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Influence of Different Protein Doses on Digestibility Levels and Food Retention in Reared Eel Fish (*Anguilla* sp.)

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ABSTRACT

The purpose of the study was to examine how various protein compositions affected the digestibilities and retention of nutrients during eel farming in controlled tanks. The average size of the eel fish samples used was 70± 1.53g/ ind. This study used a completely randomized design with four treatments and three replications. The protein content of treatment A's feed was 35%, treatment B's was 40%, treatment C's was 45%, and treatment D's was 50%. The variables that were observed included water quality, protein and fat retention, in addition to the digestibility of proteins and their overall composition. The W-Tukey Test and analysis of variance were employed in the data analysis. The feed treated with 50% protein content, or 92.01%, had the maximum protein digestibility, according to the data. The feed with a protein content of 35 and 50% had the highest level of feed digestibility of fat, which was 89.37. Additionally, the highest values of nutrition retention and extract components without nitrogen digestibilities were found in feed with 50% protein content, respectively, at 81.31 and 26.54%. In addition, the feed recorded the highest fat retention and a protein level of 14.37 at 45%. In general, every treatment used throughout the study fell within the range of water quality that is appropriate for eel fish growth and survival.

INTRODUCTION

The fish known as eels (*Anguilla* spp.) move and live their whole lives in freshwater environments, particularly rivers, before migrating to the ocean to breed **(Hovarth & Municio, 1998)**. According to **McKinnon (2006)**, there are five phases in the life cycle of an eel: Larvae (leptocephalus), eel fish seeds (glass eels), pigmented eels (elver), juvenile eels (yellow eels), and adult eels (silver eels). The fish will be prepared to breed and return to the sea after maturing into adult eel fish **(Dou & Tsukamoto, 2003; Tesch & Rohlf, 2003; Linton *et al*., 2007; Aoyama, 2009; Topan & Riawan, 2015; Arai & Kadir, 2017)**.

Eel is a fish individual with unique habitat characteristics, namely inhabiting several water conditions including freshwater, estuary and sea. The life cycle of eel is catadromous, spawning in the sea, and sunsequently the larvae migrate to rivers, and reach adulthood in freshwaters. When they are about to reproduce, the eels return to the sea to spawn **(Tesch, 2003)**. Migration is an important part of fish life cycle for the continuity of the regeneration process **(Lucas & Baras, 2001)**. The eel (*Anguilla* sp.) is a catadromous fish in which the adult fish migrate from fresh water to the sea for spawning, and the larvae return to freshwater to grow **(Hakim *et al.*, 2015)**. Catadromous fish migrate between fresh and marine waters **(Tesch *et al.*, 2003)**. According to **Silfvergrip (2009)**, young eels experience body pigmentation and are less than 200mm in size, whereas adults are more than 200mm in size.

Eel fish is a type of fish with a high level of demand in international markets, especially in Japan, Hong Kong, Germany, Italy, Taiwan and Korea, hence this fish has the potential to be an export commodity **(Affandi, 2005)**. Worldwide there are as many as 18 species of eel **(Miller *et al.*, 2009)**; in Indonesia, there are at least seven species of eel **(Fahmi *et al.*, 2012)**. Eel fish in Indonesian waters are spread across Java, Kalimantan, Bali, Sumatra, Nusa Tenggara, Sulawesi, Papua, Maluku **(Fahmi, 2015)**. Eel seeds are commonly found in river estuaries facing the open sea **(Sugianti, *et al.*, 2020)**. Environmental conditions, including water level and water temperature, affect the abundance of eel seeds **(Mckinnon & Gooley, 1998; Edeline *et al.*, 2006; Arai & Kadir, 2017)**. **Arai and Chino (2018)** stated that adult Pacific eels, *Anguilla bicolor* prefer to live in an environment with high salinity. This condition is different from *Anguilla marmorata* which can live in environments with varying salinity **(Arai & Chino, 2012)**.

The usage rate of eels tends to rise annually since they are one of the fisheries products that have significant economic worth and open markets, particularly for export **(Widyasari, 2013)**. Eel fish individual has the potential to be an export item due to its high nutritional content, which gives it significant economic worth and high demand on the global market **(Widyasari, 2013; Nafsiyah *et al.*, 2018)**. China and Japan continue to dominate the world market for eels, despite the fact that they are currently facing a problem with the decline in eel fish populations (elver/ glass eels) **(Liviawaty & Afrianto, 1998)**. A greater number of eels are being caught in the wild due to the strong demand for eels as a food source, both locally and internationally **(Fadilla *et al.*, 2022)**. Moreover, the potential for Indonesian eels is fairly significant; however, it has not been fully realized **(Chilmawati *et al.*, 2017)**. Regarding its tropical environment, Indonesia boasts natural resources that facilitate the growth of eels **(Sasongko *et al.*, 2007)**. Additionally, *Anguilla bicolor bicolor* is a variety of eel that has been widely cultivated in Indonesia, according to **Nawir *et al*. (2015)**.

The main problem in eel fish cultivation is slow growth and high feed conversion or low nutrient utilization efficiency. Eel is a type of fish whose growth is slow due to its low digestibility **(Nawir *et al.*, 2015; Perdana *et al.*, 2016; Chilmawati *et al.*, 2017)**. However, growth occurs when the amount of feed consumed is greater than the basic needs used for fish survival **(Idris, 2016)**. Eminently, efforts to overcome this problem have been made such as fish feed management. Feed that meets nutritional needs affects markedly the speed of growth **(Perdana *et al.*, 2016)**. The quality of feeding and its adequacy form one of the determining factors for increasing eel's growth **(Idris, 2016)**. Additionally, the main component of feed that is needed for aquaculture (kept aquatic animals) to grow and develop is protein **(Sahwan, 2003)**. Protein is one of the macro-nutrients that determines feed quality; it has a major role in fish growth since it is the largest body component of meat, which is around 65– 75% of the total dry weight and functions as a building material for body tissues **(Halver & Hardy, 2002)**. According to **Nawir *et al*. (2015)**, the provision of protein content and protein energy ratio in feed has a different, significant effect on survival, hepatosomatic index, ash content, and extract ingredient without nitrogen of the eel body. **Bai (2012)** stated that the optimum protein phase of the juvenile eel fish, *A. japonica*, was 44% with a protein- energy ratio of 24.1mg protein/ kJ. While, the optimum feed content for *A. marmorata* eel fish with a size of 2.29g is 50%, and for the size of 21.97g, it is 45% with the best amount of metabolic energy of 347kcal /100g **(Cheng *et al*., 2013)**. Utilization of protein content and fish growth can be optimized by giving the right protein- energy ratio **(Kaushik & Seiliez, 2010)**. An enhancement in the amount of feed consumption with an enhancement in protein content was also reported in juvenile bluefin trevally *Caranx melampygus* **(Suprayudi *et al*., 2013)**.

Fish nutritional needs are influenced by various factors, including fish species, fish age/ fish size, protein quality, feed making process, feed digestibility and environmental conditions **(Watanabe, 1988)**. The high or low specific growth rate is influenced by the feed consumed by fish **(Perdana *et al*., 2016)**. Furthermore, the digestibility of a feed is influenced by the physiological state of the fish; namely, the presence of enzymes in the digestive tract and feed particles, which will then be converted into energy and body tissue. Enzymes in the body are responsible for the digestibility of feed, viz. fat, protein and carbohydrate nutrients. These nutrients will then be used as a source of energy and growth of body tissues **(NRC, 1983; NRC, 2011)**. Food that enters the digestive tract will be digested into simple micro-sized compounds, where amino acids are hydrolyzed into simple amino acids or peptides, fats into glycerol and fatty acids and carbohydrates into simple sugars **(Halver, 1988)**.

Digestibility and protein retention are parameters of feed utilization efficiency, which are very important in eel fish cultivation, and research on these parameters has not been widely carried out. Hence, this study aimed to analyze the effect of protein content on the digestibility value and protein retention in reared eel fish.

MATERIALS AND METHODS

Making the feed, rearing of fish under study, and the analysis of the nutritional content of feed were carried out in the Nutrition Chemistry Laboratory of Politeknik Pertanian Negeri Pangkep. The research extended from May to August 2020. Fish eels samples with an average weight of 70± 1.53g/ fish were obtained from Poso Regency, Central Sulawesi. The fish specimens were adapted for 20 days before being given the test feed. Each pond was filled with 20 test fish. The test feed used was in the form of pellets with the formulation, as shown in Table (1). The test fish were given feed at satiation twice a day, namely at 05.30 and 19.00. The nutritional content of the test feed is presented in Table (1). Fish enlargement ponds used are 12 pools made of fiber, rectangular in shape with a volume of 1000L. The pond is filled with filtered well water, with a filterbag as much as half of the pond's volume. The pond is filled with water by a recirculation system. The pond is equipped with a set of aeration to supply oxygen to the test fish. The study was designed using a completely randomized design, with 4 treatments and 3 replications. The treatment involved feed with different protein content percentages as the following:

Treatment A: 35%
Treatment B: 40%
Treatment C: 45%
Treatment D: 50%

Table 1. Raw material composition and nutritional content of the test feed

Feed raw materials	Protein content			
	35% (A)	40% (B)	45% (C)	50% (D)
Casein	9	13	16	19
Fish flour	25	30	35	40
Cornflour	14	11	8	7
Soy flour	13	9	9	8
Shrimp head flour	6	6	6	6
Fine bran	17	15	10	4
Fish silage	5	5	5	5
Fish oil	4	4	4	4
Vitamins and mix minerals	2	2	2	2
Carboxymethylsellulose	5	5	5	5
Nutrition composition				
Water (%)	12,6	12,1	11,2	10,6
Crude protein (%)	36,3	40,9	45,6	50,6
Crude fat (%)	7,8	5,3	6,0	4,9
Crude fiber (%)	3,4	4,1	4,1	2,3
Extract ingredient without nitrogen (%)*	27,9	25,4	20,3	18,9
Ash (%)	12,0	12,3	12,9	12,8
Total energy (kkal/kg)	3930	3845	3974	4090

* Extract ingredient without nitrogen: determined by calculation

A protein digestibility test and total feed digestibility were conducted by adding 0.6% Cr_2O_3 indicator in the test feed, which serves as a marker following the method of **NRC (1993)**. Collection of fish faeces was carried out after 6 days of giving the test feed. The collected feces were then placed into a film bottle and stored in the freezer to maintain freshness. Afterward, they were dried in an oven at 110^{o}C for 4- 6 hours. The digestibility value of feed nutrients was calculated based on the equation of **Takeuchi (1988)**, as follows:

$$\textbf{Digestibility of nutrients} = \left(1 - \frac{a}{a'} x \frac{b'}{b}\right) x\ 100\% \qquad (1)$$

Where,
a = % Cr_2O_3 in feed
a´ = % Cr_2O_3 in faeces
b = % Nutrition in feed
b´ = % Nutrients in faeces

The protein retention value is calculated based on the equation of **Takeuchi (1988)**:

$$\textbf{NR} = \left(\frac{F-I}{P}\right) x\ 10 \qquad (2)$$

Where,
NR = Nutrition retention (%)
F = Amount of fish body nutrition at the end of rearing (g)
I = Amount of fish body nutrition at the beginning of rearing (g)
P = Amount of nutrients consumed by fish (g)

During the study, several water quality parameters were measured, including temperature (^oC), pH, dissolved oxygen (ppm), and ammonia (ppm). Other parameters, such as digestibility of protein and fat, total digestibility, retention of protein and fat, were also measured. Statistical analysis including variance was conducted if there were any differences between treatments, followed by the W-Tukey test, as indicated by **Gasperz (1991)**.

RESULTS AND DISCUSSION

1. Digestibility of feed protein

The nutrient digestibility value is expressed by the amount of nutrients in a material or energy that can be absorbed and used by fish (Aquatic animals raised). Protein digestibility in tested fish fed various protein levels is shown in Fig. (1). The protein digestibility of the tested fish fed several protein levels, namely 85.32; 88.75; 88.58 and 92.01% for feed containing protein 35 % (treatment A), 40 % (treatment B), 45 % (treatment C) and 50% (treatment D), respectively, is displayed in Fig. (1). The results of the analysis of variance showed that the test feeds with various levels of protein had a significant effect ($P< 0.05$) on protein digestibility. The results of the W-Tukey test showed that treatment A was significantly different from treatment D, while treatment B & C did not show a significant difference. The protein digestibility obtained ranged from 85.32- 92.01%, according to **NRC (1993)**. This value is within the normal range of protein digestibility (75- 95%). This means that the composition of the raw materials and the nutritional content of the test feed applied to this research meet the requirements as feed for aquaculture, especially for eel cultivation, which until now has not had commercial feed. According to **NRC (1983)**, protein digestibility is influenced by different protein content and amino acid quality in feed sources. Furthermore, according to **Idris (2016)**, the amount of calories and digestible protein in the diet are directly correlated with fish consumption. Furthermore, the amount of feed consumed, temperature, non-protein components in feed, fish type and size, treatment before and after feed preparation, protein sources, and particle size all affect how digestible the protein is **(Hasting, 1969; Choubert *et al*., 1982; Usman *et al*., 2010)**. Additionally, **Afrianto and Liviawaty (2005)** elucidated that the digestibility value of feed nutrients in fish depends on the level of fish acceptance and the enzymes inside the fish's body. Moreover, the feed protein digestibility tends to increase with increasing protein levels in the test feed. **Tillman *et al*. (1998)** stated that protein digestibility depends on the protein content in the feed, where feeds with high protein content generally have high protein digestibility, and the level of protein digestibility depends on the protein content of the feed ingredients, and the amount of protein that enters the digestive tract. **NRC (1983)** postulated that enzymes in the body are responsible for the digestion of feed which includes proteins, fats and carbohydrates. These nutrients will subsequently be used as a source of energy for growth and for maintaing body tissues.

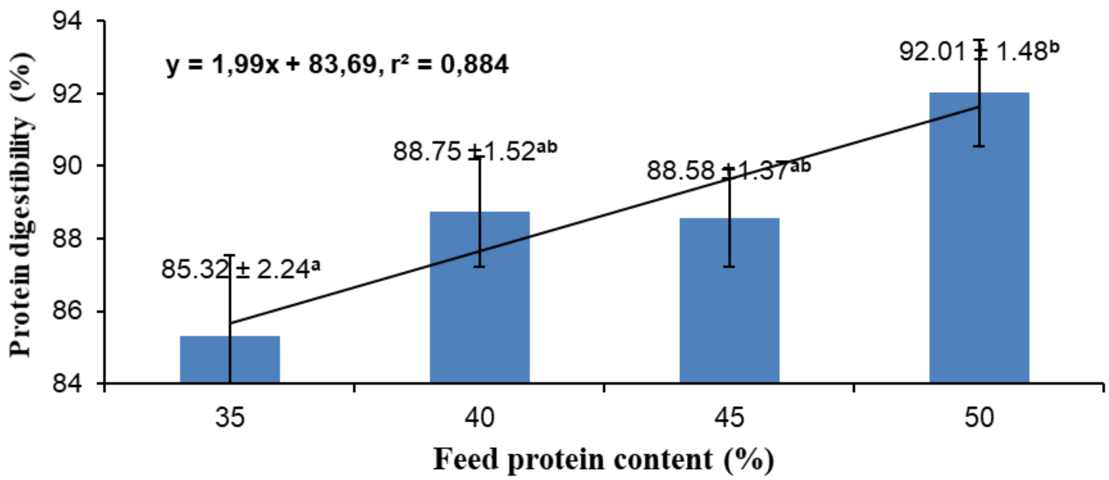

Fig. 1. Digestibility of protein (%) in test fish fed with various protein levels

Eel growth is significantly influenced by the availability of protein, and the equilibrium of the protein-energy ratio in the appropriate feed **(Nawir *et al*., 2015)**. According to **Khan and Abidi (2012)**, the availability of non-protein energy sources in the feed determines how well protein is utilized, which has an impact on growth, feed conversion, nutrient retention effectiveness, and body composition. According to **Phumee *et al*. (2009)**, feed protein intake and protein deposits have a positive correlation with feed protein content, which in turn affects body protein content. The body protein content of eel fish increased with the increase in feed protein content up to 45.38%. In this context, **Nawir *et al*. (2015)** suggested that a high body protein content also affects growth performance such as raising certain growth rates. However, optimal development and protein retention in eels are not always ensured by a high protein content in feed **(Sandiver & Yosep, 1976; Nawir *et al*., 2015)**. Furthermore, **Chilmawati *et al*. (2017)** stated that feed quality is not only determined by high protein content but also by other supporting nutritional elements, such as fat, carbohydrates, vitamins and minerals - all of these are necessary for fish health and growth. The good feed for eel fish is the fresh meat derived from fish, crustaceans and shellfish **(Matsui, 1993)**. According to **Huet (1970)**, high feed efficiency indicates efficient use of feed by fish. In such cases, only a small amount of protein is broken down to meet the energy needs, while the remaining is used for growth. In this respect, **Marzuqi *et al*. (2012)** reported that feed efficiency indicates the amount of feed that fish can consume. The feed efficiency value is not a fixed amount since this value is influenced by the feed's quality and other elements, such as fish size, kind, and water quality, as well as the quantity and timing of feeding and feed nutrition **(Chilmawati *et al*., 2017)**. Given that fish only use a tiny fraction of the energy from the feed they are given for growth, a low feed efficiency rating implies that fish need more feed to expand in weight **(Perdana *et al*., 2016)**. Investigating the amount of feed required for fish, **Zeitter *et al*., (1984)** noted that it is greatly influenced by the energy requirements released by the fish's body. It is worthy to mention that, the excessive amount of feed is not good since the leftover feed results in excessive ammonia, which evaporates in water, causing the death of fish or being stressed **(Juancey, 1982)**. The increase in amino acids causes the deamination and excretion of ammonia, which requires greater energy compared to energy for tissue growth when fish are fed high protein feed **(Guo *et al*., 2012)**. The process of excretion and catabolism of these amino acids requires a lot of energy, resulting in reduction in the allocation of protein energy to retain protein in the body **(Chilmawati *et al*., 2017)**.

2. Digestibility of feed fat

Digestibility of fat in the test fish fed various levels of protein can be seen in Fig. (2). The highest level of feed fat digestibility was obtained in feed with protein content of 35% (treatment A) and 50% (treatment D), namely 89.37. While, the lowest fat digestibility was obtained in feed with a protein content of 40% (treatment B), namely 87.05%. The results of the analysis of variance showed that the treatment of various levels of feed protein had no significant effect ($P>$ 0.05) on the digestibility of feed fat. Equivalent fat digestibility values for all tested feeds were due to relatively similar feed fat content and relatively similar rearing conditions including water quality. The need for fat feed for marine fish species is relatively higher than that required for freshwater fish **(Usman *et al*., 2010)**. Fat has the greatest energy content when compared to protein and carbohydrates **(Buwono, 2004)**. Carnivorous fish are more efficient in utilizing fat as an energy source than omnivorous or herbivorous fish. Several studies have shown that increasing feed fat levels has a significant effect on increasing fish body fat levels **(William *et al*., 2004; Du *et al*., 2005; Biswas *et al*., 2009)**. Whereas, it is not advisable to provide too much fat in feed since this may cause feed consumption to decrease **(Ling *et al*., 2006)**. According to **Takeuchi and Wattanabe (1979)**, feed with a high fat content interferes with the function of enzymes in cell membranes, resulting in low levels of protein and cell production in addition to a high feed conversion rate. Fish meal is the primary source of n-3 necessary fatty acids for fish feed, and protein can be converted to fat to cover the requirements for fat deposited in the muscles and liver for long-term energy needs **(Tacon & Metian, 2008; Nawir *et al*., 2015)**.

In addition, **Lovell (1989)** explained that, feed with an excessive energy content can restrict the amount consumed, slowing down growth. Moreover, high fat content can interfere with the activity of enzymes in cell membranes, resulting in low protein and cell synthesis and subsequently slower growth **(Takeuchi & Wattanabe, 1979)**.

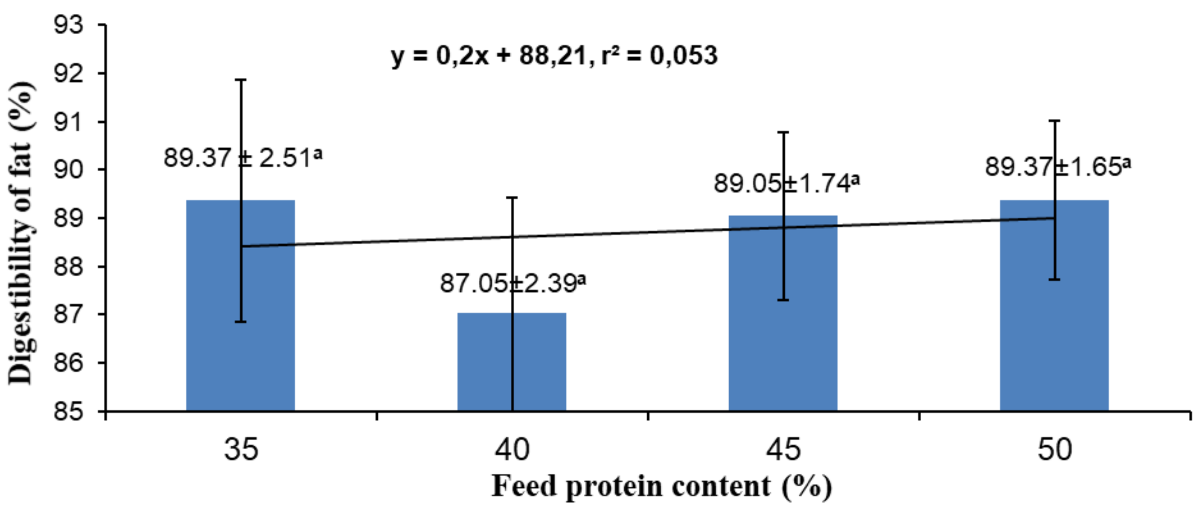

Fig. 2. Digestibility of fat in test fish fed with different levels of protein (%)

3. Digestibility of extract ingredient without nitrogen (%)

Extract ingredients without nitrogen consist of soluable carbohydrates, including monosaccharides, disaccharides and polysaccharides, which are easily soluble, ensuring high digestibility. Carbohydrates for feed are further categorized into two groups, namely crude fiber and extract ingredients without nitrogen **(Aling *et al*., 2020)**. The digestibility of extract ingredients without nitrogen in test fish fed feeds containing different levels of protein can be observed in Fig. (3). Digestibility of extract ingredients without nitrogen in the test fish that consumed feed containing 35, 40, 45 and 50% respectively, as treatments A, B, C and D, sequentially, is 67.46, 68.3, 76.94 and 81.32%. The results of the analysis of variance showed that the treatment of various levels of feed protein had a significant effect ($P< 0.05$) on the digestibility of extract ingredients, without nitrogen in the feed. The results of the W-Tukey test showed that the treatment was significantly different from treatment A, but not significantly different from treatments B and C. Treatments A and B were also not significantly different. The utilization of extract ingredients without nitrogen as an energy sparing effect can increase protein deposits to support growth performance. The content of ash and extract ingredients without nitrogen in the body of eel gave the same response, and the value of the extract ingredients without nitrogen produced was lower than the content of protein, fat, and ash **(Nawir *et al*., 2015)**. Moreover, increased protein affects the absorption or utilization of food substances, hence the digestibility of extract ingredients without nitrogen tends to increase (**Budiman *et al*., 2006)**. Remarkably, the higher the percentage of dry matter digestibility of a feed ingredient, the higher the quality of the feed ingredient (**Sondakh *et al*., 2018)**. **De Carvalho *et al*. (2010)** stated that the consumption of crude protein was highly influenced by the crude fiber content in the feed used.

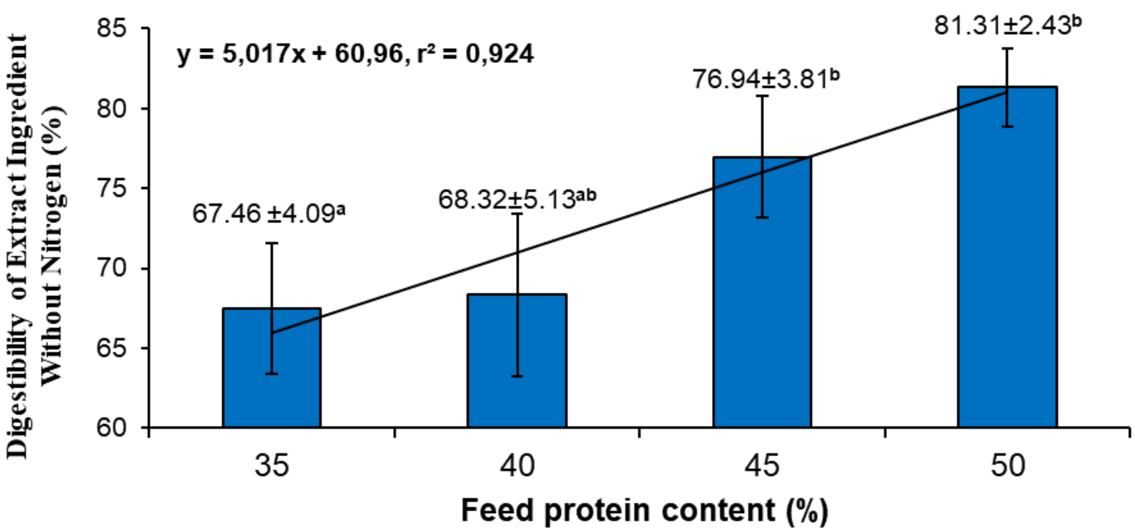

Fig. 3. Digestibility of extract ingredients without nitrogen containing various protein levels (%)

4. Protein retention

The value of protein retention in the tested fish which were fed feed containing various levels of protein is exhibited in Fig. (4). The highest feed protein retention value was obtained in feed with 50% protein content (Treatment D) of 26.54%, while the lowest protein retention was found in feed with a protein content of 35% (Treatment A) of 17.43%. This indicates that the higher the protein content in the feed, the higher the retention value. However, the protein retention value in this study was still lower than the findings **(Nawir *et al*., 2015)**. The results of analysis of variance showed that feed with various protein content had a significant ($P< 0.05$) effect on protein retention. The results of the W-Tukey test revealed that the treatment was significantly different from treatments A and B, and not significantly different from treatment C. Meanwhile, treatment A was significantly different from other treatments. Furthermore, between treatments B and C, no significant different was detected. It is worthnoting that, high protein retention in treatment D (50% protein) is related to protein digestibility and protein content of the feed. The value of specific growth rate and protein retention increases with increasing the amount of protein up to 45.38% **(Nawir *et al*., 2015)**. In general, the tendency for carnivore fish is that the higher the protein content of the feed, the higher the digestibility and protein retention. Based on the results of this study, eels were able to accumulate protein in the body as much as 26.54% for fed feed containing 50% protein, and this percentage (26.54%) is higher than the results of study by **Yudiarto *et al*. (2012)** and **Mukti *et al*. (2014)** concering the eel fish (*A. bicolor bicolor*). On the other hand, protein retention is influenced by various factors including feed protein content, amino acid balance and feed energy ratio **(Ali *et al*., 2008)**. The response to decreased growth rate of fish due to high protein was reported in *A. japonica* and *A. marmorata* eels which experienced an increase in growth rate at 45% protein content and decreased at 50% content **(Okorie *et al*., 2007; Cheng *et al*., 2013)**. The hepatosomatic index obtained at the end of the study increased with increasing growth and feed protein content although there was no significant difference **(Nawir *et al*., 2015)**. **Huet (1970)** explained the high feed efficiency as the efficient use of feed by cultivated organisms, resulting in the breaking down of only a small amount of protein to fulfil the energy needs, while the rest is used for growth **(Perdana *et al*., 2016)**. While, the energy balance is obtained from non-protein energy sources that are disproportionate cause energy requirements that come from protein to be used for maintenance processes, whereas a small part is used for growth **(Nawir *et al*., 2015)**.

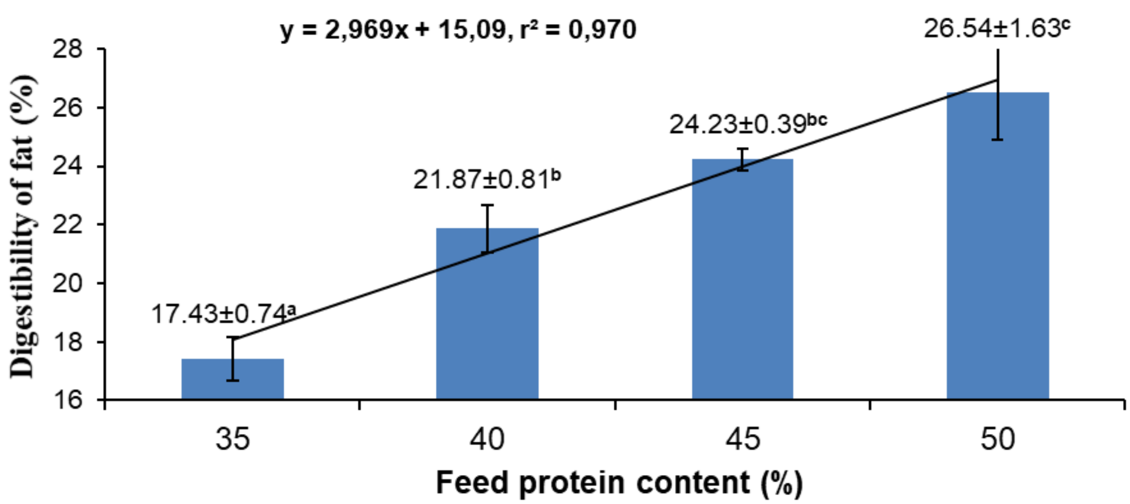

Fig. 4. Digestibility of protein in test fish fed with different levels of protein (%)

5. Fat retention

The existence of fat nutrients is very important in fish feed. The value of feed fat retention can be seen in Fig. (5). The highest fat retention was in the feed with a protein content of 45%

(treatment C), recording a fat retention value of 14.37%, while the lowest was in feed with a protein content of 40% (treatment B), with a fat retention value of 11.02%. The results of analysis of variance clarified that feed treatment with various protein content had a significant effect (*P*< 0.05) on fat retention. The results of the W-Tukey test verified that treatments A and B were significantly different from treatments C and D, whereas between treatments A and B and between treatments C and D, no significant difference was recorded. The present values are lower than the results of **Mukti *et al*. (2014)** who determined a fat retention value ranging from 16.77- 52.49%. The fat retention obtained from this study (11.02- 14.37%) is higher than that of **Yudiarto *et al*. (2012)** who recorded a value of 3.76%, but still lower than the results of the study by **Mukti *et al*. (2014)** with a fat retention value of 16.77- 52.49 %. The fat in the test fish is used as a source of energy and also as an essential fatty acid that cannot be synthesized by the body. Furthermore, essential fatty acids are needed by fish to grow and develop normally **(NRC, 1993)**. In addition, **Watanabe (1988)** added that fat functions in the absorption of vitamins A, D, E, and K. Besides, fat can be used as a nutrient substitute of protein for support growth **(Millamena *et al*., 2002)**. Eel fish cannot digest protein optimally if there is excess fat in the feed. Fat tends to be stored in the body rather than used as energy for growth due to the nature of the fat itself, which is difficult to be broken down by enzymes **(Perdana *et al*., 2016)**. Additionally, **Watanabe (2007)** noticed that a fat content of 16% could reduce the use of protein from 52 to 41% in *A. japonica* fish. **NRC (1983, 2011)** states that fat in feed can provide energy for body maintenance, allowing a greater portion of the consumed protien to be allocated for growth. While, **Stickney (1979)** postulated that the energy contained in feed derived from non-protein can affect the amount of protein used in growth. Furthermore, **Ling *et al*. (2006)** stated that the heart plays an important role in various aspects of fat metabolism, including absorption, oxidation and conversion of fatty acids to ultimately supply other tissues.

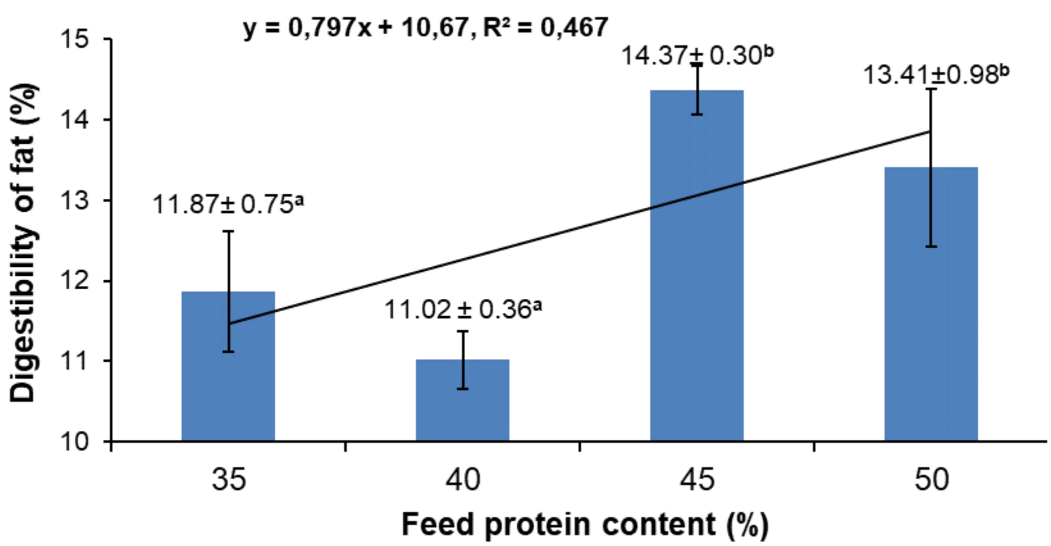

Fig. 5. Digestibility of fat in test fish fed with different levels of protein (%)

6. Water quality

Fish life is highly dependent on environmental conditions. Good water quality can support the growth, development and survival of fish **(Effendie, 2003)**. The relationship between water quality and efficiency of feed utilization is extremely tight, where the more optimal the water quality, the more optimal the efficiency of feed utilization, and an unmeasured increase in feeding rate can cause a decrease in water quality **(Boyd, 1990)**. Good water quality in fish rearing media is a very important factor for supporting the survival and growth of eels **(Fekri *et al*., 2014)**. The range of water quality for each treatment during the study can be seen in Table (2).

Table 2. The range of water quality parameters in fish rearing during the study

Protein content (%)	Parameter			
	Dissolved oxygen (ppm)	Temperature (^oC)	pH	Ammonia (ppm)
35	3,39-6,21	27-29	7,18-8,55	0,0028-0,0032
40	3,69-6,11	27-29	7,22-8,39	0,0018-0,0031
45	3,46-6,13	27-29	7,27-8,29	0,0024-0,0036
50	3,85-6,48	27-29	7,38-8,42	0,0014-0,0040

The oxygen of the fish rearing water during the study was in the range of 3.39- 6.48ppm. The value of this oxygen range is almost the same as the results of research by **Rusmaedi *et al*. (2010)** on the recirculation system of eel (*A.bicolor bicolor*) in a concrete tub which received an oxygen range of 4- 6ppm. Furthermore, proper conditions for most aquatic biota (fish) must be in the average range of more than 4ppm **(UNESCO/WHO/UNEP, 1992)**. The oxygen content in this research was still higher than the results of research of **Fekri *et al*. (2014)**, recording values ranging from 3.2 to 7.5ppm. The decrease in oxygen occurs since dissolved oxygen in water is used by bacteria to decompose ammonia into nitrite, inhibiting the growth of eel fish seeds **(Herianti, 2005)**. The oxygen value obtained sometimes reaches the minimum required to stimulate eel growth **(Fekri *et al*., 2014)**. In their study, **Bhatnagar and Devi (2013)** reported that, to increase productivity, the dissolved oxygen content in water should remain at 5ppm. Decent conditions for most aquatic biota (fish) must be in the average range of more than 4ppm. Meanwhile, the concentration of dissolved oxygen in the waters is less than 2ppm, which is a critical limit that can result in fish death **(Rusmaedi *et al*., 2010)**. The need for oxygen for eels is influenced by temperature and the speed of growth of eels fish, where the higher the temperature, the greater the oxygen demand **(Degani & Gallagher, 1985)**.

The temperature of the fish rearing water during the study was in the range of 27- 29^oC for all treatments. The temperature in this study still fulfilled the requirements for eel life. The water temperature range is equivalent to research conducted by **Rusmaedi *et al*. (2010)**, which is around 26.5- 27.8^oC. The water temperature for eel rearing is in the good category if it ranges from 28.5- 30.0^oC **(Fekri *et al*., 2014)**. Furthermore, **Badjoeri and Suryono (2013)** stated that the optimum water temperature for eel fish growth is 20- 29^oC. While, the water quality value has an optimal range and meets the needs of eel appetite at a temperature of 24- 28^oC **(Otwell & Rickards, 1982)**.

The pH value of the eel rearing water during the study was between 7.18 and 8.55. This pH value is similar to those of **Rusmaedi *et al*. (2010)** who assessed a pH range of 7.0- 7.81. Whereas, the current values are higher than those detected in the study of **Badjoeri and Suryono (2013)** who obtained a pH value of around 5. According to **EFSA (2009)**, the pH of the eel fish rearing water should be maintained at a neutral value in the range of 6.0- 8.0. **Samsudin *et al*. (2009)** deduced that eel fish are able to live at acidity levels of 4- 11. This condition is caused by the activity of microbial metabolism in water and gas exchange on the surface of the water **(Idris, 2016)**.

The ammonia condition of the eel rearing water during the research showed a range of 0.0014- 0.0040ppm. Ammonia conditions in this study are lower than the results of research conducted by **Rusmaedi *et al*. (2010)** with values ranging from 0.02 to 0.23ppm. However, **Degani and Gallagher (1985)** determined ammonia concentrations between 1- 2ppm and added that these concentrations do not cause the growth of eel fish to decrease if the pH is in the range of 6.8- 7.9. Furthermore, **Ming (1985)** stated that ammonia excretion indicates the relative amount of digested feed protein for protein synthesis or energy sources. While, an environment with high concentrations of ammonia can cause fish stress, stunted growth and even death **(Jobling, 1994)**. It was reported that, ammonia and nitrite values increased with an increase in the percentage of feed given **(Fekri *et al*., 2014)**. After supplementing by feed, ammonia excretion will grow and reach its peak a few hours later. The value of ammonia is still within the tolerance limit of <0.1ppm **(Yamagata & Niwa, 1982)**. Aquatic animals' tolerance to ammonia varies based on the species, the fish's physiological state, and the environmental factors. However, in general, the water's ammonia content shouldn't be more than 1ppm **(Ming, 1985)**. There are two types of ammonia in water, ionized (NH_4) and un-ionized (NH_3) **(Idris, 2016)**. Moreover, ammonia in the form of NH_3 is hazardous to aquatic life since it is lipophilic and readily diffuses across the respiratory membrane, in contrast to NH4, which is less able to do so **(Jobling, 1994)**. The aquatic environment's pH and temperature have an impact on ammonia toxicity, and rising pH and temperature would result in higher ammonia concentrations **(Idris, 2016)**.

CONCLUSION

The highest protein digestibility in the study was obtained in the feed treatment with protein content of 50%, namely 92.01% (treatment D). Digestibility of feed protein tends to increase with increasing levels of protein in fish feed. Meanwhile, the highest level of fat digestibility was found in feeds with protein content of 35 and 50%, namely 89.37 (Treatment A and D). Furthermore, the highest feed digestibility of extract ingredients without nitrogen was obtained from feed with a protein content of 50%, namely 81.31 (Treatment D). While, the highest feed protein retention value was in feed with 50 protein content of 26.54%. On the other hand, the highest fat retention in feed was assessed with a protein content of 45, namely 14.37% (Treatment C). Furthermore, the range of water quality for each treatment during the study was still within the appropriate range for the growth and survival of eel fish.

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