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Growth Performance and Feed Utilization of Tilapia (*Oreochromis niloticus*) Fed with Diets Containing Animal Protein Source from Expired Sausage

(Prestasi Pertumbuhan dan Penggunaan Makanan oleh Tilapia (*Oreochromis niloticus*) Diberi Diet Mengandungi Punca Protein Haiwan daripada Sosej Tamat Tempoh)

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ABSTRACT

This study aimed to evaluate the effect of diets with different levels of expired sausage flour as a source of animal protein on the growth performance of tilapia (*Oreochromis niloticus*). A completely randomized design (CRD) was used with five treatments and three replications. Furthermore, a total of 330 tilapia fish (5-7 cm and 4 ± 1.92 g) were reared in aerated 50 × 30 × 15 cm^3 aquaria with a stocking density of 22 fishes per aquarium. The feed contains an equal ratio of plant and animal protein, where the plant protein contains soy flour and rice bran flour in a ratio of 90:10. Meanwhile, the animal protein contains fish flour, added with five sausage flour levels: 0, 10, 20, 30 and 40% of the total dietary protein. The levels were then designated as A-D, respectively, while the diet with no sausage flour was used as the control (A). Feed conversion ratio (FCR) protein efficiency ratio (PER), protein retention (PR), energy retention (ER), and protein digestibility (PD) were calculated and compared among treatments. The data were analyzed using a one-way ANOVA on SPSS version 16. The results showed that diet containing 10% sausage flour significantly increased the protein efficiency ratio, protein retention, energy retention, and protein digestibility, and improve feed conversion ratio. Based on the results, 14.07% expired sausage flour protein is recommended as the best concentration for growth performance and food efficiency in tilapia.

Keywords: Expired sausage flour; growth; nutrient efficiency; tilapia

ABSTRAK

Matlamat kajian ini adalah untuk menilai prestasi pertumbuhan Tilapia (*Oreochromis niloticus*) yang diberi makan sosej tamat tempoh berbeza tahap sebagai punca makanan protein haiwan. Kajian ini menggunakan reka bentuk rawak lengkap (CRD) dengan lima rawatan dan tiga ulangan. Sebanyak 330 ekor ikan tilapia bersaiz 5-7 cm dan berat 4 ± 1.92 g telah diternak dalam akuarium berudara 50 × 30 × 15 cm^3 dengan kepadatan 22 ekor setiap akuarium. Suapan mengandungi protein tumbuhan dan haiwan (50:50), dengan protein tumbuhan terdiri daripada tepung soya dan tepung dedak padi (90:10). Tambahan pula, protein haiwan terdiri daripada tepung ikan yang ditambah dengan serbuk sosej tamat tempoh pada tahap 0, 10, 20, 30 dan 40% daripada jumlah protein pemakanan dan ditetapkan sebagai A-D masing-masing. Diet yang tidak mengandungi tepung sosej dikekalkan sebagai kawalan (A). Selepas itu, data diperoleh daripada pelbagai kumpulan rawatan dan nisbah penukaran makanan (FCR) dihitung untuk menilai prestasi pertumbuhan tilapia. Nisbah kecekapan protein (PER), pengekalan protein (PR), pengekalan tenaga (ER) dan kebolehcernaan protein (PD) juga dikira. Semua data dianalisis menggunakan ANOVA sehala. Pemakanan ikan yang mengandungi 10% protein tepung sosej yang telah tamat tempoh terbukti dapat meningkatkan nisbah kecekapan protein, pengekalan protein, pengekalan tenaga dan kebolehcernaan protein dan juga mengurangkan nisbah penukaran makanan. Khususnya, 14.07% kemasukan protein tepung sosej yang telah tamat tempoh disyorkan untuk prestasi pertumbuhan yang lebih baik dan kecekapan makanan dalam tilapia. Penyelidikan ini telah mencipta model suapan formulasi yang sesuai untuk pertumbuhan ikan tilapia dan telah berjaya mencari kandungan asid amino yang terkandung dalam sisa sosej yang telah tamat tempoh.

Kata kunci: Kecekapan nutrisi; pertumbuhan; tepung sosej tamat tempoh, tilapia

Introduction

Aquaculture is a promising industry worldwide, especially in Indonesia, where its production tends to increase up to 16.114 tons in 2018 (KKP 2018). This increase was in line with the global demand for freshwater fish which raised by 30% and need 37 million tons of fish to meet those demands (Siddiqui & Khan 2014). Most of the total demands for fish is allocated for domestic consumption. Among the fish species demand, Tilapia fish (*Oreochromis niloticus*) plays a crucial role in meeting the demand for fish (Naylor et al. 2000; Sediarti et al. 2021). The rapid rise and growth of aquaculture led to rising feed input (Amin et al. 2021; Roffeis et al. 2018). In aquaculture, feed accounts for over 50 percent of the production cost (Dwiardani et al. 2021; Karlina et al. 2013). Therefore, the impact of an increase in feed price causes decrease in farmer's profits (Webster & Lim 2001). To cope with the challenges, research interest has been directed toward the evaluation of alternative feeding sources for fish (Carneiro et al. 2017; Reynaldy et al. 2019) and the proper feeding time (Robisalmi et al. 2021).

Similar to other dried pet foods, aquaculture feed was designed to meet all nutrients requirements for growing and developing (Cho & Bureau 2001; Megawati et al. 2012; Pietoyo et al. 2021). However, a properly formulated composition of nutrients was also needed by fish to grow maximally. As reported by Younis et al. (2018), tilapia did not require only a high-protein feed to obtain maximum growth, but a proper composition of amino acid, especially lysine (Pramana et al. 2017). Lysine is one of the most limiting amino acids in fish diet, Therefore, lysine has been considered carefully as a feed formulation for increasing growth, and to increase survival rate (Zhou et al. 2007).

The need for an alternative source of fish feed was triggered by fluctuation in its price and quality (Munir et al. 2015; Priyadi et al. 2009) as well as the limited availability. Most researchers have been using soybean (Carneiro et al. 2017), moringa leaf flour (Richter et al. 2003), and fermented flour (Mo et al. 2020; Soedibya et al. 2021) as protein and carbohydrate sources. Using 10% moringa leaf flour can increase the specific growth rate (SGR) of the fishes by 2.7%, while fermented flour can provide a growth rate of 143.3 ± 9.38 as well as a food conversion ratio (FCR) of 1.59 ± 0.12. However, the high demand for these alternative sources for both human consumption and industrial use also led to an increase in their cost, hence, alternative feed ingredients from cheaper materials such as waste are the best solution to this problem.

Expired sausage can be used as a feed source. To its high nutrient content including 61.35% water, 15.95% protein, 8.41% carbohydrates, 11.77% fat, and 2.52% ash. Nutritional composition analysis also showed that expired sausages can be used as an alternative feeding source for tilapia (*Oreochromis* sp.) culture (Van Trung et al. 2011). Therefore, this study aimed to evaluate the growth performance of tilapia (*Oreochromis niloticus*) fed with different levels of expired sausage as a source of animal protein.

Materials and Methods

Experimental Design and Sample Preparation

The proximate analysis was carried out in the Laboratory of Perekayasa Hasil Perikanan, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Meanwhile, the fish feed biological assay was performed at the Laboratory of Nutrition, Faculty of Fisheries and Marine Science, Universitas Brawijaya. This study used a complete randomized design (CRD) with five treatments and three replications. The expired sausages which contained beef as the main ingredients, fats, spices, and also seasonings were obtained from food stores in Jakarta, Indonesia. Subsequently, they were washed, sliced into pieces of 1 × 1 cm size, and then ground into powder form with blender machines.

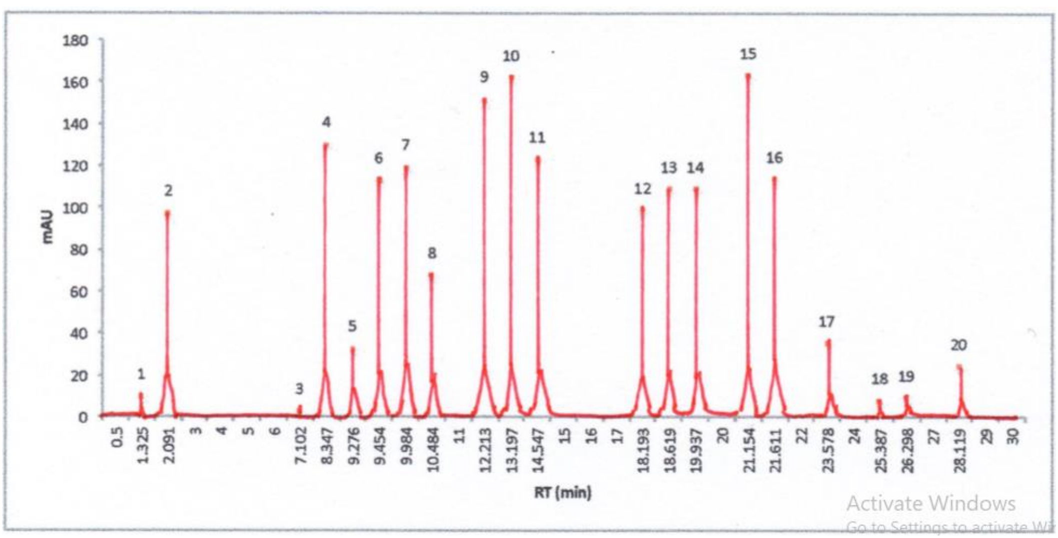
A total of 330 tilapia fish with a 5-7 cm length and 4 ± 1.92 g weight were reared in aerated 50 × 30 × 15 cm^3 aquaria with a density of 22 fishes per aquarium (Minggawati 2006; Widiastuti 2009). The total of 15 aquaria used in the present study were covered with black plastic bags, and then integrated with filters, aerators, and water pumps. Water quality including temperature, pH, oxygen and ammonia concentrations were monitored (Ghufron et al. 2020). The replacement of rearing water was carried out at the same time with faeces collection, which was done every morning and evening, exactly 20 min after feeding time. The faeces were collected using a filter with a size of 30-40 µm. Furthermore, the faeces were dried and collected in bottle.

Diet Formulation

The feed was prepared in the form of pellets and it contains 25% crude protein (Abdel-Tawwab et al. 2010; Anderson et al. 1984; Suresh & Lin 1992). Meanwhile, the proximate and amino acids were determined before the feed formulation, as shown in Table 1. The proximate analysis was carried out using several procedures, which are water content analysis, ash content analysis,

TABLE 1. Essential and non-essential amino acids content in sausage waste flour

Sample Name	Compound	Standard concentration (mg/mL)	Result (%)
Sausage waste flour	Essential Amino Acids		
	Leucine	10	2.5
	Methionine	10	1.7
	Arginine	10	2.7
	Isoleucine	10	2.8
	Phenylalanine	10	1.8
	Valine	10	2.6
	Threonine	10	2.2
	Lysine	10	3.7
	Tryptophan	10	0.8
	Histidine	10	1.2
	Non-essential amino acids		
	Asparginine	10	0.6
	Glutamine	10	0.3
	Aspartate	10	0.3
	Glutamate	10	3.7
	Serine	10	0.1
	Glycine	10	4.3
	Alanine	10	2.8
	Proline	10	2
	Cystine	10	0.6
	Tyrosine	10	1.4



HPLC chromatogram result of sausage waste flour

TABLE 2. Proteinate, amino acids, and energy content of feed ingredients used in the study

Type of material	Dietary formulations (%)				
	A (0)	B (10)	C (20)	D (30)	E (40)
Fish flour	21.98	1979	17.59	15.39	13.19
Sausage flour	0.00	6.48	12.96	19.44	25.92
Soybean flour	29.57	29.57	29.57	29.57	29.57
Bran	22.32	22.32	22.32	22.32	22.32
Tapioca flour	22.50	16.86	11.22	5.57	0.00
Cr_2O_3	0.50	0.50	0.50	0.50	0.50
CMC	0,12	1.48	2.84	4.20	5.49
Vitamin dan Mineral	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00
Amino acid essential					
Arginine	2.23	2.32	2.41	2.50	2.58
Histidine	0.54	0.59	0.63	0.68	0.72
Isoleucine	1.54	1.64	1.74	1.85	1.95
Leucine	1.91	1.96	2.01	2.06	2.11
Lysine	1.91	2.01	2.10	2.20	2.30
Methionine	1.03	1.10	1.17	1.24	1.31
Phenylalanine	1.37	1.43	1.49	1.55	1.60
Tryptophan	0.39	0.43	0.47	0.50	0.54
Threonine	1.91	1.99	2.07	2.15	2.23
Valin	1.85	1.94	2.03	2.13	2.22
Chemical analysis					
Protein (%)	26.25	26.50	26.12	26.25	26.37
Fat (%)	8.05	10.01	11.25	12.25	14.18
Ash (%)	6.57	6.48	6.38	6.29	6.19
Crude Fiber (%)	49.64	47.21	46.16	44.98	42.79
BETN (%)	56.21	53.69	52.54	51.27	48.98
DE (kcal/gram	3.76	3.85	3.90	3.95	3.99

fat content analysis, and carbohydrate content analysis according to AOAC (1995) and Aaqillah-Amr et al. (2021). The amino acid content was obtained using in HPLC based on protein percentage. Isonitrogenous and isoenergetic diets were then formulated with a total of 25% dietary protein and 3.700 kcal/kg calory. The feed contains plant and animal protein in the same ratio where the plant protein comprises soy flour and rice bran flour in a ratio of 90:10. The ratio between plant and animal protein was determined because the fish used in this experiment was tilapia, which is an omnivorous animal. On the other hand, the ratio of plant protein was determined due to the higher nutrition of soy flour to get 25% crude protein in the experimental feed. Meanwhile, the animal protein contains fish flour supplemented with various levels of powder sausage, 0, 10, 20, 30, and 40% of total dietary protein. The different levels were then designated as A-D, respectively (Table 2), while the diet with no sausage flour was used as the control (A). The animal samples were then fed twice daily at 2 and 4 pm for five weeks, and a total of 3% of biomass fish was given during the experimental period. Subsequently, the bioavailability analysis was carried out every ten days by calculating the samples' weight.

GROWTH EVALUATION

The growth performance of fish fed with the experimental diets was measured according to protocols of Abidi and Khan (2007) and Kashyap et al. (1988). Furthermore, it was calculated as a function of the weight gain with different parameters including feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), energy retention (ER), and protein digestibility (PD). Meanwhile, the feed conversion ratio (FCR) was calculated using the formula below:

$$\mathrm{FCR} = \frac{\Sigma \mathrm{P\ BK}}{\Delta \mathrm{W}}\ x\ 100\% \quad (1)$$

where FCR is the feed conversion ratio; ΣP BK is the total feed consumed (gram), and ΔW is the gain in weight of fish (gram).

Protein efficiency ratio (PER) was calculated using the formula below:

$$\mathrm{PER} = \frac{\mathrm{wt-wo}}{\Sigma \mathrm{P\ Pkn}} \quad (2)$$

where PER is the Protein Efficiency Ratio (%); W0 is the initial weight of fish (gram); Wt is the final weight of fish (gram); ΣP BK is the total dried feed (gram).

Protein retention was calculated using the formula below:

$$\mathrm{PR} = \frac{\Sigma \mathrm{Pt} - \Sigma \mathrm{P0}}{\Sigma \mathrm{PP}}\ x\ 100\% \quad (3)$$

where PR is the Protein retention (%); Σ P0 is the initial body protein (gram); Σ Pt is the final body protein (gram); and Σ PP is the total protein in feed (gram).

Energy retention was calculated using the formula below:

$$\mathrm{ER} = \frac{\Sigma\ \mathrm{GE\ t} - \Sigma\ \mathrm{GE\ 0}}{\Sigma\ \mathrm{GE\ P}}\ x\ 100\% \quad (4)$$

where ER is the energy retention (%); Σ GE 0 is the initial body energy (gram); Σ GE t is the final body energy (gram); and Σ GE P = total energy in feed (gram).

Protein digestibility was calculated using the formula below:

$$\mathrm{PD} = 100 - \left(\frac{(\mathrm{Cp\ x\ Pf})}{(\mathrm{Cf\ x\ Pp})}\ x\ 100\right) \quad (5)$$

where PD is the protein digestibility (%); Cf is the percentage Cr_2O_3 in the feces (%); Cp is the percentage Cr_2O_3 in the feed (%); Pf is the percentage protein in the faeces (%); and Pp is the percentage of protein in the feed (%).

DATA ANALYSIS

All data were analyzed using a one-way ANOVA on SPSS version 16. The statistical significance was set at the 5% probability level ($p < 0.05$) and then separated using the Least Significance Different (LSD).

RESULTS AND DISCUSSION

GROWTH PERFORMANCE OF TILAPIA (*Oreochromis niloticus*)

Table 3 shows the growth performance of tilapia (*Oreochromis niloticus*) fed diets with different levels of expired sausages, which served as an animal protein source. The survival rate of fish in the present study ranged from 84.21 - 90.00%, and there was no significant difference between the values obtained from the treatments ($P > 0.05$). Furthermore, FCR (Feed Conversion Ratio), PER (Protein Efficiency Ratio), PR (Protein Retention,) ER (Energy Retention), and PD (Protein Digestibility) were used to determine the growth performance of the fishes. Treatment B containing 10% expired sausage had the highest PER, PR, ER, and PD, but also had the lowest FCR, which was significantly different from the other treatments ($P < 0.05$).

FEED CONVERSION RATIO (FCR)

Figure 1 shows the relationship between the substitution of expired sausage flour and the feed conversion ratio of tilapia (*Oreochromis* sp.). A quadratic equation was obtained with a formula" y = 0.0012x2 – 0.0342x + 1.9163" and $R^2 = 0.84$. The value of the substitution was 14.25% with a feed conversion ratio of 1.60.

TABLE 3. Growth performance and feed utilization of tilapia (*Oreochromis niloticus*)

Parameter	Feed formulations				
	A (0%)	B (10%)	C (20%)	D (30%)	E (40%)
Survival rate (%)	84.21 ± 0.02[a]	90.00 ±0.00[a]	90.00 ± 0.00[a]	84.21 ± 0.02[a]	84.21 ±0.02[a]
Feed Conversion Ratio	1.94 ± 0.01[b]	1.60 ± 0.03[a]	1.87 ± 0.01[b]	1.88 ± 0.00[b]	2.52 ±0.20[c]
Protein Efficiency Ratio	1.96 ± 0.01[b]	2.36 ± 0.05[a]	2.05 ± 0.01[b]	2.03 ± 0.01[b]	1.51 ±0.12[c]
Protein Retention Ratio (%)	17.98 ±0.36[b]	21.62 ±0.42[a]	18.82 ±0.85[b]	18.14 ±0.74[b]	14.80 ±1.74[c]
Energy Retention (%)	15.97 ±0.44[b]	18.32 ±0.25[a]	16.55 ±0.51[b]	16.28 ±0.37[b]	12.54 ±1.49[c]
Protein Digestibility (%)	74.47 ±0.48[b]	78.92 ±0.87[a]	75.45 ±0.52[b]	74.86 ±0.81[b]	71.68 ±0.27[c]

The same letter in the same column shows no significant difference at 95% confidence level

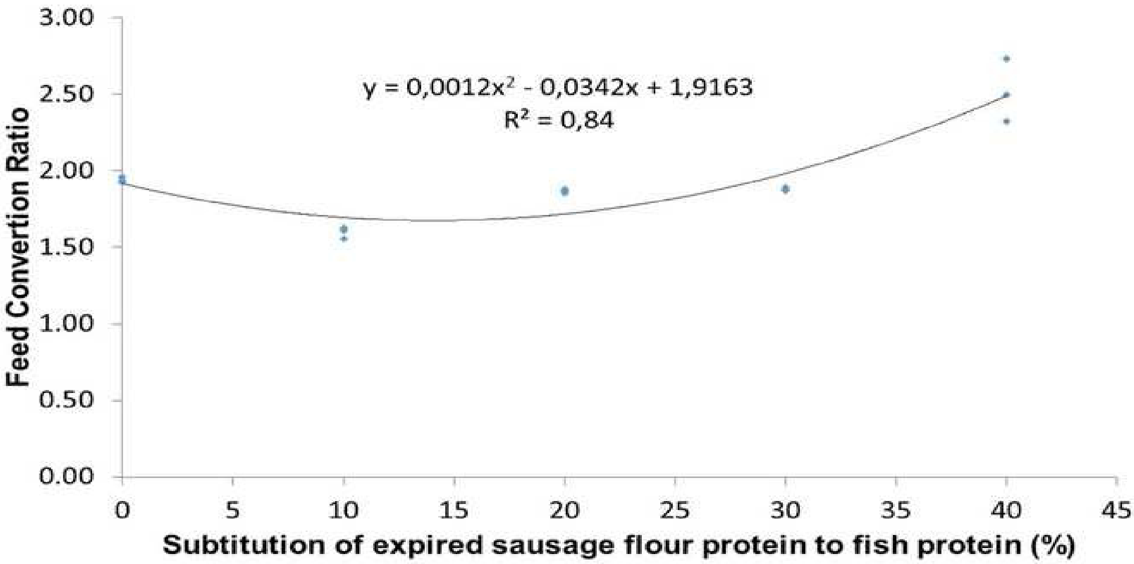


FIGURE 1. Relationship between expired sausage flour protein and fish meal protein on feed conversion ratio

PROTEIN EFFICIENCY RATIO (PER)

The relationship between the substitution of expired sausage flour protein to fish meal protein and the protein efficiency ratio of tilapia (*Oreochromis* sp.) seed shows a quadratic equation, “$y = -0.0011x^2 + 0.0314x + 2.0114$” and $R^2 = 0.83$, as shown in Figure 2. The best protein substitution was 14.27% with a protein efficiency ratio of 2.24%.

PROTEIN RETENTION

Protein retention is the absorbed protein that was used for growth as well as building/repairing damaged cells (Buwono 2000). The relationship between the protein substitution and protein retention of tilapia shows a quadratic equation with value of “$y = -0.0087x^2 + 0.2466x + 19.2242$” with $R^2 = 0.73$, as shown in Figure 3. The best substitution dose of the expired sausage was 14.17% with protein retention of 20.96%.

ENERGY RETENTION

The relationship between protein substitution of expired sausage flour and the energy retention of tilapia (*Oreochromis* sp.) shows a quadratic equation which are “$y = -0.005x^2 + 0.1359x + 17.185$” with $R^2 = 0.72$, as shown in Figure 4.

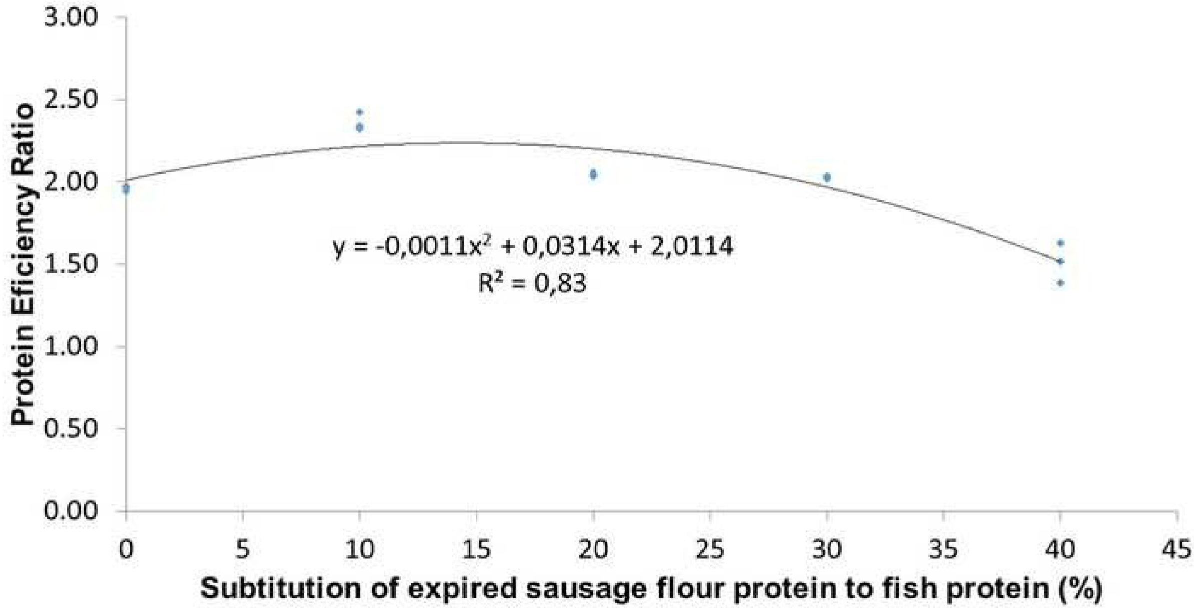


FIGURE 2. Relationship between expired sausage flour protein and fish meal protein on protein efficiency ratio

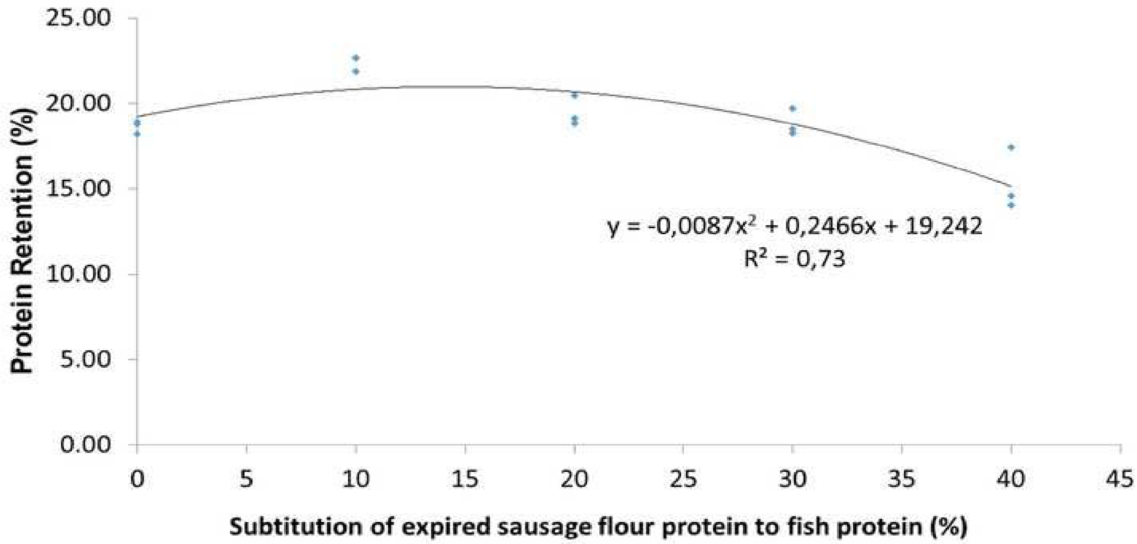


FIGURE 3. Relationship between expired sausage flour protein and fish meal protein on protein retention

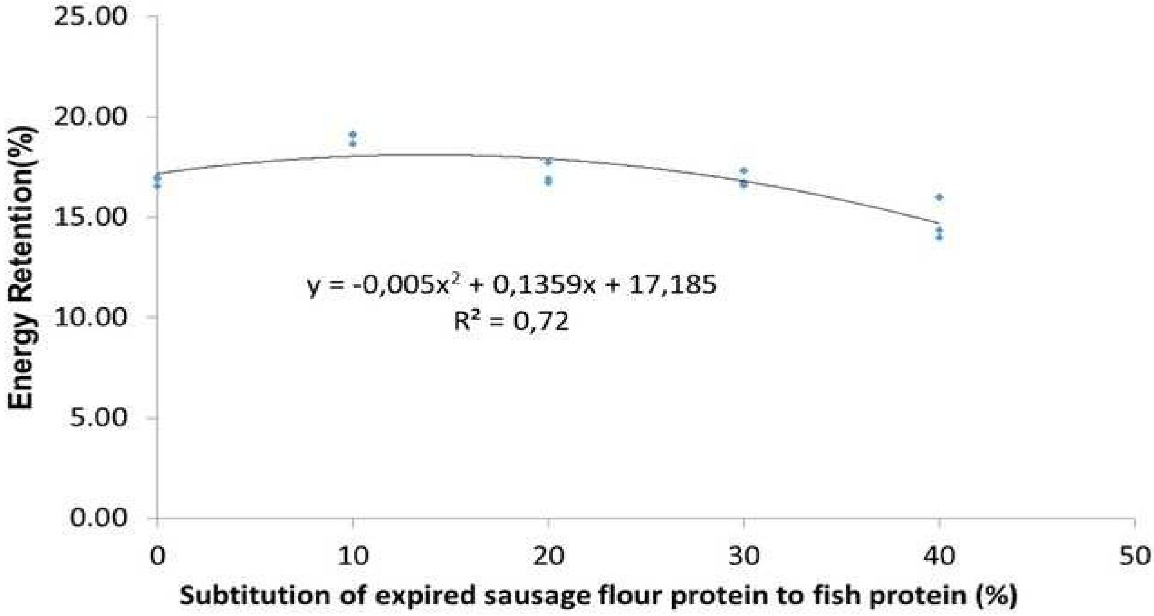


FIGURE 4. Relationship between expired sausage flour protein and fish meal protein on energy retention

The best substitution dose of the expired sausage flour protein was 13.59% with an energy retention value of 18.11%.

PROTEIN DIGESTIBILITY

Protein digestibility is an essential biochemical parameter that is used to assess the nutritional quality of an animal. Furthermore, it is widely used to evaluate the quality of protein to be hydrolyzed into amino acids by the body's digestive enzymes. The relationship between protein substitution of expired sausage flour to fish meal and the protein digestibility of tilapia (*Oreochromis* sp.) shows a quadratic equation with a formula "$y = -0.0089x^2 + 0.2579x + 75.23$" with $R^2 = 0.71$, as shown in Figure 5. The best substitution dose of the expired sausage powder was 14.48% with a protein digestibility of 77.10%.

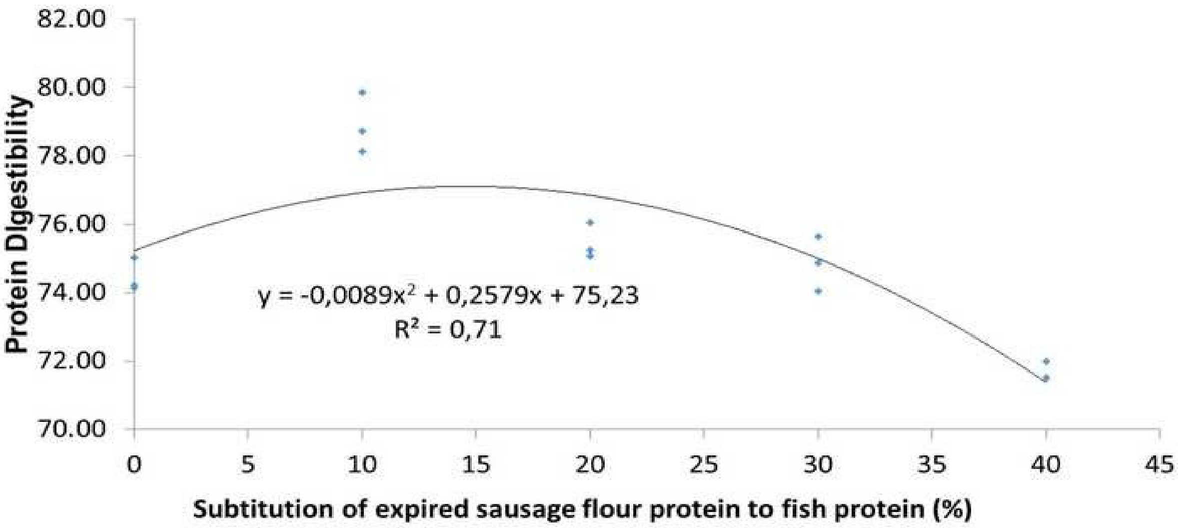


FIGURE 5. Relationship between expired sausage flour protein and fish meal protein on protein digestibility

The feed conversion ratio is an indicator that is often used in all types of farming, especially fishery (Hasan & Soto 2017). It measures the efficiency with which the bodies of livestock convert animal feed into the desired output. It can also serve as an indicator for feed or feeding strategy's efficiency (Kusuma et al. 2021). In this study, the lowest feed conversion ratio was the Treatment D (40%) with 1.60 as the value of the ratio, which indicates that 1.60 kg of feed is needed to produce 1 kg of tilapia fish. Meanwhile, the highest feed conversion value was 2.52 from the E treatment, which indicates that 2.52 kg of feed is needed to produce 1 kg of tilapia fish. These results show that the Treatment B (10%) was the best among all the treatments, because less feed was required to produce the same amount of fish weight. However, based on the quadratic regression analysis, 14.25% of expired sausage flour could be the optimum inclusion for the feed formulation.

The D treatment (40%) has the poorest feed conversion ratio because the D treatment (40%) also has the highest fat content (14.18%). This result could be due to the high fat content in the Treatment D in which tilapia only needs 5.2% of fat included in the feed formulation (Winfree & Stickney 1981). For tilapia up to 2.5 g, the optimum dietary lipid concentration was 5.2%, decreasing to 4.4% for fish up to 7.5 g. Jauncey (1982) suggested that to maximize protein utilization, dietary fat concentration should be between 8 and 10% for tilapia up to 25 g, and 6 to 8% for larger fish. Moreover, it is explained that the feed formulation, which contains 11.8% of fat for tilapia fish can cause decrease in food intake of tilapia fish, its survival, and the protein content in tilapia fish, so that it can decrease the growth of tilapia fish (Lv et al. 2021). The 4% content of fat in feed formulation can provide the optimum growth of tilapia fish. The feed conversion value obtained in the present study was relatively better than that of Handajani (2006) where 30% fermented *Azolla pinnata* flour produce a feed conversion ratio of 3.14. Yolanda et al. (2013) also reported a feed conversion ratio of 1.94, which was lower compared to this study. The conversion ratio is influenced by various factors including the quality and quantity of feed, species, size, and water quality (Ayu et al. 2019; NRC 1993).

The protein efficiency ratio is also an indicator of fish growth performance, and it can be calculated as the ratio of body weight gain in grams to the protein consumed in grams (Dewanggani et al. 2021; Ramezani 2009). The results showed that the protein efficiency ratio was higher than the value obtained by Attalla and Mikhail (2008), which explained that the use of soy flour and dried Dunaliella flour in a ratio of 50:50 can provide a 1.87 ratio in tilapia juvenile (*Oreochromis* sp.). Similarly, Handajani (2006) used rough fish flour as a substitute for fish meal in tilapia (*Oreochromis* sp.) and observed that the 75% substitution level produced the highest efficiency ratio of over 1.39. Protein efficiency ratio decreased as the dietary protein level increased, and these findings are like a value reported by Caspian Brown trout (Ramezani 2009), Bonylip Barb (Niagara e al. 2018), and Nile tilapia (*Oreochromis niloticus* L.) (Richter et al. 2003).

Furthermore, the difference in the value of PER obtained was caused by the different compositions and protein types in the formulation. The protein quality of feed ingredients is determined by their amino acids content, especially essential amino acids (Arief et al. 2016; Loekman et al. 2018; Millward et al. 2008). The result shows that the 10% expired sausage flour in the feed formulation had the highest protein efficiency and when the presence of essential amino acids in the complete feed combined with the use of 10% expired sausage flour, it can meet the essential amino acids need of the tilapia fish. Moreover, 14.27% could be the optimum inclusion of expired sausage floor based on the quadratic regression analysis. The amino acid content that has been previously known as three of the most important amino acids for tilapia fish growth are Methionine, Threonine, and Lysine, which based on Table 1 those amino acid content in Treatment B are Methionine 1.10%, Threonine 1.99%, and Lysine 2.01%. This result is close compared to the previous study (Michelato et al. 2016), which stated that based on the research, the content of methionine 1.2%, threonine 1.5%, and lysine 1.0% can provide the optimum growth for tilapia fish.

The average protein retention value in Treatment B from this study was relatively higher (21.62%) than the value on *Azolla pinnata* flour enriched with 15% probiotic microbes in the GIFT tilapia feed, which gave the best average retention of 16.48% (Soedibya 2013). Similarly, Agbo et al. (2011) reported that tilapia (*Oreochromis* sp.) with an average weight of 4.2 g, which were fed with 50% of cottonseed flour (*Gossypium hirsutum*) as a substitute for the fish meal had a 17.55% protein retention value. The high protein retention value in this study is possibly due to sufficient amino acids content in Treatment B. Lysine content in the feed formulation of Treatment B was about 2.01%, which is the optimum content for tilapia fish. The optimum content of lysine for the growth of tilapia fish is about 1.62% (Jackson & Capper 1982), and 1.51% based on

Jauncey (1982). Threonine amino acid content is one of the important limit factors for tilapia fish, which it needs 1.5% of threonine to increase the maximum growth (Michelato et al. 2016). Based on this study, the threonine content in the feed formulation of Treatment B was 1.99%, which can be one of the indicators of the high value of protein retention.

The balance between energy and protein is very important in determining fish energy retention because small energy quantity breakdowns protein to provide an energy source. This breakdown reduces the amount of protein used, which leads to the inhibition of the fish growth (Setiawati & Suprayudi 2007). Furthermore, lipid and carbohydrates are also crucial energy sources, and they affect the oxidative status of Senegalese sole. Diets containing low levels of lipid and digestible starch can reduce the fish's susceptibility to oxidation and enhance its growth rate (Jasso et al. 2020). The factors influencing the energy retention of tilapia include the level of consumed energy, digestibility, the feed's energy content, and the fish's physiological features (NRC 1993). Furthermore, all the fish's energy needs are often met by the protein, fat, and carbohydrates content of the feed, hence, the energy and protein content of feeds must be in line with their needs. The excessive protein content or energy is excreted through urine, which can pollute the rearing process (Ali & Al-Asgah 2008).

The average protein digestibility value in Treatment B was 78.92% and relatively higher than that in Handajani (2006), which used fermented *Azolla pinnata* flour on tilapia (*Oreochromis* sp.) feed and obtained the best average protein digestibility of 77.50%. However, it was relatively lower compared to Hernández et al. (2013), which used tilapia (*Oreochromis* sp.) with an average size of 0.89 g. 75% tuna fish waste hydrolysate was added to the feed formulation and protein digestibility of 87% was obtained. The cause behind this result is due to the use of tuna fish waste hydrolysate that has been through the fermentation process, which make the protein content more easily digested by tilapia and the concentration of amino acids needed by tilapia will also be more fulfilled.

Conclusion

A diet containing 10% of expired sausage flour protein can increase the protein efficiency ratio, protein retention, energy retention, protein digestibility, and it also decreases the feed conversion ratio, which makes the feed formulation in Treatment B (10%) become the most effective diet to be used for tilapia fish. Furthermore, 14.07% expired sausage flour protein is recommended as the best concentration for the growth performance and food efficiency in tilapia. This study has created an appropriate feed formulation model for the growth of tilapia and has also succeeded in determining the amino acid content of expired sausage waste and feed formulas produced.

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