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Feed digestion rates of tiger grouper *(Epinephelus fuscoguttatus)* juvenile

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Abstract. The objective of the present study was to evaluate the feed digestion of tiger grouper (*Epinephelus fuscoguttatus*) juvenile in stomach and intestine with different times. The experiment was conducted in Brackish Water Aquaculture Research Station at Ujong Batee, Aceh, Indonesia in May 2011. The observation was done at every 2 hours interval for 12 hours. The tiger grouper juveniles were obtained from the hatchery of Brackish Water Aquaculture Research Station. The samples were fed with the commercial pelleted feed of Otohime EP2®. The fish samples were taken randomly and sacrificed to obtain their stomach and intestine contents for proximate analysis of nutritional contents. The results showed that the digestion of proteins in the stomach was slower than in the intestine, while the optimum time of digestion for protein in the intestine was 10 hours post feeding, while 12 hours in the stomach. In addition, the optimum time of lipid digestion in the intestine was eight hours compared to 10 hours in the stomach. The carbohydrate components were not digested effectively by the tiger grouper juvenile.
Key Words: grouper, digestibility, pellet, proximate composition.

Introduction. Groupers are potentially important aquaculture species in Southeast Asia for their fast growth, efficient in feed conversion, and high market value (Boonyaratpalin 1997). Trash fish is presently the primary feed for grouper cultures, especially in Indonesia. However, according to Muhammadar et al (2011) the protein content of trash fish is lower than the commercial feeds. Therefore, the commercial feed is still playing a crucial role as a food source in large-scale grouper culture industry. A commercial pelleted feed has been used as one of the important feed items for grouper aquaculture since 1990. Decreasing supply, increasing cost and downstream environmental impacts (Baeverfjord et al 2006) are raising the demand for commercial feeds and in turn, a greater knowledge of the fish nutritional requirements.

Generally, meats and seed plant raw materials are formulated for fish feed (Agbugui et al 2010). However, not all materials in the feed are useful and can be digested by grouper juvenile. Since some materials have anti-nutritional factors present in legume seeds, for example, protease inhibitors, lectins, saponins and tannins limit their use as animal feed (Liener 1979). Undigested materials will be released into environment and affect the water quality and also increases the production costs. Warrer-Hansen (1982) reported that the percentage of unconsumed feed in Danish trout farm was approximately 10-30% for trash fish, moist pellets 5-10%, and 1-5% for dry feed. Lewell (1989) stated that duration time of digestion depends on fish species, fish size, composition of the meal consumed, and environmental conditions. In addition, Scocco et al (1997) and Olsson & Holmgren (2001) reported that the highest efficiency in the digestive process would be achieved only when a suitable amount of enzyme is produced, and there is enough time for completion of the hydrolysis. Hence, the objective of the present study was to evaluate the digestibility of commercial pelleted feed in the stomach and intestine of tiger grouper *Epinephelus fuscoguttatus* (Forsskål, 1775) juvenile.

Materials and Methods

Materials. The juvenile of tiger groupers were obtained from Brackish Water Aquaculture Station at Ujong Batee, Aceh, Indonesia. The experimental fish were 6-7 cm in size (total length) with 7.3-10.4 g of weights. The commercial pelleted feed of Otohime EP2® (size 2.3 mm) (Aquasonic Ltd, Australia) was used in this study.

Feeding and collection of stomach and intestine content. A total of 1000 tiger grouper juveniles were fasted for 18 hours to empty their stomach. The fish samples were then fed till satiation level (*ad libitum*) on 07:00 am. After feeding, 720 juveniles were taken randomly and transferred into four baskets (four replicates) and every basket was stocked with 180 juveniles. The digestion rate was examined for 12 hours with two-hour intervals. A total of 30 juveniles from each basket were taken randomly and sacrificed by spinal transaction. The digestive tract was removed and perforated with a small scissors to obtain the content of stomach and intestine. The perforation was done from the cardia to the pylorus organ of a stomach, while the intestine was perforated from the pylorus to the cloaca. The stomach and intestine contents were collected for proximate analysis.

Analysis of stomach and intestine contents. Proximate compositions of the stomach and intestine contents were carried out according to AOAC (1990). Dry matter was calculated by weight loss after 72 hours at 70^{o}C. Crude protein was measured using the Kjeldahl technique (AOAC 1990). Crude lipid was measured after the chloroform-methanol extraction. Samples were homogenized with a high speed homogenizer for 5 min and the lipid was determined gravimetrically after solvent separation.

Data analysis. The data were subjected to one-way analysis of variance (ANOVA), followed by comparison of means using Duncan's multiple range test to determine significance of each data (Dytham 2003; Bujel 2008). All statistical analyses were performed using SAS software 9.1 (Institute Inc., Cory, INC. 27513, USA).

Results and Discussion. Proximate compositions of stomach content within 12 hours have ranged between 43.33% to 55.10% and 5.70% to 17.28% for protein and lipid, respectively (Table 1). In addition, the proximate composition in the intestine within 0-12 hours have ranged between 34.94% to 55.10% and 3.25% to 17.28%, for protein and lipids, respectively (Table 2). The composition of protein and lipid in the stomach and intestine were significantly different during the 0-12 hours period of digestion ($p < 0.05$). However, the carbohydrate contents were not significantly different both in stomach and intestine among the time periods.

Percentage of protein content of the pellets before entering the stomach and intestine at 0 hours was 55.10%, and during the time intervals, between treatment of digestive at 0 hours and 10 hours, the declining has differed significantly ($p < 0.05$). The proteins were digested immediately after the feeding; it was indicated by decreasing of protein values between from 0 to 2 hours after feeding, for example 0 hour after feeding the protein content was 55.10%, and the value has decreased to 52.5% two hours after feeding. However, the percentage declines of protein in the interval time from zero to four hours were not significantly different ($p > 0.05$). This indicates that the digestion of protein at the first 4 hours of time interval is expected to be an adaptation period in feeding, and the digestion was continuous after this period. However, the percentage of protein in the stomach continues to decline slowly over time intervals of 6 hours to 10 hours, while the protein content has decreased significantly after 12 hours of feeding.

This results indicated that during the initial entry of food into the stomach, the digestibility of protein was slow and the longer the food in the stomach the higher digestion rate occurred. In the stomach, the protein was digested with the help of HCl (in lower pH condition) and pepsin hydrolyses and finally converted into peptides to be absorbed by the body (Lewell 1989).

Table 1

The digestibility rates of protein, lipid and carbohydrate in the stomach in different times after feeding. The mean values (Mean ± SD) in the same column followed by a different superscript indicate significant difference at $p < 0.05$

Treatment: interval times	*Proximate analysis*		
	Protein (%)	*Lipid (%)*	*Carbohydrate (%)*
0 hour	55.10 ± 1.02^{a}	17.28 ± 1.00^{a}	12.12 ± 1.48^{a}
2 hours	54.21 ± 0.44^{ab}	14.01 ± 0.97^{b}	9.40 ± 2.36^{a}
4 hours	53.85 ± 0.42^{ab}	13.61 ± 0.60^{b}	8.82 ± 1.36^{a}
6 hours	53.15 ± 0.78^{bc}	11.53 ± 0.96^{c}	8.80 ± 1.69^{a}
8 hours	52.07 ± 1.31^{cd}	11.38 ± 1.22^{c}	8.71 ± 1.92^{a}
10 hours	51.14 ± 0.98^{d}	6.02 ± 1.42^{d}	8.48 ± 3.08^{a}
12 hours	43.33 ± 0.62^{e}	5.70 ± 0.96^{d}	8.33 ± 2.99^{a}

Table 2

The digestibility rates of protein, lipid and carbohydrate in the intestine in different times after feeding. The mean values (Mean ± SD) in the same column followed by a different superscript indicates significant difference at $p < 0.05$

Treatment: interval times	*Proximate analysis*		
	Protein (%)	*Lipid (%)*	*Carbohydrate (%)*
0 hour	55.10 ± 1.02^{a}	17.28 ± 1.00^{a}	12.12 ± 1.48^{a}
2 hours	52.60 ± 1.02^{b}	13.63 ± 1.05^{b}	9.05 ± 1.47^{b}
4 hours	48.79 ± 1.19^{c}	11.75 ± 1.42^{c}	8.50 ± 1.70^{b}
6 hours	47.17 ± 0.63^{d}	9.84 ± 0.49^{d}	8.25 ± 1.59^{b}
8 hours	42.68 ± 0.51^{e}	4.57 ± 1.09^{e}	8.22 ± 1.54^{b}
10 hours	35.94 ± 0.55^{f}	3.47 ± 0.66^{e}	7.83 ± 0.91^{b}
12 hours	34.94 ± 0.53^{f}	3.25 ± 0.47^{e}	7.79 ± 0.47^{b}

Approximately 10 to 12 hours post feeding, feed in the intestine was not well digested; hence we presume that the optimum digestion time of protein in the intestine is 10 hours post feeding. According to Andersen (1998), the estimation time of the digestion process for protein in the gut of tilapia juvenile was 7.15 hours post feeding and it was considered a short time compared to other species for example, 20-60 hours and 5-17 hours for whiting *Merlangus merlangus* (Linnaeus, 1758) and burbot *Lota lota* (Linnaeus, 1758), respectively (Paakkonen & Marjomaki 1999). Moreover, Nilsson & Bronmark (2000) reported that the optimun digestion of protein for Northern pike *Esox lucius* (Linnaeus, 1758) was up to 48 hours. The rate of feed digestion is probably affected by type of feeding habit of fish and type of raw materials which was formulated in the feed. However, Humpback groupers are able to digest the protein effectively both from plants and animals resources (Laining et al 2004).

The feed lipid content in the stomach has decreased slowly and it was significantly different within four hours interval, for example the lipid content was different significantly between two and six hours post feeding, while there were no significant differences between two and four hours post feeding, even between six and eight hours after feeding. This finding is in agreement with Logothetis et al (2001) and Smith & Paulson (1974) who reported that passage time of feed in the alimentary tract of top smelt *Atherinops affinis* (Ayres, 1860) and some parrotfishes (*Scarus gibbus* (Ruppell, 1828) and *Scarus jonesi* (Streets, 1877)) were 6 hours interval, respectively. In the intestine, the lipid has started being digested at 0 hours up to 12 hours, and the lipid values have decreased abruptly then after.

The optimum lipid digestion was at 10 hours post feeding and became slower after 10 hours. In general, the lipid digestion in stomach was slower compared to in intestine. Likely protein, the rate of lipid digestion was determined by enzyme activity. According to Steffens (1989) the lipases are the most important enzymes for lipid hydrolysis in the intestine of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), however lipase activity in stomach is relatively lower.

In regard to carbohydrates, the digestion of carbohydrates in the stomach and intestine have probably occurred after 12 hours, because no significantly differences of carbohydrate contents among observed times were detected ($p > 0.05$). Generally, both in stomach and intestine, it was shown that digestion of carbohydrate took longer times than protein or lipid components. This may be caused for carbohydrate is a complex molecule and difficult to digest by the fish juvenile. Generally, freshwater and warmwater fishes appear to digest carbohydrates more effectively than marine fishes and cold water fishes (Wilson 1994). Furthermore, Laining et al (2004) reported that humpback grouper have very limited capacity to digest carbohydrate-rich products found in many plant feedstuffs, and shrimp head meal.

Conclusions. Proteins were digested effectively six 6 hours post feeding; this was slower compared to the intestine (two hours). The optimum protein digestion time was eight hours after the food entered the alimentary tract. The lipid digestion process in the stomach and intestine were approximately two hours after feeding. In addition, the carbohydrate was not effectively digested by tiger grouper juvenile.

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