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Research Paper

Evaluation of a high plant protein test diet for juvenile cobia *Rachycentron canadum* in comparison to commercial diets

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Abstract. Cobia culture is hampered by a lack of feeding protocols and optimized diets. The present study was conducted to evaluate whether a plant based protein test diet (TD) containing low amounts of fishmeal (108 g kg^{-1} diet) would support growth in juvenile cobia at similar levels as two commonly used commercial diets available in Vietnam, CD1 and CD2. The TD diet contained 206 g kg^{-1} marine (fishmeal, krill meal and fish protein concentrate) and a blend of plant ingredient and added crystalline lysine and arginine to fulfill the predicted requirements. Cobia (9.3 ± 0.1 g initial body weight) were fed to satiation for four weeks and more than tripled their initial body weight for all treatments. No significant differences in weight gain (WG), feed conversion ratio (FCR), or, protein gain, were observed between cobia fed TD or CD2. However, cobia fed CD2 deposited more lipid than cobia fed TD diet. Cobia fed TD had better WG, FCR and protein gain than cobia fed CD1, while lipid gain was less in fish fed TD. No differences in plasma amino acid profiles of 24 h-unfed cobia were observed between cobia fed any of the three diets. In summary, the results show that juvenile cobia tolerate diets with low fishmeal content provided dietary amino acid profiles are balanced towards the anticipated requirements.

Keywords: Cobia, fishmeal, plant protein.

INTRODUCTION

Cobia *Rachycentron canadum* L. (1766) is a perciform, pelagic, carnivorous marine fish. It is found in subtropical, tropical and temperate areas, except for the central and eastern Pacific (Briggs, 1960). Cobia grows rapidly and is regarded as an excellent species for aquaculture for several reasons. The species readily thrives on commercial aquafeeds (Craig and McLean, 2005), and fingerlings may reach market size of 4 to 6 kg (Chou et al., 2001) or even 6 to 10 kg (Su et al., 2000) under optimal feed and temperature condition within one year. The belly (viscera) of the fish has a high lipid content, while the fillet is lean and of high quality with white, firm and good flavored flesh suitable for the sashimi industry (Chou et al., 2001). Early research on cobia off the coast of North Carolina showed that the fish had a fast growth rate, high-quality flesh, concluded that it had a high potential for aquaculture (Hassler and Rainville, 1975). Cobia broodstock are highly fecund and induced spawning can be achieved by hormone injection (Franks et al., 2001), ambient seasonal cycles (Arnold et al., 2002; Liao et al., 2004) or photo-thermal conditioning (Kaiser and Holt, 2004). Production of fry and juvenile cobia for offshore grow-out cage culture continues to show promising results (Benetti et al., 2008; Holt et al., 2007; Liao et al., 2004). In addition, cobia tolerate a wide range of salinity (Faulk and Holt, 2006), and show a positive response to vaccination (Lin et al., 2006). Due to these desirable characteristics, cobia aquaculture has risen dramatically, especially after the success of commercial juvenile production in the late 1990s (Benetti

et al., 2008; Liao et al., 2004).

Since cobia was only recently introduced into aquaculture, documentation on the nutritional requirements of the species is still limited (Fraser and Davies, 2009; Holt et al., 2007). Some efforts have been made to determine dietary protein and lipid requirements for juvenile cobia. Chou et al. (2001) suggested that a crude protein level of 445 g kg^{-1} diet would give maximum cobia growth, while optimum dietary lipid requirement for juvenile cobia is 57.6 g kg^{-1} dry matter. Juvenile cobia fed 400 g kg^{-1} dietary crude protein showed better feed efficiencies than those fed a diet containing 500 g kg^{-1} crude protein, while dietary lipid levels (60, 120 and 180 g kg^{-1} diet) did not affect weight gain or feed utilisation (Craig et al., 2006).

Attempts have also been made to determine the digestibility of protein, lipid and phosphorous from a range of plant and animal sources in juvenile cobia. Apparent dry matter digestibility for juvenile cobia ranged 0.60 to 0.88 for animal products and corn gluten meal, and 0.59 to 0.71 for soybean meal, peanut meal, and rapeseed meal (Zhou et al., 2004). Juvenile cobia show a high capacity to utilize the phosphorus present in the ingredients, while amino acid availability reflected protein digestibility. The requirement for the indispensable amino acids, methionine, arginine and lysine, is established for cobia to be 11.9 g methionine in the presence of 6.7 g cysteine kg^{-1} dry matter, 28.2 and 23.3 g lysine kg^{-1} dry matter, respectively (Ren et al., 2012; Zhou et al., 2007; Zhou et al., 2006). Dietary taurine also has been reported to have a significant impact on growth and feed utilization in juvenile cobia, and taurine supplementation of 5 g kg^{-1} dry matter to a yeast-based diet improved both weight gain and feed efficiency (Lunger et al., 2007a).

Fishmeal is limited (FAO, 2013) and cannot be considered as a sustainable protein source for aquafeeds (Craig and McLean, 2005), which has intensified research on the replacement of fishmeal by alternative protein sources (Gatlin et al., 2007). In order to enable a sustainable expansion of farmed cobia, it is essential to have knowledge of the species-specific nutritional requirements at different life stages. In addition, information about the effects of inclusion of alternative protein sources for cobia is important for the commercial feed supply. Several alternative protein sources such as plant proteins, single cell proteins, and miscellaneous proteins have been tested in aquaculture. Studies on the replacement of fishmeal by soybean meal in the diets for cobia have shown promising results. Up to 400 g kg^{-1} of the fishmeal protein could be replaced by soybean meal protein (Chou et al., 2004) or defatted soybean meal (Zhou et al., 2005) without causing detrimental effects on weight gain and feed conversion in juvenile cobia. These findings were supported by Romarheim et al. (2008) who demonstrated that juvenile cobia fed a diet containing 335 g kg^{-1} fishmeal and 285 g kg^{-1} toasted defatted soybean meal caused no significant differences in specific growth rate or feed conversion as compared with cobia fed a fishmeal control diet.

Previously, research has demonstrated that high plant protein inclusion for other aquaculture species, such as Atlantic salmon, *Salmo solar* can be achieved (Espe et al., 2006; 2007). The aim of the present study was to evaluate whether a test diet containing high plant protein (low fishmeal) inclusion, but balanced in dietary amino acids towards the predicted requirement, would support growth in juvenile cobia comparably to two commercial diets commonly used in cobia aquaculture in Vietnam.

MATERIALS AND METHODS

Experimental diets

Two available commercial diets in Vietnam considered to provide good performance in production of juvenile cobia (Hung Van Lai, unpublished data) were selected for the experiment. One commercial diet, CD1, had 480 g protein and 74 g lipid kg^{-1} has been reported to give good growth and a low feed conversion in cobia aquaculture. The other commercial diet, CD2, contained more protein (550 g kg^{-1}) and lipid (95 g kg^{-1}) but is also considered by farmers to provide good growth in cobia. A high plant protein test diet, TD, was produced and extruded at EWOS Innovation AS, Norway. TD contained 206 g kg^{-1} of fishmeal, krill meal and fish protein concentrate, while the rest of the dietary protein was a blend of plant ingredient (730 g kg^{-1}; wheat, soy protein concentrate, sunflower meal and pea protein concentrate) blended to balance the dietary amino acids towards anticipated requirements (NRC, 2011) (Table 1). Lysine and arginine are the most limiting amino acids in most feed ingredients and particularly in plant proteins and casein. It is known that interactions of lysine and arginine may give negative effects on growth performance in fish (Berge et al., 2002; Zhou et al., 2007). Crystalline lysine and arginine were therefore added to the test diet in a balanced ratio and fulfill the requirements of juvenile cobia (Ren et al., 2012; Zhou et al., 2007). The lipid source used in the test diet was fish oil. Pellet size was 1.6 to 2.2 mm and the high plant protein test diet was extruded. Chemical analyses of the experimental diets tested are listed in Table 1.

Feeding trial

Juvenile cobia (BW 5 to 7 g) collected from Hoang Ky rearing farm in Vietnam were transferred to indoor cylindrical fiberglass tanks (1 m^3) at Duong De, Nha Trang, Vietnam. The fish were stocked at a density of 200 individuals/m^3 and fed adlib by hand at 8:00 and 17:00 with a pellet diet (480 g protein and 160 g lipid/kg diet) produced at the University of Nha Trang during an acclimatization period of 7 days.

Table 1. Dietary composition (g kg^{-1} diet) and proximate analysis (g kg^{-1} dry matter) of experimental diets.

Composition	Diets		
	CD1	CD2	TD
Fish oil			53.8
Fish meal			108
Krill meal and fish protein concentrate			98
Plant ingredients[a]			730
Lysine (78%)			7.0
Arginine			0. 9
Micronutrients			2.3[b]
Proximate analysis			
Dry matter	930	950	936
Crude protein	480	550	505
Crude fat	74.0	95.0	91.4

[a]Soya protein concentrate, pea protein concentrate, wheat protein, sunflower meal and wheat gluten (in order of inclusion high to low). Formulation of CD1 and CD2 diets were not available; Crystalline lysine (78%; DSM Ltd.co.) and arginine (100%; EVONIK Industries)
[b]Micronutrients include vitamin premix, trace element premix. Compositions of micronutrients were added to fulfill the requirement of salmon given by NRC (2011); TD, CD1 and CD2 are the test diet and two commercial diets, respectively.

After acclimatization, the juveniles were graded and 90 juveniles with mean body weight of 9.3 ± 0.1 g and 13.4 ± 0.1 cm standard body length were randomly distributed to nine experimental tanks. Each of the experimental diets was randomly assigned to three tanks. The tanks used for juvenile rearing were rectangular fiberglass tanks (0.4 × 0.5 × 0.6 m), with 110 L water, in a water recirculation system with continuous aeration. Seawater was pumped into two outdoor reservoirs (4 × 6 × 2 m), desedimented and treated with chloride (50 ppm) for 48 h. Treated seawater then be chloride test. An appropriate amount of sodium thiosulphate is required to remove any of the residual chloride from treated seawater before joining the recirculation system. Input water from a two-chamber organic filtered fiberglass tank (1 × 1 × 2 m) flowed through plastic pipes to the experimental tanks (0.1 l s^{-1}). Output water from the rearing tanks, collected via perpendicular pipes (Ø 27 mm) in the middle of each tank, passed through an organic filter set in a fiberglass tank (1 × 1.5 × 2 m) and pumped into the reservoir chamber of the filter tank before re-joining the recirculation system. The feeding trial lasted for a 4 week-period and the fish were fed using the same regime as that in the acclimation period. Just after feeding, uneaten feed was collected by feeding trays and dried at 105°C in an oven (Clayson Laboratory Apparatus Pty. Ltd.) for the calculation of food conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV). In addition, protein and lipid gain in g/ kg was calculated. Waste and feces were siphoned from the experimental tanks daily, about 30 min before morning-feeding time. Water temperature was 30.5 ± 2.3°C, salinity was 30 ± 3.1g L^{-1}, pH 7.8 to 8.3, dissolved oxygen 5.6 ± 0.5 mg L^{-1}, NH_3 ≤ 0.03 mg L^{-1}. Sea water in the recirculation system was renewed every 2 to 3 days depending on water quality analyses.

Sampling procedure

At the start of the feeding trial, juvenile cobias were individually weighed and length measured prior to distribution to each of the nine experimental tanks. Prior to any handling, the fish were anesthetized by MS 222 solution (0.4 g L^{-1}). A pooled sample of six fish was analyzed for proximate composition at the start of the experiment. At the end of the feeding trial, the fish were starved for 24 h and six fish from each tank were randomly collected and frozen for proximate analysis. Three fish from each tank were collected for sampling of body weight, standard length and organs. These were pooled, homogenized and dried out at 105°C for 24 to 36 h until constant dry weight in a moisture extraction oven (Clayson Laboratory Apparatus Pty. Ltd.) then kept in NUNC boxes in the freezer (-80 ± 3°C) until analyzed. Individual body weight to the nearest 0.1 g, standard length to the nearest 0.1 cm, and visceral organ weight to the nearest mg, were recorded in each of the sampled fish and the condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI) calculated. The other three fish collected from each tank were used for blood, liver and muscle sampling. Blood was collected from the efferent branchial artery into heparinized syringes (1 ml; 50 UI ml^{-1} blood) to vials on ice and centrifuged at 1800 x g for 10 min. Plasma was collected and de-proteinised with trichloroacetic acid (TCA, 5%, v/v, 1:1), and re-centrifuged before the de-proteinized plasma was pooled into a new vial and kept at −80±3°C

until analyzed. Pooled liver and lateral muscle samples were dried at 105°C for 24 to 36 h until constant weight in the oven then kept in NUNC boxes in the freezer (-80 ± 3°C) until analyzed. Body weight and standard length of the rest of the fish in each tank were measured and included in the growth data analyses. The samples were stored frozen until transported to NIFES for chemical analyses.

Chemical analyses

Total lipid of the diets and biological samples was analyzed gravimetrically after extraction of lipids in ethylacetate as described (Espe et al., 2006). Nitrogen was analyzed and crude protein was calculated, assuming amino acid (AA) contains 16% nitrogen (Espe et al., 2006). Total dietary AAs were analyzed after hydrolysis in 6N HCl for 22 h at 110°C. The AAs were separated on a UPLC-system (Waters Aquity UPLC BEH C18 column internal diameter of 1.7µM at a flow rate of 0.7 ml min^{-1} using the gradient offered by the supplier). The free AA composition in the de-proteinized plasma was determined on a Biochrom 20 plus Amino Acid Analyzer (Amersham Pharmacia Biotech, Sweden) equipped with a lithium column using post derivatisation with ninhydrin (Espe et al., 2006). The AAs were quantified using a standard containing the AAs of interest (Sigma Aldrich, Germany).

Calculations

Condition factor (CF) calculated as:

$$CF = \frac{\text{body weight (g)}}{[\text{standard length (cm)}]^3} x100$$

Feed conversion ratio (FCR) calculated from the amount of feed consumed (kg dry matter) and the total biomass (kg) gained:

$$FCR = \frac{\text{feed consumed (kg)}}{[\text{final biomass (kg)} - \text{initial biomass (kg)}]}$$

Percent weight gain (ΔG) calculated as the increased biomass (kg) in percent of initial biomass (kg):

$$\Delta G = \frac{[\text{final weight (kg)} - \text{intital weight (kg)}]}{\text{intital weight (kg)}} x100$$

Viscerosomatic index (VSI) calculated as follows:

$$VSI = \frac{\text{viscera weight (g)}}{\text{whole body weight (g)}} x100$$

Hepatosomatic index (HSI) was calculated as % liver of body weight to give information of lipid accumulation in the liver:

$$HSI = \frac{\text{liver weight (g)}}{\text{whole body weight (g)}} x100$$

Protein efficiency ratio (PER) was calculated as weight gain (g) for each gram protein consumed:

$$PER = \frac{[\text{final weight (kg)} - \text{intital weight (kg)}]}{\text{protein consumed (kg)}} x100$$

Protein productive value (PPV) calculated as retained protein (g) of consumed protein (g):

$$PPV = \frac{[\text{total final protein (kg)} - \text{intital protein (kg)}]}{\text{protein consumed (kg)}} x100$$

Fish protein gain and lipid gain per day calculated as:

$$\text{Protein gain} = \frac{[\text{total final protein (kg)} - \text{intital protein (kg)}]}{\text{number of fish x days of rearing}}$$

$$\text{Lipid gain} = \frac{[\text{total final lipid (kg)} - \text{intital lipid (kg)}]}{\text{number of fish x days of rearing}}$$

Statistical analysis

Data was analyzed by the statistical program SPSS for Windows (IBM® SPSS® Statictics version 19). Values are given as tank means ± SD. One way ANOVA was used to test any differences between dietary treatments using tanks as the statistical unit. If differences were obtained ($p < 0.05$) the Tukey's test was used to evaluate the differences between treatments. Prior to applying ANOVA, a Levene's test was done for testing the homogeneity of variances of the dependent variables.

RESULTS

Protein content of TD was intermediate between the two commercial feeds, while similar to lipid content of CD2, and higher than CD1 (Table 1). Dietary AAs varied among the three experimental diets (Table 2). TD contained considerably higher concentration of tyrosine, phenylalanine and arginine than CD1 and CD2, and had a higher indispensable-to-dispensable amino acids ratio (IAA/DAA). In addition, as crystalline lysine and arginine were added to TD, this diet contained more arginine than CD1 and CD2, but was comparable in lysine.

All fish readily accepted the three experimental diets and no mortalities occurred during the four-week feeding trial. The fish fed all three diets grew well and more than tripled their initial body weight during the four week trial (Figure 1). At the end of the experiment, cobia fed TD had a mean body weight of 38.7 ± 0.5 g, and a mean

Table 2. Amino acid concentration (g kg^{-1} dry matter) of the experimental diets.

Amino acids	Diets		
	CD1	CD2	TD
Glu	72.4 (150.9)	87.8 (159.7)	94.7 (187.5)
Asp	43.8 (91.2)	45.8 (83.2)	47.5 (94.2)
Pro	31.7 (66.0)	24.8 (45.2)	27.2 (53.8)
Ser	26.1 (54.4)	19.6 (35.6)	23.0 (45.6)
Ala	28.7 (59.8)	29.6 (53.9)	22.6 (44.8)
Gly	31.1 (64.9)	25.2 (45.7)	22.2 (43.9)
Tyr	11.4 (23.9)	12.1 (22.0)	15.1 (30.0)
Tau	2.0 (4.2)	3.4 (6.2)	1.2 (2.4)
OH-pro	6.1 (12.7)	4.1 (7.4)	1.5 (3.0)
Leu*	34.3 (71.5)	34.5 (62.8)	35.6 (70.5)
Lys*	29.2 (60.9)	35.8 (65.0)	34.0 (67.3)
Arg*	25.3 (52.6)	23.1 (42.0)	31.0 (61.4)
Phe*	18.1 (37.7)	17.0 (31.0)	23.0 (45.6)
Val*	23.8 (49.6)	21.9 (39.7)	22.4 (44.3)
Ile*	18.9 (39.3)	19.0 (34.5)	20.6 (40.8)
Thr*	19.0 (39.5)	18.9 (34.4)	18.3 (36.2)
His*	9.1 (19.0)	8.9 (16.1)	10.6 (21.0)
Met*	8.2 (17.1)	11.9 (21.7)	8.8 (17.4)
Sum AA	437.2 (910.9)	440.0 (800.1)	458.2 (907.2)
IAA/DAA	0.74	0.77	0.80

Numbers in parentheses are g amino acid/kg protein; tryptophan was not analyzed; amino acids (AA) followed by an asterisk (*) are considered indispensable for fish; IAA/DAA is the ratio of indispensable to dispensable amino acids; taurine was not included in Sum AA or the IAA/DAA ratio;

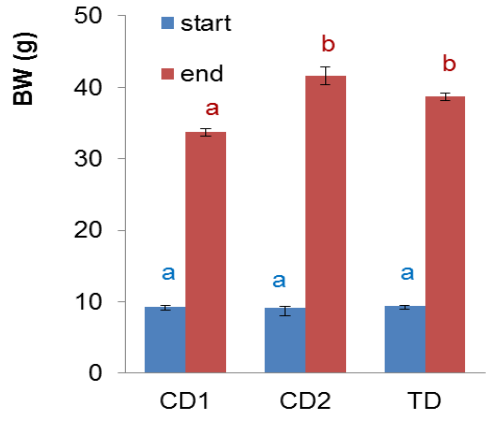

Figure 1. Mean body weight (BW) of cobia at the begining (start) and the end (end) of the trial; vertical bar on the top of columns indicate SEM; column not sharing letters in common indicate significant differences (*P < 0.05).*

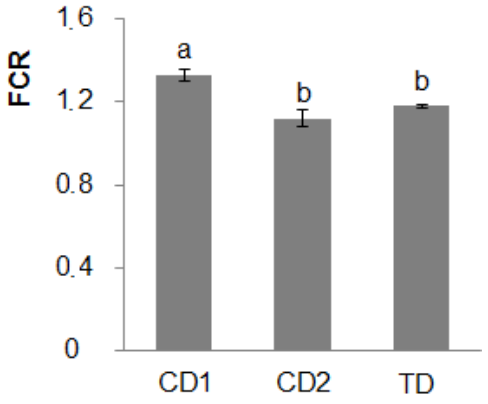

Figure 2. Feed conversion ratio (FCR) in cobia fed different experimental diets; vertical bar on the top of columns indicate SEM; column not sharing letters in common indicate significant differences (*P < 0.05).*

standard length of 20.0 ± 0.1cm. Feed conversion ratio (FCR), percent weight gain (ΔG), and protein gain/fish/day in cobia fed TD were 1.18, 303.4% and 0.16, respectively. No differences in growth performance, FCR or ΔG were observed between juvenile cobia fed TD and the cobia fed CD2 (Figures 2 and 3). However, cobia fed TD had a lower whole body lipid content than those fed CD2 ($P < 0.05$) which resulted in a higher lipid gain in the fish fed CD2 than those fed TD (Table 4 and Figure 3). Conversely, juvenile cobia fed TD obtained higher mean weight, protein gain/fish/day and had a better FCR than the cobia fed CD1. As with the CD2, cobia fed CD1

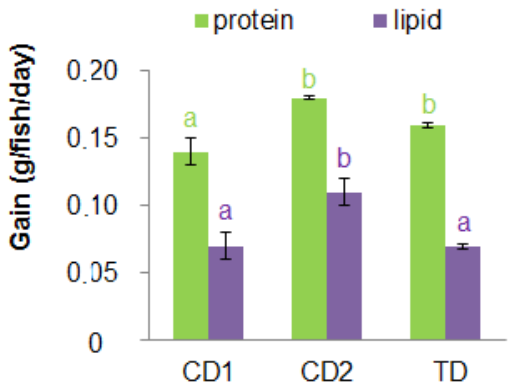

Figure 3. Mean protein and lipid gain (g/fish/day) in cobia fed different experimental diets; vertical bar on the top of columns indicate SEM; column not sharing letters in common indicate significant differences (*P<0.05)*.

Table 3. Concentration of free amino acid (µmol/100 ml) in plasma of cobia fed the three experimental diets for four weeks and then starved for 24 h.

Amino acids	Diets		
	CD1	CD2	TD
Gly	78.2±4.2	84.6±9.5	84.9±5.4
Ala	57.4±6.8	66.4±3.5	54.8±0.7
Pro	8.8±3.0	6.0±2.0	17.0±7.6
Gln	15.5±1.5	16.2±1.2	15.8±0.4
Glu	13.7±2.1	16.8±0.6	14.5±1.1
Asn	12.9±0.9	11.8±1.5	14.5±0.7
Ser	11.2±0.9	13.0±1.6	12.0±0.7
Tyr	5.8±0.6	5.4±0.5	5.7±0.8
Asp	3.4±0.4	3.8±0.8	4.8±0.5
Thr*	21.9±1.6	38.6±8.5	21.2±2.2
Lys*	20.8±3.5	20.5±3.4	19.8±1.7
Val*	16.0±1.1	15.6±0.0	15.7±1.8
Leu*	15.7±1.6	15.2±0.8	15.1±1.8
Met*	8.5±0.5	12.4±2.1	8.8±0.9
Ile*	9.0±0.7	9.1±0.4	8.0±0.9
Phe*	8.3±0.6	8.4±0.2	7.4±1.0
His*	6.6±0.5	6.9±0.8	6.8±0.4
Arg*	3.7±0.5	3.4±0.4	4.6±0.9
Trp*	1.7±0.1	1.3±0.1	1.1±0.2
Total AA	319.1±28.0	355.5±18.4	332.7±7.2
IAA	112.2±9.9	131.4±6.9	108.7±10.2
DAA	206.9±18.5	224.1±14.0	224.0±3.2
IAA/DAA	0.5±0.0	0.6±0.0	0.5±0.1

Values are presented as mean ± SEM; amino acids (AA) followed by an asterisk are considered indispensable for fish; cysteine was not detected from the plasma samples due to its low concentration.

had higher liver lipid content in the body and thus lipid accretion was higher than in those fish fed TD.

Total plasma free amino acid (FAA) concentration in cobia fed TD, CD1 and CD2 at 24 h postprandial was 332.7, 319.1 and 355.5 µmol/ml, respectively (Table 3). Plasma free indispensable AAs consisted mainly of threonine, lysine, valine, and leucine, while dispensable free AAs comprised dominantly glycine and alanine. However, no differences in plasma AA profiles were observed in cobia fed any of the diets in the current

Table 4. Weight gain (ΔG, % of initial BW), condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), protein and lipid (g/kg) of whole body, muscle, and liver, protein efficiency ratio (PER) and protein productive value (PPV) of juvenile cobia fed the experimental diets

Growth parameters	Diets		
	CD1	CD2	TD
ΔG	269.7±12.2^{b}	391.3±16.1^{a}	303.4±4.5^{ab}
CF	0.47±0.01	0.48±0.01	0.48±0.00
VSI	12.70±0.29	12.64±0.38	12.44±0.22
HSI	3.25±0.18	3.46±0.16	3.23±0.10
Body protein	149.3±5.2	151.0±4.0	148.8±3.2
Body lipid	75.1±3.5^{b}	82.2±2.0^{b}	63.1±2.3^{a}
Muscle protein	178.3±4.6	175.8±1.1	177.7±3.6
Muscle lipid	27.6±2.4	29.7±1.9	24.7±1.1
Liver lipid	420.4±14.6^{a}	418.1±6.5^{ab}	372.0±10.4^{b}
PER	1.56±0.03	1.63±0.06	1.66±0.02
PPV	0.24±0.01	0.25±0.01	0.25±0.00

Values are presented as mean of three tanks ± S.E.M; means in the same row with different superscripts are significantly different ($P < 0.05$).

experiment.

Neither did condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI), protein efficiency ratio (PER) and protein productive values (PPV) differ between fish fed any of the diets in the current experiment (Table 4).

DISCUSSION

Animal tissues and protein sources are abundant in taurine and OH-proline, but less in phenylalanine (Espe et al., 2012; Park et al., 2002). The taurine and OH-proline levels in CD1 and CD2 were considerably higher than in TD. Taurine and OH-proline are present in considerable higher concentration in diets with higher fishmeal inclusion and reduces as fishmeal is replaced with plant protein ingredients (Espe et al., 2012). Therefore, the higher concentration of taurine and OH-proline, but lower phenylalanine indicates a higher inclusion of animal protein sources in both CD1 and CD2.

Growth performance of cobia fed TD was comparable to those fed CD2, but better than those fed CD1. Concentration of proteins and lipids in TD and CD2 were higher than in CD1, which may have caused better growth performance in those fish fed the two former diets. In addition, ingredients used for formulations of TD and CD1 and CD2 may differ in terms of sources of macro components (e.g., protein and lipid quality) or micronutrients resulting in differences in feeding efficiencies, digestibility and growth performance. This may result in better growth performance and improved FCR obtained in fish fed TD and CD2.

In the present experiment, TD contained as low as 108 g kg^{-1} fishmeal, but still provided good growth performance and feed utilization in juvenile cobia. Previous studies have shown that fish species respond differently to replacement of fishmeal by alternative protein sources in the diets. A total replacement of fishmeal by soybean protein concentrate supplemented with methionine (Kaushik et al., 1995) or plant protein diet added crystalline AAs to balance the diets towards AA requirements (Rodehutscord et al., 1995) did not negatively affect growth and nutrient utilization in rainbow trout. Red drum *Sciaenops ocellatus* fed diet of 900 g kg^{-1} soybean meal (McGoogan and Gatlin, 1997) or gilthead seabream *Sparus aurata* fed 750 g kg^{-1} plant ingredients (De Francesco et al., 2007) performed as well as counterparts fed fishmeal based diets. While, Atlantic salmon fed a plant based diet containing 50 g kg^{-1} fishmeal performed equally well as fish fed the fishmeal-based control diet (Espe et al., 2007). In cobia, a replacement of up to 400 g/kg fishmeal protein by soybean meal protein (Chou et al., 2004) or by defatted soybean meal (Zhou et al., 2005) did not affect weight gain and FCR in juvenile cobia. A diet containing 335 g kg^{-1} fishmeal feed and 285 g toasted defatted soybean meal/kg feed resulted in specific growth rate or FCR in cobia comparable to those fed the fishmeal control (Romarheim et al., 2008). Similar results were reported by Lunger et al., (2007b), although a blended diet containing 80 g kg^{-1} fishmeal diet caused a retardation in weight gain and feed efficiency in cobia (Lunger et al., 2007b). Results from the current study suggested that cobia have adapted well the plant-based protein test diet.

One of the concerns when replacing fishmeal protein with alternative protein sources in aquafeeds is related to imbalances between indispensable amino acids (IAAs) and dispensable amino acids (DAAs), causing reduced growth. Rainbow trout *Oncorhynchus mykiss* perform

better when fed diets with balanced AA profiles than when fed imbalanced AA diets (Yamamoto et al., 2000). Weight gain, FCR and nitrogen retention in rainbow trout generally increased when dietary the ratio of IAAs to DAAs increased from 0.30 to 1.33, but performance decreased when the ratio increased above 1.94 (Green et al., 2002). Similar results are also reported in gilthead sea bream (Gomez-Requeni et al., 2003). TD in the current study had an IAAs to DAAs ratio of 0.80 comparable to CD2, while CD1 had a lower ratio. This may contribute to the better growth performance obtained in cobia fed TD and CD2, compared to fish fed CD1.

Neither HSI, VSI nor CF differed in the juvenile cobia fed any of the diets in the current experiment. This is in accordance with that reported by Zhou et al. (2005) on cobia fed high soybean protein diets and Espe et al. (2006) on Atlantic salmon fed plant-based protein diets. Atlantic salmon fed plant protein-based diets deposited significantly less lipid than those fish fed fishmeal-based diet (Espe et al., 2006), while European sea bass *Dicentrarchus labrax* deposited more lipid when fed high plant protein diets (Kaushik et al., 2004). In cobia muscle lipid are reported to increase as dietary soybean meal increased (Chou et al., 2004) or being unaffected (Zhou et al., 2005). In the present experiment, cobia fed TD deposited less lipids in the whole body than that in cobia fed CD1 or CD2, and less or equal lipid deposition in liver. Our findings support the hypothesis that there are differences between species in the amount of fishmeal that can be replaced which partly are likely related to their natural life history and food choices in nature.

The concentrations of plasma FAAs in cobia at 24 h postprandial in the current study compare well to that reported for cobia in a previous study in which the fish were fed different levels of lizardfish silage (Mach and Nortvedt, 2011). Plant ingredients, e.g. wheat gluten or maize gluten may influence the retention efficiency of crystalline lysine that has been reported in rainbow trout (Tran et al., 2007). In addition, added crystalline amino acids in the diet may be absorbed more quickly than protein bound amino acids released from the digestion of soybean protein (Ambardekar et al., 2009). The asynchrony of FAAs released from diets for absorption could reduce the efficiency of protein synthesis if added crystalline lysine and arginine are absorbed and catabolized before the protein bound amino acids are released. This asynchronization will reflect in the pattern of changes in plasma FAAs. Generally, concentrations of plasma FAAs increase and peak following feed intake, and then return to the pre-feeding levels (Ambardekar et al., 2009; Karlsson et al., 2006; Murai et al., 1987; Thebault, 1985). The time point that most of the plasma FAAs are assumed to return to the pre-feeding levels in cobia was about 24 h (Mach and Nortvedt, 2011). Had the inclusion of the plant ingredients and added crystalline lysine and arginine in TD caused negative effects on absorption and utilization of amino acids, cobia fed this diet would require longer time for digestion and absorption compared to cobia fed CD1 and CD2. If this was the case, higher concentrations of plasma FAAs would be expected in cobia fed TD, compared to cobia fed CD1 and CD2 at 24 h postprandial. However, concentrations of plasma FAAs in cobia fed TD were similar to cobia fed both CD1 and CD2 24 h postprandial, and comparable to that reported by Mach and Nortvedt (2011). These results suggested that cobia had utilized well TD as well as both CD1 and CD2 in the current study.

Plant protein diets can be utilized in fish providing the dietary amino acids are balanced towards the requirement (Espe et al., 2006; 2007). This implies that the amino acid profile has a more important role than crude dietary composition. In the present experiment, cobia fed TD showed comparable or better than those fed CD2 or CD1 in concentrations of plasma FAAs at 24 h postprandial, growth performance, lipid and protein deposition, and PPV, suggested that the plant proteins used are deposited sufficiently. This needs further studies to clarify. However, results from the present study indicate that juvenile cobia can tolerate diets with low fishmeal inclusion provided dietary amino acid profiles are balanced towards the anticipated requirements. Thus, TD as used in the present experiment is as a good starting point for further requirement studies in this species (Nguyen et al., 2013; 2014) and for producing a low fishmeal-based diet for cobia farming.

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