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Aquaculture 229 (2004) 325–333

Aquaculture

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Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*

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Received 3 January 2003; received in revised form 2 May 2003; accepted 5 May 2003

Abstract

An 8-week feeding trial was conducted with cobia to determine the amount of soybean meal that could replace fish meal in formulated diets without reducing growth. Juvenile cobia (initial mean weight, 32 g) were fed 48% crude protein diets in which dietary protein was supplied by brown fish meal or a mixture of hexane extracted soybean meal and the fish meal, resulting in 10%, 20%, 30%, 40%, 50% and 60% of fish meal protein being replaced by soybean protein. The fish readily accepted all seven experimental diets and no fish died during the trial. Detrimental effects on growth performance were obvious when half of the fish meal protein was replaced by soybean protein. There existed a significant difference in fish weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) when the replacement level for fish meal protein was increased from 40% to 50%, indicating that up to 40% of fish meal protein can be replaced by soybean meal protein without causing reduction in growth and protein utilization. On the other hand, quadratic regression analysis shows a growth optimum at 16.9% replacement of fish meal protein by soybean meal protein. Lipid concentrations in the cobia muscle increased significantly as dietary soybean meal increased. Muscle concentrations of free threonine and histidine decreased as use of the soybean meal increased in the diets. Since methionine concentration in the test diets decreased from 2.52 to 1.36 g 16 g^{-1} N as the soybean meal protein replacement level was increased from 0% to 60% while all other essential amino acids remained relatively constant, dietary requirement of methionine was calculated assuming it was equally available between the two proteins. The broken-line model analysis based on fish weight gain

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0044-8486/$ - see front matter
doi:10.1016/S0044-8486(03)00395-8

shows a breakpoint when dietary methionine + cystine concentration was 2.66 g 16 g^{-1} N or 1.28 g 100 g^{-1} diet.

Keywords: Cobia; *Rachycentron canadum*; Hexane extracted soybean meal; Brown fish meal; Replacement; Methionine; Nutrition

1. Introduction

Aquaculture of the cobia is gaining popularity in Taiwan and other Asia Pacific countries. The cobia is a carnivorous pelagic fish. It can grow with good feed conversion efficiency in offshore net cage systems from fingerling to 4–6 kg marketable size in 1 year. Its white flesh, which is suitable for sashimi consumption, is highly prized. Although commercial feeds have been widely used to replace trash fish as cobia production increases, cost-effective feeds especially formulated to maximize growth and health of this species are yet to be developed because of the paucity of nutritional requirement research. At present, commercial feeds based on formulas similar to Asian sea bass or grouper feeds result in acceptable growth in cobia net-cage culture with feed conversion ratios ranging from 1.5 to 1.8. Proximate analysis of a cobia sinking pellet feed manufactured by a Taiwanese feed mill revealed a crude protein concentration of 45.3%, crude lipid concentration of 16.0% and ash concentration of 11.0%.

Chou et al. (2001) found that the most suitable dietary protein level for maximum growth of juvenile cobia was 44.5% when fish meal and casein were the sole protein sources. In their study, levels of dietary protein were achieved by progressively replacing casein (47.6–19.1%) with α-starch while fish meal remained constant at 30.1%. The cobia grew from 33 to 283 g in 8 weeks when fed the best performing diet. When the cobia were fed test diets containing fish meal (31%), casein (11.1%) and soy protein (11.5%) in a second trial (Chou et al., 2001), a similar growth performance was observed (from 41 to 328 g in 8 weeks). Use of soy protein in the diets was readily accepted by the cobia.

Soybean meals are widely used as the most cost-effective alternative for high-quality fish meal in feeds for many aquaculture animals (Storebakken et al., 2000) because of high protein content, relatively well-balanced amino acid profile, reasonable price and steady supply of soybeans. Different soybean products, such as soy protein concentrate, extracted and toasted (defatted) soybean meal, full-fat soybean meal, or low oligosaccharides soybean meal (Refstie et al., 1998), tended to produce contrasting fish growth because of the varied quantities of antinutrient compounds and/or lowly digestible carbohydrates present in these products. Different fishes show varied sensitivity to soy antinutrients. In Atlantic salmon and rainbow trout, soybean meal has been found to cause distinct morphological alternations in the intestine, in addition to impaired growth and protein utilization; and the effects escalate with increasing dietary level (Krogdahl and Bakke-McKellep, 2001). Dietary soybean meal also appears to stimulate immune responses because of inflammation in the distal intestine (Krogdahl et al., 2000). Other fishes are not as sensitive to soybean antinutrients as the salmonids. Red drum (Reigh and Ellis, 1992) and Japanese flounder (Kikuchi, 1999) can effectively grow on diets containing almost equal amounts of fish meal and soybean meal without adversary effects.

Although incorporating soy protein in cobia diets at a modest level (11.5%) has proven feasible (Chou et al., 2001), the feasible and cost-effective use of defatted soybean meal, which is generally more economical than other soy products, remains unknown. The purpose of the present study was to quantify the maximum level of the soybean meal that could be incorporated in the diets of the juvenile cobia without reducing growth. Commercial hexane extracted soybean meal was used to partially replace brown fish meal and the resultant growth and changes in fish body composition and muscle amino acid profiles were evaluated.

2. Materials and methods

Seven isonitrogenous (crude protein concentration, 48%) experimental diets (Table 1) were compared. Diets 100/0–40/60 were formulated to produce diets in which 0 (Diet 100/0), 10 (90/10), 20 (80/20), 30 (70/30), 40 (60/40), 50 (50/50) and 60% (40/60) of protein from fish meal was replaced by that from soybean meal. The fish meal was brown fish meal (Biobio, Chile; crude protein concentration = 67.8%) and the soybean meal was commercial hexane extruded soybean meal (crude protein concentration = 42.5%). Fish oil and soybean oil were added, respectively, to compensate for differences in lipid composition of the fish meal and soybean meal. All ingredients were thoroughly mixed and wet-extruded as pellets, dried at 45 °C and refrigerated at 4 °C until fed. Proximate composition analyses according to standard methods (AOAC, 1995) indicated that all diets were more or less equal in protein (determined as Kjeldahl $N \times 6.25$) and lipid concentration (Table 1). Ash concentration decreased as the fish meal level decreased. Essential amino acids concentrations (exclusive of tryptophan) of the experimental diets were approximately equal except methionine. Methionine concentration decreased from 2.52 g 16 g^{-1} N in Diet 100/0 to 1.36 g 16 g^{-1} N in Diet 40/60 (Table 1).

The growth trial was conducted at the Tungkang Marine Laboratory. Cobia (*Rachycentron canadum*) juveniles were from a single egg mass and fed a commercial seabass feed until they reached a mean weight of about 32.3 g. Prior to the experiment, fish were individually weighed and distributed to each of 21 fiberglass tanks (1 m in diameter, water depth 30 cm) so the total weight of eight fish in each tank was almost equal. Each tank was supplied with sand-filtered seawater at a flow rate of 2–2.5 l per min. Water temperature was 28 ± 0.5 °C and salinity was 32 ± 1‰ during the growth trial. Each dietary treatment was randomly assigned to three tanks. The fish were fed twice daily by hand as much as they would consume in 30 min at 0900 and 1600 h. The feeding trials lasted for 8 weeks. Fish were bulk-weighed biweekly and individually at the end of the experiment. At the end of the feeding trial and overnight after the last feeding, three fish from each tank were randomly sampled and frozen for proximate analysis according to standard method

Table 1
Ingredient, proximate and essential amino acid composition of the experimental diets

	Diet[a]						
	100/0	90/10	80/20	70/30	60/40	50/50	40/60
Ingredient composition, g/kg dry diet							
Fish meal	447.8	403.0	358.2	313.4	268.7	223.9	179.1
Soybean meal	0	71.4	142.8	214.3	285.7	357.1	428.6
Fish oil	63.7	67.7	71.4	75.8	79.8	83.8	87.9
Soybean oil	8.6	7.2	5.7	4.3	2.9	1.5	0
Cellulose	175.5	146.3	117.5	87.8	58.5	29.3	0
Constant ingredients[b]	304.4	304.4	304.4	304.4	304.4	304.4	304.4
Proximate composition (n = 3), g 100 g^{-1} diet							
Crude protein	47.3	47.8	48.1	49.4	48.5	46.1	49.4
Crude lipid	12.3	12.2	12.5	12.7	12.5	14.9	13.9
Ash	23.9	22.8	21.2	15.2	12.6	12.2	7.7
Essential amino acid composition (n = 3), g 16 g^{-1} N							
Met	2.52	2.32	2.06	1.94	1.76	1.51	1.36
(Met + Cys)	3.25	3.12	2.88	2.72	2.65	2.37	2.23
Lys	5.76	5.72	5.67	5.62	5.65	6.40	5.38
Phe + Tyr	8.23	8.31	8.26	8.31	8.40	7.59	8.30
Thr	4.19	4.09	4.05	4.02	4.26	4.09	3.92
Ile	3.90	3.87	3.92	4.00	4.01	4.06	4.12
His	2.92	2.83	2.75	2.72	2.68	2.74	2.51
Val	4.42	4.30	4.35	4.41	4.38	4.37	4.39
Leu	9.45	9.47	9.36	9.39	9.61	7.28	9.29
Arg	5.07	5.11	5.25	5.34	5.54	6.06	5.52

* Vitamin/mineral mixture (values are in g 100 g^{-1} diet): choline chloride, 0.20; ascorbic acid, 0.1; mineral premix**, 0.2; vitamin premix***, 0.2; K_2HPO_4, 0.3 and $Ca(H_2PO_4)_2$, 1.0.
** Mineral premix (values are in g 100 g^{-1} mineral premix): $MgSO_4 \cdot 7H_20$, 13.3; $AlCl_3 \cdot 6H_2O$, 0.7; $ZnSO_4 \cdot 7H_2O$, 2.0; KI, 0.005; $FeSO_4 \cdot 7H_2O$, 3.5; $CuSO_4 \cdot 5H_2O$, 1.0 and NaCl, 5.0.
*** Vitamin premix (values are in g kg^{-1} vitamin premix): retinoic acid, 6.0 (M IU); vitamin D_3, 1.0 (M IU); all-rac-α-tocopherol, 50.0; vitamin K_3, 2.5; thiamin, 10.0; riboflavin, 15.0; pyridoxine, 7.5; cyanocobalamin, 0.025; pantothenic acid, 27.5; nicotinamide, 60.0; biotin, 0.5; folic acid, 2.5 and inositol, 100.0; supplied by Hoffmann La Roche, Taiwan.
[a] Fish meal protein/soybean meal protein.
[b] Constant ingredients (g/kg dry diet): wheat gluten, 71.4; corn gluten, 163.7; squid oil, 10; vitamin/mineral mixture*, 20; α-starch 9.3 and carboxymethylcellulose, 30.

(AOAC, 1995). Two to five fish from each treatment were also randomly selected and a portion of the lateral muscle from each fish was sampled. Muscle free amino acid profiles were quantified (Cross et al., 1975) using an amino acid analyzer (Beckman 6300 System).

Weight gain (g/fish), feed conversion ratio (FCR), protein efficiency ratio (PER), net protein utilization (NPU) and body composition data (muscle proximate composition and free amino acid concentration) were analyzed (Statistical Analysis System, SAS Institute, Cary, NC) for statistical significance ($P < 0.05$) by ANOVA (Snedecor and Conchran, 1978). Individual differences between dietary treatments were determined by Duncan's new multiple range test. A quadratic regression analysis (Snedecor and Conchran, 1978)

Table 2
Weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) of the cobia fed experimental diets in which fish meal protein (FM) was partially replaced by soybean meal protein (SBM)[a]

Diet (FM/SM)	Weight gain (g/fish)	FCR[b] (g dry feed/g gain)	PER[c] (g gain/g protein)	NPU[d] (g body protein/g protein)
100/0	107.7 ± 1.2^{ab}	1.28 ± 0.01^{c}	1.81 ± 0.02^{ab}	0.35 ± 0.01^{ab}
90/10	107.4 ± 3.2^{ab}	1.29 ± 0.04^{c}	1.79 ± 0.05^{ab}	0.35 ± 0.02^{ab}
80/20	114.5 ± 0.7^{a}	1.19 ± 0.01^{c}	1.93 ± 0.01^{a}	0.38 ± 0.00^{a}
70/30	104.3 ± 3.5^{b}	1.29 ± 0.04^{c}	1.73 ± 0.06^{bc}	0.33 ± 0.02^{bc}
60/40	107.2 ± 3.5^{ab}	1.28 ± 0.04^{c}	1.78 ± 0.06^{ab}	0.34 ± 0.02^{b}
50/50	90.2 ± 3.4^{c}	1.48 ± 0.06^{b}	1.61 ± 0.06^{c}	0.31 ± 0.02^{c}
40/60	81.8 ± 2.2^{c}	1.65 ± 0.04^{a}	1.35 ± 0.03^{d}	0.25 ± 0.01^{d}

[a] Initial average body weight was approximately 32.3 g. Values are means ± S.E. Means within a given column with different superscript letters are significantly different ($P<0.05$). No fish died during the 8-week trial.

[b] FCR = feed supplied (g, DW)/body weight gain (g).

[c] PER = body weight gain (g)/protein fed (g).

[d] NPU = body protein gain (g)/protein fed (g).

was conducted to analyze the weight gain of the cobia in response to soybean replacement.

3. Results

All fish readily accepted the experimental diets and no fish died during the 8-week feeding trial. Weight gains, protein efficiency ratio (PER) and net protein utilization (NPU) were significantly reduced while feed conversion ratio (FCR) significantly increased as the proportion of soybean meal in the diets increased (Table 2). A significant difference between Diets 100/0–60/40 and Diets 50/50 and 40/60 was observed in the growth performance parameters, indicating that up to 40% of fish meal protein could be replaced by soybean meal protein without causing reduction in growth and protein utilization. At this replacement level, the diet contained 26.9% fish meal and

Table 3
Muscle proximate composition (dry weight basis) of the cobia fed experimental diets in which fish meal (FM) protein was partially replaced by soybean meal protein (SBM)[a]

Diet (FM/SBM)	Protein (g 100 g^{-1})	Lipid (g 100 g^{-1})	Ash (g 100 g^{-1})
100/0	85.4 ± 0.9^{ab}	2.37 ± 0.31^{c}	8.5 ± 0.1^{ab}
90/10	85.6 ± 1.5^{a}	2.53 ± 0.52^{c}	8.6 ± 0.1^{a}
80/20	84.8 ± 1.0^{ab}	1.81 ± 0.57^{c}	8.4 ± 0.1^{abc}
70/30	81.9 ± 1.2^{b}	4.94 ± 1.21^{ab}	7.9 ± 0.2^{cd}
60/40	82.4 ± 0.4^{ab}	3.47 ± 0.47^{bc}	8.3 ± 0.1^{abc}
50/50	82.6 ± 0.9^{ab}	5.16 ± 0.49^{ab}	7.7 ± 0.3^{d}
40/60	82.3 ± 0.2^{ab}	5.90 ± 0.10^{a}	8.1 ± 0.1^{bcd}

[a] Means (± S.E., $n=3$) within the same column with different superscript letters are significantly different ($P<0.05$).

Table 4
Mean concentrations (nmol g^{-1}) of free amino acids in dorsal muscle of the cobia fed experimental diets in which fish meal protein (FM) was partially replaced by soybean meal protein (SBM)

Amino acid	Diet (FM/SBM)[a]						
	100/0	90/10	80/20	70/30	60/40	50/50	40/60
n	5	3	3	3	3	3	2
Met	0.39	0.27	0.37	0.38	0.34	0.33	0.38
Lys	1.44	1.48	1.09	1.09	1.06	1.87	1.36
Trp	0.13	0.11	0.11	0.11	0.14	0.14	0.13
Phe	0.42	0.37	0.37	0.35	0.42	0.42	0.43
Thr	3.04^{ab}	3.25^{a}	2.97^{ab}	2.28^{dc}	2.83^{abc}	2.53^{bcd}	1.92^{d}
Ile	0.49	0.43	0.38	0.39	0.45	0.48	0.49
His	2.39^{a}	2.17^{a}	2.11^{a}	2.40^{a}	1.56^{ab}	1.11^{b}	1.12^{b}
Val	1.09	1.00	0.93	0.94	1.15	1.20	1.04
Leu	1.19	1.09	1.02	1.04	1.22	1.27	1.19
Arg	0.34	0.32	0.26	0.63	0.40	0.56	0.45

[a] Means within the same row with different superscript letters are significantly different ($P<0.05$).

28.6% soybean meal. As the highest weight gain was observed in the group fed Diet 80/20 (Table 1), analysis by quadratic regression shows a growth optimum at 16.9% replacement of fish meal protein by soybean meal protein.

Partial replacement of dietary fish meal by soybean meal also significantly affected the muscle proximate composition of the cobia (Table 3). On a dry weight basis, lipid concentration in the muscle increased as dietary soybean meal level increased, while muscle ash level varied without a definite trend. Although muscle protein concentration showed a decreasing trend with increasing dietary soybean meal level, the trend was not definite. Except for threonine and histidine, concentration of all free essential amino acids in the cobia muscle was not affected by the dietary treatments (Table 4). There was a significant ($P<0.05$) decrease for threonine and histidine concentrations in the free amino acid pool as the replacement level increased.

4. Discussion

Different fishes respond differently to dietary soybean supplementation (Refstie et al., 2000). Omnivorous freshwater fishes such as carp when fed diets with defatted soybean meal as the sole protein source grew as well as when fed fish meal diets if the limiting essential amino acids were supplemented (Viola et al., 1982). Tilapia, which is also omnivorous, showed reduced growth as well as feed and protein efficiencies when fish meal in the diets was totally replaced by defatted soybean meal (Shiau et al., 1989). However, partial replacement, up to two thirds, of fish meal protein with soybean meal protein did not compromise growth (Shiau et al., 1990). Carnivorous blue catfish grew well on diets with 70% of crude protein from toasted soybean meal and 30% from fish meal (Webster et al., 1992), indicating a great tolerance to soybean diet. Channel catfish also grow well on diets high in soybean (Mohsen and Lovell, 1990). In marine carnivorous fish, yellowtail showed reduced growth and feed efficiency when of fish meal protein replacement by soybean meal protein was greater than 20% (Shimeno et al., 1993). Juvenile red drum and Japanese flounder were shown to tolerate replacement of the protein from fish meal with defatted soybean meal up to 50% (Reigh and Ellis, 1992) and 45% (Kikuchi, 1999), respectively. Two salmonids, rainbow trout and Atlantic salmon, appear to be as sensitive to soy replacement as yellowtail. Soy replacement as little as 25% may reduce growth, feed efficiency and protein retention in rainbow trout (Kaushik et al., 1995). Unlike yellowtail, cobia appear to have better tolerance for dietary defatted soybean meal. Up to 40% of fish meal protein can be replaced by defatted soybean meal protein in cobia diets without compromising fish growth and feed efficiency. At the highest replacement level (60%), weight gain of the cobia was 20% less than the all fish meal group, from 110 to 82 g/fish (Table 2). No apparent abnormality in morphology and behavior was observed throughout the growth trial.

In addition to high levels of protein, soybeans contain approximately 30% carbohydrate, of which oligosaccharides make up 10% and non-starch polysaccharides (NSP) make up the remaining 20% (Storebakken et al., 2000). These soy carbohydrates are not digestible by monogastric animals and some components show antinutritional effects in fish. As the soy replacement level increased, the concentration of soy carbohydrates in the test diets increased and ash content decreased (Table 1). Since the brown fish meal contains approximately 18% ash, the successive replacement of ash from the fish meal with carbohydrates from the soybean meal would render digestibility of non-protein components more favorable to the high fish meal groups, even if the antinutritional effects of soybeans are not considered. It is also possible that reduction in digestible energy for the diets containing soybean meal also played a role in limiting fish growth. In the test diets, fish oil from fish meal and supplemental fish oil constituted the bulk of dietary lipids for the cobia. A small amount ($< 1\%$) of soybean oil was added just to compensate for the low level of residual oil in the defatted soybean meal. Although a high content of 18:2n-6 in soy oil may cause metabolic competition with n-3 fatty acids (Storebakken et al., 2000), which are required in relatively high levels by marine finfish, this should not be the case in the present study.

Soybeans contain several antinutritional factors that are known to affect fish growth and health (Storebakken et al., 2000; Hendricks, 2002). Protease inhibitors inhibit trypsin and basic proteases in fish with considerable species variation. Most of the inhibitor activity is reduced in defatted soybean meal because of steaming in toasters during the oil extracting processes. It is further lost during feed pelleting in which high pressure and moisture are applied. In the present study, the feed pellets were cold extruded. Reduction of the inhibitors during the extrusion was unlikely. The test fish did not seem to show adverse response to the experimental diets. Another soy component phytate binds phosphorus and divalent or trivalent ions and reduces their availability to fish. In the present study, as a common practice, inorganic phosphorus was supplemented in the mineral premix to ensure its sufficiency. Other antinutrients such as lectins and saponins also exist in soybeans. In Atlantic salmon, pathological changes in the mucosa of the distal intestine have been found when using full-fat or defatted soy meals in the diet (Baeverfjord and Krogdahl, 1996). An antigenic soy peptide was hypothesized to cause an allergic reaction and subsequent abnormality including enteritis.

Storebakken et al. (2000) suggested that studies with soybean meals in the diets of a new fish species in aquaculture should include investigations on the intestinal morphology of the fish.

As soybean replacement level increased, the supply of some essential nutrients was compromised. Methionine, which showed a continuous decline from 2.52 to 1.36 g 16 g^{-1} N as soybean meal level increased from 0% to 60%, would become the first limiting amino acid to affect growth of the cobia. In contrast to methionine, all other essential amino acids were maintained at fairly constant levels among the test diets (Table 1). As the test diets were all isoenergetic and isonitrogenous, a broken line model analysis (Robbins, 1986) thus was performed to examine the response of fish weight gain to dietary sulfur amino acid (methionine and cystine) concentration. The result showed a breakpoint when dietary methionine + cystine concentration was 2.66 g 16 g^{-1} N, suggesting that juvenile cobia require dietary methionine + cystine at a level of 2.66% of dietary protein or 1.28 g of diet to achieve optimum growth. The requirement was obtained assuming methionine was equally available between the fish meal and the soybean meal. Although the present study was not designed to quantify essential amino acid requirements of the cobia, the obtained value could offer a preliminary estimate of the methionine requirement before a legitimate study could be conducted. At this requirement level, the dietary need of the juvenile cobia for methionine is comparable to or in the low range of the requirements by other marine carnivorous finfish (Wilson, 2002).

Acknowledgements

The research was partly supported by grants 90AS-1.1.4-FA-F1(1), 91AS-1.1.5-AI-A1(02) and 91AS-1.1.5-FA-F1 from the Council of Agriculture, Taiwan, Republic of China. We thank C.Y. Tsai of the Joint Center for High Valued Instruments, National Taiwan University for assistance in amino acid analyses.

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