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Juvenile cobia (*Rachycentron canadum*) can utilize a wide range of protein and lipid levels without impacts on production characteristics

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Abstract

Two, 6 week feeding trials were conducted to evaluate the impacts of protein and lipid on weight gain, feed efficiency ratio values and biological indices of juvenile cobia (*Rachycentron canadum*). Utilizing a 2×3 factorial design, experimental diets containing two levels of crude protein (CP; 40 and 50%) and three levels of lipid (6, 12 and 18%), providing 14.4, 15.1 and 16.6 kJ available energy/g dry diet (calculated), respectively, were formulated for use in both feeding trials. In the first trial, cobia (initial weight 49.3 g per fish) was randomly assigned to one of the six experimental diets and fed to apparent satiation twice daily. At the end of the first trial, weight gain in cobia was not significantly impacted by protein levels with values ranging from 333% (50% CP) to 353% (40% CP). However, lipid significantly ($P<0.05$) affected weight gain with fish fed the diet containing 18% total lipid returning the lowest growth of 293%. Feed efficiency ratio values were not significantly impacted by dietary protein or lipid levels and ranged from 0.46 (50% CP/18% lipid) to 0.51 (50% CP/6 and 12% lipid). Survival was significantly impacted by protein and lipid with fish fed the diets containing 50% CP and 18% lipid having lower ($P<0.05$) survival rates of 90%.

In the second trial, smaller fish were utilized (7.4 g average initial weight) under identical experimental conditions and dietary formulations. Weight gain was not significantly affected by protein or lipid levels and ranged from 1099% in fish fed the diet containing 40% CP/18% lipid to 1305% in fish fed the diet containing 50% CP/12% lipid. Feed efficiency ratio values, visceral somatic and hepatosomatic indices were significantly affected by protein and/or lipid. Muscle and liver lipid were impacted by dietary lipid ($P=0.0203$ and 0.0012, respectively). Muscle protein was significantly impacted by dietary protein levels, while liver protein was affected by both main effects. Dietary protein and lipid had no impact on muscle ash.

These data suggest that juvenile cobia can thrive on a wide range of protein and lipid levels, as well as a range of protein to energy ratios. Positive impacts of optimizing the protein component in terms of economic and environmental concerns, coupled with the ability to maintain the rapid growth rates this species are renowned for at lower dietary lipid levels, point towards beneficial consequences of further refinement of commercial cobia production feeds.

Keywords: Cobia; Protein; Energy; Lipid; Health

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1. Introduction

Cobia represents a species of increasing interest to aquaculture due to its impressive growth performance (Chou et al., 2001; Lunger et al., 2006). This species also expresses many other favorable production-related characteristics which include spawning in captivity (Caylor et al., 1994; Arnold et al., 2002; Faulk and Holt, 2006), high survival post-weaning (Kaiser and Holt, in press), ability to withstand shifts in salinity (Faulk and Holt, 2006) and responsiveness to vaccination (Lin et al., 2006). Cobias also adapt to confinement and readily accept commercially-available extruded diets (Craig and McLean, 2005). Importantly, this species produces a high quality fillet suitable for the sashimi and white table cloth restaurant businesses which presently enhances their market value. However, while a fledgling cobia aquaculture industry already exists; future expansion that capitalizes on the true potential of this species will be heavily dependent upon increasing our general knowledge of its basic nutritional and environmental requirements.

Quantitative nutritional requirements for a species with such intense global interest are severely lacking. While optimal protein and lipid levels have been determined (Chou et al., 2001) and a few investigations involving utilization of alternate protein sources in the species (Chou et al., 2005; Lunger et al., 2006) and impacts of dietary lipid (Wang et al., 2005) have been conducted, very little information is available as to the specific nutrient requirements of cobia. Indeed, currently there is no information for specific requirements of cobia for any of the essential amino acids, essential fatty acids or other key nutrients so necessary for the successful and economically viable culture of cobia on a commercial level.

As protein represents the most expensive component of aquafeeds (Cho et al., 2005; Craig and McLean, 2005; Miller et al., 2005), from an economic standpoint it is vitally important that protein be utilized for the synthesis of muscle tissue and not for metabolic energy (Williams et al., 2003; Ozorio et al., 2006). Protein sparing by non-protein energy sources has been documented in a wide range of species (Serrano et al., 1992; Shiau and Peng, 1993; Thoman et al., 1999; Azevedo et al., 2002), but protein sparing by lipid has been best documented in the salmonids (Einen and Roem, 1997; Refstie et al., 2001; Azevedo et al., 2002). The ability to utilize lipid rather than protein as an energy source can lead to a decreased loss of ingested protein by catabolism (Refstie et al., 2001; Williams et al., 2003), thereby potentially reducing nitrogenous waste input into culture systems (Miller et al., 2005). This could have dramatic impacts on environmentally sensitive receiving waters or effluents from high density recirculating aquaculture systems (RAS).

It has been well established that protein to energy ratios in aquafeeds have significant impacts on fish performance (Nematipour et al., 1992; Lee et al., 2000; Azevedo et al., 2002; Cho et al., 2005). Fish feed to satisfy their energy requirements, and if dietary energy is insufficient, i.e. a high protein to energy ratio, feed consumption is increased (Mathis et al., 2003) and dietary protein will be utilized for metabolic energy, not only resulting in an inefficient use of an expensive dietary component, but also contributing to nitrogenous wastes in effluent waters. As well, if dietary energy is too high, i.e. a low protein to energy ratio, feed consumption is reduced, and as a result, fish will reduce feeding with resultant growth reduction due to an intake reduction of other essential nutrients (Cho et al., 2005). Additionally, excessive dietary energy can result in increased lipid deposition (Nematipour et al., 1992) that can negatively impact the health and well being of the cultured animal (Craig et al., 1999). Clearly, accurate protein to energy ratio values are necessary to optimize dietary formulations for any cultured species. This is particularly so for rapidly growing animals such as cobia, not only from an economic standpoint of cost-effective diet formulations, but also from a final product quality perspective.

The present study was undertaken to investigate the impacts of varying protein and lipid levels, and thus, protein to energy ratios, on weight gain, feed efficiency ratio values and other production characteristics of juvenile cobia. Two separate feeding trials involving identical diets and RAS were conducted to further our understanding of protein to energy ratios and optimal protein and lipid levels in dietary formulations for cobia.

2. Materials and methods

Feeding trials were conducted at the Virginia Seafood Agricultural Research and Extension Center (VSAREC) in Hampton, VA, and the Virginia Tech Aquaculture Center (VTAC) in Blacksburg, VA in identically constructed and operated RAS. The RAS incorporated 24, 110 l glass aquaria connected to a KMT-based (Kaldnes Inc, Providence, RI) fluidized-bed biofilter for conversion of ammonia to nitrate, a bubble bead filter (Aquaculture Technologies Inc., Metaire, LA) was used to eliminate solids (uneaten feed, fecal material, mucus and other fish waste), a protein skimmer (R and B Aquatic Distribution, Waring, TX) for removal of ultra-fine particulates and dissolved materials, and a 40 W UV sterilizer (Emperor Aquatics, Pottstown, PA) for disinfection. The aquaria and sump water were oxygenated using diffusion air lines connected to a 1 hp Sweetwater remote drive regenerative blower (Aquatic Ecosystems, Apopka, FL). Water temperature and dissolved oxygen (DO) were monitored daily using a YSI-85 Series dissolved oxygen meter (YSI Inc., Yellow Springs, OH). Total ammonia nitrogen (TAN) was monitored daily by spectrophotometric analysis and nitrite and nitrate levels were quantified once weekly (Hach Inc., Loveland, CO). Throughout the trial, photoperiod was maintained at a 12 h photophase–scotophase using automated timers with lighting derived from banks of commercial fluorescent tubes positioned 1.8 m above the experimental system.

Following a one-week acclimation to the RAS, fish were sorted by weight and subsequently randomly distributed into the 24 aquaria (trial 1: five fish per tank, average initial weight 49.3 g/fish; trial 2: ten fish per tank, average initial weight 7.4 g/fish). Aquaria were then randomly assigned to one of six dietary groups (triplicate tanks, $n=15$ in trial 1 and $n=30$ in trial 2). Water temperature was maintained at 26+2 C through ambient air heating/cooling and salinity held at 20 ppt in trial 1 at the VSAREC with double filtered seawater from Hampton Bay and 15 ppt in trial 2 through the addition of synthetic sea salt (Crystal Sea Marine Mix®, Marine Enterprises International, Inc. Baltimore, MD) to well water at the VTAC. Water quality parameters for both systems during each trial were as follows: dissolved oxygen > 6.50 mg/l, TAN < 0.30 mg/l, nitrite < 0.45 mg/l and pH ≥ 7.8.

2.1. Diets and feeding

Six experimental diets were formulated and manufactured for each of the 2×3 factorial feeding trials at the VTAC. Two levels of crude protein (CP; 40 and 50% dry weight) and three levels of lipid (6, 12 and 18% dry weight), providing 14.4, 15.1 and 16.6 kJ available energy/g dry diet (calculated), respectively (Table 1) were utilized as the experimental diets. Protein was provided by solvent-extracted Special Select® menhaden fish meal (Omega Protein, Hammond, LA) which reduced endogenous lipid levels to approximately 1.15% dry weight (Table 1). Dietary lipid was added back into the diets in the form of menhaden fish oil (Omega Oils, Reedville, VA). In order to stimulate feeding rhythmicity, diet palatability, and appetite, fish were fed to apparent satiation for 1 week with a conditioning diet providing 50% CP and 12% lipid (dry weight basis). At trial start, fish were fed 7% body weight/day of the respective diets, decreasing to 4% by the termination of the trial. Diets were hand fed twice daily at 09.00 and 16.00 h. Hand feeding, while labor intensive and time-consuming, was employed instead of automatic feeders in order to monitor feeding behavior and health status of the experimental animals and to guard against uneaten diet accumulation and resultant wastage. Both feeding trials were of 6 week duration.

Table 1
Composition of the experimental diets (g/100 g dry diet)

Ingredient	Diet 1 40/6	Diet 2 50/6	Diet 3 40/12	Diet 4 50/12	Diet 5 40/18	Diet 6 50/18
Fish Meal[1]	52.5	65.7	52.5	65.7	52.5	65.7
Dextrin[2]	21.5	11.5	23	13	19	9
Lipid[3]	5.4	5.3	11.4	11.3	17.4	17.3
Mineral[4]	4	4	4	4	4	4
Vitamin[5]	3	3	3	3	3	3
CMC[2]	1	1	1	1	1	1
Cellufil[2]	12.6	9.5	5.1	2	3.1	0
Protein[6]	40	50	40	50	40	50
Lipid[6]	6	6	12	12	18	18
Energy [6]	14.4	14.4	15.1	15.1	16.6	16.6
P:E[7]	31.9	39.8	26.6	33.2	24.0	30.0

[1]Special Select® Menhaden fish meal, Omega Proteins, Hammond, LA. Solvent-extracted with hexane according to Lochmann and Gatlin, 1993. Crude protein 76.2% (dry), crude lipid 1.14% (dry).
[2]US Biochemical Corporation, Aurora, IL.
[3]Omega Oils, Reedville, VA.
[4]ICN Corporation, Costa Mesa CA.
[5]See Moon and Gatlin (1991).
[6]Calculated.
[7]mg protein/ kJ.

2.2. Analytical procedures

During the trial, fish were group weighed weekly to enable adjustment of feeding rate to account for weight gain. Upon trial termination for the preliminary trial at the VSAREC, only weight gain (percent increase from initial weight), feed efficiency (FE) ratio values (g gain/g fed) and survival (%) were determined from each individual aquaria ($n=3$/treatment). For the second feeding trial at the VTAC, these parameters were again measured, as well as visceral somatic index (VSI), hepatosomatic index (HSI), and muscle ratio (MR). These indices were recorded for 3 fish randomly taken from each tank ($n=9$ per treatment). Additionally, these same three fish ($n=9$ per treatment) were bled via caudal venipuncture with heparinized syringes, hematocrit values obtained and the plasma separated from whole blood for plasma protein determination.

Total lipid was determined gravimetrically (Folch et al., 1957), and crude protein and moisture following standard methods (AOAC, 1990). Muscle and liver samples were taken from 3 fish per tank ($n=9$ per treatment) for proximate compositional analysis.

All data were subjected to factorial analysis of variance and, where appropriate, means were compared by Duncan's Multiple Range Test with differences considered

Table 2
Weight gain, feed efficiency ratio, and survival of juvenile cobia in the first six-week feeding trial

Dietary Treatment		Gain[1]	FE[2]	Survival (%)
Protein	Lipid			
40	6	355	0.47	100
40	12	362	0.50	100
40	18	340	0.48	100
50	6	377	0.51	100
50	12	376	0.51	100
50	18	245	0.46	80
ANOVA *P*>*F*				
Protein		0.3190	0.3338	0.0001 *40>50*
Lipid		0.0097 *12, 6>18*	0.1262	0.0001 *6, 12>18*
*P*L*		0.0466	0.2535	0.0001
Pooled SE		23.16	0.02	0.00

[1]Percent increase from initial weight.
[2]g gained/g fed.

significant at the $P \leq 0.05$ level (SAS Inc., Cary, North Carolina).

3. Results

3.1. Weight gain, feed efficiency and survival

3.1.1. Trial 1

There were no significant effects of protein on weight gain nor protein or lipid on FE ratio values in trial 1 which utilized larger juvenile cobia (49.3 g/fish average initial weight; Table 2). Weight gain in cobia fed the diets containing 40% CP was 353% (percent increase from initial weight) compared to 333% for fish fed the diets containing 50% CP. Weight gain ranged from 369% in fish fed the diets containing 12% dietary lipid to the lowest return ($P<0.05$) with respect to growth of 293% in fish fed the high lipid diets containing 18% total lipid. Fish fed the diets containing 6% lipid returned intermediate weight gains of 366%. Feed efficiency ratio values were 0.48 and 0.50 for fish fed the diets containing 40 and 50% crude protein, respectively. When grouped by lipid levels, FE ratio values ranged from 0.47 in fish fed the diet containing 18% lipid to 0.51 in fish fed the diet containing 12% dietary lipid. Fish fed the diet containing 6% lipid returned intermediate FE ratio values of 0.50. Survival was significantly impacted by protein and lipid (Table 2) with fish fed the diets containing 50% CP and 18% lipid having lower ($P<0.05$) survival rates of 90%. This was due to the loss of a single fish in each of the three replicate aquaria that were assigned to the diet containing 50% CP and 18% lipid. These mortalities also resulted in a significant interaction between protein and lipid in terms of survival.

3.1.2. Trial 2

Weight gain was not significantly impacted by either protein or lipid in the second trial (Table 3). Weight gain in fish fed the diets containing 40% CP was 1156% compared to 1237% in fish fed the 50% CP diets. When grouped by lipid levels, weight gain was 1198%, 1226% and 1166% for the 6, 12 and 18% lipid diets, respectively. Feed efficiency ratio values were significantly impacted by protein level, with fish fed the 40% CP diets having higher FE values (0.76) compared with those fed the diets containing 50% CP (0.72). Dietary lipid did not significantly affect FE, with values of 0.74, 0.75 and 0.75 for fish fed the 6, 12 and 18% lipid diets, respectively. There was a significant interaction between protein and lipid ($P=0.0209$) with FE values, most likely due to two extreme FE values in fish fed the diets containing 40 and 50% CP at the 6% lipid level (Table 3). Survival was excellent in the second feeding trial with no mortalities recorded during the 6 week period.

3.2. Biological indices

Biological indices were all significantly affected by protein and lipid (Table 3). The VSI was significantly

Table 3
Weight gain, feed efficiency ratio, visceral somatic index, hepatosomatic index and muscle ratio of juvenile cobia in the second, six-week feeding trial

Dietary treatment		Gain[1]	FE[1]	VSI[2]	HSI[3]	MR[4]
Protein	Lipid					
40	6	1223	0.80	9.13	2.25	30.2
40	12	1147	0.74	10.33	2.71	32.9
40	18	1099	0.76	9.98	2.19	29.7
50	6	1173	0.68	9.34	2.06	26.2
50	12	1305	0.75	9.43	2.02	34.1
50	18	1232	0.74	9.07	2.01	32.0
ANOVA Pr>*F*						
Protein		0.1289	0.0372 *40>50*	0.0020 *40>50*	0.0001 *40>50*	0.8556
Lipid		0.6159	0.9155	0.0042 *18, 12>6*	0.0036 *12, 18>6*	0.1240
*P*L*		0.2124	0.0209	0.8972	0.1461	0.5208
Pooled SE		60.4	0.02	0.67	0.22	1.58

[1]Percent increase from initial weight.
[2]g gained/g fed.
[3]Visceral mass weight/total body weight * 100.
[4]Liver weight/total body weight * 100.
[5]Fillet weight/total body weight * 100.

impacted by both protein and lipid, with a higher VSI observed in fish fed the diets containing 40% CP (10.4) compared with 9.3 for fish fed the diets containing 50% CP. Fish fed the lower lipid diets (6%) had significantly lower VSI (9.1) than those fed the diets containing 12 and 18% (10.2 and 10.3, respectively). Hepatosomatic indices also were significantly impacted by both dietary protein and lipid (Table 3), with highest values recorded in fish fed the diets containing 40% CP (2.9) and lowest values in fish fed the 50% CP diets (2.08). The HSI was lower ($P<0.05$) in fish fed the low lipid diet (2.24) with higher ($P<0.05$) HSI being recorded in fish fed the 12 and 18% lipid diets (2.67 and 2.57, respectively). Muscle ratio (MR) was not significantly impacted by dietary protein with similar values being recorded for fish fed the 40 and 50% CP diets (32.5 and 32.6%). However, fish fed the diets containing 6% lipid had higher ($P<0.05$) MR (33.6%) than fish fed diets containing 18% lipid 1 (31.1%). Fish fed the diets containing 12% lipid returned an intermediate response for MR of 32.9%.

3.3. Tissue analyses and hematological parameters

Muscle and liver tissue compositions were both affected by protein and lipid (Table 4). Muscle and liver protein levels were significantly impacted by dietary protein, with fish fed the 40% CP diets having less muscle and liver protein (20.2 and 9.6% wet weight, respectively) than fish fed the 50% CP diets (21.4 and 11.1% wet weight, respectively). Fish fed the 6, 12 and 18% lipid diets had similar muscle protein (20.6, 21.2 and 20.6% wet weight, respectively) and liver protein (10.7, 9.4 and 10.4% wet weight, respectively). Muscle and liver lipid concentrations were significantly impacted by dietary lipid, with increasing lipid levels with increasing dietary lipid. Fish fed fish fed the diets containing 6% lipid had the lowest muscle (1.3% wet weight) and liver (21.6% wet weight) lipid when compared with fish fed the diets containing 12 and 18% lipid (1.9 and 2.6, respectively for muscle lipid; 29.0 and 29.9, respectively for liver lipid). Fish fed the diets containing 40 and 50% CP had similar values for muscle lipid (2.2 and 1.6% wet weight, respectively) and liver lipid (28.1 and 25.5% wet weight, respectively). There was no significant impact of dietary protein or lipid on muscle dry matter or muscle ash (Table 5). Muscle dry matter ranged from 23.2 to 24.2% while muscle ash ranged from 6.1% in cobia fed the diets containing 6% lipid to 6.4% in fish fed the diets containing 18% lipid. Packed cell volume also was not significantly impacted by dietary protein or lipid levels (Table 5) and ranged from 40.1% in fish fed the 40% CP/6% lipid diet to 35.5% in fish fed the 50% CP/18% diet. Plasma protein levels (Table 5) were significantly affected by dietary lipid ($P=0.0187$) with cobia fed the diets containing 6% lipid (4.4%) having higher plasma protein levels than those fed the diets containing 18% lipid (3.8%). Cobia fed the diets containing 12% lipid returned intermediate plasma protein levels of 4.1%.

Table 4
Percent muscle and liver lipid, muscle and liver protein in biological samples taken from juvenile cobia after the second, six-week feeding trial

Dietary treatment		Muscle lipid	Liver lipid	Muscle protein	Liver protein
Protein	Lipid				
40	6	1.37	24.14	19.70	9.87
40	12	1.82	30.67	20.78	9.14
40	18	3.35	29.41	20.02	9.74
50	6	1.16	18.98	21.65	11.58
50	12	1.93	27.32	21.52	10.01
50	18	1.85	30.32	22.43	11.02
ANOVA Pr>*F*					
Protein		0.1283	0.1170	0.0379 *50>40*	0.0032 *50>40*
Lipid		0.0203 *(18>6)=12*	0.0012 *18, 12>6*	0.8163	0.0838
*P*L*		0.1489	0.2761	0.6468	1.0000
Pooled SE		0.40	1.84	0.80	0.74

All values are on a wet weight basis.

Table 5
Muscle dry matter and ash, packed cell volume (PCV) and plasma protein concentrations from juvenile cobia after the second, six-week feeding trial

Dietary treatment		Muscle dry matter	Muscle ash	PCV	Plasma protein
Protein	Lipid				
40	6	23.6	6.73	40.1	4.71
40	12	23.9	6.36	36.1	4.12
40	18	24.1	6.58	36.2	3.78
50	6	23.2	5.92	37.9	4.00
50	12	23.8	6.50	37.3	4.00
50	18	24.2	6.35	35.5	3.88
ANOVA Pr>*F*					
Protein		0.5493	0.7287	0.7577	0.0907
Lipid		0.0532	0.6048	0.3818	0.0187 *(6>18)=12*
*P*L*		0.6255	0.6895	0.7411	0.0589
Pooled SE		0.29	0.29	2.24	0.16

4. Discussion

Cobia juveniles performed equally well on a wide variety of protein and lipid levels, as data from both the preliminary trial and main feeding trial returned similar results. As well, protein to energy levels ranging from 24 to 40 mg protein/kJ returned similar results for weight gain and FE and juvenile cobia performed equally well when fed diets containing available energy levels ranging from 14 to 17 kJ/g dry diet. The significantly higher FE in cobia fed diets containing 40% CP most likely indicates full utilization of the dietary protein component in feeds containing lower levels of protein. The protein requirement for juvenile cobia has been determined to be 44.5% CP (dry weight basis; Chou et al., 2001) utilizing fish of approximately 33 g initial weight. These authors also determined the optimal lipid level for maximal growth in cobia to be 5.76%, also on a dry weight basis. Our data certainly support the findings of Chou et al. (2001) that juvenile cobia fed diets containing 40% CP (dry weight basis) performed equally well to those fed diets containing 50% CP, both in terms of weight gain, and statistically higher FE values, in two separate feeding trials. Although the industry in the US currently is feeding commercial diets in excess of 58% crude protein and 15% lipid (dry weight basis), grow out facilities in Taiwan and other Asian countries are utilizing diets providing approximately 48% CP and 18% lipid (dry weight basis). While the dietary protein level being utilized in Taiwanese facilities is more in line with the established requirements than that being utilized at United States production facilities, the higher dietary lipid level is being utilized to customize products for the lucrative sashimi market. Clearly these data suggest that the current formulations utilized in commercial culture of cobia in the US are over-formulated with respect to protein. As protein is the most expensive component of aquafeeds, especially when fish meal serves as the predominant protein source (Azevedo et al., 2002; Miller et al., 2005), such over-formulation serves only to reduce the economic viability of the industry. Additionally, environmental concerns must enter the equation especially when considering net pen and RAS operations. As excess protein is catabolized for energy and thus excreted as ammonia and nitrogen, elevated levels of dietary protein that do not enhance production characteristics should be avoided (Azevedo et al., 2002). For net pen operations, this could lead to hypernutrification and subsequent eutrophication of receiving waters, while for RAS-based operations, this excess nitrogen must be eliminated from the system, resulting in increased operational costs and/or decreased production due to water quality issues.

Considering dietary lipid, it is evident that cobia, like many other fish, are amenable to body compositional changes with respect to dietary manipulations (Lochmann and Gatlin, 1993; Craig et al., 1995, 1999; Gaylord and Gatlin, 2000; Rasmussen et al., 2000b). The higher lipid diets utilized in Asia are for the fattening of the belly area–the highest priced and most desired cut for the sashimi market. Excess dietary lipid clearly does not contribute to more rapid, or even more cost-effective growth, as indicated by our study, as well as those of others (Chou et al., 2001; Wang et al., 2005). However, higher dietary lipid contributes to tailoring final product quality of cobia for the sashimi market. Excessive dietary lipid concentration in aquafeed formulations is not without risks however, and can present several problems, especially when utilizing marine fish oils. Diets that are higher in energy content often result in excessive lipid deposition in many species (Hanley, 1991; Nematipour et al., 1992; Serrano et al., 1992; Craig et al. 1995; Rasmussen et al., 2000a,b), which may be indicative of inefficient nutrient utilization (Craig et al., 1999). As well, excess dietary energy can result in decreased feed consumption and ultimately, reduced growth (Wang et al., 2005). In the present study, the higher lipid diets resulted in increased lipid deposition in the muscle component. However, a disproportionate share of lipid accumulation occurred in the hepatic tissue (almost 30% wet weight). Excessive lipid deposition in the liver and/or peritoneal cavity can result in lower health status of the animal (Flood et al., 1996; Tucker et al., 1997; Craig et al., 1999; Mathis et al., 2003) and poor dress-out if the deposition is in the form of intraperitoneal fat. Dietary lipid in the present study significantly lowered plasma protein levels, which could point towards lowered overall health status (Lunger et al., 2006). Additionally, juvenile cobia also accumulated lipid in the liver with increasing dietary lipid levels, which is in agreement with Wang et al. (2005) who observed similar results with cobia. The lipid accumulation in the liver could indicate potential for serious health issues for cultured cobia with the possibility of decreased resistance to disease. Increased hepatic lipid stores can negatively impact the health status of fish, leading to higher levels of oxidative stress (Craig et al., 1999; Kiron et al., 2004). Additionally, higher dietary lipid levels, especially those high in highly unsaturated fatty acids, such as marine fish oils, are well known to increase the α-tocopherol requirement in fish (Watanabe et al., 1981; Cowey et al., 1983; Roem et al., 1990; Kiron et al., 2004; Lin and Shiau, 2005). Since the quantitative vitamin E requirement of cobia is not known, excessive dietary lipid levels should be avoided, especially if they do not contribute to increased production performance. Finally, the costs of excessive dietary lipid inclusion in aquafeeds for cobia must be addressed. As fish oil will continue to increase in price as the aquaculture industry continues to rely upon this lipid source for aquafeeds for higher level marine carnivores, inclusion rates must be controlled if economic viability of the industry is to be maintained (Craig and McLean, 2005). Since the present study, as well as those by Chou et al. (2001) and Wang et al. (2005), indicate no protein-sparing effect of lipid in juvenile cobia, excessive dietary lipid should be avoided for economic as well as animal health concerns. Clearly, the data from the present studies, as well as those of Chou et al. (2001) indicate optimal dietary lipid levels for juvenile cobia range between 6 and 12% lipid on a dry weight basis. However, development of high lipid "finishing" diets for production sashimi products necessitates further research.

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