Digestibility of moringa leaf meal (*Moringa oleifera*) feed in milkfish (*Chanos chanos*)

[1]Muhaimin Hamzah, [1]Wellem H. Muskita, [1]Agus Kurnia, [2]Anshar

[1] Department of Aquaculture, Faculty of Fisheries and Marine Science, Halu Oleo University, Southeast Sulawesi, Indonesia; [2] Study Programme of Aquaculture, Food Ingredient and Medicine, Avicenna Health Technology Institute, Southeast Sulawesi, Indonesia. Corresponding author: M. Hamzah, mhamzah@uho.ac.id

Abstract. This study is aimed to evaluate the effect of substitution of soybean meal (*Glycine max*) with moringa leaf meal (*Moringa oleifera*) on their digestibility level in milkfish (*Chanos chanos*). A total of 120 milkfish juveniles (initial weight: 7.81±0.85 g) were distributed into 12 glass tanks (10 juveniles tank^{-1}) with a size 60 × 50 × 40 cm and were reared for 60 days. Four experimental diets were prepared based on a gradual substitution of soybean meal (SM) with *M. oleifera* leaf meal (MLM) in the diet: namely, 100% SM (Diet A), 75% SM and 25% MLM (Diet B), 50% SM and 50% MLM (Diet C) and 25% SM and 75% MLM (Diet D). All the experimental diets were incorporated with 0.5% chromium oxide (Cr_2O_3) as an indicator to determine their digestibility level in the fish. The feces were collected one hour after the fish feeding. Statistically, it was shown that the fish fed diets with a different SM levels of substitution with MLM had significantly different protein digestibility and total digestibility degrees. The protein digestibility values of the fish ranged between 82.41 and 86.81% and the total digestibilty of the fish ranged between 56.36 and 62.19%. An increase in MLM in the diet was inversely correlated with the digestibility of the feed in the fish. A substitution of SM with MLM up to 50% in the diet revealed no change of digestibilty in the fish. However, the MLM incorporation of more than 50% in the diet resulted in a decrease in protein digestibility in the fish. Overall, this study concluded that a substitution of SM with MLM, as vegetable protein sources, could be done up to 50% without compromising the digestibility of milkfish fed.
Key Words: protein digestibility, soybean meal, milkfish juveniles, chromium oxide, fish feces.

Introduction. Milkfish (*Chanos chanos*), one of the most sought-after brackishwater aquaculture comodities in Indonesia, is a species with a high tolerance towards environmental changes within the tropical water and also with a strong resistance towards pests and diseases (Male et al 2019). According to statistic data from Indonesia Fisheries, in 2017 the production of *C. chanos* in Indonesia was of 636.825 MT (Nainggolan et al 2018) and it is expected to increase, since *C. chanos* is not only consumed as a protein source in human food, but is also used for baits to capture tuna fish (Syamsuddin 2010).

A possible strategy to increase *C. chanos* production is to alter the rearing method from extensive to intensive fish culture. The intensification in high density *C. chanos* culture relies on feed formulated in the pellet form (Aslamyah et al 2018). Feed quality is determined by the quality of feed ingredients, particularly of the protein, lipid, carbohydrate, mineral and vitamin and other essential nutritions (Maslang et al 2018).

Generally, the vegan protein source that is often used in feeds is soy bean meal (Hardy 2010), due to its vegetable protein nutritive value, digestibility and balanced amino acid profile (Lovell 1988). Furthermore, soybean meal is also a source of vitamin A, E, K and of several minerals (calcium, magnesium, iron, phosphor, manganese, copper, selenium, and potassium). Hence, it's not suprising that soybean meal is commonly used as feed ingredient for many fish species, in fish culture (Storebakken et al 2000). However, the use of soybean meal (SM) as fish ingredient competes with its use as food for human being and thus alternative vegetable protein sources should be investigated for their potential as fish feed ingredient.

One feed ingredient that can potentially replace SM as alternative vegetable protein source is moringa (*Moringa oleifera*) leaf. Makkar & Becker (1997) stated that *M. oleifera* is of a great interest for its leaves, flower or fruit that can be used as food ingredient. Some studies showed that *M. oleifera* leaf meal (MLM) contain similar nutritional values with SM. According to many sources, *M. oleifera* leaf (ML) can contain various crude protein levels: 22.75% (Melo et al 2013), 26% (Makkar & Becker 1996), 32% (Soliva et al 2005), 25.95% (Idowu et al 2017), 26.40% (Shiriki et al 2015), 27.1% (Fuglie 1999) or 27.69% (Hussain et al 2018). Other studies have shown that ML contains levels of vitamin C at equivalent to 7 oranges, vitamin A equivalent to 4 carrots, calcium equivalent to 4 glasses of milk, potassium equivalent to 3 bananas and protein equivalent to 2 yoghurts (Mahmood 2011). MLM contains also antioxidants and antimicrobials (Das et al 2012).

As reported in literature, the digestibility of MLM in pangasius (*Pangasius bocourti*), ranges between 69.43-81.95%. The protein digestibility ranges between 87.37 and 91.87% when fish is fed with a diet containing 0-200 g kg^{-1} of MLM (Puicha et al 2017). The aim of this study is to evaluate the digestibility level of *C. chanos* fed with different contents of MLM in the diet.

Material and Method. This experiment was conducted from October 2018 to January 2019 in the Laboratory of Fish Hatchery and Production Faculty of Fisheries and Marine Science, at the Halu Oleo University. Meanhile, the proximate analysis and Cr_2O_3 level analysis in feed and feces were performed in the Laboratory of Fish Nutrition Department of Aquaculture, at the Faculty of Fisheries and Marine Science of the IPB University. A total of 120 *C. chanos* juveniles (initial weight: 7.81±0.85 g) were reared in 12 glass tanks (size: 60 × 50 × 40 cm), filled with filtered marine water (at a salinity of 25-27 ppt and at a temperature of 25-27°C). The fish were reared by using a recirculating system. Four experimental diets were formulated to be iso-nitrogenous (CP:35%) (Table 1). Four experimental diets were prepared to contain different levels of substitutions of soybean meal with *M. oleifera* leaf meal in the diet. The experimental diets were 100 % SM (diet A), 75% SM and 25% MLM (diet B), 50% SM and 50% MLM (diet C) and 25% SM with 75% MLM (diet D). All the diets were incorporated with 0.5% of Cr_2O_3, as an indicator to determine the feed digestibility.

Table 1

Formulation of experimental diets and results of proximate analysis of the feed

Ingredients	*Experimental diets (g 100 g^{-1})*			
	A	*B*	*C*	*D*
Fish meal	22	22	22	22
Shrimp meal	22	22	22	22
Soybean meal	25	18.75	12.5	6.25
Moringa leaf meal	0	6.25	12.25	18.75
Corn meal	11	11	11	11
Rice bean meal	11	11	11	11
Tapioca meal	4.5	4.5	4.5	4.5
Sago meal	3	3	3	3
Fish oil	0.5	0.5	0.5	0.5
Squid oil	0.5	0.5	0.5	0.5
Mineral and vitamin mix	0.5	0.5	0.5	0.5
Proximate analysis (%)				
Crude protein	38.38	37.94	37.28	35.66
Crude lipid	8.02	5.9	6.6	4.56
Moisture	7.34	8.68	8.15	10.18
Crude ash	11.88	12.26	12.52	14.22
Fiber	5.72	5.85	5.49	5.8

The experiment was conducted using a completely randomized design with four treatments and three replications.

The first step of this experiment was the preparation of *C. chanos* to be reared. The *C. chanos* juveniles (initial weight: 7.81±0.85 g) that had been adapted to a rearing condition were distributed into 12 glass tanks (10 fish $tank^{-1}$). The fish were reared for 60 days and fed twice a day (08:00 a.m. and 04:00 p.m.), at satiation. One hour after feeding, the feces were collected to determine the feed digestibility. Collected feces were put in small bottle and kept in a freezer. Feces collecting was conducted during the entire experimental period (60 days). After the feeding trial, the feces were dried, their Cr_2O_3 level was determined and they underwent a proximate analysis. The determination of Cr_2O_3 level and the proximate analysis were also performed on the experimental diets.

The two parameters determined were total digestibility and protein digestibility. Calculation of the two parameters followed the formula proposed by Maynard & Loosly (1969) as follows:

$$CPD = 100 - 100 \times [\frac{Id}{If} x \frac{Nf}{Nd}]$$

$$TD = 100 - [100 \times \frac{Id}{If}]$$

Where:
CPD - crude protein digestibility(%);
TD - total digestibility (%);
Id - % Cr_2O_3 in the diet;
If - % Cr_2O_3 in the feces;
Nf - % protein in the feces;
Nd - % protein in the diet.

All of the data were analyzed by using ANOVA. If there was anydifference in the 5% significance level, a Tuckey test would follow. The data were processed by using Microsoft Excel Programme and Minitab Version 19.

Results. Figure 1 presents the results of the crude protein digestibility calculations for *C. chanos* fed with a different dosage of MLM substitute for SM in the diet. The highest CPD was determined for the diet A (86.81%), followed by the diet C (86.21%) and by the diet B (84.82%), and eventually by the diet D (82.41%). Results of ANOVA analysis showed that there was a significant difference ($p<0.05$) in the crude protein digestibility values in *C. chanos*. The Tuckey test showed that the CPD of the fish fed with diet A was no significantly different of the CPD in the fish fed with diets B and diet C, however it was significantly different of the CPD in the fish fed with diet D. Moreover, the CPD of the fish fed with diets B and C weren't significantly different of the CPD in the fish fed with diet D.

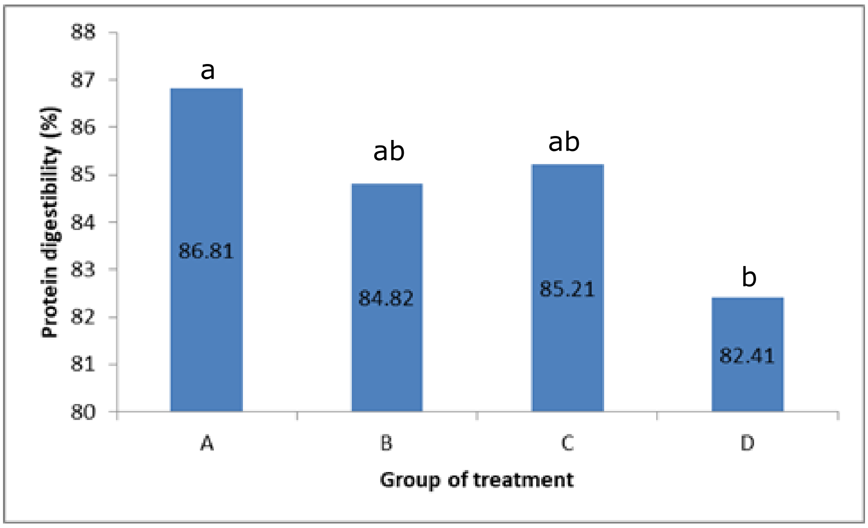

Figure 1. Crude protein digestibility in *Chanos chanos* fed with different of substitutions of SM with MLM in their diet.

Figure 1 presents the results of the total digestibility calculations for *C. chanos* fed with a different dosage of MLM substitute for SM in the diet. The highest total digestibility of dry materials was determined for the diet C (62.19%), followed by the diet B (60.84%) and by the diet A (60.68%), eventually by the diet D (56.36%). Results of ANOVA analysis showed that the different substitution levels of SM with MLM in the diet caused significant differences in the total digestibility in *C. chanos*. The Tuckey test showed that the total digestibility values of diets A, B and C were significantly different of the total digestibility values of the diet D in, in the observed fish specimens.

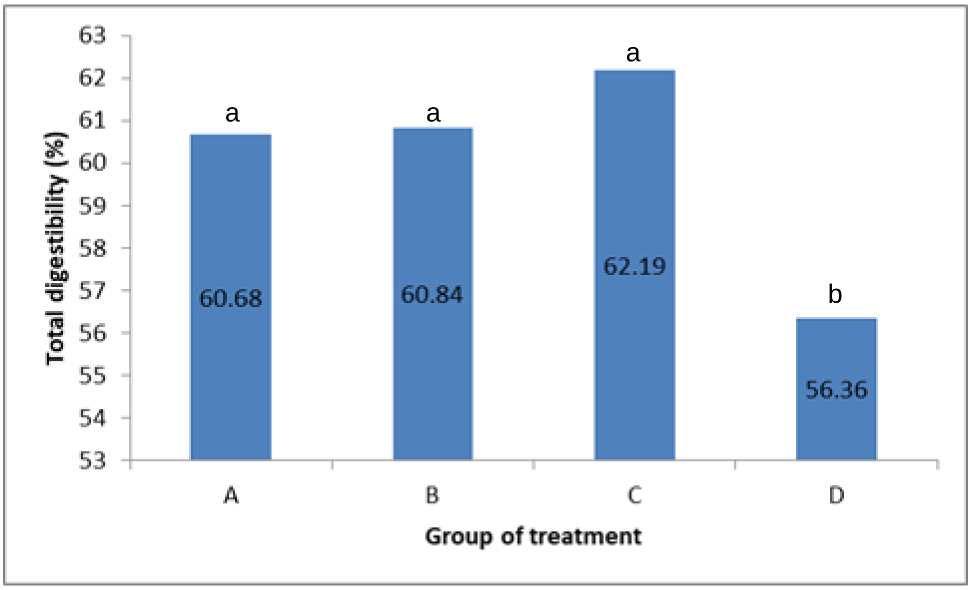

Figure 2. Total digestibility in *Chanos chanos* fed with different substitutions of SM with MLM in their diet.

Discussion. Utilization of MLM as fish feed ingredient has been studied by some fish nutritionist such as Yuangsoy & Masumoto (2012) in feed for carp (*Cyprinus carpio*); Puicha et al (2017) in feed for *P. bocourti*; Idowu et al (2017) in feed for catfish juveniles (*Clarias gariepinus*); David-Oku et al (2018) in feed for *C. gariepinus*; Hussain et al (2018) in feed for *Lobeo rohita* juveniles; Maslang et al (2018) in feed for nile (*Oreochromis niloticus*); and Anshar et al (2018) in feed for *C. chanos*. These studies showed that, generally, MLM has the potential to be used asingredient in fish feed.

This study found that increasing the MLM level in diets implies a decrease of protein digestibility in fish (Figure 1). A substitution of 75% of SM with MLM in the diet (treatment D) resulted in the lowest shrimp protein digestibility and was significantly different of the other treatments (A, B and C). This might be caused by a decrease in the protein contribution from SM, thus affecting the overall protein digestibility. SM has a very high digestibility and its amino acid profile is very well balanced (Lovell 1988), therefore decreasing the SM content in a diet could reduce the protein digestibility of the diet. This finding is related to the experimental results obtained by Puicha et al (2017), where it was found that protein digestibility of bocourti's catfish decreased as the MLM content in the diet increased. Similarly to the protein digestibility determinations, the proximate analysis showed that the protein content in the diet decreased as the MLM content in the diet increased. The lowest crude protein concentration was found in diet D (Table 1).

According to this experiment, the protein digestibility of the tested diets ranged between 82.41 and 86.81%. These values were higher than the protein digestibility results found by Hussain et al (2018), that ranged between 47.23-74.03% for *Lobeo rohita* juvenile fed with MLM at 0, 10, 20, 30, and 40% of the diet content. However, the protein digestibility results found in this study were still lower than the results of protein digestibility found by Puicha et al (2017), in the range of 89.56-91.87%, in *P. bocourti* fed with diets at MLM concentrations of 0, 100, 150 and 200 g kg^{-1}.

In this study, it was observed that the total digestibility values were relatively similar in the treatments A, B and C. However, a decrease was found in the treatment D, which was significantly different of the treatments A, B, and C (Figure 2). This result indicated that MLM could substitute SM in the diet up to a content of 50%. Substitution of more than 50% SM with MLM in the diet can cause a decrease of the total digestibility in the fish. In other words, MLM cannot entirely substitute SM as vegetable protein source in the *C. chanos* diet. The low total digestibility in the fish fed with the diet D (75% MLM) might be caused by a higher carbohydrate content in MLM, compared to the SM. In our previous study, we found that carbohydrate content in MLM was 39.17%, while Burrsens et al (2011) found that the carbohydrate content in SM was 28%. A high carbohydrate content in an ingredient could decrease the activity of cellulose enzyme in fish, so that their feed digestibility also decreases. Liao et al (2015) revealed that feed digestibility is affected by the presence of the enzyme and by its activity level in the digestive tract of fish.

The total digestibility found in the present study ranged between 56.36-62.19%. This finding is lower than the values of Puicha et al (2017), which ranged between 69.43-81.95% in *P. bocourti* fed with a diet at MLM concentrations of 0, 100, 150, and 200 g kg^{-1}. The potential cause might be the different species used as a sample in the experiment. Pangasius catfish is a freshwater fish that has higher capability to efficiently utilize carbohydrate, compared to the *C. chanos*, which are brackish water fish.

Conclusions. A substitution of soybean meal with *M. oleifera* leaf meal up to 50% in the diet revealed no change of digestibilty in *C. chanos*. However, the *M. oleifera* leaf meal incorporation of more than 50% in the diet resulted in a decrease in protein digestibility in the fish. Overall, this study concluded that substitution of soybean meal with *M. oleifera* leaf meal as a vegetable protein can be done with a maximum substitution of 50%.

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Authors:
Muhaimin Hamzah, Halu Oleo University, Faculty of Fisheries and Marine Science, Department of Aquaculture, 93231 Southeast Sulawesi, Indonesia, e-mail: mhamzah@uho.ac.id
Wellem H. Muskita, Halu Oleo University, Faculty of Fisheries and Marine Science, Department of Aquaculture, 93231 Southeast Sulawesi, Indonesia, e-mail: wellemhenrikmuskita@uho.ac.id
Agus Kurnia, Halu Oleo University, Faculty of Fisheries and Marine Science, Department of Aquaculture, 93231 Southeast Sulawesi, Indonesia, e-mail: aguskurnia@uho.ac.id
Anshar, Avicenna Health Technology Institute, Study Programme of Aquaculture, Food Ingredient and Medicine, 93116 Southeast Sulawesi, Indonesia, e-mail: ansharefka@yahoo.com

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