Available online at www.sciencedirect.com

SCIENCE DIRECT®

Aquaculture 249 (2005) 439–447

Aquaculture

www.elsevier.com/locate/aqua-online

Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (*Rachycentron canadum*)

Ji-Teng Wang[a], Yong-Jian Liu[a,*], Li-Xia Tian[a], Kang-Sen Mai[b], Zhen-Yu Du[a], Yong Wang[a], Hui-Jun Yang[a]

[a]*Institute of Aquatic Economic Animals, School of Life Science, Sun Yat-sen University, 135 Xinggang West Road, Guangzhou 510275, PR China*

[b]*College of Fisheries, Ocean University of China, Qingdao 266003, PR China*

Received 24 February 2005; received in revised form 14 April 2005; accepted 18 April 2005

Abstract

A study was undertaken to evaluate the effect of the dietary lipid level on growth, feed utilization, lipid deposition and lipid metabolism by cobia juveniles. Three isonitrogenous diets containing 47% crude protein with increasing dietary lipid levels 5%, 15% and 25% (DM, dry matter) were fed to satiety to triplicate groups of 20 fish (7.71 g) for 6 weeks. At the end of the feeding trial, fish fed diets containing 5% and 15% lipid showed a higher growth than those fish fed with 25% lipid. Though daily feed intake (DFI) decreased with increasing dietary lipid, there was no significant difference in daily energy intake (DEI) among treatments. As dietary lipid level increased, energy retention (EI), daily energy gain (DEG), daily lipid intake (DLI), daily lipid gain (DLG), viscerosomatic index (VSI), intraperitoneal fat ratio (IPF) and body lipid content increased dramatically and the 25% group had the highest values. Hepatosomatic index (HSI) and muscle lipid content were higher at 25% lipid group than 5% lipid group, but no significant different was found between 15% and 25% lipid group. Activities of G6PD and ME were reduced with increasing lipid intake, but activities of IDH and 6PGDH did not change among groups. In conclusion, high dietary lipid levels above 15% produced little practical benefit because of higher fat accretion in cobia.

Keywords: Cobia; Lipid level; Growth; Body composition; Feed utilization

1. Introduction

Cobia are large, migratory, coastal pelagic fish of the monotypic family Rachycentridae and are widely distributed in tropical, subtropical and warm temperate seas with the exception of the eastern Pacific

* Corresponding author. Tel.: +86 020 84110789; fax: +86 020 84110789.

E-mail address: Ls59@zsu.edu.cn (Y.-J. Liu).

0044-8486/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2005.04.038

(Briggs, 1960; Shaffer and Nakamura, 1989). The cobia is an important and popular recreational species in many parts of the world. The mostly white meat of the fish is served in restaurants as steak or as raw fish, sashimi (Chou et al., 2001) because of its excellent quality and taste with few bones. The cobia is regarded as having the greatest potential among all candidate species for offshore cage culture in China. With the recent success of artificial propagation and larval production, culture of cobia is fast gaining economic importance in Southeast Asia and China because of their fast growth, efficient feed conversion and high market value. The cobia can attain 4–6 kg in 1 year under the present net culture conditions. Despite the commercial success of cage culture of cobia, little information is known about their nutritional requirements.

Lipid is an important component of the diet, both as the source of energy and essential fatty acids (Watanabe, 1982). It can also spare dietary protein from use as energy and limit ammonia production (Bromley, 1980; Vergara et al., 1999). On the other hand, excessive energy in diets can lead to decrease feed consumption (reducing total protein intake) and reduce the utilization of other nutrients resulting to reduced growth (Page and Andrews, 1973; Watanabe, 1982; Daniels and Robinson, 1986; Ellis and Reigh, 1991). Moreover, high dietary lipid can also affect carcass composition due to an increase in lipid deposition (Bromley, 1980; Hillestad and Johnsen, 1994). Therefore, the increase of dietary lipid levels should be carefully evaluated and determined.

It is desirable to increase the muscular lipid concentration through feeding high-fat diets because cultured cobia is consumed mainly as sashimi (Chou et al., 2001). In practice, commercial cobia feeds usually include about of 15–16% crude lipid (Liao et al., 2004). However, optimal lipid level for this species has been estimated to be around 5.87% of diet (Chou et al., 2001). In Atlantic salmon, current diets containing upwards of 30% lipid have been shown to significantly improve feed and protein utilization efficiencies and to reduce nitrogen excretion (Hardy, 1999). Therefore, the objective of this study was to evaluate the effect of increasing dietary lipid level from 5% to 25% on growth performance, feed utilization, lipid deposition, lipid metabolism by cobia juveniles at 28.5 °C.

2. Material and methods

2.1. Experimental diets

Prior to use, all feed ingredients were analyzed for proximate composition and the data obtained were used as a basis for making the required formulation. Three isonitrogenous diets with 47% crude protein (CP) were formulated to provide three dietary fat levels at 5.0%, 15.0% and 25.0% (dry basis). Ingredient and proximate composition of the experimental diets are presented in Table 1. White fish meal and casein were used as protein sources. Fish oil and corn oil were used as lipid sources. Corn starch and α-starch were used as carbohydrate sources. A commercial fish mineral/vitamin pre-mix was added to the diets at a level of 2.4%. The diets were bound using 3% CMC.

All dry ingredients including CMC were thoroughly mixed using a dough mixer, after which, oil and sufficient distilled water were added to form a soft dough (approximately 60% dry matter, DM). The experimental diets were produced through a 3-mm die using a mono-screw extruder. The diets were

Table 1
Formulation and composition of experimental diets

	L5	L15	L25
Ingredient (g/100 g)			
White fish meal[a]	45	45	45
Casein	16.80	16.80	16.80
α-starch	10	10	10
Fish oil[a]	0.36	7.03	13.70
Corn oil	1.67	5.00	8.33
Corn starch	20.77	10.77	0.77
Vitamin mix[b]	1.5	1.5	1.5
Mineral mix[c]	0.9	0.9	0.9
CMC	3	3	3
Proximate composition (g/100 g dry matter)			
Moisture	15.91	16.35	12.71
Crude protein	47.91	47.17	47.12
Crude lipid	5.05	16.97	26.12
Ash	9.33	9.15	9.48
Gross energy (kJ/g)	16.61	18.46	21.02

[a] Imported from New Zealand, supplied by Inc. Chenyi, China.
[b] According to Chou et al. (2001), supplied by Inc. Chenyi, China.
[c] Mineral premix consisted of (g/kg premix): $MnSO_4 \cdot 7H_2O$, 399; $Ca(H_2PO_4)_2$, 3; $AlCl_3 \cdot 6H_2O$, 21; $ZnSO_4 \cdot 7H_2O$, 60; KI, 0.15; K_2HPO_4, 0.6; $FeSO_4 \cdot 7H_2O$, 105; $CuSO_4 \cdot 5H_2O$, 30; NaCl, 150 and cellulose, 231.25; supplied by Chenyi, China.

dried at air temperature about 28 °C overnight (to about 85% DM) and stored at −20 °C until used. Samples of each diet were taken for chemical analyses.

2.2. Experimental fish

Juvenile cobia (*Rachycentron canadum*) were obtained from a commercial fish farm (Longsheng, Zhanjiang, China) and maintained at the nutrition laboratory of Marine Beeding Seed Base Of State Project (863) In The South, Zhanjiang, China. Before initiation of the feeding trials, they were acclimated to laboratory conditions for a 14-day period by feeding a commercial diet, fish were selected according to the health condition and body weight and individually weighed. Sample fish were not fed for 24 h prior to the start of growth study. They were then randomly allocated to 9 1000-l cylindrical fiberglass tanks at a stocking density of 20 fish (7.71 g/fish) per tank. Three replicate groups of fish were used for testing each diet. The feeding experiment lasted for 6 weeks. During the 42-day feeding period, fish were hand-fed with experimental diets to apparent satiation twice a day (08:30 and 17:30 hours). Pellets were distributed slowly, allowing all fish to eat. All fish were bulk-weighed and counted at the start of the experiment and then again at 3 weeks and at the conclusion of 6 weeks feeding study.

2.3. Rearing system

A flow-through system was used, and each tank was supplied with a constant flow of sand-filtered seawater (15 l min^{-1}). Water quality parameters were monitored during the experimental period. Water temperature was maintained at 28.5 ± 1.2 °C, salinity was held at 30‰, dissolved oxygen levels were kept greater than 7 mg l^{-1}. Fish were subjected to a natural photoperiod regime and all tanks had similar light conditions. Each tank was fitted with mesh screens matched to the size of animals as they grew. Two airstones in each tank provided aeration. The tanks were cleaned fortnightly.

2.4. Sample collection and chemical analysis

Eighteen fish from an initial pool of fish were sampled and kept frozen for chemical analyses of whole body composition. At the end of the growth trial, five fish from each tank were used for whole body determination of proximate composition and gross energy. Before sampling, fish were starved for 48 h. After being stunned, eight fish per tank were individually weighed and the digestive glands and muscle (dorsal fillets without skin) were rapidly removed. The viscera, liver and intraperitoneal fat were weighed for calculation of hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitoneal fat ratio (IPF). Six livers were collected for analyses of enzyme activities (glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase and malic enzyme). Samples were frozen in liquid nitrogen and stored at −70 °C for subsequent analysis.

All chemical composition analyses of diets, whole body and tissues were conducted according to the methods specified by the AOAC (1984). Crude protein (N × 6.25) was determined by the Kjeldahl method using an Auto Kjeldahl System (Buchi B-324/K-437, Switzerland). Moisture was determined by drying to a constant weight in an oven at 105 °C. Crude fat was extracted using ether after acid hydrolysis. Ash was determined by a muffle furnace at 550 °C for 7 h. Gross energy contents were analyzed using an adiabatic bomb calorimeter (model WHR-15, Changsha, China, calibrated with benzoic acid). Tissue lipid was extracted using chloroform: methanol (2:1 v/v), according to the method of Folch et al. (1957).

2.5. Enzyme assay

Preparation of the liver samples was the same as in a previous study (Hung et al., 1989). Soluble protein content of liver homogenates was determined by the Lowry method using a Sigma kit (kit no. P 5656). The activities of lipogenic enzymes were assayed on supernatant fractions: glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) according to Glock and McLean (1953) as modified by Kawaga et al. (1964), malic enzyme (ME; EC 1.1.1.40) according to Wise and Ball (1964), and NADP-dependent isocitrate dehydrogenase (ICDH, EC 1.1.1.42) according to Bernt and Bergmeyer (1974). The activities of lipogenic enzymes were determined by measuring the production of NADPH by reading the change in

absorbance at 340 nm per unit time in the assayed solutions. One unit of enzyme activity (IU), defined as the amount of enzyme that produces 1 μmol of NADPH per min at an assay temperature of 24.0 °C, is expressed as units mg^{-1} of hepatic soluble protein and tissue activity as units g^{-1} of liver tissue (wet weight).

2.6. Statistical analysis

All data are presented as mean ± SD of three replicate groups, unless otherwise specified. Data were analyzed by one-way analysis of variance (ANOVA) to test the effects of experimental diets. Where significant differences were found ($P<0.05$), a Duncan New Multiple Range Test was used to rank the groups. All statistical analyses were performed using the SPSS 11.0 for Windows.

3. Results

3.1. Growth performance and biometric parameters

No fish died during the growth trial. Dietary lipid level significantly ($P<0.05$) effected weight gain and feed efficiency of the cobia (Table 2). The SGR varied between 6.38 ± 0.09% and 6.65 ± 0.11% day^{-1}. The final body weight (FBW), weight gain (WG), daily feed intake (DFI) and specific growth rate (SGR) were significantly lower in the 25% group than in the 5% and 15% groups ($P<0.05$), while there was no significant difference in fish fed diets containing 5% and 15% lipid. The high dietary lipid group (25%) had the best feed conversion ratio (FCR) and protein retention efficiency (PRE). The results tended to show that feed efficiency was generally improved by increasing dietary lipid. Body condition indices were also influenced by dietary lipid level (Table 2). Both VSI and IPF were elevated significantly by increased dietary lipid levels ($P<0.05$). The HSI values of fish fed 15% and 25% lipid were significantly higher than those fed 5% lipid diet.

3.2. Whole body and tissue composition

Table 3 shows the effect of dietary lipid level on fish body and tissue composition. Increasing dietary lipid led to elevated tissue and whole body lipid levels. The lipid content (as % of wt.) and gross energy (GE, kJ g^{-1} wt.) content of whole body increased ($P<0.05$) in direct proportion to the lipid level in the diets. Whole body moisture concentrations were inversely related to lipid. Crude ash of whole

Table 2
Growth, feed utilization and biometric parameters of cobia fed with experimental diets at the end of growth trial

	L5	L15	L25
IBW(g/fish)	7.71 ± 0.16	7.71 ± 0.16	7.71 ± 0.16
FBW(g/fish)	126.05 ± 5.35[a]	125.87 ± 1.61[a]	112.40 ± 1.91[b]
DFI (g 100 g $fish^{-1}$ day^{-1})[a]	5.04 ± 0.20[a]	4.93 ± 0.29[a]	4.21 ± 0.14[c]
WG (% g/100g)[b]	1537.56 ± 75.64[a]	1525.16 ± 62.58[a]	1357.34 ± 54.16[b]
SGR (%/day)[c]	6.65 ± 0.11[a]	6.64 ± 0.09[a]	6.38 ± 0.09[b]
FCR (g/g)[d]	1.01 ± 0.04[a]	0.98 ± 0.05[a]	0.89 ± 0.03[b]
PER[e]	2.07 ± 0.07[b]	2.17 ± 0.11[b]	2.40 ± 0.03[a]
VSI (%)[f]	11.45 ± 0.53[c]	13.59 ± 0.22[b]	14.70 ± 0.57[a]
HIS (%)[g]	4.01 ± 0.08[b]	4.97 ± 0.05[a]	5.02 ± 0.32[a]
IPF (%)[h]	0.71 ± 0.05[c]	1.26 ± 0.05[b]	1.54 ± 0.02[a]

Values (mean ± standard deviations of three replications) in the same row with different superscripts are significantly different ($P<0.05$).
[a] Daily feed intake = 100 × feed offered (g)/average total weight (g)/days.
[b] Weight gain = 100 × (final body weight − initial body weight)/initial body weight.
[c] Specific growth ratio = 100 × ln(final weight/initial weight)/days of the experiment.
[d] Feed conversion ratio = g dry feed consumed/g wet weight gain.
[e] Protein efficiency ratio = fish wet weight gain/protein intake.
[f] Viscerosomatic index = 100 × (viscera weight/whole body weight).
[g] Hepatosomatic index = 100 × (hepatosomatic weight/whole body weight).
[h] Intraperitoneal fat ratio = 100 × (IPF weight/whole body weight).

Table 3
Body and tissue proximate compositions in juvenile cobia fed with diets containing different lipid levels (in % of wet weight basis)

	L5	L15	L25
Initial body fat	4.46 ± 0.18	4.46 ± 0.18	4.46 ± 0.18
Whole body			
Moisture	71.11 ± 0.39[a]	67.54 ± 0.84[b]	66.30 ± 0.10[c]
Crude protein	17.40 ± 0.14[a]	16.40 ± 0.40[a]	13.46 ± 1.00[b]
Crude lipid	9.23 ± 0.30[c]	13.93 ± 0.69[b]	20.31 ± 1.94[a]
Ash	2.74 ± 0.04	2.73 ± 0.08	2.77 ± 0.13
Energy (kJ g^{-1}	7.10 ± 0.18[c]	8.49 ± 0.20[b]	9.58 ± 0.04[a]
Dorsal muscle			
Moisture	75.42 ± 0.73[a]	72.51 ± 2.42[a,b]	71.13 ± 0.58[b]
Crude protein	19.69 ± 0.97	19.19 ± 0.71	18.86 ± 0.01
Crude lipid	3.20 ± 0.87[b]	6.58 ± 2.14[a]	8.74 ± 0.35[a]
Ash	1.37 ± 0.01	1.42 ± 0.15	1.35 ± 0.04
Liver			
Moisture	49.98 ± 2.24[a]	43.86 ± 0.94[b]	41.28 ± 2.59[b]
Crude protein	8.87 ± 0.44[a]	7.23 ± 0.32[b]	6.54 ± 0.16[c]
Crude lipid	42.41 ± 5.79[b]	57.95 ± 6.11[a]	51.79 ± 4.12[a,b]

Values (mean ± standard deviations of three replications) in the same row with different superscripts are significantly different ($P<0.05$).

body composition was similar in all treatments. Dorsal muscle lipid content was significantly higher in fish fed with 15% and 25% lipid diets than in with 5% group ($P<0.05$). Liver lipid content was significantly higher in fish fed with 15% lipid diets than in the 5% group ($P<0.05$), while there was no significant difference in fish fed diets containing 15% and 25% lipid. Muscle protein content was unaffected by the dietary lipid level.

3.3. Energy retention and deposition of nitrogen and lipid

Nitrogen, energy and lipid utilization of cobia fed with experimental diets are reported in Table 4. *N* intake and *N* gain (g kg ABW^{-1} day^{-1}) were significantly lower in fish fed with 25% lipid diet than those fed with other diets ($P<0.05$). The percentage of *N* retention for fish fed with diets containing 5% lipid was significantly higher than for those fed 25% lipid ($P<0.05$), but no significant difference in fish fed with 15% or the 5% or 25% lipid was observed.

Table 4
Nitrogen, energy and lipid utilization by juvenile cobia fed with experimental diets

	L5	L15	L25
ABW[a]	66.88 ± 2.68[a]	66.81 ± 0.71[a]	60.06 ± 0.88[b]
Nitrogen			
Intake (g kg ABW^{-1} day^{-1})[b]	3.25 ± 0.13[a]	3.11 ± 0.18[a]	2.77 ± 0.09[b]
Gain (g kg ABW^{-1} day^{-1})[c]	1.19 ± 0.02[a]	1.11 ± 0.02[a]	0.89 ± 0.07[b]
Retention (percentage NI)[d]	36.57 ± 1.41[a]	35.92 ± 2.64[ab]	32.09 ± 1.62[b]
Energy			
Intake (kJ kg ABW^{-1} day^{-1})[e]	7.04 ± 0.27	7.73 ± 0.45	7.72 ± 0.25
Gain (kJ kg ABW^{-1} day^{-1})[f]	3.06 ± 0.09[c]	3.68 ± 0.10[b]	4.13 ± 0.03[a]
Retention (percentage EI)[g]	43.50 ± 2.23[c]	47.68 ± 2.18[b]	53.50 ± 1.47[a]
Lipid			
Intake (g kg BW^{-1} day^{-1})[h]	2.14 ± 0.08[c]	7.00 ± 0.41[b]	9.60 ± 0.31[a]
Gain (g kg ABW^{-1} day^{-1})[i]	4.02 ± 0.12[c]	6.13 ± 0.32[b]	8.91 ± 0.84[a]

Values (mean ± standard deviations of three replications) in the same row with different superscripts are significantly different ($P<0.05$).

[a] Average body weight = (initial body weight + final body weight)/2.

[b] Daily nitrogen intake = feed intake nitrogen / ABW × days.

[c] Daily nitrogen gain = (final body weight × final body nitrogen − initial body weight × initial body nitrogen) / ABW × days.

[d] Nitrogen retention = 100 × daily nitrogen gain / daily nitrogen intake.

[e] Daily energy intake = feed intake energy / ABW × days.

[f] Daily energy gain = (final body weight × final body energy − initial body weight × initial body energy) / ABW × days.

[g] Energy retention = 100 × daily nitrogen gain / daily nitrogen intake[a]. Daily lipid intake = feed intake lipid / ABW × days.

[h] Daily nitrogen gain = (final body weight × final body lipid − initial body weight × initial body lipid) / ABW × days.

[i] Lipid retention = 100 × daily lipid gain / daily lipid intake.

The energy intake (g kg ABW^{-1} day^{-1}) were identical among groups. However, energy gain and energy retention significantly increased ($P<0.05$) with dietary lipid levels due to the increasing energy density of the diets. Fish fed 5%, 15% and 25% lipid diets showed energy retention levels of $43.50 \pm 2.23\%$, $47.68 \pm 2.18\%$ and $53.50 \pm 1.47\%$, respectively. Lipid intake and lipid gain (g kg ABW^{-1} day^{-1}) significantly increased ($P<0.05$) as dietary lipid level increased, the highest values (9.60 ± 0.31, 8.91 ± 0.84) were found in fish fed with diet with the highest lipid level.

3.4. Activities of lipogenic enzymes

Data on the activity of the four NADPH-generating dehydrogenases assayed in juvenile cobia liver are reported as IUg^{-1} liver or per mg protein in Table 5. 6PGDH and IDH were not affected by dietary treatment. Of the two regulatory enzymes in the lipogenic pathway, G6PD and ME activities were affected by the treatment ($P<0.05$). Hepatic malic enzyme and glucose-6-phosphate dehydrogenase (G6PD) activities (IUg^{-1} liver) were higher in fish fed with 5% lipid diet than the fish fed with 25% lipid diet ($P<0.05$). Moreover, the activity of G6PDH was over sixfold higher than that of ME in fish fed with 15% and 25% lipid diets.

Table 5
Activities of hepatic glucose-6-phosphate dehydrogenase (G6PDH), phosphogluconate dehydrogenase (PGD), malic enzyme (ME) and isocitrate dehydrogenase (IDH) from cobia fed with experimental diets

	L5	L15	L25
Glucose-6-phosphate dehydrogenase			
IU/g liver	9.93 ± 2.80^{a}	$8.04 \pm 1.33^{a,b}$	4.07 ± 2.31^{b}
IU/mg protein	5.15 ± 1.78	5.88 ± 0.77	3.57 ± 2.14
Phosphogluconate dehydrogenase			
IU/g liver	7.20 ± 1.94	5.97 ± 2.36	5.96 ± 1.87
IU/mg protein	3.64 ± 0.54	4.44 ± 1.87	5.13 ± 1.46
Malic enzyme			
IU/g liver	6.46 ± 1.26^{a}	2.66 ± 2.00^{b}	0.56 ± 0.14^{b}
IU/mg protein	3.22 ± 0.32^{a}	$1.96 \pm 1.43^{a,b}$	0.49 ± 0.11^{b}
Isocitrate dehydrogenase			
IU/g liver	11.11 ± 2.82	5.57 ± 3.68	8.75 ± 7.18
IU/mg protein	5.55 ± 1.22	4.19 ± 2.77	7.66 ± 6.47

Values (mean ± standard deviations of three replications) in the same row with different superscripts are significantly different ($P<0.05$).

4. Discussion

Lipids play an important role as a source of dietary energy for marine fish. They are well utilized by fish and necessary for normal growth and development. In cobia, diets with lipid exceeding 19% have been rarely tested. In the present experiment, the value of WG was excellent and exceeded those reported in several previous studies on cobia (Chou et al., 2001, 2004). This is probably due to higher initial weight of the fish in the previous experiment (30–40 vs. 7.71 g in the present experiment).

No significant difference was found between weight gain of the fish fed diets of 5% and 15% lipid. This is in agreement with Chou et al. (2001), showing no significant growth enhancement when lipid levels were increased beyond 6% to the highest level tested 18%. In this study, weight gain of fish fed with the 5% and 15% lipid diets were significantly higher than for the 25% lipid diet and a dietary lipid level in excess of 15% had a negative influence on growth. When fish are fed a diet containing excess energy, growth may be reduced due to reduced feed consumption. The present study showed that the increase in dietary lipid level was associated with a decline in feed intake. The DFI of fish fed with highest lipid diets was significantly lower than those fed with the 15 and 5% lipid diets (mean ± SD of 4.21 ± 0.14 versus 4.93 ± 0.29, 5.04 ± 0.20 g 100 g fish^{-1} day^{-1}, respectively). When expressed in terms of energy, differences in intake were similar among 25%, 15% and 5% groups (7.72 ± 0.25, 7.73 ± 0.45, 7.04 ± 0.27 kJ kg ABW^{-1} day^{-1}, respectively). Our observations also provide support for the contention that fish fed to satiation can adjust feed intake to meet energy requirements (e.g., Page and Andrews, 1973; Marais and Kissil, 1979; Jobling and Wandsvik, 1983; Ellis and Reigh, 1991; Kaushik and Medale, 1994; Ogata and Shearer, 2000; Lupatsch et al., 2001). This finding agrees with other studies that high dietary lipid level might decrease feed intake and reduce fish growth (Page and Andrews, 1973; Ellis and Reigh, 1991; Lee et al., 2002).

The protein sparing effect of lipid has been studied by numerous researchers. In salmonids, up to 30% lipid diets have been shown to improve feed and protein utilization efficiencies and to reduce *N* excretion (Beamish and Medland, 1986; Hillestad and Johnsen, 1994; Helland and Grisdale-Helland, 1998; Torstensen et al., 2001). Such protein-sparing effects have been reported in many fish species fed high energy diets containing lipid as a major energy source. However, some authors also have observed no protein sparing effect of lipid (McGoogan and Gatlin, 1999; Thoman et al., 1999; Vergara et al., 1999). Results of the present study suggest no sparing of protein by dietary lipid, too. The increase of dietary lipid level from 15% to 25% did not improve growth. This agrees with previous results in this species (Chou et al., 2001). In the present experiment, increasing dietary lipid did not result in increased *N* retention (%). *N* retention of fish fed with 5% lipid diet was higher than that of fish fed with 25% lipid diet. Increasing dietary lipid level did, however, result in increased lipid gain (g kg^{-1}feed), energy retention (%) and final whole-body lipid content. These results indicate that cobia tend to increase their lipid deposition with increasing fat levels in diets.

The correlation between dietary lipid and body lipid concentrations has been well studied. Too much dietary lipid may result in excessive fat deposition in the visceral cavity and tissues. Otwell and Rickards (1981) suggested that total body-fat content is an important determining factor of consumer acceptance and greater fat deposition occurring in viscera (led by high-energy diets) can reduce the commercial value of the product. Additionally, there was an apparent decrease in body protein content when fish were fed diets with high lipid levels (Page and Andrews, 1973; Reinitz and Hitzel, 1980; Williams and Robinson, 1988; Bjerkeng et al., 1997). Over the course of the 6-week experiment, the whole body lipid content of the fish increased from an initial concentration of 4.46 ± 0.18 g $100g^{-1}$ to mean values of 9.23, 13.93 and 20.31 g $100g^{-1}$, respectively, for the 5%, 15% and 25% lipid diet. Similar results have also been reported in other fish species (e.g. Bromley, 1980; Watanabe, 1982; Hillestad and Johnsen, 1994; Vergara et al., 1996; Helland and Grisdale-Helland, 1998; Jover et al., 1999; Peres and Oliva-Teles, 1999). In this study, both VSI and IPF were affected significantly by dietary fat level. The viscera somatic index (VSI) and IPF increased significantly with increasing lipid level in the diets, 11.45 ± 0.53, 0.71 ± 0.05 for 5%; 13.59 ± 0.22, 1.26 ± 0.05 for 15% and 14.70 ± 0.57 and 1.54 ± 0.02 for 25% group, respectively. VSI percentage is an important trait directly affecting the yield in the cobia production. Different from the results observed in other fish fed high lipid diets (Peres and Oliva-Teles, 1999; Lee et al., 2002), muscular mass plays the important contribution to body fat deposit in the present study for cobia. Fish fed with 15% lipid diet have significantly higher percentage muscle lipid and liver lipid (mean 6.58 ± 2.14, 57.95 ± 6.11) than fish fed with 5% lipid diets (mean 3.20 ± 0.87, 42.41 ± 5.79), but no significant difference between the 15% and 25% groups (mean 8.74 ± 0.35, 51.79 ± 4.12). HSI followed a similar trend as liver lipid. Similar results have been reported for eel (Otwell and Rickards, 1981; Gallagher et al., 1984; García Gallego et al., 1993) and salmon (Sheehan et al., 1996; Hemre and Sandnes, 1999). These results suggested that cobia seem to store lipid in muscle and viscera as dietary lipid content increased from 5% to 15% and mainly deposited lipid in adipose tissue and muscle as dietary lipid content exceeded 15%.

In fish, hepatic tissue is the preferential site of de novo fatty acid synthesis (Lin et al., 1977a,b; Likimani and Wilson, 1982). NADPH is essential for hepatic fatty acid biosynthesis; Malic enzyme, G6PD, P6GDH and IDH are involved in catalyzing production NADPH. The highest activities of hepatic malic enzyme and G6PD were found in fish fed with 5% lipid diet (6.46 ± 1.26, 9.93 ± 2.80 IU g^{-1} liver), and no significant difference was found between those fed with 15% (2.66 ± 2.00, 8.04 ± 1.33 IU g^{-1} liver) lipid diet and 25% (0.56 ± 0.14, 4.07 ± 2.31 IU g^{-1} liver) lipid diet (Table 5). In other teleosts, it has been reported that the activities of G6PD and ME were depressed by elevated levels of dietary lipid (Årnesen et al., 1993; Shimeno et al., 1993). Our results agree with this phenomenon. With regard to the two key regulatory enzymes in the lipogenic pathway, the activity of G6PD was over 6 times higher than that of ME in fish fed with 15% and 25% lipid diets. It suggested that the cytoplasmic reducing equivalents NADPH are mainly provided by the pentose phosphate pathway. The results were in accordance with data from studies with coho salmon (Lin et al.,

1977a,b), seabass (Iniesta et al., 1985) and rainbow trout (Walzem et al., 1991; Hung and Storebakken, 1994). The nature of dietary carbohydrate supply is also known to affect the enzyme activities. It has been confirmed in many fish species that lipogenesis is stimulated by high-carbohydrate diets and conversely suppressed by high-lipid dietary (Henderson and Sargent, 1981; Brauge et al., 1995; Catacutan and Coloso, 1997). In the present experiment, apparent better conservation of dietary lipid in fish fed with 5% lipid diet may have been due to the carbohydrate content being the highest. The results of the present study show that cobia juvenile prefer to use protein as a dietary energy source than lipid.

In conclusion, high dietary lipid levels above 15% produced little practical benefit because of higher fat accretion in cobia under the experimental condition used in this study. Muscle was also found to be a major fat deposition site in cobia. The fat utilization in cobia needs further investigation.

References

AOAC, 1984. Official Methods of Analysis, 14th edn. Association of Official Analytical Chemists, Washington, DC, USA.

Årnesen, P., Krogdahl, A., Kristiansen, I.Ø., 1993. Lipogenic enzyme activities in liver of Atlantic salmon *Salmo salar* L. Comp. Biochem. Physiol. 105B, 541–546.

Beamish, F.W.H., Medland, T.E., 1986. Protein sparing effects in large rainbow trout, *Salmo gairdneri*. Aquaculture 55, 35–42.

Bernt, E., Bergmeyer, H.U., 1974. Isocitrate dehydrogenase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis, vol. 2. Academic Press, New York, NY, pp. 624–627.

Bjerkeng, B., Refstie, S., Fjalestad, K.T., Storebakken, T., Rødbotten, M., Roem, A.J., 1997. Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. Aquaculture 157, 297–309.

Brauge, C., Corraze, G., Medale, F., 1995. Effects of dietary levels of carbohydrate and lipid on glucose oxidation and lipogenesis from glucose in rainbow trout, *Oncorhynchus mykiss*, reared in freshwater or seawater. Comp. Biochem. Physiol. 111A, 117–124.

Briggs, J.C., 1960. Fishes of worldwide (circumtropical) distribution. Copeia 1960 (3), 171–180.

Bromley, P.J., 1980. Effect of dietary protein, lipid and energy content on the growth of turbot *Scophthalmus maximus*. Aquaculture 19, 359–369.

Catacutan, M.R., Coloso, R.M., 1997. Growth of juvenile Asian seabass, *Lates calcarifer*, fed varying carbohydrate and lipid levels. Aquaculture 149, 137–144.

Chou, R.L., Su, M.S., Chen, H.Y., 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 193, 81–89.

Chou, R.L., Her, B.Y., Su, M.S., Hwang, G., Wu, Y.H., Chen, H.Y., 2004. Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. Aquaculture 229, 325–333.

Daniels, W.H., Robinson, E.H., 1986. Protein and energy requirements of juvenile red drum (*Sciaenops ocellatus*). Aquaculture 53, 243–252.

Ellis, S.C., Reigh, R.C., 1991. Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum *Sciaenops ocellstus*. Aquaculture 97, 383–394.

Folch, J., Lees, M., Stanley-Sloane, G.H., 1957. A simple method for the isolation purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–507.

Gallagher, M.L., Kane, E., Beringer, R., 1984. Effect of size on composition of the American eel (*Anguilla rostrata*). Comp. Biochem. Physiol. 78, 533–536.

García Gallego, M., Hidalgo, M.C., Suárez, M.D., Sanz, A., Higuera de Ia, M., 1993. Feeding of the European eel *Anguilla anguilla*: II. Influence of dietary lipid level. Comp. Biochem. Physiol. 105A, 171–175.

Glock, G.E., McLean, P., 1953. Further studies on the properties of glucose-6-phosphate dehydrogenase and 6-phosphoglucose dehydrogenase in rat liver. Biochem J. 55, 400–408.

Hardy, R.W., 1999. Problems and opportunities in fish feed formulation. Aquac. Mag. 25, 56–60.

Helland, S.J., Grisdale-Helland, B., 1998. The influence of replacing fish meal in the diet with fish oil on growth, feed utilization and body composition of Atlantic salmon (*Salmo salar*) during the smoltification period. Aquaculture 162, 1–10.

Hemre, G.I., Sandnes, K., 1999. Effect of dietary lipid level on muscle composition in Atlantic salmon *Salmo salar*. Aquac. Nutr. 5, 9–16.

Henderson, R.J., Sargent, J.R., 1981. Lipid biosynthesis in rainbow trout, *Salmo gairdneri*, fed diets of differing lipid content. Comp. Biochem. Physiol. 69C, 31–37.

Hillestad, M., Johnsen, F., 1994. High-energy/low-protein diets for Atlantic salmon: effects on growth, nutrient retention and slaughter quality. Aquaculture 124, 109–116.

Hung, S.S.O., Storebakken, T., 1994. Carbohydrate utilization by rainbow trout is affected by feeding strategy. J. Nutr. 124, 223–230.

Hung, S.S.O., Fynn-Aikins, F.K., Lutes, P.B., Xu, R., 1989. Ability of juvenile white sturgeon (*Acipenser transmontanus*) to utilize different carbohydrate sources. J. Nutr. 119, 727–733.

Iniesta, M.G., Cano, M.J., Garrido-Pertierro, A., 1985. Properties and function of malate enzyme from *Dicentrarchus labrax* L. liver. Comp. Biochem. Physiol., B 80, 35–39.

Jobling, M., Wandsvik, A., 1983. An investigation of factors controlling food intake in Arctic charr, *Salvelinus alpinus* L. J. Fish Biol. 23, 391–404.

Jover, M., Garcia-Gomez, A., Tomas, A., De la Gandara, F., Perez, L., 1999. Growth of Mediterranean yellowtail (*Seriola dumerilii*) fed extruded diets containing different levels of protein and lipid. Aquaculture 179, 25–33.

Kaushik, S.J., Medale, F., 1994. Energy requirement, utilization and dietary supply to salmonids. Aquaculture 124, 81–97.

Kawaga, Y., Kawaga, A., Shimazono, N., 1964. Enzymatic studies on the metabolic adaptation of hexose monophosphate shunt in rat liver. J. Biochem. 56, 364–371.

Lee, S.M., Jeon, I.G., Lee, J.Y., 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture 211, 227–239.

Liao, I.C., Huang, T.S., Tsai, W.S., Hsueh, C.M., Chang, S.L., Leaño, E.M., 2004. Cobia culture in Taiwan: current status and problems. Aquaculture 237, 155–165.

Likimani, T.A., Wilson, R.P., 1982. Effects of diet on lipogenic enzyme activities in channel catfish hepatic and adipose tissue. J. Nutr. 112, 112–117.

Lin, H., Romsos, D.R., Tack, P.I., Leveille, G.A., 1977a. Influence of dietary lipid on lipogenic enzyme activities in coho salmon *Oncorhynchus kisutch* (Walbaum). J. Nutr. 107, 846–854.

Lin, H., Romsos, D.R., Tack, P.I., Leveille, G.A., 1977b. Effects of fasting and feeding various diets on hepatic lipogenic enzyme activities in coho salmon *Oncorhynchus kisutch* Walbaum. J. Nutr. 107, 1477–1483.

Lupatsch, I., Kissil, G.Wm., Sklan, D., Pfeffer, E., 2001. Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream (*Sparus aurata* L.). Aquac. Nutr. 7, 71–80.

Marais, J.F.K., Kissil, G. Wm., 1979. The influence of energy level on the feed intake, growth, food conversion and body composition of *Sparus aurata*. Aquaculture 17, 203–219.

McGoogan, B.B., Gatlin, D.M., 1999. Dietary manipulations affecting growth and nitrogenous waste production of red drum *Sciaenops ocellatus*: I. Effects of dietary protein and energy levels. Aquaculture 178, 333–348.

Ogata, H.Y., Shearer, K.D., 2000. Influence of dietary fat and adiposity on feed intake of juvenile red sea bream *Pagrus major*. Aquaculture 189, 237–249.

Otwell, W.S., Rickards, L.W., 1981. Cultured and wild American eels, *A. rosfrata*: fat content and fatty acid composition. Aquaculture 26, 67–76.

Page, J.W., Andrews, J.W., 1973. Interaction of dietary levels of protein and energy on channel catfish (*Ictalurus punctatus*). J. Nutr. 103, 1339–1346.

Peres, H., Oliva-Teles, A., 1999. Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles *Dicentrarchus labrax*. Aquaculture 179, 325–334.

Reinitz, G., Hitzel, F., 1980. Formulation of practical diets for rainbow trout based on desired performance and body composition. Aquaculture 19, 243–252.

Shaffer, R.V., Nakamura, E.L., 1989. Synopsis of biological data on the cobia, *Rachycentron canadum* (Pisces: Rachycentridae). U.S. Dep. Commer., NOAA Tech Rep NMFS 82 [FAO Fisheries Synopsis 153], 21 p.

Sheehan, E.M., O'Connor, T.P., Sheehy, P.J.A., Buckley, D.J., FitzGerald, R., 1996. Effect of dietary fat intake on the quality of raw and smoked. Ir. J. Agric. Food Res. 35, 37–42.

Shimeno, S., Ming, D.C., Takeda, M., 1993. Metabolic response to dietary carbohydrate to lipid ratios in *Oreochromis niloticus*. Nippon Suisan Gakkaishi 59, 827–833.

Thoman, E.S., Davis, D.A., Arnold, C.R., 1999. Evaluation of growout diets with varying protein and energy levels for red drum (*Sciaenops ocellatus*). Aquaculture 176, 343–353.

Torstensen, B.E., Lie, O., Hamre, K., 2001. A factorial experimental design for investigation of effects of dietary lipid content and pro- and antioxidants on lipid composition in Atlantic salmon (*Salmo salar*) tissues and lipoproteins. Aquac. Nutr. 7, 265–276.

Vergara, J.M., Robaina, L., Izquierdo, M.S., De la Higuera, M., 1996. Protein sparing effect of lipids in diets for fingerlings of gilthead seabream. Fish. Sci. 62, 624–628.

Vergara, J.M., López-Calero, G., Robaina, L., Caballero, M.J., Montero, D., Izquierdo, M.S., Aksnes, A., 1999. Growth, feed utilization and body lipid content of gilthead seabream (*Sparus aurata*) fed increasing lipid levels and fish meals of different quality. Aquaculture 179, 35–44.

Walzem, R.L., Storebakken, T., Hung, S.S.O., Hansen, R.J., 1991. Relationship between growth and selected liver enzyme activities of individual rainbow trout. J. Nutr. 121, 1090–1098.

Watanabe, T., 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. 73B, 3–15.

Williams, C.D., Robinson, E.H., 1988. Response of red drum to various dietary level of menhaden oil. Aquaculture 70, 107–120.

Wise, E.M., Ball, E.G., 1964. Malic enzyme and lipogenesis. Proc. Natl. Acad. Sci U S A 52, 1255–1263.