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## Crude protein and amino acids content in some common feeds of tiger grouper (*Epinephelus fuscoguttatus*) juvenile

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**Abstract.** The objective of present study was to determine the nutrient contents of natural feed of tiger grouper (*Epinephelus fuscoguttatus* (Forsskål, 1775)) especially for crude protein and amino acids content. Five types of feed which have been commonly used by farmers were tested in this study, namely shrimp (*Mesopodytes sp.*), Love Larva® pellet, EP2® pellet, anchovies (*Stolephorus spp.*) and tilapias (*Oreochromis mossambicus* (Peters, 1852)). The result showed that the average content of crude protein were 46.51% in shrimp, 50.16% in pellet Love Larva®, 55.00% in pellet EP2®, 52.54% in anchovies and 45.16% in tilapias. The result also showed that EP2® pellet comprised significantly higher protein content than other diets ( $p < 0.05$ ). The major essential amino acid in all of five feed types were Lysine (2.2 to 3.7%), Leucine (2.5 to 3.6%) and Arginine (2.4 to 3.4 %). The major non essential amino acid in all five feed types were Glutamic acid (range from 4.7 to 6.5%) and Aspartic acid (range from 3.1 to 4.7%). Pellet EP2® had highest amino and Aspartic acid compared to other diets whereas higher Tyrosine was detected in shrimp.

**Key Words:** Aceh Province, type of feeds, crude protein, amino acids content.

**Introduction.** The tiger grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775) is one of the important fishery commodities in Asian countries. It is very popular for seafood, and is cultured intensive and extensively in Malaysia, Indonesia and Hongkong (Teng et al 1978; Liu & Mitcheson 2008; Sugama et al 2008). In Indonesia especially in Aceh Province, extensive and semi intensive aquaculture is commonly practice and trash fish is usually used as feed item for the fishes.

The commercial pellets are the most effective feed for grouper because it comprises all nutritional components required by the fish. The most common commercial feed are EP2® and Love Larva® pellets. However, the pellets are rarely used by farmers because its price is relatively high and considered costly for traditional aquaculture or extensive and semi-intensive scales. Therefore, the development of mass culture of grouper in Indonesia is still infancy.

In Aceh Province, Indonesia, most farmers used shrimp (*Mesopodytes sp.*), anchovies (*Stolephorus spp*) and tilapias (*Oreochromis mossambicus* (Peters, 1852)) as alternative feeds to reduce the production cost of grouper. However, the composition of nutrients content of these natural feeds were unknown. Moreover, the composition of common artificial feeds (EP2® and Love Larva® pellets) were not reported yet. Therefore, in the present study we attempt to evaluate the nutrients content of some natural and artificial feeds which have been commonly used for tiger grouper culture with the objective of obtaining the best alternative feed for tiger grouper juvenile.

## Material and Method

**Sampling.** Protein and amino acid profiles of five feeds of grouper (*E. fuscoguttatus*) i.e. shrimp (*Mesopodytes* sp.), Love Larva® and EP2® pellets, anchovies (*Stolephorus* spp) and tilapias (*O. mossambicus*) were evaluated. The feeds were collected from the nearby farm and transported to laboratory. The feeds were then washed and dried at room temperature prior to further analysis.

**Crude Protein and Amino Acids Analysis.** Protein content of the dried feeds (shrimp, Love Larva® and EP2® pellets, anchovies and tilapias) were determined using Kjeldahl method (AOAC 1990). A total of 0.2 grams of powdered samples were added with 6N hydrochloric acid (HCl) in borosilicate test tubes. The tubes were covered with parafilm and masked tape tightly. The samples were placed in a furnace at temperature of 110°C for 24 hours. The samples were then cooled at room temperature for 20 minutes. A total of 400 µL of alpha amino butyric acid (AABA) was added into sample and filtered with Sartorius stedim filter paper (Biotech, 1288) into 100 mL flask. The samples were then diluted with deionized water and then filtered with aqueous syringe nylon filter 0.2 µm. A total of 2 mL filtered sample were cooled at -20°C prior to amino acid analysis using HPLC standart procedure.

**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). All statistical analyses were performed using SPSS software v14 (SPSS Inc., Chicago, IL, USA).

## Results and Discussion

**Crude protein.** The ANOVA test showed that the effect of different feed types on the crude protein levels were significant ( $p < 0.05$ ). The Duncan multi range test showed that the higher crude protein content was found in EP2® pellet and it was significantly different than other diets ( $p < 0.05$ ). However, the crude protein content in the shrimp was not significantly different than tilapia ( $p > 0.05$ ) (Figure 1).

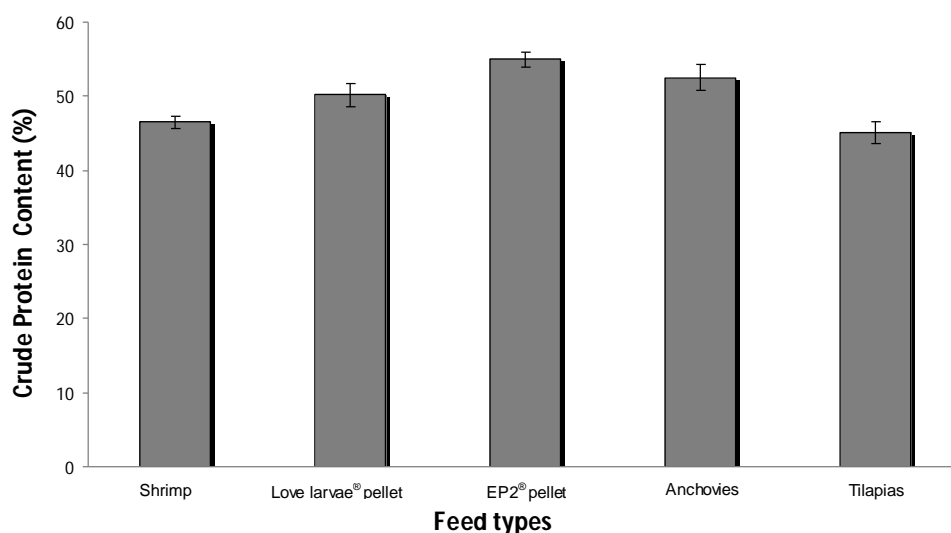


Figure 1. Crude protein content of five feeds for juvenile tiger grouper (different letters indicate significant different at  $p < 0.05$ ).

The average of crude protein content (CP%) of the feeds were 46.51%, 50.16%, 55.00%, 52.54% and 45.16% for shrimp, Love Larva® pellet, EP2® pellet, anchovies and tilapia, respectively. According to Lewell (1989) the optimum protein levels for fish have ranged from 25% to 50%. Therefore, in general the crude protein contents in tested diets were higher and sufficient for the fish. According to Docan et al (2011) the feed

quality is an important factor to ensure the growth and survival of the cultured fishes. Moreover, the carnivorous fishes need higher protein requirements than herbivorous or omnivorous. For instance, Malabar grouper (*Epinephelus malabaricus* (Bloch & Schneider, 1801)) needs protein with a range from 40% to 50% and 45-52% for other grouper spp. (Boonyaratpalin 1997).

Furthermore, the optimum dietary protein level for *E. malabaricus* was ranged from 40% to 50 (Teng et al 1978). In addition, humpback grouper (*Cromileptes altivelis* (Valenciennes, 1828)) and Malabar grouper (*E. malabaricus*) required a dietary protein of 51% and 55%, respectively (Usman et al 2005; Tuan & Williams 2007). Hence, we assumed that shrimp, anchovies and tilapia were suitable for alternative diet of tiger grouper juvenile. This is in agreement with Bombeo-Tuburan et al (2001) who reported that *Oreochromis sp.* can be used as a feed for juvenile grouper (*Epinephelus coioides* (Hamilton, 1822)). In addition, Sugama et al (2008) reported that shrimp (*Mesopodytes sp.*) can be consumed effectively by the grouper juvenile. However, further studies are needed to evaluate the role of these diets on the survival rate and growth performance of tiger grouper.

**Essential amino acids.** Essential amino acid profiles of five feeds were showed in Table 1. The major amino acids in the feeds were Lysine (2.2 to 3.7%), Leucine (2.5 to 3.6%) and Arginine (2.4 to 3.4 %). In general, the EP2<sup>R</sup> has higher essential amino acids level compared to other diets.

The results showed that the alternative feeds tested in this study had nine types of essential amino acids. However, the values of essential amino acids were not significantly different among the feeds for Arginine, Threonine, Valine, Methionine, Isoleucine, Leucine, and Phenylalanine ( $p > 0.05$ ). The higher Histidin and Lysine were found in the EP2<sup>®</sup> pellet. There was significantly difference between EP2<sup>®</sup> pellet with other diets ( $p < 0.05$ ), however, no significantly differences were observed among the EP2<sup>®</sup> pellet shrimps, Love Larva<sup>®</sup> pellet and anchovies for Lysine ( $p > 0.05$ ), however, these values were significantly different than tilapia ( $p < 0.05$ ) (Table 1).

Actually, fishes require ten types of essential amino acids namely; Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Theorine, Tryptophan and Valine (Chau et al 2008; Lewell 1989). However, only nine esesensial amino acids were recorded in this study where Tryptophan was not detected by this method. However, the level of each essential amino acids requirement are different from species to species. For instance, juvenile of red sea bream (*Pagrus major* (Temminck & Schlegel, 1843)) requires Histidine, Lysine and Leucine of 12.8 mg<sup>-1</sup>, 40.6 mg<sup>-1</sup> and 41.1 mg<sup>-1</sup>, respectively (Koshio 2002). For Nile tilapia, the Histidine, Lysine and Leucine requirements were 1.7%, 5.1% and 2.8-3.6%, respectively (Santiago & Lewell 1988). However, the Histidine, Lysine and Leucine requirements for wild juvenile of estuary grouper (*E. coioides*) were 1.62%, 6.49% and 6.16%, respectively (Alava et al 2004; Toledo et al 2004).

**Non essential amino acids.** The profile of seven non essential amino acids (Aspartic acid, Serine, Glutamic acid, Proline, Glycine, Alanine and Tyrosine) from five tested feeds (shrimp, Love Larva<sup>®</sup> and EP2<sup>®</sup> pellet, anchovies and tilapia) are presented in the Table 2. The major non essential amino acid in all feeds were Glutamic acid (ranged from 4.7 to 6.5%) and Aspartic acid (ranged from 3.1 to 4.7%). The Duncan's multi range test showed that there were no significantly differences among the feeds for Serine, Glutamic acid, Proline, Glycine and Alanine ( $p > 0.05$ ). The higher Aspartic acid was found in the EP2<sup>®</sup> pellet, but it was not significantly different than shrimp, Love Larva<sup>®</sup> pellet and anchovies ( $p > 0.05$ ), while the higher Tyrosine was detected in shrimp, but it was not significantly different than Love Larva<sup>®</sup> pellet, EP2<sup>®</sup> pellet and anchovies ( $p > 0.05$ ). Overall, EP2<sup>®</sup> pellet had higher contents of Aspartic acid, Serine, Glutamic acid, Proline and Alanine, while shrimp and anchovies had higher content in Tyrosine and Glycine, respectively.

Table 1

Percentage of essential amino acid contents in the various alternative feeds of tiger grouper (*E. fuscoguttatus*).  
Mean of values in the same column followed by a different superscript are significant different ( $p < 0.05$ ).

Feed	HIS	ARG	THR	VAL	MET	LYS	ILE	LEU	PHE
A	0.85±0.1 <sup>a</sup>	2.46±0.4 <sup>a</sup>	1.59±0.3 <sup>a</sup>	2.22±0.3 <sup>a</sup>	1.09±0.2 <sup>a</sup>	2.59±0.3 <sup>ab</sup>	2.15±0.3 <sup>a</sup>	3.08±0.4 <sup>a</sup>	1.97±0.3 <sup>a</sup>
B	1.03±0.2 <sup>ab</sup>	3.19±0.1 <sup>a</sup>	1.87±0.1 <sup>a</sup>	2.19±0.1 <sup>a</sup>	1.37±0.0 <sup>a</sup>	2.94±0.1 <sup>ab</sup>	2.19±0.0 <sup>a</sup>	3.27±0.1 <sup>a</sup>	1.20±0.0 <sup>a</sup>
C	1.03±0.2 <sup>ab</sup>	3.19±0.1 <sup>a</sup>	1.87±0.1 <sup>a</sup>	2.19±0.1 <sup>a</sup>	1.37±0.0 <sup>a</sup>	2.94±0.1 <sup>ab</sup>	2.19±0.0 <sup>a</sup>	3.27±0.1 <sup>a</sup>	1.20±0.0 <sup>a</sup>
D	0.96±0.3 <sup>ab</sup>	3.21±1.0 <sup>a</sup>	1.99±0.6 <sup>a</sup>	2.16±0.7 <sup>a</sup>	1.41±0.4 <sup>a</sup>	3.14±0.9 <sup>ab</sup>	1.97±0.6 <sup>a</sup>	3.23±1.0 <sup>a</sup>	1.86±0.6 <sup>a</sup>
E	0.88±0.0 <sup>a</sup>	2.40±0.1 <sup>a</sup>	1.52±0.1 <sup>a</sup>	1.95±0.1 <sup>a</sup>	1.09±0.0 <sup>a</sup>	2.16±0.2 <sup>a</sup>	1.67±0.1 <sup>a</sup>	2.54±0.2 <sup>a</sup>	1.51±0.0 <sup>a</sup>

Note: A = shrimp, B = Love Larva<sup>®</sup> pellet, C = EP2<sup>®</sup> pellet, D = anchovies, E = tilapias, HIS = Histidine, ARG = Arginine, THR = Threonine, VAL = Valine, MET = Methionine, LYS = Lysine, ILE = Isoleucine, LEU = Leucine, PHE = Phenylalanine.

Table 2

Percentage of non essential amino acid contents in the various alternative feeds of tiger grouper (*E. fuscoguttatus*).  
Mean of values in the same column followed by a different superscript are significant different ( $p < 0.05$ ).

Feed	ASP	SER	GLU	PRO	GLY	ALA	TYR
A	3.86±0.6 <sup>ab</sup>	1.58±0.3 <sup>a</sup>	5.69±0.7 <sup>a</sup>	1.75±0.4 <sup>a</sup>	2.45±0.3 <sup>a</sup>	2.59±0.3 <sup>a</sup>	1.67±0.2 <sup>b</sup>
B	4.13±0.1 <sup>ab</sup>	1.82±0.1 <sup>a</sup>	5.67±0.2 <sup>a</sup>	1.90±0.1 <sup>a</sup>	2.16±0.1 <sup>a</sup>	2.40±0.1 <sup>a</sup>	1.59±0.2 <sup>ab</sup>
C	4.72±0.4 <sup>b</sup>	1.94±0.0 <sup>a</sup>	6.54±0.1 <sup>a</sup>	2.14±0.1 <sup>a</sup>	2.81±0.0 <sup>a</sup>	2.99±0.0 <sup>a</sup>	1.54±0.0 <sup>ab</sup>
D	3.89±1.1 <sup>ab</sup>	1.93±0.6 <sup>a</sup>	5.87±1.7 <sup>a</sup>	2.10±0.7 <sup>a</sup>	2.89±1.0 <sup>a</sup>	2.65±0.8 <sup>a</sup>	1.53±0.3 <sup>ab</sup>
E	3.11±0.1 <sup>a</sup>	1.44±0.0 <sup>a</sup>	4.72±0.2 <sup>a</sup>	2.10±0.1 <sup>a</sup>	3.18±0.1 <sup>a</sup>	2.69±0.3 <sup>a</sup>	1.06±0.1 <sup>a</sup>

Note: A = shrimp, B = Love Larva<sup>®</sup> pellet, C = EP2<sup>®</sup> pellet, D = anchovies, E = tilapias, ASP = Aspartic acid, SER = Serine, GLU = Glutamic acid, PRO = Proline, GLY = Glycine, ALA = Alanine, TYR = Tyrosine.

According to Ghassem et al (2009) Glutamic acid and Aspartic acid were the most important essential amino acids for *Channa striatus* (Bloch, 1793), *Pangasianodon hypophthalmus* (Sauvage, 1878), *Clarias batrachus* (Linnaeus, 1758) and *Oreochromis niloticus* (Linnaeus, 1758). According to Lewell (1989) insufficient essential amino acids in the feed can be replaced by non-essential amino acid. For example, Cysteine can replace about 60% of the Methionine. In addition, Tyrosine can replace around 50% of the Phenylalanine requirement for channel catfish.

**Conclusions.** The average content of crude protein contents (CP%) established in the feeds were shrimp (46.51%), Love Larva® pellet (50.16%), EP2® pellet (55.00%), anchovies (52.54%) and tilapia (45.16%). The EP2® pellet has highest protein content and it was significantly different compare to other diets. The major essential amino acid in all of the feeds were Lysine (2.2 to 3.7%), Leucine (2.5 to 3.6%) and Arginine (2.4 to 3.4 %). On average, EP2® pellet had highest amino acid level compared to other diets. The major non essential amino acid in the feeds were Glutamic acid (range from 4.7 to 6.5%) and Aspartic acid (range from 3.1 to 4.7%). In addition, the higher Aspartic acid was found in the EP2® pellet, while the higher Tyrosine was detected in shrimp.

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