorders in rats since when fed a lysine-deficient diet, they had a higher content of liver fat and reduced ß-oxidation (Tanphaichitr *et al*., 1976).

Previous studies also showed that supplementing either methionine or lysine in diets of both Atlantic salmon and rainbow trout reduced the hepato-somatic index and the peritoneal and body lipid contents (Walton *et al*., 1984; Espe *et al*., 2007; Gaylord *et al*., 2007; Espe *et al*., 2008). Direct dietary supplementation with carnitine also improved the hepato-somatic and viscero-somatic indices and the condition factor of yellow croaker (Sang *et al*., 2012). In contrast, replacing fishmeal diets with yeast-fermented soybean meal up to 50% had no effect on the condition factor and hepato-somatic index of black sea bream (Zhou *et al*., 2011). These diets might contain sufficient amounts of these two essential amino acids for this species, especially the 50% replacement diet, which still contains 30% dietary fishmeal. There is evidence that using plant protein sources such as soybean meal to replace fishmeal reduces the lipogenic enzyme activity in European sea bass. This led to a lower lipid content in the flesh of this species (Dias *et al*., 2005; Messina *et al*., 2007), possibly because of imbalances of methionine and lysine, as observed for Asian sea bass in the present study.

The present study showed that reducing the amount of dietary fishmeal led to a lower ash content in the flesh of Asian sea bass, as it did with Nile tilapia (Cao *et al*., 2008; Koumi *et al*., 2011). This may have been because dietary fishmeal is a main source of some macro-minerals, particularly Ca and P (National Research Council, 1993) which directly affect bone formation. Decreased bone protein was observed in this study when fish were fed the low fishmeal diets. Nevertheless, it seemed that using 10% dietary fishmeal reduced the dietary ash content to approximately 11% but did not affect the growth of Asian sea bass, implying that essential minerals such as Ca and P were still available at sufficient levels. This was in agreement with the results of Chaimongkol and Boonyaratpalin (2001), who reported that Asian sea bass fed a diet that yielded only 9–12% dietary ash had similar growth to those fed diets that contained higher ash levels of 20–24%.

Supplementing the diet with betaine in the present study increased the feed intake, as it did with rainbow trout (Tiril *et al*., 2008), and reduced the hepato-somatic and viscero-somatic indices, as observed in yellow croaker (Sang *et al*., 2012). Betaine may compensate for deficiencies of dietary methionine and lysine in carnitine production, by acting as a methyl donor (Kidd *et al*., 1997).

However, the present study showed that there is no necessity to supplement the 10% fishmeal diet with betaine, because this did not improve the specific growth rate or the survival rate and also led to a higher feed conversion ratio and nitrogen loading, and a reduced protein efficiency ratio and protein retention compared to that without supplementation. It further decreased the flesh protein and increased the flesh lipid contents. A better solution would be to balance dietary methionine or lysine by using the commercially available synthetic source. This would have the benefit of both increasing fish growth and improving physical qualities.

CONCLUSIONS

This study showed that fishmeal in the diet for Asian sea bass can be reduced to 10% without an adverse impact on the growth, survival rate and feed utilization. It decreased the nitrogen loading and flesh lipid content, but increased the hepato-somatic and viscero-somatic indices and the condition factor. These indices and the condition factor were decreased when the 10% fishmeal diet was supplemented with 1% betaine, but this did not lead to an improvement in the growth, survival rate, feed utilization or flesh biochemical qualities.

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