Utilization of fish-processing waste as replacement of fish meal in aquafeed

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Abstract. Fishmeal is the most expensive component of aquaculture feed, thus finding cheaper alternative protein sources become a major challenge in aquafeed industries. This study aimed at investigating the effect of fishmeal replacement with a fish waste meal of fish processing industries on growth performances and nutrient utilization of Nile tilapia (*Oreochroms niloticus*). Fish-waste of albumin-extracted Snakehead was collected, dried and grounded to produce a fine meal. The result showed that pellets produced from the fish-waste meal showed better growth, higher nutrient utilization and survival rate than the commercial pellet, $p<0.05$. The best Specific growth rate (SGR), feed utilization efficiency and survival rate (SR) were obtained from the tilapia receiving pellet in T3 or 60% fish-waste meal, with length and weight gain of 4.00 ± 0.11 cm and 7.99 ± 0.10 g respectively. In addition, the protein digestibility recorded from diet 3 (T3) was also significantly higher, 76.4%. However, no significant effect was observed in terms of survival rate (all 100%) and feed conversion ratio (1.44-1.53). These results suggest that the fish waste from fish processing industries can be used as fish meal replacements in aquafeed industries.

1. Introduction

Feed is the costliest component in aquaculture industries, accounting for 60-70% of total production cost [1]. In addition, feed quality is also very critical to the growth of cultured fish. In general, the nutrient requirement for fish is 32-45% protein, 4-28% lipid, 10-30% carbohydrate, 1-2.5% mineral, and 1-2.5% vitamin [2]. The most common protein source for aquafeed is fish meal, due to high protein content (~65-70%) and high digestibility. However, the use of fish meals has been criticized due to the high dependency on wild fish catch and also expensive. Thus, protein sources with relatively cheap prices become a major interest in aquafeed research nowadays [3].

Several studies suggest using plant materials and waste from fish processing. Fish waste produced from albumin extraction of snakehead fish in PT. Royal Medicalink Pharmalab or commonly referred to as snakehead fish extract waste (LEIG) has been considered potential material for protein sources due to its high protein content, ~50%. Research on the utilization of fish processing waste as fish pellets conducted by Nurhayati & Asti, stated that pellet with a composition of 60% fish waste

consisting of tail and fins, was able to increase growth in length and weight of African catfish by 20.97%. Similarly, Firmansyah [4] documented that utilization of fish processing waste (fuming), was able to increase growth by 14.56% and the protein content by 6.06%. The previous studies suggest that wastes from fish processing industries can be used as potential ingredients for protein sources of aquafeed. However, optimal usage of the fish waste meal in aquafeed has not been studied.

Thus, the present study was aimed to determine optimal usage of fish waste meal in aquafeed for Nile tilapia, *Oreochromis niloticus*. In addition, the physical characteristics of the produced pellet, nutrient digestibility and its effect on the growth as well as survival rates of Nile tilapia, were also evaluated.

2. Material and Methods

This research was conducted at the Biology Laboratory, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Laboratory of Nutrition Analysis and Fish Feed, Universitas Airlangga, Integrated Testing Laboratory of the Universitas Pembangunan Nasional (UPN) Veteran Jawa Timur and Green House Sukolilo Park Regency Surabaya.

2.1. Preparation of Fish-Waste Meal

Fish waste was collected from a local Pharmalab industry which extracted the medicinal content of snakehead fish, *Channa striata*, for drug purposes. The waste was firstly dried using an oven at a temperature of 70±5°C to reach a water content of ~10%. Then it was grounded to produce a fine flour using a grinding machine. The produced meal was tested for the total volatile base nitrogen (TVB-N) to determine the meal quality. The assay was carried out according to the Indonesian standard for fish meal, SNI 01-4495-1998 method and carried out at the Laboratory of Nutrition Analysis and Fish Feed, Universitas Airlangga, Surabaya.

2.2. Pellet Production

Pellet production was carried out according to the modified method of Nurhayati and Febiyani. Basal ingredients consisted of 85% rice bran, 10% tapioca meal, and 4% vitamin mix. The basal ingredients were then mixed with fish waste meal according to each treatment. T1 (50% basal component: 50% fish waste meal), T2 (45% basal component: 55% fish-waste meal) and T3 (40% basal component and 60% fish-waste meal). All components were afterwards mixed and fermented with *Saccharomyces cerevisiae* for 24h. The fermented dough was then pelleted (diameter of ~2mm) and dried in an oven at a temperature of 60ºC. The proximate composition of all diets was presented in table 1.

Table 1. Proximate content of produced pellet.

Proximate	Treatments				
	T0[a] (%)	T1[b] (%)	T2[c] (%)	T3[d] (%)	National standard (SNI 01-4087-2006; %)
Moisture	9.23	8.31	8.95	7.57	≤12
Ash	9.48	13.61	15.06	15.57	≤13
Crude Protein	34.91	44.26	47.84	51.21	≥30
Crude Fat	4.6	2.98	4.98	5.17	≥5
Crude Fibre	5.45	5.86	5.59	5.95	≤6
Carbohydrate	41.78	30.84	23.17	20.48	-

[a] T0/ Control (commercial pellet).
[b] T1/ Treatment 1 (fishwaste meal 50%).
[c] T2/ Treatment 2 (fishwater meal 55%).
[d] T3/ Treatment 3 (fishwaste meal 60%).

The nutritional content of pellets, in general, was quite similar especially moisture, fat, and fibre was quite similar which were 7-9%, 4-5% and 5% respectively. However, protein content in the fish waste

pellet was higher than the control, ~35 in the commercial pellet (T0) and 44-51% in the fish waste pellet. But carbohydrate content appeared to be smaller in the produced pellet compared to commercial pellet in the control.

2.3. Feeding Experiment

Nile tilapia, *Oreochromis niloticus*, with an average weight of 5.01±05 g or 7-8 cm in length, were assigned into four groups (T0, T1, T2 and T3) with triplicates of experimental units. Each experimental unit consisted of an aquarium (60x30x30 cm) and was stocked with 8 tilapia. Each aquarium was filled with water and installed aeration supply. The fish were cultured for 30 days and fed at 3% of the fish's body weight three times daily.

2.4. Measurement Parameters

The observed parameters were pellet characteristics, proximate analysis of pellets, proximate analysis of meat, initial and final body weight, initial and final length, feed conversion ratio, feed digestibility and survival rate.

Pellet characters including buoyancy, stability in water, hardness and durability were also measured. Determination of buoyancy was performed by floating 10 pellet samples for 180 minutes [5]. Determination of stability in water by measuring the percentage ratio of dry weight and wet weight [6]. Determination of pellet hardness by dropping the load at a certain height on the pellets and then calculating the pellets that pass during the sieve [7]. Determination of the speed of rupture with a span of 5 minutes and then emphasis is placed on the pellet sample that is inserted into the water container [7].

The proximate analysis consists of moisture, ash, fat, protein, fibre and carbohydrate content. Determination of moisture content was carried out gravimetrically based on the difference in sample weight after and before the sample was dried. Determination of ash content is divided into dry ash and wet ash. Determination of crude protein content can be done using the Kjeldahl method with the principles of titration and stoichiometry. Quantitative fat analysis was carried out using the soxhlet extraction method and calculated gravimetrically. Determination of crude fibre content using the soxhlet extraction method. Determination of carbohydrates using the by difference method [8].

The growth of the fish body by measuring the difference between the initial length and the final length as well as the initial and final weight. Survival Rate (SR) for 30 days, Feed Conversion Ratio (FCR), Digestibility test using total collecting methods, namely collecting faeces for 10 days then measuring the difference between feed protein and faecal protein then divided by feed protein [9].

2.5. Data Analysis

Data of fish body length and weight, feed conversion ratio (FCR) and survival rate were analyzed using Analysis of Variance (ANOVA) and Tukey's follow-up test to determine the effect of significant differences between each combination on the measurement parameters as previously described by Amin et al [10].

3. Results

3.1. Physical Properties of the Producing Pellet

The physical analysis of the pellets included buoyancy, stability in water, hardness and durability in water were presented in Table 2. As the control (T0), commercial pellet 9 Hi-Pro Vite 781-1) was also used as a comparison.

Tabel 2. Physical properties of the produced pellet.

Measurement parameters				
Treatments	**Buoyancy (min)**	**Stability (%)**	**Hardness (%)**	**Durability in water (min)**
T0[a]	180	99.50	98.5	60
T1[b]	5	99.12	98	15
T2[c]	5	99.11	97.5	15
T3[d]	8	87	97.5	15

[a] T0/ Control (commercial pellet).
[b] T1/ Treatment 1 (fishwaste meal 50%).
[c] T2/ Treatment 2 (fishwater meal 55%).
[d] T3/ Treatment 3 (fishwaste meal 60%).

In addition, the present study assessed the total Volatile Base Nitrogen (TVB-N) of the fish waste meal and obtained the value of 9.17mg N / 100 g. The levels of TVB-N in the fish meal are still in good condition, though based on SNI 2715: 2013. It was below the maximum standard for fish pellet ingredient.

3.2. Proximate analysis of meat of Nile tilapia

The nutritional content of fish meat was presented in Table 3. In general, protein content in the fish meat fed on fish-waste pellet was 2% higher than that of fish receiving commercial pellet. While other components including moisture, ash, and crude fat appeared to be similar, 60-62%, 4%, 9% and 3% respectively. While there were slight differences in crude fibre and carbohydrate contents.

Table 3. Proximate analysis of Nile tilapia meat, *O. niloticus*.

Proximate	**Composition**				
	T0[a] (%)	**T1[b] (%)**	**T2[c] (%)**	**T3[d] (%)**	**National standard (%)**
Moisture	60.01	60.89	62.75	62.42	79.7
Ash	4.81	4.74	4.11	4.6	-
Crude Protein	9.03	9.1	9.3	9.49	18.7
Crude Fat	3.25	3.3	3.37	3.18	1
Crude Fibre	0.13	0.1	0.7	0.9	-
Carbohydrate	22.77	21.87	20.4	20.22	0

[a] T0/ Control (commercial pellet).
[b] T1/ Treatment 1 (fishwaste meal 50%).
[c] T2/ Treatment 2 (fishwater meal 55%).
[d] T3/ Treatment 3 (fishwaste meal 60%).

3.3. Growth of meat of Nile tilapia

The results showed that the use of fish waste meal significantly affected the growth of fish in terms of weight and length, $p<0.05$. Fish receiving pellet in treatment 3 had the highest gain in terms of length and weight, 3.83 ± 0.09 cm and 10.1 ± 0.12 g respectively as shown in Table 5. In addition, the result showed that Nile tilapia reared in all treatments had a high survival rate, 100% in all treatments indicating good survival rates in growth rearing condition for *O. niloticus*.

Table 5. Growth and survival rate of *O. niloticus* fed with different concentrations of fish waste meal for 30 days.

Parameters	**Treatment**			
	T0	**T1**	**T2**	**T3**
Initial Weight (g)	5.01 ± 0.5	5.01 ± 0.5	5.01 ± 0.5	5.01 ± 0.5

Initial length (cm)	7.5 ± 0.23	7.5 ± 0.23	7.5 ± 0.23	7.5 ± 0.23
Final length (cm)	9.6 ± 0.02[a]	10.11 ± 0.11[b]	10.69 ± 0.11[c]	11 ± 0.09[d]
Lengt gain (cm)	2.52 ± 0.2[e]	3 ± 0.12[f]	3.52 ± 0.1[g]	3.83 ± 0.09[h]
Final weight (g)	13.64 ± 0.18[i]	13.95 ± 0.09[ij]	14.25 ± 0.18[j]	15.1 ± 0.12[k]
Weight gain (g)	8.6 ± 0.19[l]	9 ± 0.09[lm]	9.2 ± 0.19[m]	10.1 ± 0.12[n]
SR (%)	100 ± 0.00[o]	100 ± 0.00[o]	100 ± 0.00[o]	100 ± 0.00[o]
FCR	1.68 ± 0.18[b]	1.5 ± 0.01[ab]	1.46 ± 0.03[ab]	1.41 ± 0.07[a]

3.4. Feed Conversion Ration (FCR)

The result showed that FCR was significantly affected by treatments, $p<0.05$. The lowest FCR value was found in fish fed on pellet with 60% fish-waste meal (T3), FCR value of 1.41 ± 0.07. While the highest FCR value was found in the treatment of T0 pellets with an FCR value of 1.68 ± 0.18. The FCR values in fish receiving T1 and T2 were 1.5 ± 0.01 and 1.46 ± 0.03, respectively. Based on Table 6, it is known that the FCR value of T0 is not significantly different from T1, T2 and T3. While T1 and T2 have FCR values that are significantly different from T3.

3.5. Protein Digestibility

The digestibility value of *O. niloticus* fish in T0 treatment was 70.3%, T1:77.7%, T2:78.9% and T3 80.4%. The highest digestibility value is at T3 and the lowest is at T0 as shown in Table 7.

Table 7. Protein digestibility of feed produced from fish waste in Nile tilapia, *O. niloticus*

Parameter	**T0[a]**	**T1[b]**	**T2[c]**	**T3[d]**
Protein content in feed (%)	34.91	44.26	47.84	51.21
Protein content in fish feces (%)	10.36	9.87	10.11	10.03
Digestibility (%)	70.3	77.7	78.9	80.4

[a] T0/ Control (commercial pellet).
[b] T1/ Treatment 1 (fishwaste meal 50%).
[c] T2/ Treatment 2 (fishwater meal 55%).
[d] T3/ Treatment 3 (fishwaste meal 60%).

4. Discussion

The Fish-waste meal collected from a commercial fish processing industry had a high protein content, therefore it can be used as fish feed ingredients. The TVBN on the fish waste meal was 9.17 mg N/100 g indicating that the TVB-N levels were in good condition based on standard TVBN value for commercial fish meal according to Indonesian standard (SNI 2715:2013), less than 150 mg/100 g [11]. Furthermore, physical characteristics such as stability and hardness were quite similar to that of commercial pellet. However, durability and buoyancy of the produced pellet were lower compared to commercial pellet, 5-8 min durability and 15 for durability. This result might be because a pelleting machine used in the present study was of lower quality compared to the pelleting machine in the industries [12, 13].

Nutrient content of fish-waste pellet especially crude protein was higher than that of commercial pellet. According to Cho and Lovell [14], high protein content may suggest high quality feed. Similarly, Masitoh [15] stated that higher protein content in feed increased the body's protein retention and subsequently improved fish growth. Low protein in the feed may slow down fish growth. The protein in the body's tissues will be used to maintain more important body tissue functions [16].

Higher protein content in the produced pellet was in line with the better growth of Nile tilapia in the present study. The best growth was obtained from Nile tilapia receiving pellet with 60% fish-waste

meal, indicated by higher total weight gain and length gain. A previous study by Marzuqi et al [17] also reported the higher the protein content of feed improved the final weight value of fish. It was explained that higher protein supply via feed increased protein supply for building blocks of tissues [18]. The high growth rate of pellets in the T3 treatment indicates that the energy requirements for the maintenance of life processes and other functions have been met [19]. The low growth of fish fed on T0 pellets was due to the low protein content compared to the four treatments. The high protein content in T3 pellets also affected the proximate meat of *O. niloticus* fish. The results showed that the highest protein content was in fish receiving the T3 pellet (9.49%), ~0.5% higher than that of fish fed on a commercial diet.

The same result was reported by Webster et al [20] where the protein content of fish meat was directly proportional to the protein content of the feed given [20]. Protein in the diet is hydrolyzed by the pepsin enzyme into polypeptides, which are then passed on by trypsin and chymotrypsin into dipeptides. Then, the pancreatic carboxyl peptidase cleaves the carboxyl group and the intestinal mucosal aminopeptidase cleave the N group on the peptide chain test. Then the peptides are broken down into single amino acids by the dipeptidase found on the brush border. These amino acids then are absorbed by erythrocyte cells and distributed by the blood to organs and tissues [19]. On the other hand, lower protein content in feed might limit amino acid supply and resulted in slower growth [18]. Fish fed a diet with low protein content may interfere with enzyme activity. Furthermore, there will be disturbances in energy metabolism so that the protein synthesis process will be disrupted and eventually growth will be low [19].

The present study also showed higher protein digestibility in fish-waste pellets compared to a commercial diet. The highest value was obtained from the T3 feed with a value of 80.4%, while the lowest was at commercial feed (~70.3%). These results indicate that T3 pellets can be digested well by the digestive system of fish. This is because the T3 pellet contains a lot of fish-waste meal, which was 60%. This is similar to a study by Hadiwiyoto [26] in which higher fish meal inclusion in the diet leads to better protein deposition in fifth flesh due to being more easily digested and effectively absorbed by the fish body. According to Afrianto and Liviawaty [27], in principle, the growth and digestibility of fish to artificial feed given depends on the level of fish acceptance of the enzymes it has. The presence of the fermentation process also affects digestibility. The addition of yeast in feed ingredients used in the fermentation process can cause good and beneficial effects, such as improving the quality of feed ingredients both in terms of nutrition and digestibility [28].

If the digestibility is good, it will cause weight gain in tilapia. Protein is broken down into amino acids that are easily digested by fish [28]. The better protein digestibility was also confirmed by lower FCR value in the fish receiving fish-waste pellet. The lowest FCR was obtained from fish fed on the T3 pellet (1.41). While the highest FCR value was found in fish fed on the commercial pellet (T0: 1.68). The FCR value of fish from T3 pellets is classified as having a good FCR value. Accordingly, Sopha et al [25] reported that the better protein digestibility of feed, the higher protein that can be absorbed and utilized for the growth and formation of new tissues.

The survival rate of *O. niloticus* fish from all treatments reaches 100%. This value indicates that all experimental fish were cultured in optimal environmental conditions [21], besides the met of all nutrient requirements. In addition, Panggabean [24] explained that environmental conditions mostly represented by water quality was one of the determining factors for the success of fish farming. Water quality can significantly affect the survival and growth rates of fish. Furthermore, the survival of fish is very dependent on the adaptability of fish to diets quality, fish health status, stocking density [22]. The nutritional content of the pellets given in the study in all treatments, in general, met the standards of SNI 01-7242-2006. This is in accordance with Adewumi et al [23], that the survival rate of fish is

influenced by the nutrients contained in the fish feed given. Overall, both water quality and water condition are optimal in the present experiment, therefore, having a good survival rate.

5. Conclusion

The fish-waste meal could be a potential replacement for fish meal in aquaculture feed. The fish-waste pellet had good physical quality including buoyancy, stability in water, hardness and durability in rearing water. *In vivo* studies also confirmed that fish fed on the fish-waste pellet had a better growth rate, feed conversion ratio. The best fish-waste pellet was pellet with 60% fish-waste inclusion. In addition, the fish-waste pellet also gave a good survival rate (100%) suggesting it good nutritional composition for Nile tilapia.

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