

## Evaluation of protein and lipid on feed of Tilapia *Oreochromis niloticus*

### Evaluasi kebutuhan protein pakan dan lemak pada ikan nila *Oreochromis niloticus*

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#### ABSTRACT

The goal of this study was to discover the optimal protein and lipid levels for increasing the growth performance of Nile tilapia *Oreochromis niloticus*. A 2×2 randomized factorial design was used for this research, and two treatment factors were used: the protein level (28% and 32%) and the lipid level (6% and 8%). So, there were four test treatments and carried out with three replications. Nile tilapia with an average body weight of  $5.60 \pm 0.06$  g were grown in a  $900 \times 45 \times 35$  cm<sup>3</sup> aquarium at a density of 20 fish/aquarium. The experimental fish were grown for 60 days and fed three times daily until they appeared to be satisfied. Parameters evaluated in this study were growth performance and blood chemistry. The fish fed with diet 32;8 showed significantly higher growth than other treatments. Plasma protein in the fish fed with 32;6 diets were higher than those of plasma triglycerides, high density lipoprotein, and low density lipoprotein content were the same among the treatments. The result shows that feed 28;8 has the best protein efficiency ratio and protein retention.

Keywords: *Oreochromis niloticus*, protein sparing effect, lipid, feed

#### ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui kadar protein dan lipid yang optimum dalam meningkatkan kinerja pertumbuhan ikan nila *Oreochromis niloticus*. Penelitian ini menggunakan rancangan acak faktorial 2×2 dengan dua faktor perlakuan yaitu kadar protein (28% dan 32%) dan kadar lipid (6% dan 8%). Jadi terdapat empat perlakuan uji dan dilakukan dengan tiga kali ulangan. Ikan nila dengan rata-rata bobot badan  $5,60 \pm 0,06$  g dipelihara dalam akuarium berukuran  $900 \times 45 \times 35$  cm<sup>3</sup> dengan kepadatan 20 ekor/akuarium. Ikan dipelihara selama 60 hari dan diberi makan tiga kali sehari secara *at satiation*. Parameter yang dievaluasi dalam penelitian ini adalah kinerja pertumbuhan dan kimia darah. Ikan yang diberi perlakuan dengan pakan 32;8 menunjukkan pertumbuhan yang lebih tinggi dibandingkan perlakuan lainnya. Protein plasma pada ikan yang diberi pakan 32;6 lebih tinggi dibandingkan dengan trigliserida plasma, HDL dan LDL rendah yang sama antar perlakuan. Hasil penelitian menunjukkan bahwa pakan 28;8 mempunyai nilai protein efisiensi rasio dan retensi protein terbaik.

Kata kunci: *Oreochromis niloticus*, efek hemat protein, lemak, pakan

## INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) represents an economically promising species for farmed fish production, ranking third and contributing to 9% of the world's total fish production in 2020 (FAO, 2022). However, a significant challenge in Nile tilapia aquaculture is the high cost of feed, which can account for up to 80% of the total expenses (Wardani, 2020). Feed production costs are mainly driven by the protein content source and other essential nutrients. Among these nutrients, protein and lipid are crucial for fish nutrition. Protein plays a significant role in fish feed composition, contributing significantly to the higher cost of fish diets compared to lipid and carbohydrates (Hua *et al.*, 2019; El-Wahab *et al.*, 2020).

The quantity and quality of protein in the feed have a significant impact on fish growth rate, survival rates, and overall production costs in aquaculture (Daniel, 2017; Syahailatua *et al.*, 2017). Insufficient protein in the feed can lead to inadequate supply for the formation of new body tissues (Carneiro *et al.*, 2017). Conversely, excessive protein or mismatched protein quality in the feed might not be utilized for body protein synthesis, leading to its excretion as nitrogen waste, mainly in the form of ammonia (Handajani *et al.*, 2018; Xia *et al.*, 2015). Protein use in feed depends on both quantity and quality, as well as the availability of lipids and carbohydrates for energy (Xie *et al.*, 2017).

Lipid is a preferable source of energy compared to carbohydrates due to the limited ability of fish to metabolize carbohydrates (NRC, 2011). Balanced lipid content in the feed reduces the utilization of protein as an energy source, making protein more efficient for growth and enabling a protein-sparing effect (Thirunavukkarasar *et al.*, 2022). Lipids are critical for fish growth and health as they provide essential fatty acid and aid in absorption, transfer and metabolism of lipid-soluble vitamins and carotenoids (Jiang *et al.*, 2015). However, excessively high lipid levels can result in lipid deposition in fish bodies, leading to reduced feed intake (Xie *et al.*, 2017), decreased digestibility (Sandre *et al.*, 2017), impaired fish growth (Guo *et al.*, 2019), liver degeneration, and decreased quality at harvest time (NRC, 2011).

Therefore, improving feed efficiency in fish can be achieved by providing feed with an appropriate ratio of protein to lipid. Numerous

studies on Nile tilapia feed have indicated that feeds containing 29.8% protein with an energy-to-protein ratio (E/P) of 8.5 kcal/g have shown optimal growth (Wulanningrum *et al.*, 2019). Suprayudi *et al.* (2022) reported that commercial feeds with 29.23% protein and 6% lipid exhibited the best growth performance, feed consumption, and protein retention compared to other treatments. Other studies showed that feeds with 30% protein and 11% lipid (Ye *et al.*, 2016). Therefore, this study aims to evaluate the impact of different protein contents (28% and 32%) and lipid contents (6% and 8%) in Nile tilapia feed to achieve optimal utilization of feed protein and lipid content.

## MATERIALS AND METHODS

### Feed and experimental fish preparation

All feeds employed fish meal, meat bone meal, and soybean meal as the principal protein sources. The formulation of the feed started with a proximate analysis of the raw materials. All raw materials were weighed according to requirements, mixed in a homogenizer, and then formed into pellets. The feed pellets were dried in an oven set to 35°C for 24 hours. Protein and fat levels were determined through proximate analysis of the feed. Table 1 shows the composition of the feed raw ingredients.

### Experiment design

This study employed a factorial design with four treatments and three replications. The feed treatments included two levels of protein content, 28% and 32%, combined with two levels of lipid content, 6% and 8%.

### Fish culture and data collection

The fish with average initial weight of 110–112 g were reared in 12 aquariums measuring 90×45×35 cm with a density of 20 individuals per aquarium. Feeding was administered thrice daily at 08:00 AM, 12:00 PM, and 04:00 PM *ad libitum*. Throughout the rearing period, water quality was maintained within optimal conditions: temperature (27.74–29.24°C), dissolved oxygen (3.54–4.68 mg/L), and pH (7.01–7.51). A 70% water change was conducted three times a day. Each aquarium was equipped with a thermometer to maintain a stable temperature, aeration to ensure dissolved oxygen, and a top filter to reduce turbidity.

Water temperature was measured daily in the morning and evening, while pH and dissolved oxygen were measured three times during the rearing period: at the beginning, on day 40, and on day 60. At the start and end of the rearing period, fish biomass was measured, and proximate body analysis was performed. Biomass measurement was performed after a 24 hours fasting period. After weighing the biomass at the end of the rearing period, blood biochemical tests were performed on selected experimental fish from each treatment.

### Chemical analysis

Chemical analysis comprised proximate analysis of feed and fish bodies based on the

AOAC (2012) standards. Proximate analysis was performed at both the beginning and end of the rearing period. The parameters measured in this study compared initial and final biomass, survival rate, specific growth rate, feed efficiency (NRC, 2011), protein efficiency ratio, protein retention, lipid retention, triglycerides, HDL, LDL, and cholesterol. Sampling occurred at the beginning of the rearing period and on day 60, with three samples for each treatment.

### Data analysis

This study employed a factorial randomized design (FRD) with four treatments and three replications. Statistical analysis was performed on growth performance and blood biochemical

Table 1. The content of the test feed formulation, as well as the test feed approximate results.

Raw materials	Treatment of protein and lipid (%)			
	28;6	28;8	32;6	32;8
Composition of raw materials (%)				
Fish Meal	2.00	2.00	2.00	2.00
Meat bone meal	4.50	4.50	5.00	5.00
Soybean meal	19.50	21.00	29.00	29.00
Industrial wheat flour	28.20	25.22	20.60	18.50
Pollard	26.70	26.30	21.80	21.00
Fish oil	0.80	1.68	0.80	1.60
Crude palm oil	0.70	1.30	0.70	1.30
Corn gluten meal	3.60	3.60	3.60	3.60
Copra meal	4.00	4.00	6.00	6.00
Poultry meat meal (PMM)	3.10	3.00	3.50	4.50
Monocalcium phosphate	3.00	3.00	3.00	3.00
Soya lecithin	0.50	1.00	0.50	1.00
Lysine	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50
Vit C	0.05	0.05	0.05	0.05
Vit E	0.03	0.03	0.03	0.03
Choline Chloride	0.02	0.02	0.02	0.02
Antioxidant	0.02	0.02	0.02	0.02
Vit Mix	0.20	0.20	0.20	0.20
Min mix	0.20	0.20	0.20	0.20
Filler	1.68	1.68	1.78	1.78
Polymethylolcarbamide (PMC)	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Protein	28.01	28.11	31.98	32.09
Lipid	6.15	7.99	6.14	8.05
Gross Energy (kkal GE/100 g)	367.68	374.39	369.94	377.36
Calorie/ protein (C/P) ratio	13.13	13.32	11.57	11.76

analysis. Data were tabulated in Microsoft Office Excel 2016 and analyzed using SPSS 24.0. Differences were considered significant based on the results of the analysis of variance with a 95% confidence interval. The Duncan's test was applied if significant differences were found at the 95% confidence interval.

## RESULTS AND DISCUSSION

### Result

#### Growth performance

The growth performance showed significant differences in biomass weight (Wt), daily growth rate (LPH), feed efficiency (EP), protein efficiency ratio (PER), and protein retention due to different treatments. The two-way ANOVA results indicated that protein had an impact on final weight (Wt), daily growth rate (LPH), and

feed efficiency (EP), whereas lipid influenced feed efficiency (EP), protein efficiency ratio (PER), and protein retention (RP). However, there was no interaction between the two factors, protein and lipid.

#### Blood biochemistry

Table 3 shows an analysis of Nile tilapia blood profiles, including total cholesterol, triglycerides, HDL, and LDL levels. The protein content in the diet had a significant effect on total blood cholesterol levels ( $p < 0.05$ ).

#### Body chemical composition

The proximate analysis of Nile tilapia body composition is presented in Table 4. The protein content, fat content, moisture content, and glycogen content in the body were not significantly affected by the protein and lipid content in the

Table 2. The growth performance of Nile tilapia fed with protein and lipid level.

Parameter	Treatment				Two-way ANOVA		
	P28L6	P28L8	P32L6	P32L8	P	L	P*L
B <sub>0</sub> (g)	110.91 ± 2.00	112.59 ± 0.74	112.04 ± 0.99	112.10 ± 1.11	-	-	-
B <sub>t</sub> (g)	531.79 ± 37.89 <sup>a</sup>	537.47 ± 15.07 <sup>b</sup>	566.95 ± 35.19 <sup>b</sup>	578.00 ± 27.34 <sup>b</sup>	*	-	-
SGR (%/day)	2.77 ± 0.06 <sup>a</sup>	2.89 ± 0.11 <sup>ab</sup>	2.95 ± 0.06 <sup>b</sup>	2.98 ± 0.09 <sup>b</sup>	*	-	-
SR (%)	93.33 ± 7.64	93.33 ± 7.64	83.33 ± 7.64	95.00 ± 0.00	-	-	-
FE	58.88 ± 1.36 <sup>a</sup>	74.78 ± 3.26 <sup>bc</sup>	71.94 ± 2.23 <sup>b</sup>	78.99 ± 5.46 <sup>c</sup>	*	*	-
PER	2.18 ± 0.05 <sup>a</sup>	2.76 ± 0.12 <sup>b</sup>	2.38 ± 0.07 <sup>a</sup>	2.65 ± 0.18 <sup>b</sup>	-	*	-
PR (%)	36.56 ± 0.23 <sup>a</sup>	45.32 ± 1.66 <sup>b</sup>	39.50 ± 0.37 <sup>a</sup>	44.43 ± 3.33 <sup>b</sup>	-	*	-
LR (%)	49.06 ± 26.90	46.44 ± 28.94	53.17 ± 37.79	38.72 ± 27.06	-	-	-

Note: P28L6 = protein 28% and lipid 6%; P28L8 = protein 28% and lipid 8%; P32L6 = protein 32% and lipid 6%; P32L8 = protein 32% and lipid 8%; B<sub>t</sub> = final biomass; SGR = specific growth rate; SR = survival rate; FE = feed efficiency; PER = protein efficiency ratio; PR = protein retention; LR = lipid retention. Different superscript in the same row indicated significant differences ( $P < 0.05$ ). Different superscript in the same row indicated significant differences ( $P < 0.05$ ).

Table 3. Blood biochemistry of tilapia *Oreochromis niloticus*.

Parameter	Treatment				Two-way ANOVA		
	P28L6	P28L8	P32L6	P32L8	P	L	P*L
TG, mg/dL	281.00 ± 17.44 <sup>a</sup>	314.67 ± 18.01 <sup>b</sup>	322.00 ± 8.89 <sup>b</sup>	320.00 ± 12.12 <sup>b</sup>	*	-	-
HDL, mg/dL	55.67 ± 4.51 <sup>bc</sup>	50.67 ± 7.09 <sup>ab</sup>	63.00 ± 5.00 <sup>c</sup>	43.67 ± 2.08 <sup>a</sup>	-	*	*
LDL, mg/dL	21.80 ± 2.36 <sup>a</sup>	27.07 ± 1.72 <sup>a</sup>	26.50 ± 2.00 <sup>a</sup>	27.67 ± 5.97 <sup>a</sup>	-	-	-
CLO, mg/dL	133.67 ± 9.24 <sup>a</sup>	140.67 ± 12.34 <sup>a</sup>	170.67 ± 8.62 <sup>b</sup>	135.33 ± 4.62 <sup>a</sup>	*	-	*

Note: TG = Triglyceride; HDL = high-density lipoprotein; LDL = low-density lipoprotein; CLO = cholesterol. Different superscripts in the same row indicated significant differences ( $p < 0.05$ ). Different superscripts in the same row indicated significant differences ( $p < 0.05$ ).

feed ( $p>0.05$ ). The body's fat content, protein content, and moisture content did not increase significantly when the protein and lipid content in the feed increased ( $p<0.05$ ).

### Discussion

The fish's growth performance increased as the protein and fat content in the feed increased. The treatment with 32% protein and 8% fat resulted in greater growth compared to other treatments. This suggests that higher protein and fat contents lead to increased fish weight growth. Ye *et al.* (2016) increasing dietary protein or lipids level can improve feed efficiency, with a mix of both resulting in optimal growth. However, in research Thirunavukkarasar *et al.* (2022), feeds containing 35% protein and 10% fat showed significantly higher weight growth ( $p<0.05$ ) compared to other protein and fat contents in Nile tilapia. Thus, higher feed protein leads to increased breakdown of amino acids for energy in various metabolic reactions, thereby reducing protein availability for growth.

In this study, higher fat content increased the utilization of protein for growth. Haidar *et al.* (2018), found that consuming a high protein diet can lead to catabolism, which reduces protein efficiency. Protein utilization is reflected in the protein efficiency ratio and protein retention. The best protein retention was found in the treatment with 28% protein and 8% fat ( $45.32 \pm 1.66\%$ ). This indicates that fish can convert protein from feed into stored protein more efficiently, thus showing a linear relationship with the protein efficiency ratio ( $2.76 \pm 0.12$ ). Based on the treatments, higher fat content significantly increased the protein efficiency ratio and protein retention.

Xu *et al.* (2015) showed that increasing feed fat from 8% to 16% enhanced the protein efficiency ratio in Manchuria trout at protein levels of 40–45%. In this study, the protein efficiency ratio increased with the increase in feed fat from 6%

to 8%. The interpretation of protein efficiency ratio and protein retention in this research shows that treatment with 8% fat in the feed resulted in higher protein utilization compared to treatment with 6% fat. Higher lipid levels in the feed can improve protein utilization, aiding in improved nutrient utilization and growth in Nile tilapia. This demonstrates that fat content in feed can enhance the utilization of non-protein energy (protein-sparing effect). Feed energy significantly impacts the protein requirements of fish. Reducing the use of protein as an energy source in feed can be achieved by using appropriate non-protein energy sources, particularly fat (Thirunavukkarasar *et al.*, 2022).

Utilizing fat as an energy source can decrease the use of protein as an energy source, thereby optimizing protein use for growth. Additionally, this study also shows increased feed efficiency with consistent increases in protein and fat levels, consistent with research in Manchuria trout (Xu *et al.*, 2015), brown trout (*Salmo trutta fario*) (Wang *et al.*, 2018) and Israeli carp (*Cyprinus carpio*) (Aminikhoei *et al.*, 2015). Lipoproteins are the most common lipid transport form in fish (Wang *et al.*, 2018). Feed protein and fat content affect serum/plasma cholesterol concentrations (NRC, 2011; Jiang *et al.*, 2015). Providing high fat (8%) at 28% protein did not show a significant increase in cholesterol but increased triglyceride levels. Some authors reported that blood TG levels have an inverse relationship with feed protein content (Jin *et al.*, 2015) but have a positive correlation with fat content (Li *et al.*, 2017; Cao *et al.*, 2019).

This was evident in this study, where TG levels increased significantly with higher fat levels in 28% protein feed. Moreover, increasing the protein level in feed to 32% showed no significant difference compared to the 28:8 treatment. HDL and LDL indicate the transport of different lipoprotein-containing fatty acids. The HDL value increased in the P32L6 treatment, suggesting the effect of added protein on HDL

Table 4. Protein, lipid, and moisture content of the whole-body tilapia.

Parameter	Treatment			
	28;6	28;8	32;6	32;8
Protein (%)	$17.10 \pm 1.05$	$18.62 \pm 0.51$	$17.81 \pm 1.41$	$17.31 \pm 1.37$
Lipid (%)	$4.14 \pm 1.88$	$4.01 \pm 2.04$	$3.68 \pm 2.14$	$3.66 \pm 2.38$
Moisture (%)	$73.41 \pm 2.41$	$75.03 \pm 1.83$	$73.65 \pm 2.62$	$74.12 \pm 2.26$



values, where HDL is dominated by lipoprotein content. However, the P32L8 treatment showed a different trend. Most of the research on Nile tilapia demonstrate increase of HDL level in line with increased content of the lipid on the feed (Imlani *et al.*, 2024; Paul *et al.*, 2022; Xie *et al.*, 2017) it is still unclear how the increase of dietary lipid content produce lower HDL level in this study, therefore additional indepth metabolic study is required to explain this phenomenon especially since blood biochemical parameters are highly sensitive to fish body physiological processes and many external factors can affect this field research.

The entire fish body's chemical composition is greatly influenced by the nutritional profile of the consumed diet (Chowdhury *et al.*, 2021; Singha *et al.*, 2020). This study found no significant differences between all treatments, indicating that lower protein and higher fat did not negatively affect body chemical composition, especially fat accumulation in the flesh. The P28L8 treatment showed higher values for increased crude protein (CP) and body lipid content compared to other treatments. In the protein-sparing effect of lipids, the majority of the energy would come from dietary lipids, allowing protein to be used purely for growth with minimum inevitable catabolism. This leads to enhanced growth and nutrient utilization (Thirunavukkarasar *et al.*, 2022). Furthermore, based on prior study, the increase in total body fat content could be related to the supply of higher-fat feed, which results in accumulation of fat in the body (Sagada *et al.*, 2017).

## CONCLUSION

The optimal protein and lipid utilization was found in the treatment with 28% protein and 8%. This treatment demonstrate the reduce the use of protein as an energy source based on increased values of protein retention, protein efficiency ratio, and final body proximate. The 8% fat content in feed demonstrates a protein-sparing effect.

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