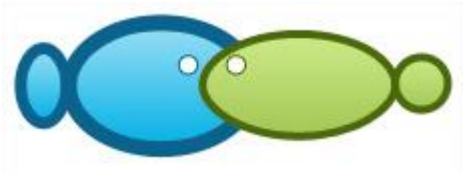

Evaluation of digestibility and ammonia excretion of fish meal and fish silage fed to juvenile Indonesian shortfin eel (*Anguilla bicolor*)

[1,2]Hany Handajani, [3]Widanarni, [3]Mia Setiawati, [3]Tatag Budiardi, [4]Sujono

[1] Department of Fisheries, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Indonesia; [2] Study Program of Aquaculture Science, Graduate School, Bogor Agricultural University, Indonesia; [3] Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia; [4] Department of Animal Science, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, East Java, Indonesia. Corresponding author: Widanarni, widanarni@yahoo.com

Abstract. This study aimed to analyze Apparent Digestibility Coefficient Ingredient (ADC_{ing}) of fish meal and fish silage processed biologically by lactic acid bacteria, digestibility of feed, apparent digestibility of protein, ingredients, ammonia excretion, specific growth rate, feed efficiency, and protein efficiency ratio in eel (*Anguilla bicolor*) culture. The study used Completely Randomized Design with three dietary treatments with three replications. Feed used in this study were reference diet (100% commercial feed), test diet I: 70% commercial feed +30% fishmeal (FM), and test diet II: 70% commercial feed + 30% fish silage (FS) processed biologically by lactic acid bacteria. Each experimental unit contained 20 fry with the average weight of 7.5±0.189 g, maintained in an aquarium of 48 L water. Feed was given of paste form with the addition of 0.6% Cr_2O_3 as digestibility indicator. Fry were cultured for 40 days, while faeces were collected for 20 days to analyze the nutrient content. Feeding was conducted at satiation, 3 times a day. Total ammonia nitrogen (TAN) level was measured every hour during six hours. Analysis result showed that apparent digestibility coefficient ingredient value of test diet II (30% FS) which was 77.469±0.045% was higher than test diet I (30% FM) which was 72.372±0.064%. The highest digestibility value of feed and ADC_{diet} protein were found in test diet II (30% FS), those were 76.592±0.014% and 96.557±0.514%, respectively. The lowest ammonia excretion produced was obtained in test diet II, namely 0.540±0.128 mg TAN (g fish hour)$^{-1}$. The highest specific growth rate, feed efficiency and protein efficiency ratio were found in test diet II (30% FS), those were 44.4±1.706 g, 40.018±1.096%, and 0.802±0.0219, respectively. Based on the result of this study, it was concluded that the best ADC_{ing}, feed digestibility and ADC_{diet} (protein), ammonia excretion, specific growth rate, feed efficiency and protein efficiency ratio was found in test diet II (30% FS).
Key Words: digestibility, ammonia excretion, fish silage, lactic acid bacteria, eel.

Introduction. The scarcity and the high price of fish meal pushes the needs to evaluate other feed ingredients as protein source. Several studies had been conducted to identify alternative protein sources which allowed the reducing of feed costs (Glencross & Hawkins 2004; Naylor et al 2000; FAO 2014). The nutritional value of fish feed depends on the quality of proteins from the ingredients used in feed formulation (Glencross et al 2007; Li et al 2009; Hu et al 2013).

Trash fish is an intact fish that had been unfit for human consumption. Fish meal derived from trash fish is rich in amino acids, energy, fatty acids and minerals, and it contains attractants that can increase fish appetite (Chandrapal 2007). Fish waste is a by-product of catches which is not economically valuable or the remnants of fish processing, which if left alone will pollute the environment. Utilization of fish waste as silage is a technology that does not cause environmental pollution, it is easy to proceed and cheap. The fish silage product is a liquid made from trash fish or remnants of fish processing industry hich is liquefied resembling porridge by enzymes contained in the fish itself through a fermentation process with the aid of an acid or microbe which is

purposely added (FAO 2010). Biologically processed fish silage contains high nutritional value with 39.9% protein content (Ramirez et al 2008) or higher 45.95% (Handajani et al 2013); so it can be used as an ingredient for fish feed, with expectations that it can replace fish meal of which price is constantly increasing.

Eel (*Anguilla* spp.) is a widely consumed fishery commodity in many countries in the world, especially in East Asian countries (Japan, Taiwan, Korea, China and Hong Kong), European countries (Italy, Germany, France and Netherlands) and USA (FAO 2014). Production capacity of Indonesian shortfin eel (*Anguilla bicolor*) in Indonesia was projected to reach 120,000 tons, but the production realization was only 5,000 tons or around 4% of total capacity (KKP 2014). Eel is a carnivorous fish that require high protein in feed, so that it impact on feed cost. Feed necessity is the largest capital investment in this business, which can reach 70% of the total fish maintenance, so feed is the most expensive necessity (Suprayudi et al 2012). Therefore, it is necessary to search for alternative feed ingredients as fish meal substitute for a cheaper price. Decent fish feed ingredients are evaluated based on nutritional value, also on digestibility value of the ingredients and on the generated waste (Glencross et al 2007). Studies about the use of fish silage as fish feed ingredient have been conducted before. In the study conducted by Samaddar et al (2011, 2015), fish silage had a high digestibility, protein digestibility in Nile tilapia (*Oreochromis niloticus*) was 80.6%, in catfish was 81.9%, and in *Labeo rohita* was 82.94%. Tanuja et al (2017) stated that total digestibility in *Labeo rohita* was 58,4% and protein digestibility was 74%. Nevertheless, until now there have been no studies using fish silage as a constituent of eel feed. Therefore, this study was necessary to be conducted to analyze the digestibility of biologically processed with lactic acid bacteria, total digestibility and apparent digestibility of protein, apparent digestibility ingredients, ammonia excretion, and specific growth rate, feed efficiency and protein efficiency ratio of eel (*Anguilla bicolor*).

Material and Method

This study was conducted at Wet Fisheries Laboratory, proximate analysis and feed digestibility were conducted at Fish Nutrition Laboratory, University of Muhammadiyah Malang, Malang, East Java, Indonesia. The time period was from February to April 2017.

Tested fish. In this experiment there were nine units experiment (aquarium). Each experimental unit contained 20 fry with the average weight of 7.5±0.189 g, maintained in an aquarium with a size of 60x30x40 cm filled with 48 L of water. Eel individuals were obtained in an eel cultivator in Tulungagung East Java, Indonesia. Prior to treatment, eel were adapted to laboratory condition for two weeks in a 100×100×100 cm fiber tub and fed with a 45% protein content feed. During adaptation period, the health of fry should be cared, so that it was fit enough to be used as a test fish. One day (24 hours) prior to feeding treatment, the test fry were fasted to eliminate the effect of previous feed, then fish were weighed to record the initial weight (Takeuchi 1988). Fry were cultured for 40 days.

Preparation of fish silage. Trash fish was obtained from fishermen in Sendang Biru Port, South Malang, East Java. Fresh solid fish waste consists of head, bone, entrails and meat milled into one. Molasses was obtained from Kebon Agung Malang sugar mill. Before using it, molasses heated until it boiled, to kill microorganisms present in molasses. *Lactobacillus* sp. was used as starter, cultivated in MRS (Man Rogosa and Sharpe) medium at 37°C for 24 hours until the concentration reached $1x10^9$ cfu mL^{-1}. Freshwater waste after grinding were mixt with molasses (20% of wet weight) and inoculated with *Lactobacillus* sp. (5% of dry weight). Silage was stored for 5 days under anaerobic condition to reach a product with pH 4 (modification of Ramirez et al 2008).

Preparation of tested diet. Test diet used in this study, P1: reference diet (100% commercial feed), P2: test diet I (70% commercial feed and 30% fish meal), P3: test diet II (70% commercial feed and 30% fish silage). Composition and proximate analyzes of

the experimental feed were presented in Table 1 and Table 2. Cr_2O_3 was added into the feed (0.6%) as an indicator of digestibility (Takeuchi 1988).

Table 1

Composition of reference and test diet (g kg^{-1})

Ingredients	*Reference diet*	*Test diet I*	*Test diet II*
Commercial feed	964.0	664.0	664.0
Fish silage	0	0	300.0
Fish meal	0	300.0	0
CMC*	30.0	30.0	30.0
Cr_2O_3	6.0	6.0	6.0
Total	1000	1000	1000

*Carboxymethil cellulose as binder in fish feed.

Table 2

Proximate analysis result of reference and test diet

Nutritional value	*Reference diet*	*Test diet I*	*Test diet II*
Water content (%)	7.41	8.31	9.47
Ash content (%)	9.35	8.79	9.23
Crude protein (%)	44.99	49.89	47.29
Crude fat (%)	11.87	12.73	9.16
Crude fiber (%)	1.56	1.62	1.54
Free nitrogen extract (%)	32.23	26.97	32.78
GE (kcal 100 g^{-1} feed)*	495.67	509.62	485.33
Energy:protein (kcal g^{-1} $protein^{-1}$)	11.017	10.215	10.263

*Gross energy = (5.6 x protein) + (9.4 x fat) + (4.1 x BETN) (Bureau et al 2002).

Faeces collection and digestibility measurement. The fry were adapted in an aquarium for 3 days. Every morning before the fry were fed, the water was changed (20% of the total volume). Feeding was conducted at satiation, three times a day (Watanabe 1988). On the next day, faeces collection began for each treatment (reference diet and test diet). Faeces were collected in a 50 mL bottle and stored in freezer. Faeces collection was conducted every 2 hours to avoid nutrients cleansing and it was conducted for all 20 days of cultivation.

Feed and faecal samples were smoothed for analysis. Proximate analysis was conducted at Fish Nutrition Laboratory University of Muhammadiyah Malang, Indonesia. Water content was determined by drying the sample at 105°C to get the constant weight, whereas ash content was determined using a furnace (Furnace THERMOLYNE 47900; at 600°C for 4 hours). Protein content (Nx6.25) was conducted based on Kjeldahl method (demolition by FOSS TecatorTM, distilled by Kjeltec FOSS 2100, titration by JENCONS Digitate Pro). Lipid content was determined by extracting with petroleum ether (FOSS SoxtecTM 2055), whereas crude fiber was determined by gravimetric method, reacting the sample with acid and base to separate fiber fraction and other components. Proximate analysis referred to AOAC (2012) method. Wet destruction method was conducted to determine the Cr_2O_3 content in diet and faeces, and the absorbance was detected using spectrophotometer with a wavelength of 350 nm (Takeuchi 1988).

Ammonia excretion. Measurements of ammonia excretion was carried out in a closed aquarium container (40x30x30 cm) filled with 8 L of water. Prior to observation, fry were fasted for one day. The next day, fry were fed and tested until it was full and given time for 30 minutes. Furthermore, the fry were moved into other aquarium, 3 fry/aquarium. Observations of ammonia level was conducted every hour from the beginning o hour to the sixth hours. Observation at the 0 hour was conducted to water stock just before the fish was put into the aquarium. Water sample was taken every hour for six hours and total ammonia concentration (total ammonia nitrogen, TAN) was analyzed by Nessler

method with a portable HANNA C200 spectrophotometer (HANNA Instrument, Co, Italy) (Yigit et al 2003).

Water quality measurement included temperature, pH and DO and was done by using YSI 556 meters (YSI Incorporated 1725, Yellow Springs, OH, USA).

Experimental design and data analysis. This study was conducted with experimental method in laboratory using complete randomized design. This study used three (3) test diet treatments with 3 replications. The data obtained was tabulated with MS Office Excel 2013 programme. ANOVA with SPSS version 22 programme was used to find out the effect of treatment on each tested parameter. If there was any significant different, another analysis was conducted using Duncan test method to see the difference between treatments.

Data collection. Parameters of this experiment included total digestibility, apparent digestibility of proteins, apparent digestibility of ingredients, ammonia excretion, survival rate, specific growth rate, feed efficiency, protein efficiency ratio and feed conversion. Water quality observed during maintenance period was temperature, dissolved oxygen (DO) and pH using APHA measurement method (2006). Calculation of digestibility was conducted using the following the formula (Glencross et al 2007):

$$\text{Total digestibility (\%)} = \left\{\frac{1 - \text{Cr}_2\text{O}_3 \text{ in diet (\%)}}{\text{Cr}_2\text{O}_3 \text{ in faeces (\%)}}\right\} \text{ x } 100$$

Apperent Digestibility Coefficient (ADC_{diet})

$$ADC_{diet} \text{ (\%)} = \left\{1 - \frac{\text{Cr}_2\text{O}_3 \text{ in diet}}{\text{Cr}_2\text{O}_3 \text{ in faeces}} \text{ x } \frac{\text{protein in faeces}}{\text{protein in diet}}\right\} \text{ x } 100$$

Apparent Digestibility Coefficient Ingredient (ADC_{ing})

$$ADC_{ing} \text{ (\%)} = \left\{\frac{\text{digestibility of test diet } - \text{ 0,7 digestibility of reference}}{0,3}\right\}$$

Calculation of ammonia excretion was conducted using the following formula (Almendras 1994):

Ammonia excretion level (mg TAN (g fish hour)$^{-1}$) = [(N_6-N_0) x V]/W/T

N_6 = TAN concentration at the sixth hours (mg L^{-1})
N_0 = TAN concentration at the 0 hour (at the beginning) (mg L^{-1})
V = volume of medium water (8 L)
W = fish weight (g)
T = time interval (6 hours).

Results. Digestibility data of feed ingredients and ammonia excretion which included total digestibility, apparent digestibility of protein, apparent digestibility of ingredients, and ammonia excretion are shown in Table 3.

Table 3

Total digestibility, apparent digestibility coefficient and ammonia excretion

Parameter	*Reference diet*	*Test diet I*	*Test diet II*
Total digestibility (%)	76.217±0.1152^{b}	75.063±0.1924^{a}	76.592±0.1339^{c}
ADC_{diet} (protein) (%)	94.573±0.724^{a}	95.326±0.063^{b}	96.559±0.514^{c}
ADC_{ing} (%)	-	72.372±0.064	77.469±0.045
Ammonia excretion (mg TAN (g fish hour)$^{-1}$)	0.995±0.242^{b}	0.879±0.137^{ab}	0.540±0.128^{a}

The different letters in each row showed real difference (P<0.05).

Analysis of Variance (ANOVA) results showed that total digestibility, ADC_{diet} protein and ammonia excretion value were significantly different between treatments ($P<0.05$). The highest total digestibility and ADC_{diet} protein were found in test diet II (Fish Silage) those were 76.592% and 96.559%, respectively. For total digestibility, the second highest value was found in test diet I (Fish Meal), followed by reference diet, whereas for ADC_{diet} protein the second highest value was found in feed reference then followed by test diet I. The lowest level of ammonia excretion was found in test diet II. For digestibility, fish silage ingredients value was 7.04% higher than fish meal. The lowest ammonia excretion value was found in test diet II which was 0.54 (mg TAN (g fish hour)$^{-1}$), this value was 45.73% lower compared to reference diet.

Table 4

Eel growth performance for 40 days

Parameter	*Reference diet*	*Test diet I*	*Test diet II*
SR (%)	100±0.00	100±0.00	100±0.00
FI (g fish^{-1})	108.249±1.971	113.029±2.709	110.927±1.301
SGR (%)	0.320±0.052^{ab}	0.264±0.083^{a}	0.463±0.072^{b}
FE (%)	29.226±1.498^{a}	34.193±1.058^{b}	40.018±1.096^{c}
PER (%)	0.649±0.033^{a}	0.723±0.022^{b}	0.802±0.021^{c}
FCR	3.427±0.171^{c}	2.926±0.089^{b}	2.5±0.068^{a}

The different letters in each row showed real difference of each treatment ($P<0.05$). SR (survival rate), FI (feed intake), SGR (specific growth rate), FE (feed efficiency), PER (protein efficiency ratio), FCR (Feed conversion rate).

ANOVA results showed that SGR, FE, PER and FCR value were significantly different between treatments ($P<0.05$), while SR and FI did not vary significantly ($P>0.05$). The highest SGR, FE and PER values were found in test diet II (FS), followed by test diet I (FM) and reference diet. The lowest FCR was found in test diet II (FS), followed by the test diet I and reference diet.

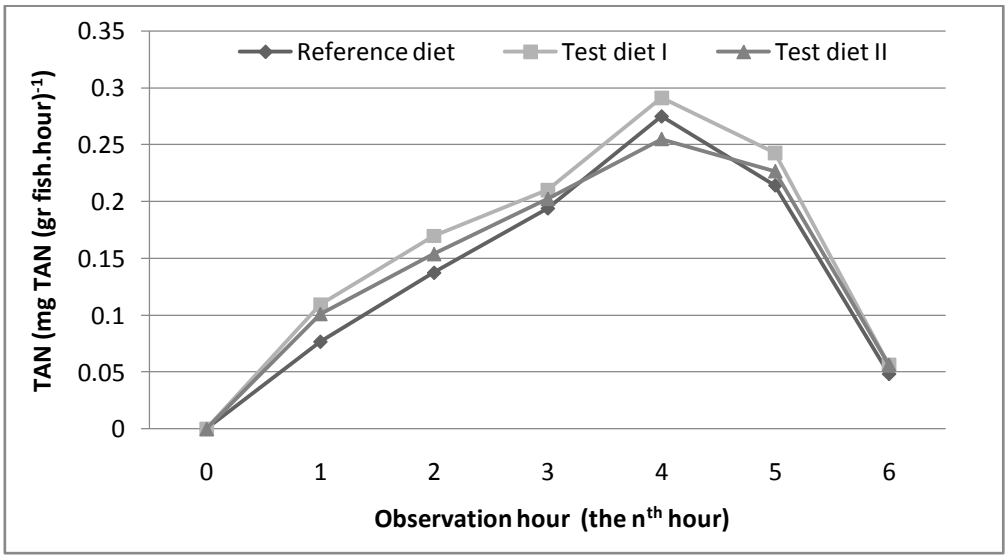

Figure 1. Total ammonia nitrogen (TAN) rate during observation time.

The rate of TAN value at all treatments reached the highest value at the 4th hour after feeding. Then at the 5th and 6th hours, TAN value for all treatments tended to decrease. TAN value on reference diet ranged from 0.00121-0.2751 (mg TAN (g fish hour)$^{-1}$), test diet I (FM) ranged from 0.00121-0.2711 (mg TAN (g fish hour)$^{-1}$), in test diet II (FS) ranged from 0.00121-0.2468 (mg TAN (g fish hour)$^{-1}$).

Water quality, temperature, pH and DO values were within optimum range for all treatments.

Table 5

Water quality parameters during cultivation

Parameter	*Treatment*			*Optimum limit (reference)*
	Reference diet	*Test diet I*	*Test diet II*	
Temperature (°C)	28.3-29.5	28.5-30.0	28.5-29.4	28-33 [1]
pH	7.3-7.5	7.4-7.7	7.3-7.6	6-8 [2]
DO (mg L^{-1})	4.5-5.1	4.7-5.5	4.7-5.4	> 3.0 [3]

[1] Luo et al (2013), [2] Tseng & Wu (2004), [3] Herianti (2005).

Discussion. The main components in evaluating feed ingredients are ingredients characterization, ingredients digestibility, palatability, nutrients utilization and function (Glencross et al 2007). Nutrient digestion is a crucial early stage in evaluating feedstuff potential for aquaculture species. Information on digestibility value of the feed content is needed to maximize the growth of fish by considering nutritional requirements and discarded metabolic products (Booth & Allan 2003; Glencross et al 2007). Digestibility shows the amount of nutrient composition absorbed and used for growth and metabolic processes (NRC 2011).

In this study, feed digestibility value of fish silage feed was higher than fish meal feed and reference diet. Ingredients digestibility (ADC_{ing}) value of tested fish silage was 77.47%, total digestibility was 76.59% and protein digestibility (ADC_{diet}) was 96.56%. Digestibility value was higher compared with referenced feed which was always used in cultivation of eel and also higher than fish meal contained feed.The result of this study was in accordance with the study of Tanuja et al (2017), which examined the use of fish silage as feed material in *Labeo rohita*. The result showed that feed containing 100 g kg^{-1} of fish silage had total digestibility of 58.4% and protein digestibility value of 74%, both values higher compared with control feed of which total digestibility was 52.78% and protein digestibility was 70.5%. In tilapia fish, the results of using 50% fish silage yields presented a total digestibility reached to 85.9% and protein digestibility reached 86.6% (Fagbenro & Jauncey, 1998). According to NRC (2011), a good protein digestibility coefficient ranges from 75 to 95%.

Fish silage had higher digestibility value because the feed ingredients had been fermented using lactic acid bacteria, making the material easier to decompose into simpler components, so that it was easier to digest. This result was in accordance with the study by Ramirez et al (2008) and Lian et al (2005) which stated that protein hydrolysis in feed ingredients could help food digestion process, due to the release of peptides and free amino acids from polypeptide bond, so chemo-attractants had the potential to stimulate nutrients in carnivorous animals. Total digestibility described the digestibility of all nutrients digested by fish. A high total digestibility value was due to the quality of feed ingredients. Total digestibility was also given by the amount of carbohydrates contained in feed ingredients, because carbohydrates contribute with 50-80% of dry substance (Suryaningrum et al 2017). Protein quality of feed ingredients determined the growth performance, so the protein digestibility was the most proper parameter to analyze. Protein quality was related to amino acid composition and digestion, so that protein digestibility indicated amino acid digestibility (Koprucu & Ozdemir (2005). Setiawati et al (2016) stated that certain bioactive compounds contained in feed ingredients could be suspected as anti-nutrients, the high content of those would inhibit nutrient absorption because of fish limitations in digestion, because digestive enzymes could not work properly.

The amount of feed intake in this study did not show any significant difference between treatments. It showed that the feeds had a good palatability and had similarity. Feed palatability was determined by the taste, smell and color which were the physical factors of the feed (Mokoginta et al 1999). The survival rate of all treatments was equal to 100% due to water quality condition during maintenance and was within the optimum limits. In addition, feeding treatment provided was suitable for the eel. The increase of fish weight was influenced by the quality of given feed. Results of this study showed that the weight of individual fish in all treatments increased. It proved that nutrient content

consumed by fish exceeded its needs for body maintenance and the excess was used for growth. Absolute growth, feed efficiency and protein efficiency ratio parameters of test diet II (FS) had the highest values. The lowest FCR was found in test diet II (FS). Test diet I (FM) had higher protein content but had the lowest SGR, FE, and PER value. This is probably due to protein content in test diet I (FM) (49.89%) which was higher than reference diet and test diet II (FS). Excess protein in test diet I (FM) showed that high protein content does not always have a positive correlation in the increase of SGR, FE, PER value in eel. The increasing of amino acids caused deamination and ammonia excretion that required greater energy than energy for tissue growth when fish were fed with high protein feed (Guo et al 2012). Excess protein in feed was allegedly used as energy for amino acid deamination process and nitrogen excretion that is wasted into water. Excretion process and catabolism of amino acids required a lot of energy so that the allocation of proteins energy for protein retention in the body would be reduced. Eel protein requirement was 40-50% of the given feed (Tibbetts et al 2001; Tibbetts et al 2000; Chiu and Pan 2002). According to Nawir et al (2015), weight fry of 6.5-7.5 g required 45.38% protein in the feed to support its growth, while feed containing 49.60% protein content produced lower growth than feed containing 45.38% protein content.

The lowest ammonia excretion value was found in test diet II (FS) treatment. The highest TAN value was showed at the 4^{th} hour after feeding for all treatments, then at the 5^{th} and 6^{th} hours, TAN value tended to decrease. According to Bureau (2004), around 80-90% of nitrogen metabolic waste released by fish in the form of ammonia. The amount of ammonia released by fish are mainly influenced by the amount, quality and type of protein contained in the feed. Feed containing excess amino acids also affected the amount of ammonia production. Feed with less amino acid content caused more ammonia excretion. Nitrogen content consumed by fish was excreted as ammonia (NH_3) form, which immediately ionizes into ammonium ions ($NH4^+$) in the water. The rate of TAN value reached the highest value at the 4^{th} hour after feeding for all treatments. Then at the 5^{th} and 6^{th} hours TAN value for all treatments tended to decrease. Ammonia excretion level of 111.6 g and 40.0 g $fish^{-1}$ size catfish were 0.008±0.003 and 0.011±0.003 mg TAN (g catfish hour)$^{-1}$ respectively (Gunadi et al 2013). This value was almost the same as ammonia excretion level of turbot (*Psetta maeotica*) with 45% protein content artificial feed which was 0.00698 mg TAN (g fish hour)$^{-1}$ (Yigit et al 2005a). California halibut (*Paralichthys californicus*) fed with 43-45% protein content continuously for 12 hours during the day produced 91-113 mg TAN (kg fish day)$^{-1}$ ammonia or equivalent to 0.0038-0.0047 mg TAN (g fish hour)$^{-1}$ (Merino et al 2007). Similar result was reported by Yigit et al (2005b), which stated that a 100 g $fish^{-1}$ size Black Sea turbot had ammonia excretion rate equivalent to 0.0042 mg N (g fish hour)$^{-1}$, while a 42 g $fish^{-1}$ fish had ammonia excretion level equivalent to 0.0048 mg N (g fish hour)$^{-1}$. In catfish with a size of 111.6 g and 40.0 g, the peak of ammonia excretion occurred at the 5^{th} hour after eating and decreased afterward (Gunadi et al 2013). In California halibut (*P. californicus*) the greater peaks of ammonia excretion of larger fish occured longer (Merino et al 2007). The peak of ammonia excretion in 4-20 g of halibut fish lasted for 4-6 hours after feeding, while in 112-199 g of halibut, the peak of ammonia excretion lasted for 10-12 hours after feeding. Meanwhile, daily production of ammonia in a 0.09-3.8 kg white sturgeon fish (*Acipenser transmontatus*) ranged from 1.5-27.6 mg TAN kg^{-1} of fish per hour (equivalent to 0.0015-0.0276 mg TAN (g fish hour)$^{-1}$ with ammonia production peak in within 2-6 hours after feeding (Thomas and Piedrahita 1998).

Conclusions. This study showed that the use of test diet II (fish silage) on eel feed leaded in higher values of total digestibility, protein digestibility, ingredient digestibility and growth performance compared to fish meal and commercial feed. In addition, the use of fish silage on eel feed could reduce the waste of cultivation due to the low of ammonia excretion.

Acknowledgements. The authors gratefully acknowledge to Indonesian Ministry of Research and Technology of Higher Education for grants during the study.

References

Almendras J. M. E., 1994 Ammonia excretion rates of the sea bass, *Lates calcarifer*, in fresh and sea water. The Israeli Journal of Aquaculture-Bamidgeh 56(2):76-82.

Booth M. A., Allan G. L., 2003 Utilization of digestible nitrogen and energy from four agricultural ingredients by juvenile silver perch *Bidyanus bidyanus*. Aquaculture Nutrition 9:317-326.

Bureau D. P., Kaushik S. J., Cho C. Y., 2002 Bioenergetics. In: Fish nutrition. Halver J. E., Hardy R. W. (eds), 3rd edition, Academic Press, San Diego CA (US), p. 8.

Bureau D. P., 2004 Factors affecting metabolic waste outputs in fish. In: Avances en nutricion acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola, 16-19 Noviembre, 2004, Cruz Suarez L. E., Marie R. D., Lopez N. M. G., Villarreal D., Scholz U., Gonzalez M., Hermosilla, Sonora, Mexico, p. 7.

Chandrapal G. D., 2007 Status of trash fish utilization and fish feed requirements in aquaculture – India. Proceedings of the regional workshop on low value and trash fish in the Asia-Pacific region, 7-9 June 2005, Hanoi, Vietnam, pp. 1-7.

Chiu S. T., Pan B. S., 2002 Digestive protease activities of juvenile and adult eel (*Anguilla japonica*) fed with floating feed. Aquaculture 205(1-2):141-156.

Fagbenro O. A., Jauncey K., 1998 Physical and nutritional properties of moist fermented fish silage pellets as a protein supplement for tilapia *Oreochromis niloticus*. Animal Feed Science Technology 71:11-18.

Glencross B. D., Hawkins W. E., 2004 A comparison of the digestibility of several lupin (*Lupinus* spp.) kernel meal varieties when fed to either rainbow trout (*Oncorhynchus mykiss*) or red sea bream (*Pagrus auratus*). Aquacuaculture Nutrition 10:65-73.

Glencross B. D., Booth M., Allan G. L., 2007 A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition 13:17-34.

Guo Z., Zhu X., Liu J., Yang Y., Lan Z., Xie S., 2012 Effects of dietary protein level on growth performance, nitrogen and energy budget of juvenile hybrid sturgeon *Acipenser baerii* × *A. gueldenstaedtii*. Aquaculture 338:89-95.

Gunadi B., Harris E., Supriyono E., Budiardi T., 2013 [The digestibility of protein and ammonia excretion in the maintenance of catfish (*Clarias gariepinus*)]. Jurnal Akuakultur Indonesia 12(1):62-69. [In Indonesian].

Handajani H., Hastuti S. D., Sujono, 2013 [The use of various organic acids and lactic acid bacteria to the nutritional value of fish waste]. Jurnal Depik 2(3):126-133. [In Indonesian].

Herianti I., 2005 [Environmental engineering to spur development of *Anguilla bicolor*]. Jurnal Oseanologi Limnologi Indonesia 37:25-40. [In Indonesian].

Hu L., Yun B., Xue M., Wang J., Wu X., Zheng Y., Han F., 2013 Effects of fishmeal quality and fish meal substitution by animal protein blendon growth performance, flesh quality and liver histology of Japan esesea bass (*Lateolabrax japonicas*). Aquaculture 372-375:52-61.

Koprucu K., Ozdemir Y., 2005 Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). Aquaculture 250:308-316.

Lian P. Z., Lee C. M., Park E., 2005 Characterization of squid-processing by product hydrolysate and its potential as aquaculture feed ingredient. Journal of Agriculture and Food Chemistry 53:5587-5592.

Luo M., Guan R., Li Z., Jin H., 2013 The effects of water temperature on the survival, feeding, and growth of the juveniles of *Anguilla marmorata* and *Anguilla bicolor pacifica*. Aquaculture 400-401:61-64.

Merino G. E., Piedrahita R. H., Conklin D. E., 2007 Ammonia and urea excretion rates of California halibut (*Paralichthys californicus*, Ayres) under farm-like conditions. Aquaculture 271:227-243.

Mokoginta I., Takeuchi T., Suprayudi A. M., Wiramiharja Y., Setiawati M., 1999 [Effect of different carbohydrate sources on feed digestibility, feed efficiency and growth of

gouramy (*Osphronemus gouramy* Lac)]. Jurnal Ilmu-Ilmu Perairan dan Perikanan Indonesia IV(2):13-19. [In Indonesian].
Nawir F., Utomo N. B. P., Budiardi T., 2015 The growth of eel fed with different protein level and protein-energy ratio. Jurnal Akuakultur Indonesia 14(2):128-134.
Naylor R. L., Goldburg R. J., Primavera J. H., Kautsky N., Beveridge M. C. M., Clay J., Folke C., Lubchenco J., MooneyH., Troell M., 2000 Effect of aquaculture on world fish supplies. Nature 405:1017-1024.
Ramirez H. S., Arias L., Prado A., Shirai K., 2008 Utilization of fisheries by-catch and processing wastes for lactic acid fermented silage and evaluation of degree of protein hydrolysis and *in vitro* digestibility. Revista Mexicana de Ingeniería Química 7(3):195-204.
Samaddar A., Mondal K., Kaviraj A., 2011 Evaluation of fermented mixture containing fish offal meal in compound diets for the freshwater catfish *Mystus vittatus* (Bloch). Proceedings of the Zoological Society 64(2):117-123.
Samaddar A., Kaviraj A., Saha S., 2015 Utilization of fermented animal by-product blend as fishmeal replacer in the diet of *Labeo rohita*. Aquaculture Reports 1:28-36.
Setiawati M., Jusadi D., Laheng S., Suprayudi M. A., Vinasyiam A., 2016 The enhancement of growth performance and feed efficiency of Asian catfish, *Pangasianodon hypophthalmus* fed on *Cinnamomum burmannii* leaf powder and extract as nutritional supplementation. AACL Bioflux 9(6):1301-1309.
Suprayudi M. A., Edriani G., Ekasari J., 2012 [Evaluation of fermented product quality of various local by-products of local agro-industry: its effect on digestibility and growth performance of juvenile carp (*Cyprinus carpio*)]. Jurnal Akuakultur Indonesia 11(1):1-10 [In Indonesian].
Suryaningrum L. H., Jusadi D., Setiawati M., Sunarno D., 2017 Nutrient composition and apparent digestibility coefficient of *Ulva lactuca* meal in the Nile tilapia (*Oreochromis niloticus*). AACL Bioflux 10(1):77-86.
Takeuchi T., 1988 Laboratory work-chemical evaluation of dietary nutrients. In: Fish nutrition and mariculture. Watanabe T. (ed), pp. 179-233, Department of Aquatic Bioscience, University of Fisheries, Tokyo (JP).
Tanuja S., Kumar A., Nayak S. K., 2017 Effect of dietary intake of acid ensiled fish waste on the growth, feed utilization, hematology and serum biochemistry of rohu *Labeo rohita* (Hamilton, 1822) fingerlings. Indian Journal of Animal Research 51(3):501-505.
Thomas S. L., Piedrahita R. H., 1998 Apparent ammonia-nitrogen production rates of white sturgeon (*Acipenser transmontatus*) in commercial aquaculture systems. Aquacultural Engineering 17:45-55.
Tibbetts S. M., Lall S. P., Anderson D. M., 2000 Dietary protein requierement of juvenile American eel (*Anguilla rostrata*) fed practical diets. Aquaculture 186:145-155.
Tibbetts S. M., Lall S. P., Anderson D. M., 2001 Optimum dietary ratio of digestible protein and energy for juvenile American eel (*Anguilla rostrata*) fed practical diets. Aquaculture Nutrition 7(4):213-220.
Tseng K. F., Wu K. L., 2004 The ammonia removal cycle for a submerged biofilter used in a recirculating eel culture system. Aquacultural Engineering 31:17-30.
Watanabe T., 1988 Nutritional energetics. In: Fish nutrition and mariculture. Watanabe T. (ed), pp. 79-92, JICA Textbook the General Aquaculture Course, Tokyo.
Yigit M., Koshio S., Aral O., Karali B., Karayuce S., 2003 Ammonia nitrogen excretion rate - An index for evaluating protein quality of three feed fishes for the black sea turbot. The Israeli Journal of Aquaculture-Bamidgeh 55(1):69-76.
Yigit M., Erdem M., Aral O., Karaali B., 2005b Nitrogen excretion patterns and postprandial ammonia profiles in black sea turbot *Scophthalmus maeoticus* under controlled conditions. The Israeli Journal of Aquaculture-Bamidgeh 57:231-240.
Yigit M., Ergun S., Turker A., Karaali B., Bilgin S., 2005a Using ammonia nitrogen excretion rates as an index for evaluating protein quality of prawns in turbot *Psetta maeotica* nutrition. Turkey Journal of Veterinary and Animal Science 29:1343-1349.

*** APHA [American Public Health Association], 2006 Standard methods for the examination of the water and wastewater. 22nd edition, American Public Health Association, Washington, DC.
*** AOAC [Association of Official Analytical Chemists], 2012 Official method of analysis of the Association of Official Analytical Chemist. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.
*** FAO [Food and Agriculture Organization of the United Nations], 2014 *Anguilla japonica* (Temminck & Schlegel, 1847). Cultured Aquatic Species Information Programme, Fisheries and Aquaculture Department.
*** FAO [Food and Agriculture Organization of the United Nations], 2010 Fishery and aquaculture statistics. 2010 FAO yearbook annuaire, A-1 & A-6.
*** KKP [Ministry of Marine and Fisheries], 2014 [Marine and fishery statistics data in 2014]. Kementerian Kelautan dan Perikanan RI, Jakarta 63 p. [In Indonesian].
*** NRC [National Research Council], 2011 Nutrient requirement of fish and shrimp. National Academic Press, Washington DC.

Received: 3 January 2018. Accepted: 16 April 2018. Published online: 21 April 2018.
Authors:
Hany Handajani, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Fisheries, Indonesia, East Java, Malang 65144, Jalan Raya Tlogomas no 246; Bogor Agricultural University, Graduate School, Study Program of Aquaculture Science, Indonesia, West Java, Dramaga Campus, Bogor 16680, e-mail: handajani@umm.ac.id; hanyhandajani.umm@gmail.com
Widanarni, Bogor Agricultural University, Faculty of Fisheries and Marine Science, Department of Aquaculture, Indonesia, West Java, Jalan Agathis, IPB Dramaga Campus, Bogor 16680, e-mail: widanarni@yahoo.com
Mia Setiawati, Bogor Agricultural University, Faculty of Fisheries and Marine Science, Department of Aquaculture, Indonesia, West Java, Bogor 16680, Jalan Agathis, IPB Dramaga Campus, e-mail: miasetia@apps.ipb.ac.id
Tatag Budiardi, Bogor Agricultural University, Faculty of Fisheries and Marine Science, Department of Aquaculture, Indonesia, West Java, Bogor 16680, Jalan Agathis, IPB Dramaga Campus, e-mail: tatagbdp@yahoo.com
Sujono, University of Muhammadiyah Malang, Faculty of Agriculture and Animal Science, Department of Animal Science, Indonesia, East Java, Malang 65144, Jalan Raya Tlogomas no 246, e-mail: sujono.umm64@yahoo.com

How to cite this article:
Handajani H., Widanarni, Setiawati M., Budiardi T., Sujono, 2018 Evaluation of digestibility and ammonia excretion of fish meal and fish silage fed to juvenile Indonesian shortfin eel (*Anguilla bicolor*). AACL Bioflux 11(2):495-504.