Marine Finfish Aquaculture Network

Effects of the partial substitution fish oil by soybean oil in the diets on muscle fatty acid composition of juvenile cobia *(Rachycentron canadum)*

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The cobia *(Rachycentron canadum)* is a carnivorous fish. It can grow with good feed conversion efficiency in offshore net cage systems from fingerling to marketable size (4 – 6 kg) in 1 year. Cobia are provided with high-energy feeds. At present, commercial feeds for cobia contain lipid levels around 15 – 24%[1,2].

Aquaculture feeds depend heavily on animal ingredients[3] and fish oil is the main lipid source used in such feeds, especially in those formulated for carnivorous species. The demand for marine fish oil (MFO) in aquafeeds is continually increasing and may exceed 75% of the global supply by the year 2010[4]. In order to maintain the rapid growth of the global aquaculture industry, it has been becoming increasingly crucial for the aquaculture feed industry to evaluate alternatives to fish oils for coming decades[5].

Soybean oil is the world's largest source of vegetable oil and contains higher levels of poly-unsaturated fatty acids than others, such as rapeseed oil or palm oil, but lacks eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with linoleic acid (18: 2n-6) dominating at approximately 51–64% (NRC, 1993). A number of studies has shown that soybean oil can partially replace fish oils[6,7], without reducing growth and feed efficiency. However soybean oil does not cover the essential fatty acid requirements of many marine fishes. It changes the final balance of dietary fatty acids in the feed and changes the fatty acid composition of fillet[8], thereby reducing flesh quality[7].

The objective of the this study was to determine the effects of substituting soybean oil for fish oil in diets on muscle fatty acid composition of juvenile cobia.

Materials and methods

Six isonitrogenous experimental diets (45 % crude protein; 20 % lipid) were formulated to produce diets in which 0% (D0), 20% (D20), 40% (D40), 60% (D60), 80% (D80) and 100% (D100) of fish oil was replaced by soybean oil. All the dry ingredients were mixed until homogenous in a mixer, and then water and lipid were added and mixed. 2.0 mm and 3.0 mm diameter pellets were wet- extruded for cobia different growth stage, air-dried to about 100 g kg^{-1} moisture and sealed in vacuum–packed bags and frozen stored (-20°C) until feeding. Samples of all diets were subjected to proximate composition and fatty acids analysis; the results are presented in Tables 1 and 2.

Table 1: Experiment diets composition (g kg^{-1} diet)

Ingredients	Diets					
	D0	D20	D40	D60	D80	D100
Fish meal[a]	450	450	450	450	450	450
Shrimp head meal	260	260	260	260	260	260
Wheat meal	30	30	30	30	30	30
Gluten	120	120	120	120	120	120
Fish oil[b]	100	80	60	40	20	0
Soybean oil[c]	0	20	40	60	80	100
Vitamin C	10	10	10	10	10	10
Vitamin Premix	20	20	20	20	20	20
Mineral	10	10	10	10	10	10
Proximate composition						
Crude protein	45.89	45.06	44.81	45.70	44.55	42.75
Lipid	20.28	20.12	20.25	20.14	20.67	19.95
Ash	6.16	4.75	5.01	5.34	5.16	4.94
Moisture	9.54	9.72	10.81	10.65	11.92	11.40

a: Vietnam fish meal: Crude protein = 600 g kg^{-1}, b: Vietnam fish oil; c: Soybean oil from Tuong An company (Vietnam).

Table 2: Main fatty acid composition of diets (% total fatty acids)

Fatty acid	Diets					
	D0	D20	D40	D60	D80	D100
18:2n-6	0.66	1.60	2.29	3.40	3.39	6.29
18:3n-3	0.14	0.06	0.06	0.07	0.07	0.11
20:4n-3	0.05	0.05	0.00	0.05	0.04	0.05
20:5n-3	0.56	0.49	0.36	0.35	0.32	0.24
22:6n-3	1.85	1.64	1.21	1.19	1.10	0.86
∑SFA	8.72	8.30	7.66	8.11	7.72	8.70
∑MUFA	1.79	1.52	1.31	1.39	1.28	1.44
∑n-3	2.89	2.50	1.81	1.84	1.69	1.40
∑n-6	0.71	1.64	2.29	3.44	3.43	6.33
n-3/n-6	4.07	1.52	0.79	0.53	0.49	0.22
∑HUFA	2.47	2.19	1.57	1.59	1.46	1.16
EPA/DHA	0.30	0.30	0.30	0.30	0.29	0.28

Fish rearing

Juvenile cobia *(Rachycentron canadum)* was obtained from a farm in Nha Trang (Khanh Hoa, Vietnam). Fish were acclimated and fed with a commercial diet (45 % crude protein, 20 % lipid) for 2 weeks before starting of the trial, and then fish (initial mean weight 12.69 g) were randomly distributed to each of 18 tanks with 15 fish per tank. Fish were fed to satiation in 30 min, twice daily at 08:00 h and 16:00 h. The feeding trial lasted for 8 weeks. Temperature and salinity in tanks were monitored daily, while pH and ammonia and oxygen were monitored once per three days. Animals were kept under natural photoperiod conditions. During the experimental period, temperature was 28 - 32°C, salinity was 26 – 30 ‰, pH: 7.8 - 8.5, ammonia was lower than 1 mg L^{-1} and dissolved oxygen was not less than 4.5 mg L^{-1}.

Sample collection and analysis methods

At the end of the 8-week feeding trial, fish in each tank were individually weighed and sampled for muscle analysis 24 hours after the last feeding. Three fish from each tank were randomly sampled and frozen at -30°C for muscle fatty acid analysis.

Fatty acid composition was determined in diets and muscle of fish. Lipid extraction was according to Folch et al., 1957 and fatty acids were transformed to methyl esters. Fatty acids were separated by gas chromatography (GC 6890A, Agilent, USA) using equipped with a FID detector, and using a HP-FFAP (0.25 mm x 25 m) capillary column with nitrogen as the carrier gas. The injector and detector temperatures were both kept at 250°C. The column temperature was programmed initially at 120°C for 1 min, 12°C/min to 150°C, 10°C/min to 180°C, 0.5°C/min to 184°C, 4°C/min to 190°C and to a final temperature of 210°C. Fatty acid methyl esters were identified by comparison to external standards (SIGMA).

Results were expressed as mean ± standard of deviation (SD) and group mean difference were compared using one – way ANOVA. When there were differences, the group means were further compared with Duncan's multiple range test. All computations were performed with SPSS 12.0. A significant level of $P < 0.05$ was employed at all cases.

Results

All feeds were readily accepted, fish survival rates over 93 % were recorded in all treatments. The muscle fatty acid composition clearly reflected that of the dietary lipids. Table 3 shows the muscle fatty acid profiles. There were no significant differences on SFA in muscle of cobia. Levels of n-3 HUFA in muscle were reduced significantly from 3.41 % to 1.73 % and levels of MUFA increased significantly from 3.77 % to 10.19 % while replacement of fish oil by soybean oil increased from 0 % to 100 %. The EPA/DHA was not significantly affected by replacing levels.

Discussion

Inclusion of vegetable oils in fish diet modifies the body fatty acid profiles, and this effect that is more evident in marine fish species because of their limited ability to convert 18C fatty acid to longer polyunsaturated fatty acid[9]. In this present study, there was a notable increase in muscle levels of both 18:2n-6 and 18:3n-3 as the soybean oil in the diets was increased. Similar results have been reported for other species such as seabream *Sparus aurata*[7,10] sharpsnout seabream *Diplodus puntazzo*[11]. These authors also reported a strong dependence of muscle fatty acid composition on the experiment diet that fish has received. In addition, replacement of dietary fish oil by soybean oil had resulted in lower levels of n-3 PUFA especially EPA and DHA in fish muscle. Fish oil replacement by soybean oil in diet reduced n-3 PUFA fatty acids and increased the n-6 PUFA fatty acids in cobia muscle. Content of essential fatty acids such as EPA, DHA in diets reduced the as the soybean oil in the diets was increased and influential right up to the concentration of EPA, DHA in the muscle of the fish. This suggests that these are fatty acids necessary for cobia, a similar composition to that obtained on several marine fish species such as seabream[7,8,10], humpback grouper *Cromileptes* altivelis[13]. n-3/n-6 ratio in the muscle of the fish is strongly affected by n-3/n-6 ratio in feeds. According to Sargent *et al*.[14], n-3 and n-6 series fatty acids play a role as substrates for some enzymes related to lipid metabolism in the body of fish, so the balance of the n-3/n-6 is very important for the growth of fish. In present experiments, the n-3/n-6 ratio in the fish muscle decreased rapidly from 11.43 to 1.80, while this ratio in the feed decreased from 4.07 to 0.22. This may be the cause of changes in the biochemical composition of the fish muscle. Saturated fatty acids are a major muscle component, which may be the reason why they increased in fish muscle. These

Table 3: Fatty acid composition of muscle from cobia fed different diets (% total fatty acids)

Fatty acid	Diets					
	D0	D20	D40	D60	D80	D100
14:0	0.67^{c}	0.57^{b}	0.55^{b}	0.52^{b}	0.43^{a}	0.37^{a}
16:0	4.59	4.31	4.44	4.69	4.41	4.51
18:0	6.62^{a}	6.63^{a}	7.03^{ab}	7.71^{bc}	7.69^{bc}	8.38^{c}
20:0	0.57^{b}	0.52^{ab}	0.47^{a}	0.54^{ab}	0.48^{ab}	0.45^{a}
22:0	0.17^{c}	0.17^{bc}	0.14^{ab}	0.16^{bc}	0.13^{a}	0.13^{a}
24:0	0.26^{e}	0.23^{d}	0.22^{cd}	0.20^{c}	0.16^{b}	0.13^{a}
14:1n-5	0.18^{c}	0.16^{b}	0.15^{b}	0.15^{b}	0.13^{a}	0.12^{a}
16:1n-7	1.68^{c}	1.46^{b}	1.41^{b}	1.34^{b}	1.14^{a}	1.01^{a}
18:1n-9	1.55^{a}	2.92^{b}	3.98^{b}	5.65^{c}	6.90^{d}	8.90^{e}
24:1n-9	0.37^{e}	0.32^{d}	0.30^{cd}	0.26^{c}	0.21^{b}	0.16^{a}
18:2n-6	0.27^{a}	0.42^{b}	0.55^{b}	0.74^{c}	0.86^{c}	1.08^{d}
18:3n-3	0.08^{a}	0.09^{ab}	0.11^{bc}	0.12^{c}	0.13^{c}	0.15^{d}
20:2n-6	0.07	0.07	0.07	0.08	0.08	0.08
20:3n-3	0.33^{bc}	0.30^{abc}	0.37^{c}	0.28^{abc}	0.24^{ab}	0.20^{a}
20:4n-3	0.08	0.07	0.07	0.06	0.06	0.05
20:5n-3	0.75^{d}	0.64^{c}	0.59^{c}	0.57^{c}	0.46^{b}	0.38^{a}
22:6n-3	2.58^{e}	2.25^{d}	2.11^{cd}	1.93^{c}	1.61^{b}	1.30^{a}
SFA	12.89	12.43	12.85	13.81	13.30	13.95
MUFA	3.77^{a}	4.86^{ab}	5.84^{bc}	7.41^{cd}	8.32^{de}	10.19^{e}
n-3	3.77^{d}	3.35^{c}	3.24^{c}	2.97^{c}	2.50^{b}	2.08^{a}
n-6	0.34^{a}	0.50^{b}	0.61^{b}	0.82^{c}	0.94^{c}	1.17^{d}
HUFA	3.41^{e}	2.96^{d}	2.76^{cd}	2.56^{c}	2.13^{b}	1.73^{a}
n-3/n-6	11.43^{e}	6.95^{d}	5.28^{c}	3.78^{b}	2.72^{ab}	1.80^{a}
EPA/DHA	0.29	0.29	0.28	0.30	0.29	0.29

Data in the same row with different superscripts differ at P < 0.05

fatty acids is well presented in both the diets and the muscle, it would appear that the muscle can make good use of it, mainly as an energy source.

This study shows that soybean oil replacement modified the fish muscle fatty acid profile, reducing the levels of EPA, DHA and increasing the levels of LA, LNA. It would be interesting to analyse the effects of replacement fish oil by soybean oil in cobia diets for longer period of time.

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