Enrichment of commercial feed with plant proteins for *Oreochromis niloticus* diet: digestibility and growth performance

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Abstract. In fish farming, feed cost contributes 50 to 70% of the fish production cost. Fish can grow optimally if their nutrient needs are available, including protein, fat, carbohydrates, vitamins, and minerals. Approximately 70% of the fish feed ingredients in Indonesia are imported, while the demand for and production of the meal (fish and shrimp) in Indonesia reached 1.650.000 tons in 2018. Based on these problems, it is necessary to look for alternative raw materials, especially local raw materials. The quality of fish feed ingredients is determined from their nutritional value and digestibility, feed nutrition retention, and waste, which could be used as alternative nutrition, such as pumpkin seeds and sunflower seeds. This study aimed to evaluate the nutritional quality of the pumpkin and sunflower seeds, consisting of the digestibility value, total digestibility, and protein digestibility of pumpkin seeds and sunflower seeds in Nile tilapia *Oreochromis niloticus*. This study used a completely randomized design (CRD) containing three treatments in different feed ingredients: feed made from pumpkin seed flour, the meal made from sunflower seed flour, and commercial feed and was repeated three times. The variables were measured, including material digestibility, total digestibility, protein digestibility, growth rate, protein retention, fat, and feed conversion ratio. The results showed that pumpkin seed feed gave the highest yield compared to commercial feed and sunflower seed feed. Meanwhile, the digestibility of materials, total digestibility, and protein digestibility did not show significant differences between treatments. To sum up, the growth performance of *O. niloticus* was higher in pumpkin seeds than sunflower seeds, and no differences in the digestibility value of the ingredients, the total digestibility, and the protein digestibility between pumpkin seeds and sunflower seeds in *O. niloticus*.
Key Words: handmade feed, pumpkin seeds, sunflower seeds, tilapia.

Introduction. In 2018, fish and shrimp feed production in Indonesia reached 1.65 million tonnes. The feed consists of 1.3 million tonnes of fish feed, and the remaining 350,000 tonnes is shrimp feed. Feed raw materials are needed to produce feed with this amount, but currently, around 70% of raw materials still rely on imports (Anonymous 2019). Raw materials which are imported products include fish meal, soybean flour, and pollard. A review conducted by Ningrum et al (2018) showed that Indonesia's soybean imports amounted to 2.585 million tonnes in 2018, then it has been projected to increase in 2020 by 4.13%. Efforts to reduce dependence on imported raw materials have been conducted to find local-based alternative raw materials. Several studies have shown that local raw materials such as fermented *Azolla* (Handajani 2011), oil palm (Pamungkas et al 2011), copra (Suprayudi et al 2013), cocoa pod husks (Jusadi et al 2013), leaves of lamtoro (Fitriliyani 2010), rubber seeds (Suprayudi et al 2013; Suprayudi et al 2017), cassava flour (Onuoha et al 2020) could be used as feed raw materials. However, the nutritional value of those raw materials is still insufficient for fish feed. Therefore, it is necessary to look for other alternative raw materials with protein value and availability are abundant.

Moreover, one factor that determines the success of fish culture production is the availability of high-quality feed. The need for feed is the most considerable production cost, reaching 70% of the total fish maintenance (Suprayudi et al 2013; Suprayudi et al

2017; Ayisi 2021). Fish could grow optimally if their nutrient needs are provided, namely protein, fat, carbohydrates, vitamins, and minerals (Ljubojević et al 2015). Of all them, protein has a vital role in the growth, development of fish body tissues and is a source of energy for aquatic animals (Jobling 2016). However, the protein price is high, so the feed is one determinant of the high cost of fish feed (Jiang et al 2016; Hua et al 2019). The amount and quality of feed protein will affect fish's growth and survival rate (Hua et al 2019). If the protein in the feed is lacking, the supply of protein for retention and forming new body tissue will not be sufficient.

Conversely, if the excess feed protein or protein quality is not following the needs, the protein is not used to synthesize fish body protein. It will be excreted as nitrogen waste, especially in ammonia (Suprayudi et al 2013). Fish may utilize dietary protein as an additional energy source to satisfy maintenance before growth in circumstances where the dietary P/E ratio is imbalanced and available non-protein energy is insufficient, resulting in decreased growth and production (Jiang et al 2016). Based on these problems, it is necessary to look for alternative raw materials, especially local raw materials. Feed raw materials must meet several criteria, including abundant availability, relatively low prices, easy to digest by fish, have good nutritional content, and do not compete with humans needs, such as food (Handajani et al 2018; Suprayudi et al 2013). Furthermore, Glencross et al (2007) explain fish feed ingredients' quality apart from their nutritional value. It could also be based on the digestibility value of the material, the level of feed nutrition retention, and the resulting waste of the agro-industrial activities such as pumpkin (*Cucurbita moschata*) seeds and sunflower (*Helianthus annuus*) seeds.

Yellow squash (*C. moschata*) could grow widely throughout the world in the lowlands and highlands with an altitude between 0 and 1,500 m above sea level. It is an annual creeping vegetable that will die after bearing fruit. Furthermore, the pumpkin seeds are oval, flat, and have not been utilized by the community (Hendrasty 2003; Patel 2013). They are rich in protein, fiber, minerals, fatty acids, carotenoids, and phytosterols (Glew et al 2006; Lestari & Meiyanto 2018; Syed et al 2019). The primary fatty acids in pumpkin seeds are palmitic, stearic, oleic, and linoleic (Kim et al 2012). According to Manda Devi et al (2018), pumpkin seed kernels provided moderate minerals, especially P, Mg, and K.

Moreover, the seed contained methionine and tryptophan, which were the most limiting amino acids. In contrast, the seed also had significant amounts of arginine, glutamic, and aspartic acids. Therefore, pumpkin seed kernels could be utilized for fish meals because of their high lipid and protein content and fatty acid and amino acid compositions (Loukos et al 2003; Kanyandenge 2017). In the same case, protein, unsaturated fats, fibre, vitamins (particularly E), selenium, copper, zinc, folate, iron, and other nutrients are all found in the common sunflower seed, which is farmed and consumed all over the world (Guo et al 2017). It has been estimated that sunflower seeds contain roughly 20% protein, which provides the sulfur and nitrogen for seedling development after germination (Youle & Huang 1978). Moreover, according to González-Pérez et al (2005), the sunflower seed contains two types of storage proteins: 11S globulins and napin-type 2S albumins, 60% of which are water-soluble 2S albumins and the rest 11S globulins. In another review, the seed also provides aspartic acid, glutamic acid, and arginine of 10.50 g, 26.91 9, 9.75 g/100 g protein, respectively (Adeleke & Babalola 2020). In addition, leucine, phenylalanine, tyrosine, cysteine, and methionine, the essential amino acids, make up a total of 2.30 g to 12.20 g, 1.30 g to 8.20 g, 0.83 g to 4.30 g, 0.40 g to 6.40 g, and 0.30 g to 2.10 g of protein per 100 g of the material analyzed (Dotto & Chacha 2020). Consequently, it has been used as a feed additive for improving fish feed quality in some fish cultivation, such as *Oreochromis niloticus* (Soltan 2002; Soltan et al 2015; Hassaan et al 2018), *Cyprinus carpio* (Rahmdel et al 2018), and *Oncorhynchus mykiss* (Greiling et al 2018a).

Based on the nutritional value of the pumpkin and sunflower seeds waste, these two ingredients are potential fish feed supplements. However, until now, no information has been obtained about the digestibility of pumpkin and sunflower seeds in fish. As a result, we need a study on pumpkin seeds and sunflower seeds in fish feed. The evaluation was carried out through a digestibility test and the effect of adding pumpkin and sunflower seeds to the feed on the growth performance of *O. niloticus*.

Material and Method

Research methods. This study was conducted in an indoor laboratory from July to October 2020, employing an experimental approach with a completely randomized design (CRD). There were three treatments in T1: reference feed (commercial), T2: feed made from pumpkin seed flour, and T3: feed made from sunflower seeds, and each treatment was repeated three times.

Preparation of test feed. The feed treatment consisted of T1: 94.6% commercial feed mixed with tapioca flour (3%) and Cr_2O_3 (0.6%), while T2 consisted of 66.4% commercial feed, 30% of pumpkin seed flour, 3% of tapioca flour, and 0.6% Cr_2O_3, and T3 was made from 66.4% commercial feed, 30% sunflower seed, 3% of tapioca flour, and 0.6% Cr_2O_3. The meals were formulated according to the nutritional needs of *O. niloticus* (Table 1). Cr_2O_3 was added as an indicator of digestibility (Takeuchi 1988). Tapioca is commonly used in the food industry, such as improving the taste and texture of various foods and foods that need consistency or stickiness (Shigaki 2016).

Table 1

Composition of commercial and treatment feed

Raw material	*T1*	*T2*	*T3*
Commercial feed (g)	96.4	66.4	66.4
Pumpkin seed flour (g)	0	30	0
Sunflower seed flour (g)	0	0	30
Tapioca flour (g)	3	3	3
Cr_2O_3 (g)	0.6	0.6	0.6
Total	100	100	100

Fish treatment. *O. niloticus* were obtained from the Umbulan Fish Seed Center, Pasuruan, East Java, Indonesia. There were 250 *O. niloticus* (7 g to 10 g), 150 samples for treatment, and 100 samples for stock. Afterward, they were reared in an aquarium (60 cm × 30 cm × 40 cm) with 48 L of water and were acclimatized under laboratory conditions for two weeks with commercial pellets (35% protein) in Aquaculture Laboratory, Department of Aquaculture, University of Muhammadiyah Malang, Indonesia. During the adaptation period, the health and vitality of the fish were considered so that it was suitable for test animals. Afterward, 2 days before the feeding treatment, the fishes were fasted to remove the remaining feed in their intestine.

Data collection and digestibility measurement. *O. niloticus* were adapted to the experimental aquarium for 3 days. Every morning before the feeding process, the water medium was changed to 20% of the total volume. The feeding process was conducted with a frequency of three times a day (Watanabe 1988). On day 2, feces of all treatments were collected for each treatment and then dried at 60ºC for 48 h. After that, dried feces were kept in a film bottle and stored in the refrigerator. The feces collection was carried out every 2 h to avoid leaching of nutrients for 20 days maintenance period.

Dietary and fecal samples were pulverized for analysis before proximate analysis would be conducted. The proximate analysis was carried out at the Fish Nutrition Laboratory, University of Muhammadiyah Malang, Indonesia. All proximate analyses followed the methods from AOAC (2012). The moisture content was determined by drying samples at 105ºC until constant weight, while the ash content was measured using a furnace (Furnace THERMOLYNE 47900; at 600ºC for 4 h). According to the Kjeldahl method, the protein content was evaluated using various equipment, such as crushing via FOSS TecatorTM; distillation by Kjeltec FOSS 2100; titration using JENCONS Digitate Pro.

Furthermore, the lipid content was analyzed by extracting petroleum ether (FOSS SoxtecTM 2055). Meanwhile, crude fiber was calculated by the gravimetric method by reacting the sample into acids and bases to separate the fiber fraction and other components. The wet crushing method was used to determine the levels of Cr_2O_3 in feed and feces. The absorbance was detected using a spectrophotometer with a wavelength of 350 nm (Takeuchi 1988).

$$\text{Total digestibility (\%)} = \left\{1 - \frac{\text{feed } Cr_2O_3 \text{ (\%)}}{\text{feces } Cr_2O_3 \text{ (\%)}}\right\} \times 100$$

$$\text{Protein digestibility (\%)} = \left\{1 - \frac{\text{feed } Cr_2O_3 \times \text{feces protein}}{\text{feces } Cr_2O_3 \times \text{feed protein}}\right\} \times 100$$

$$\text{Digestibility of ingredients (\%)} = \left\{\frac{\text{feed digestibility} - 0.7 \text{ digestibility of referent feed}}{0.3}\right\} \times 100$$

Moreover, the growth performances and survival rate of *O. niloticus* were also observed, including growth rate (GR), specific growth rate (SGR), absolute length growth (AL), and survival rate (SR). They were determined via a formulation from Dangeubun et al (2019) study:

$$GR\ (g) = Wt - W0$$

where: GR = growth rate (g);
W0 = initial weight (g);
Wt = final weight (g).

$$SGR\ (\%\ d^{-1}) = \frac{W0\text{-}Wt}{t}$$

where: SGR = specific growth rate (% d^{-1});
W0 = initial weight (g);
Wt = final weight (g);
t = time (day).

$$AL\ (cm) = Lt - L0$$

where: AL = absolute lenght (cm);
L0 = initial lenght (cm);
Lt = final lenght (cm).

$$SR\ (\%^{1}) = \frac{\text{Live fish}}{\text{Dead fish}} \times 100$$

where: SR = survival rate (%).

Statistical analysis. All data were evaluated using analysis of variance (ANOVA) provided in the SPPS program. Furthermore, the data that showed differences among treatments were analyzed using Duncan's Multiple Range Test.

Results. In this study, the proximate analysis was intended to know the nutrient content in the treatment feed, including water, ash, protein, crude fat, crude fiber, Nitrogen free-material, and total energy (Table 2). T3 could be considered the best treatment, while the lowest nutrition content was identified in T1. The quality level of feed treatments was determined from their protein and fat content. However, the highest nutrient content could not be judged to generate the best data at the end of the period.

At the time of observation, the result data shown in Table 3 illustrates *O. niloticus* for 30 days after feed treatment application. Overall, the data present statistically significant differences ($p < 0.05$) among treatments, proved with various notations. Moreover, T2 contributes the highest values of most parameters, including SGR, GR, and AL, while T1 was determined the worse treatment to improve *O. niloticus* growth performances.

Table 2

Results of the proximate analysis of the test feed

Nutritional value	*T1*	*T2*	*T3*
Water content (%)	9.36	8.07	8.58
Ash content (%)	10.75	10.30	10.30
Crude protein (%)	23.62	26.84	23.28
Crude fat (%)	7.98	17.28	20.92
Crude fibre (%)	10.67	9.06	8.10
Nitrogen-free material (%)	37.62	28.45	28.82
Total energy (kcal g^{-1})	361.53	429.38	445.18
Energy:protein (kcal g^{-1} $protein^{-1}$)	15.31	15.99	19.12

Nitrogen-free material = 100% - (% crude protein +% crude fat +% crude fibre +% water content +% ash).
Total energy = (5.6 x protein) + (9.4 x fat) + (4.1 x nitrogen-free material) (Bureau & Hua 2010).

Table 3

Performance of tilapia for 30 days

Parameter	*T1*	*T2*	*T3*
SGR (%)	0.81 ± 0.01^{a}	0.87 ± 0.01^{c}	0.82 ± 0.06^{b}
GR (g)	4.07 ± 0.14^{a}	4.74 ± 0.20^{c}	4.19 ± 0.21^{b}
AL (cm)	0.73 ± 0.10^{a}	0.99 ± 0.16^{c}	0.94 ± 0.05^{b}
SR (%)	86	86	86

SGR: specific growth rate, GR: growth rate, AL: the absolute length, SR: survival rate. The different letters in the same row indicate significant differences among treatments.

Furthermore, SGR data showed that T2 was the best treatment, accounting for 0.87±0.01%, followed by T3 with 0.82%. Meanwhile, the bottom position belonged to T1 with 0.81±0.01%. There was the same pattern in the GR results. T2 had the highest value, reaching 4.74±0.20%, 0.55%, and 0.67% higher than T3 and T1, respectively. Moreover, the AL data also had precisely the same interpretation, referring to SGR and GR data. The AL calculation revealed that the lowest level was in T1 (0.73±0.10%), while T2 placed the top position (0.99±0.16%). However, interestingly, SR had no different result among treatments after applying treatment for 30 days.

Figure 1 reveals the data, including total digestibility, protein digestibility, and material digestibility of *O. niloticus* after 30 days of feed treatment. All values are presented in percentage. In general, the data mainly determined no significant difference ($p > 0.05$), such as total digestibility and protein digestibility. On the other hand, there was a significant difference in ingredient digestibility.

Similar notation (a) was obtained from statistical analysis in total digestibility and protein digestibility. It means that various feed treatments did not impact those parameters ($p > 0.05$). Furthermore, the ingredient digestibility parameter also revealed a significant value among treatments. Although, the T2 and T3 (73.16±0.19% and 72.51±0.48%, respectively) came with a higher ingredient digestibility than the control group (T1).

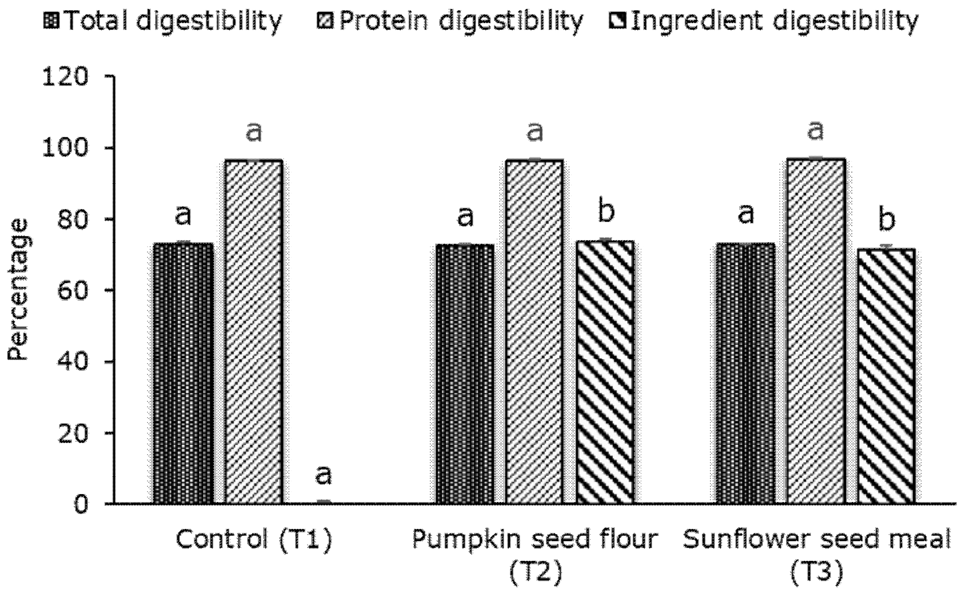

Figure 1. Graph of total digestibility, protein digestibility, and material digestibility in the treatment.

Discussion. Pumpkin seed and sunflower seed flours were found to have a positive impact on the nutritional content of feeds based on proximate analysis. For instance, there was an incline in the crude protein and fat in T2 (26.84% and 17.28%, respectively) and T3 (23.28% and 20.92%, respectively). All feed treatments still had over 5% of crude fiber, which possibly negatively impacted *O. niloticus* nutrient digestibility significantly (Table 2).

In the actual case, the crude fiber level takes responsibility for the feed quality regarding the absorption of nutrients. It is commonly used to examine the nutritional profile of compound feeds (Sun et al 2019). In a study by Adamidou et al (2011), the sharpsnout seabream's (*Diplodus puntazzo*) growth and nutrient digestibility were not affected by the presence of fiber in the diet at levels up to 5%. According to Handajani & Widodo (2010) there is an issue with the fish's capacity to break down crude fiber. For instance, *O. niloticus* can only absorb 10% of the crude fiber content. In another study, Bou et al (2014) also proved that feeding a diet with up to 18% fiber did not affect sea bream (*Sparus aurata*) growth performance. Therefore, dietary fiber concentrations between 3 and 5% could become helpful to fish development. In contrast, excessive fiber quantities could decrease the apparent digestibility of dry matter and the other nutrients' efficiency ratio (Altan & Korkut 2011; Grundy et al 2016).

Regarding those discoveries, the present study assumed no significance of total digestibility, protein digestibility, and ingredient digestibility because of the high concentration of crude fiber (> 5%) in feed treatment. It means that the addition of pumpkin seed and sunflower seed flour did not diminish crude fiber, so that the control feed (T1) and feed treatment (T2 and T3) still had crude fiber at the same level (Figure 1). Due to the higher amount of fiber in the diet, there is a much greater percentage of waste excreted, which leads to lower protein intake (Santoso & Putri 2013). Hardy (2002) stated that fish could not absorb the nutrients of feed ingredients optimally because of the high crude fiber value. In a similar study conducted by Ramos et al (2012), all agro-industrial by-products, including aerial part of cassava, mesquite bean, cotton, cocoa, soursop, and palm cake, came with an adverse effect for *O. niloticus* digestibility, whereas the crude fiber was over 200 g kg^{-1}. Furthermore, in many cases, one of the major difficulties to fish feed raw materials is anti-nutritional chemicals in vegetable foods, for example, crude fiber (Suprayudi et al 2013). Therefore, the decrease in crude fiber indicates that the quality of the feed matter has improved. This is because the low-value crude fiber will make the feed ingredients more digestible (Hepher 1988; Vitanti et al 2021). In addition, the feed ingredients digestion could be affected by several factors, including ration composition, fish condition, feed ingredients form and differences between individuals (Chemello et al 2020; Hatmaya 2008).

Moreover, fish growth is a process of an organism's length and weight development in a certain period. Those activities are influenced by several factors, including the quality and quantity of feed, fish age, and water quality of media (Lugert et al 2016; Jisr et al 2018). The present study discovered that T2 and T3 showed better results than *O. niloticus* growth performances, such as GR, SGR, and AL, while T1 was a worse treatment. Those results were opposite with *O. niloticus* protein digestibility, which had no difference among treatments ($p > 0.05$). However, T2 still could boost *O. niloticus* growth higher than others. According to Rasmussen & Jokumsen (2009), protein digestibility and SGR were shown to have a strong positive relationship. As a result, this study believed that crude fat plays a vital role in assisting *O. niloticus* growth (Table 3). Based on Table 2, T2 and T3 had almost three times quantities of crude fat from T1, accounting for 7.98%. Dietary lipids are essential for the growth of fish because they provide energy for the organism and act as transporters for fat-soluble vitamins in the body (Peng et al 2008). Furthermore, the lipid could improve the protein efficiency through increasing non-protein energy sources, providing more protein available for fish development (Xu et al 2001). The digestibility lipid improved rainbow trout growth performance (initial weight = 7 g fish^{-1}) (Bureau et al 2002). In another study, 250 g of pumpkin seed cake claimed American catfish's specific growth rate and absolute weight, accounting for 345.7 g and 422±34.9 g, respectively (Greiling et al 2018b). Based on those results, that the pumpkin seed is considered could be applied for triggering *O. niloticus* growth.

In addition, Table 3 also reveals that the SR had no significant difference among treatments, reaching 86% of each treatment at the end of the period. That percentage means that pumpkin seed and sunflower seed did not act as toxicants for *O. niloticus* body.

Conclusions. The application of pumpkin seed and sunflower seed presents a positive result, such as *O. niloticus* growth performances and commercial feed quality compared to feed reference. The growth performance of *O. niloticus* was higher generated by pumpkin seeds than sunflower seeds. However, there were no significant differences in the digestibility value of the ingredients, the total digestibility, and the protein digestibility between pumpkin seeds and sunflower seeds in *O. niloticus*.

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