VOLUNTARY FEED INTAKE AND TRANSITION OF INGESTA IN THE GASTROINTESTINAL TRACT OF JUVENILE COBIA (*Rachycentron canadum*) FED DIFFERENT DIETS

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ABSTRACT

This study aimed to evaluate the palatability of a plant based protein diet (BL/A), with high inclusion levels of plant protein sources but balanced in lysine to arginine ratio (1.1), compared to two locally commercial pellets CD1 (Uni-President, Ltd.) and CD2 (INVE, Ltd.), and the transition of ingesta in juvenile cobia. Juvenile cobia were fed to satiety with each of the three diets had equal feeding rate of 5.3-5.4±0.3% BW (for a meal). No differences in stomach filling occurred between cobia fed the PBD diet and those fish fed the CD1 or CD2 diet. Gastric evacuation rates in cobia were performed as an exponential relationship, and were estimated as the function $V_T=V_0\ e^{-b(x)}$ *(*V_T*, volume of feed at time T;* V_0*, volume of feed at time 0; b, the instantaneous evacuation rate; and x, time postfeeding;* $R^2>0.95$*). Between 77 to 80% of the stomach contents were evacuated to the lower parts of the gastrointestinal tract at 8 h, and most of consumed feed (98%) was emptied out of the stomach at 16 h postfeeding. This was supported by the fact that cobia had good appetite in the 2nd feeding of the day. Time required for the return of appetite in cobia was within 8 h after feeding to satiation.*

Key words: Cobia, lysine, arginine, evacuation

I. Introduction

Cobia, *Rachycentron* canadum Linnaeus (1766), is the only species of the family Rachycentridae, and is widely distributed in subtropical, tropical and temperate areas, except for the central and eastern Pacific (Briggs, 1960). This species has many favorable production- related characteristics, such as rapid growth, and thus is regarded as a good candidate species for aquaculture. Under optimal feed and temperature condition cobia fingerlings can reach the marketable size of 4-6 kg (Chou, Su, & Chen, 2001) or even 6-10 kg (Su, Chien, & Liao, 2000) within a year. Further, cobia is highly marketable prized because of its high quality with white, firm and good flavored flesh that is also suitable for the sashimi industry (Chou et al., 2001). However, since cobia was only recently introduced into aquaculture documentation on the nutritional requirement of the species is still limited. Cobia culture is hampered by a lack of good feeding protocols and nutritionally optimized diets.

Chou and coworkers reported that protein concentration of 445 g kg^{-1} dry matter diet would give maximum growth in cobia, while optimum dietary lipid for juvenile cobia was found to be 57.6 g kg^{-1} dry matter (Chou et al., 2001). Replacement of fishmeal by plant protein sources, the nowadays dominant protein ingredient in aquaculture diets, shows promising results. In cobia, up to 400 g kg^{-1} of fishmeal can be replaced with soybean meal without negatively affect growth and feed conversion ratios (Chou et al., 2004; Zhou, Mai, Tan, & Liu, 2005). Plant ingredients may not well balanced in indispensable amino acids profiles that consequently reduce growth performance in fish (Rumsey, Siwicki, Anderson, & Bowser, 1994). Amongst in the indispensable amino acids in fish, lysine and arginine concentrations and/or its proportion in the diets are often taken into consideration when fishmeal protein is replaced by plant

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protein sources in aquafeeds (Venero, Davis, & Lim, 2008). Concentrations of lysine and arginine are often low in gluten or corn-based proteins and in casein (Venero et al., 2008). In addition to protein turnover, lysine and arginine are involved in a range of metabolic and physiological functions (Harpaz, 2005; Walton, Cowey, & Adron, 1984). Lysine affects collagen synthesis, as its hydroxylation product, hydroxylysine, is necessary for formation of the intermolecular crosslinks in collagen (Eyre, 1980; Piez & Likins, 1957). Arginine is the precursor for synthesis of nitric oxide, urea, polyamines, proline, glutamate, creatine and/or agmatine (Hird, 1986; Wu & Morris, 1998). Further, arginine participates in the regulation of extra-endocrine signaling pathways including AMP-activated protein kinase (AMPK) and the target of rapamycin, TOR (Jonsson et al., 2006; Yao et al., 2008), as well as immune functions (Li, Yin, Li, Kim, & Wu, 2007; Wu, Jaeger, Bazer, & Rhoads, 2004) and reproductive performance in mammals (Mateo et al., 2007). Additionally, lysine and arginine are assumed to share and/or compete for the same trans-membrane carrier systems. The metabolism and utilization of one of the amino acid affects the other and may give negative effects on fish growth (Berge, Sveier, & Lied, 2002). Although, mechanism of absorption, metabolism and utilization of lysine and arginine in cobia is yet to be determined.

In cobia the requirement of lysine and arginine requirement for maximized weight gain, specific growth rate and protein efficiency ratio is reported to be 23.8 and 28.2 g kg^{-1} diet, respectively (Ren, Ai, & Mai, 2012; Zhou, Wu, Chi, & Yang, 2007). Plant based protein diets may lead to imbalance in lysine to aginine ratio, and thus resulting in poor palatability, reduced palatability and/or digestibility, that consequently reduce growth performance in fish (Dabrowski, Arslan, Terjesen, & Zhang, 2007; Nguyen, Jordal, Buttle, Lai, & Rønnestad, 2013; Nguyen, Rønnestad, Buttle, Lai, & Espe, 2014). Understanding the rate of digestion in association with gastric evacuation rate may help to predict the return of appetite (Riche, Haley, Oetker, Garbrecht, & Garling, 2004), and figure out appropriate feeding strategies for better feed intake and feed efficiency by administering food as soon as appetite has returned (Grove, Loizides, & Nott, 1978; Lee, Hwang, & Cho, 2000). In the present study cobia with a plant based protein diet (BL/A), with high inclusion levels of plant protein sources but balanced in lysine to arginine ratio (1.1), compared to two locally commercial pellets CD1 (Uni-President, Ltd.) and CD2 (INVE, Ltd.). The aim of this study is to evaluate the palatability of these diets, and feed intake and the transition of ingested feed in the gastrointestinal tract of juvenile cobia postfeeding.

II. Materials and methods

1. Experimental diets

Two locally commercial diets pellets CD1 (Uni-President, Ltd.) and CD2 (INVE, Ltd.), and a plant based protein diet (BL/A) produced and extruded by EWOS Innovation AS, Norway were used in the present experiment (Table 1). The CD1 diet contained 480 g protein and 74 g lipid kg^{-1} dry matter, while the CD2 diet contained 550 g protein and 95 g lipid kg^{-1} dry matter). The BL/A diet 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). Appropriate amount of crystalline lysine and arginine were added in the BL/A diet in a balanced ratio and fulfill the requirements of juvenile cobia (Ren et al., 2012; Zhou et al., 2007). The pellet size was 1.6 mm.

Table 1. Formulation (g kg^{-1} dry matter basis) of the experimental diets

Ingredients	Diets		
	BL/A	CD1	CD2
Fish oil	53.8	-	-
Marine ingredient[a]	206.9	-	-
Plant ingredient[b]	730	-	-
Lysine	7.0	-	-
Arginine	0. 9	-	-
Micronutrients [c]	2.3	-	-
Proximate analysis		-	-
Dry matter	936	930	950
Crude protein	505	480	550
Crude fat	91.4	74	95
Ash	69	-	-
Energy (MJ/kg)	20	-	-

[a] Fish meal, krill meal and fish protein concentrate (in order of inclusion high to low).
[b] Soya protein concentrate, pea protein concentrate, wheat protein, sunflower meal and wheat gluten.
[c] Micronutrients include vitamin premix, trace element premix. Compositions of micronutrients were added to fulfill the requirement of Atlantic salmon according to National Research Council (1993); Crystalline lysine (78%; DSM Ltd.co.) and arginine (100%; EVONIK industries)

2. Experimental fish and water-circulation tanks

Juvenile cobia (500 juveniles of 3.0-5.0 g body weight), purchased from a local hatchery in Nha Trang, Vietnam, were transported and acclimatized in a fiberglass tank (5 m^3) at the Center for Aquatic Animal Health and Breeding Studies (Nha Trang University) for a period of one week. During acclimatization, the fish were fed ad libitum by hand at 8:00 and 17:00 with a pellet diet (480 g protein and 160 g lipid kg^{-1} diet) produced at the University of Nha Trang. After the acclimatization period, cobia were sorted out and fish of similar BW (8.0±0.1 g) were used for the experiment. The fish were randomly distributed into the experiment tanks.

The experimental tanks used were rectangular fiberglass tanks (0.4x0.5x0.6 m), with 110 L water filled, setting under a water recirculation system with continuous aeration. Each of the diets were randomly assigned to three tanks. Input water from a filtered fiberglass tank (1.0x1.0x2.0 m) went through plastic pipes to rearing tanks (0.2 L $second^{-1}$). Output water from the rearing tanks was collected by perpendicular pipes (Ø 27 mm) in the middle of each tank. Output water was then filtrated in a fiberglass tank (1.0x1.5x 2.0 m), before it were pumped back in to the filtered fiberglass tank (for input water). Seawater was pumped into a reservoir (24 m^3), and was desedimented and chloride treated before coming into the recirculation system. Water in the recirculation system was renewed every 2-3 days depended on environmental parameter analyses. In experiment I, water temperature was 30.5±2.3 °C (mean±SD), salinity was at 30±3.1 g L^{-1}, pH at 7.8-8.3, oxygen at 3.8±0.5 mg L^{-1} and $NH_3 \leq 0.1$ mg L^{-1}. While, these parameters for water in experiments II and III were 29.2±2.8 °C, salinity was 28±3.1 g L^{-1}, pH 7.8-8.3, oxygen 4.6±0.5 mg L^{-1}, $NH_3 \leq 0.03$ mg L^{-1}. The experimental tanks were covered by a fishing-net on the top to prevent any cobia jumping out of the experimental system.

3. Feeding trial and sampling procedure

Feeding trial: One hundred and eighty juvenile cobia (8.0±0.1 g) were distributed in to fifteen tanks (12 individuals/tank) and starved for 24 h. The juvenile cobia were randomly assigned to the three diets. Cobia were fed *ad lib* by hand at the morning meal at 8:00 for sampling during 24 h periprandial. Fifty four unfed cobia were also included as a reference (control group).

Sampling procedure: Prior to exposure to any sampling, juvenile cobia were anesthetized by MS-222 solution (0.4 g L^{-1}). Individual body weight and total length were measured to the nearest 0.1 g and 0.1 cm. Six fed cobia the CD1-, CD2- and BL/A diet, were dissected for collection of ingesta and chyme from the stomach, midgut and hindgut at just before feeding and at each of the following time postfeeding 0.25, 0.5, 1, 2, 4, 8, 16 and 24 h. Six unfed cobia from the control group were also dissected for collection of chyme in the GI-tract at the above sampling points. Therefore, control fish had fasted for 48 h at the time of the final sampling. The fish's GI-tract was dissected and carefully separated in stomach, midgut and hindgut to avoid loss of content (Fig. 1). Chyme and ingesta in these segments were carefully collected and transferred onto pre-weight aluminum foils. The collected contents in the GI-tract were dried at 105 °C in the oven (Clayson Laboratory Apparatus Pty. Ltd.) for 24 h for determining dry weight basic.

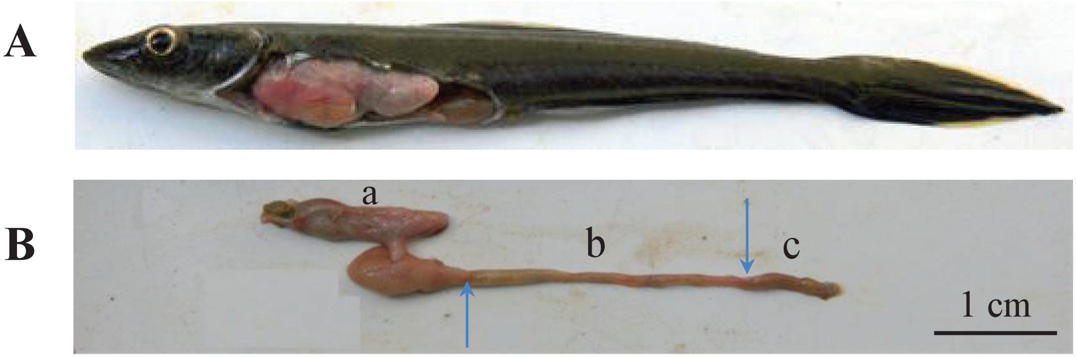

Fig. 1. Schematic diagram showing the dissection of cobia for collecting samples.

A*, the juvenile cobia with body cavity opened;* ***B*** *stomach (a); midgut (b); hind gut (c).During dissection, the gut was carefully stretched out, then the hindgut was identified from the GI terminus to the first folded-gut site, and the midgut was identified between the hindgut and the outlet of the stomach (pylorus).*

4. Statistical analysis

Data was analyzed by the statistical program SPSS for Windows (IBM® SPSS® Statistics version 24). Values are given as tank means ± SEM (standard error of the mean). ANOVA was used to test any differences between dietary treatments. 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.

III. Results and discussions

Juvenile cobia showed high appetite when they were offered the two commercial diets, and the plant-based protein test diet with balanced lysine to arginine ratio (BL/A). Analysis of the contents from the stomach indicated that juvenile cobia had a feeding rate of 5.3±0.3% BW for CD1-, and BL/A diet, and slightly higher for the CD2 diet (5.4±0.4% BW). No significant differences in stomach filling occurred between cobia fed the BL/A diet and the two commercial diets. Dry matter in the stomach of unfed cobia was stable as a minimum level (1.88-2.83 mg or 0.03-0.04% BW) within the time of the experiment. Significantly higher stomach filling in fed cobia compared to unfed cobia indicated the good palatability of the plant-based protein diet and both the commercial diets.

Gastric evacuation rates in juvenile cobia fed three diets (Fig. 2) could be fitted by the exponential function $Y_T=V_0\ e^{-b(x)}$ (V_T, volume of feed at time T; V_0, volume of feed at time 0; b, the instantaneous evacuation rate; and x, time postfeeding; $R^2>0.95$). One hour after a single meal, most of the ingesta was still in the stomach (89; 88 and 91% estimated from dry matter basic for CD1-, CD2- and BL/A diet, respectively), with only a small fraction transferred to the midgut (MG) and hindgut (HG). Stomach was gradually emptying, and

36-41 % of ingested feed was transferred to the further parts of the GI-tract at the 4 h after a meal. Between 77 to 80% of stomach contents was evacuated to the lower parts of the GI-tract at 8 h, and most of consumed feed (98%) was emptied out of the stomach around the 16 h postfeeding (Fig. 2). Based on gastric evacuation results at 8 h postfeeding, it could be inferred that the return of appetite in cobia was within this period after being fed to satiation.

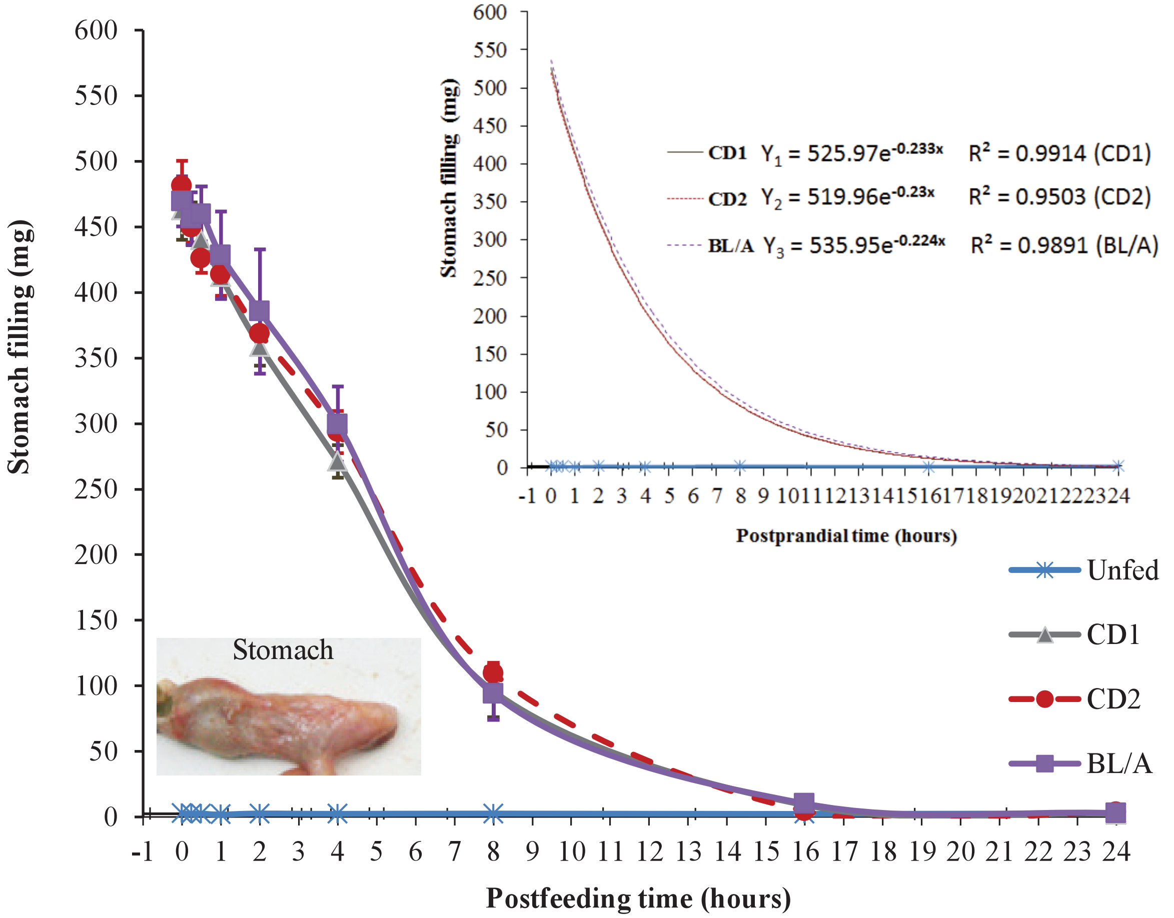

Fig. 2. Stomach filling (dry mass) in juvenile cobia fed different diets postfeeding.

Data are presented as means (n=6) at selected time points after feeding. Sampling started from time 0/ just after cobia fed to statiety. Vertical bar indicates ±SEM. The upper graph (insert) shows calculated gastric evacuation based on exponential fit for each diet. The equation for the relationship between stomach content (Y) over time (x) postpradial in cobia fed the CD1 diet was $Y = 0.526e^{-0.233x}$ *(*$R^2 = 0.9914$*); CD2 diet,* $Y = 0.536e^{-0.23x}$ *(*$R^2 = 0.9891$*); and BL/A diet,* $Y = 0.520e^{-0.23x}$ *(*$R^2 = 0.9503$*).*

Dry contents of chyme in the MG gradually increased and peaked at 4-6 h postfeeding, and then gradually declined to the level close to the unfed cobia at 16 h postfeeding (Fig. 3). No significant differences in the chyme content (dry mass) in midgut of juvenile occurred between cobia fed the BL/A diet and the two commercial diets.

At the 0.5 h postfeeding, content of the chyme in the HG rapidly increased to the highest level observed during the study, and stabilized at this level within the 4-16 h, followed by a rapid decrease to the minimum level similarly to unfed cobia around the 24 h postfeeding (Fig. 3).

It should be noted that there was a methodological challenge regarding sampling the complete contents of the GI-tract. The pyloric caeca is a complex compartment, and despite the relatively large appearance the intraluminal volume of each caecum was very small and impossible to empty. The chyme

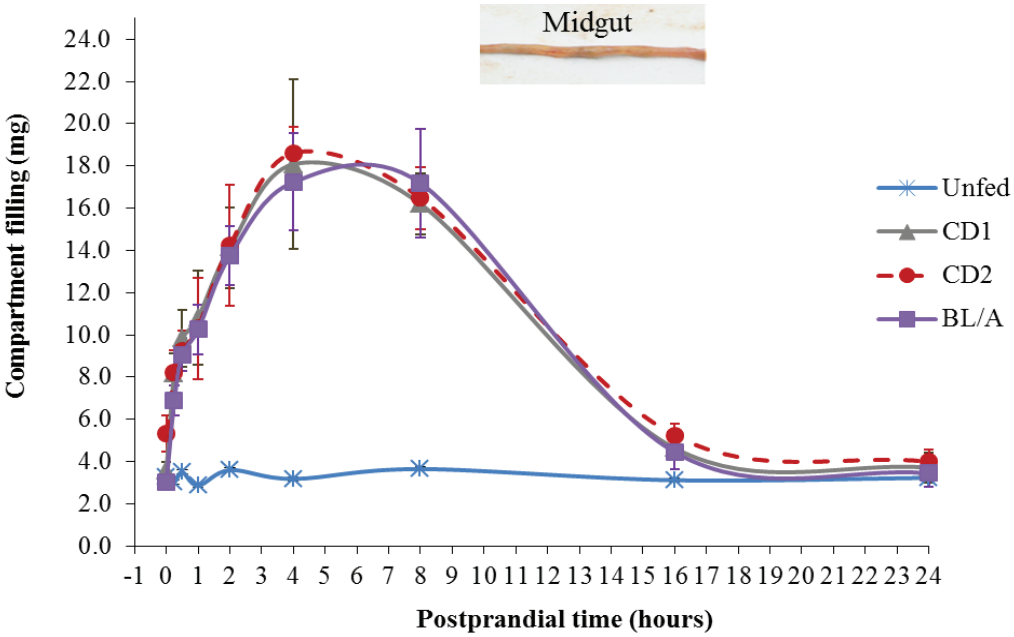

Fig. 3. Chyme content (dry mass) in midgut of juvenile cobia fed different diets postfeeding.

Data are presented as means (n=6) at selected time points after feeding. Sampling started from time 0/ just after cobia fed to statiety. Vertical bar indicates ±SEM.

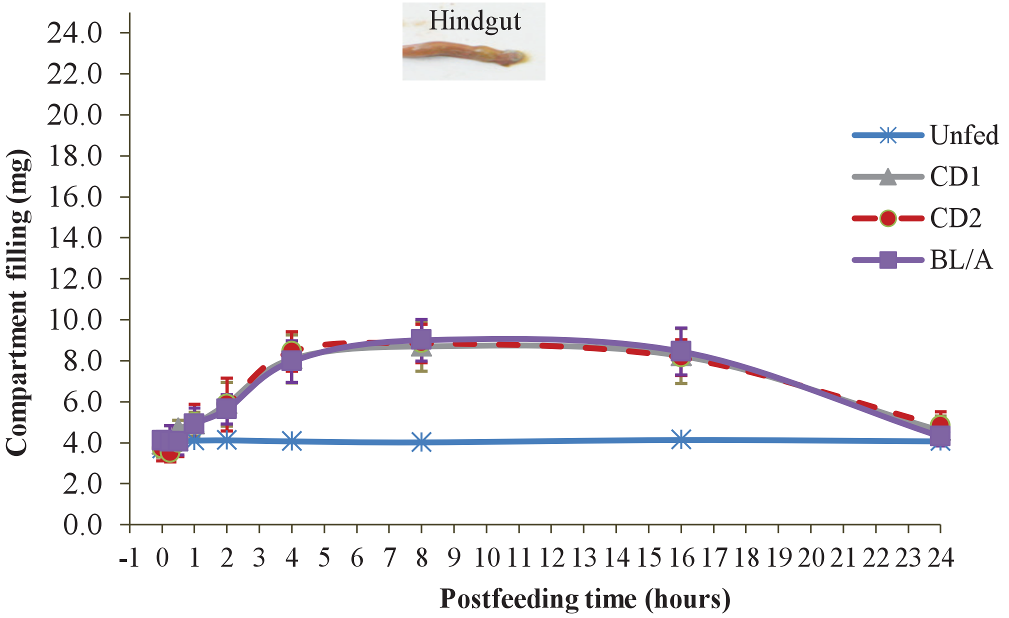

Fig. 4. Chyme content (dry mass) in hindgut of juvenile cobia fed different diets postfeeding.

Data are presented as means (n=6) at selected time points after feeding. Sampling started from time 0/ just after cobia fed to statiety. Vertical bar indicates ±SEM.

stored in the caeca appeared to be relatively small when stripping was tested, but these trials resulted in crushed tissue and unreliable and mixed matter (tissue and chyme). Also, the remaining content from GI-tract in 24-h and 48- h starved cobia shows that there was still some leftover chyme (unfed, Figs 2, 3, 4). The composition of this is not known, but might probably be indigestible matter with some bile due to the yellow color.

In the present experiment, cobia had consumed 5.3-5.4% body weight (BW) when they were first offered the CD1-, CD2- and BL/A diet. This indicated good palatability of all three diets when compared to the recommendations made by Sun and coworkers (2006) that feeding rate should be from 9% BW day-1 in cobia 10–20 g (41); and reduced to 2–3% BW day-1 in cobia of 100–200 g BW for better growth and feed efficiency (42). Replacement of fishmeal by plant protein sources in the diets may lead to imbalance in lysine to aginine ratio, and thus resulting in reduced palatability and/or digestibility, that consequently reduce growth performance in fish (Nguyen et al., 2013; Nguyen et al., 2014; Rumsey et al., 1994). In order to maximize growth and feed utilization in fish fed plant-based protein feed, a blend of plant protein ingredients is formulated in combination with supplementation of crystalline amino acids. By doing so, dietary amino acid profiles fulfill the requirement and/or mimic the amino acid profiles of the fishmeal-based diets (Espe, Lemme, Petri, & El-Mowafi, 2006; Espe, Mowafi, & Ruohonen, 2012). Understanding the rate of digestion in association with gastric evacuation rate may help to predict the return of appetite (Riche et al., 2004), and figure out appropriate feeding strategies for better feed intake and feed efficiency by administering food as soon as appetite has returned (Grove et al., 1978; Lee et al., 2000). Several mathematical models have been proposed to estimate gastric evacuation rate, for example linear model (Bromley, 1988; Tyler, 1970), exponential model (He & Wurtsbaugh, 1993; Riche et al., 2004; Stubbs, 1977), square root or linear model (Jobling, 1987; Lambert, 1985; Pandian, 1967). Though, there is still controversial as to which model would be the most appropriate applicable one due to the variation of factors affecting gastric evacuation rate. For example, Jobling (1987) proposed that small particles of a low energy density, e.g. zooplankton, were exponentially evacuated, while large particles of high energy density, e.g. fish prey, were linearly evacuated (Jobling, 1987). Plotting the gastric evacuation curves for the data obtained in the pilot experiment indicates an exponential relationship between the stomach content and the time postfeeding in cobia (Fig. 2). These findings were in line with the model proposed that cobia show gastric evacuation rate in an exponential function (He & Wurtsbaugh, 1993; Riche et al., 2004; Stubbs, 1977). However, feed makers were not available in the present study and cobia were fed pellets to satiety only one meal, thus the precision of the estimated model is limited. Further studies using inert indicators such as titanium dioxide (TiO_2) or ferric oxide (Fe_2O_3) (Riche et al., 2004; Richter, Luckstadt, Focken, & Becker, 2003) in combination with different diet composition and feeding regimes are required to accurately estimate the gastrointestinal transit kinetics in cobia.

The evacuation time of the ingesta through the GI-tract is in association with the absorption of nutrients following feeding (Dabrowski, 1983; Fletcher, 1984; Talbot, Higgins, & Shanks, 1984). Generally, cold water fish require longer time to achieve complete digestion than warm water fish species, consequently warm water fish show shorter evacuation time of ingesta though the GI-tract compared to cold water fish (Smith, 1989). Atlantic salmon showed gut transit time of 60 h (Talbot et al., 1984), while this in hybrids sarotherodon, *Oreochromiss niloticus x Sarotherodon areus* was 24 h (Ross & Jauncey, 1981). Time required for gastric evacuation in common dab, *Limanda limanda*, and black rockfish, *Sebastes melanops* was 15 h and 76 h, respectively (Brodeur, 1984; Fletcher, 1984). In the present experiment, about 80% of the stomach content had been evacuated to the lower part of the GI-tract at the 8 h postfeeding. The return of appetite is closely related to the GI emptying (Huebner & Langton, 1982; Sims, Davies, & Bone, 1996; Vahl, 1979). Hunger in satiety feeding fish recovers when 80-90% of the stomach content has been evacuated (Grove et al., 1978; Riche et al., 2004; Valen, Jordal, Murashita, & Rønnestad, 2011), as orexigenic signals in the GI-tract may increase when most of the content in the stomach evacuates, while anorexigenic signals decrease accordingly (Valen et al., 2011). Results from the pilot experiment, together with literature data suggested that the appetite in cobia have returned 8 h after satiety feeding. This was supported by the fact that cobia had good appetite in the 2nd feeding of the day. Time required for the return of appetite in cobia was within 8 h after feeding to satiation.

IV. Conclusion

Juvenile cobia had a high voluntary feed intake when offered the two commercial diets and the plant-based protein test diet. When fish were fed to satiation, most of the content in the stomach was emptied within 8 h post-feeding. This suggests that cobia tolerate moderate to high levels of plant ingredient inclusion. Further studies using nutrient markers are required to totally elucidate the utilization of crystalline lysine and arginine as well as the antagonism (if any) at absorptive and post-absorptive levels and catabolism of these two AAs in cobia.

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