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PROTEIN LEVEL EFFICIENCY OF TILAPIA *Oreochromis niloticus* FEED WITH SELENOMETHIONINE SUPPLEMENTATION

Efisiensi Tingkat Protein Pakan Ikan Nila *Oreochromis niloticus* dengan Suplementasi Selenometionin

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ABSTRACT

One of the nutrients that play an important role for fish growth is protein, one of which is in the formation of body tissues. The amount and quality of protein in fish feed affects growth performance, feed utilization, water quality and of course the price of the feed itself. Excessive feed protein or whose quality does not meet the needs will be excreted as nitrogen waste, especially in the form of ammonia. This study aimed to reduce protein consumption in tilapia (Oreochromis niloticus) diets through selenomethionine supplementation. The test feed was designed using two different protein concentrations: 26% and 30%. Selenomethionine supplementation levels were 0 mg/kg (control) for 30% feed protein, and 3 mg/kg and 6 mg/kg Se for 26% feed protein. This study used a completely randomized design (CRD) with three treatments and three replicates. Tilapia with an average initial weight of 8.05 ± 0.25 g were reared in an aquarium measuring 100×50×50 cm^3 with a stocking density of 15 fish per aquarium. The fish were reared for 60 days and fed three times a day until full. The results showed that supplementation of 3 mg/kg Selenomethionine to 26% feed protein was better than either the control or 6 mg/kg Se supplementation. Supplementation of 3 mg/kg Se to 26% feed protein level significantly increased feed intake (FI), final biomass weight (Bt), feed conversion ratio (FCR), feed efficiency (FE), protein retention (PR), and daily growth rate (DGR) in tilapia. A 100% survival rate was recorded in all treatment groups.

Keywords: Feed, Protein Efficiency, Selenomethionine, Tilapia

ABSTRAK

Salah satu nutrien yang memainkan peranan penting bagi pertumbuhan ikan adalah protein, salah satu peranannya yakni dalam pembentukan jaringan tubuh. Jumlah dan kualitas protein dalam pakan ikan berpengaruh terhadap kinerja pertumbuhan, pemanfaatan pakan, kualitas air dan tentunya harga pakan itu sendiri. Protein pakan yang berlebih atau yang kualitasnya tidak sesuai dengan kebutuhan akan diekskresikan sebagai buangan nitrogen terutama dalam bentuk amonia. Penelitian ini bertujuan untuk mengurangi konsumsi protein pada makanan ikan nila

(*Oreochromis niloticus*) melalui suplementasi selenometionin. Pakan uji dirancang menggunakan dua konsentrasi protein berbeda: 26% dan 30%. Tingkat suplementasi Selenomethionine yakni 0 mg/kg (kontrol) untuk protein pakan 30%, dan 3 mg/kg dan 6 mg/kg Se untuk protein pakan 26%. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan tiga perlakuan dan tiga ulangan. Ikan nila dengan bobot awal rata-rata 8,05 ± 0,25 g dipelihara dalam akuarium berukuran 100×50×50 cm^3 dengan padat tebar 15 ekor per akuarium. Ikan-ikan tersebut dipelihara selama 60 hari dan diberi pakan tiga kali sehari sampai kenyang. Hasil penelitian menunjukkan bahwa suplementasi 3 mg/kg Selenomethionine ke dalam protein pakan 26% lebih baik dibandingkan perlakuan lainya baik itu kontrol ataupun suplementasi 6 mg/kg Se dalam pakan. Suplementasi 3 mg/kg Se terhadap kadar protein pakan 26% secara signifikan meningkatkan asupan pakan (FI), berat biomassa akhir (Bt), rasio konversi pakan (FCR), efisiensi pakan (FE), retensi protein (PR), dan laju pertumbuhan harian (DGR) pada ikan nila. Tingkat kelangsungan hidup 100% tercatat di semua kelompok perlakuan.

Kata Kunci: Efisiensi Protein, Ikan Nila, Pakan, Selenometionin

INTRODUCTION

Protein is an important ingredient crucial for tissue development and proliferation in organisms (Halver, 1989). The price of protein in feed substantially affects the total cost of fish feed. The amount and quality of dietary protein directly influence fish growth rates, survival rates, and production expenses in aquaculture. Inadequate dietary protein undermines the availability necessary for the production of new bodily tissues. In contrast, high or inadequate protein levels result in ineffective use for tissue synthesis, leading to nitrogen excretion, predominantly as ammonia (Pattipeilohy et al., 2020). Selenium (Se) has recently attracted significant interest in animal nutrition as an essential micromineral (Lin, 2014; Baidya & Murthy, 2015). Selenium and selenoproteins are essential for the antioxidant defense mechanisms of organisms. Research indicates that elevated selenium levels mitigate oxidative stress induced by oxidized fish oil (Malandrakis et al., 2014). Furthermore, it has been observed for the first time that the mRNA expression of several selenoproteins, including gpx1, selenof, selenon, selenoi, selenoo, selenoe, msrb1, sephs2, selenot, selenot2, and selenop, is influenced by the interaction between selenium and oxidized fish oil (Liu et al., 2016). Selenium is a key component of the enzyme glutathione peroxidase (Rotruck et al., 1973), crucial for cellular defense against oxidative damage by promoting the reduction of hydrogen peroxide and lipid peroxides (Watanabe et al., 1997). Selenium is an essential trace element that functions as a constituent of iodothyronine deiodinase enzymes. Iodothyronine deiodinase (ID), a selenoprotein, facilitates the transformation of thyroxine (T4) into the biologically active thyroid hormone, 3,5,3'-triiodothyronine (T3) (Brown & Arthur, 2001). Thyroxine levels modulate insulin production, with increased insulin levels enhancing glucose uptake into cells, thus channeling protein-derived energy towards growth activities (Pattipeilohy et al., 2017).

Fish serve as a superior source of selenium for humans, possessing greater amounts of selenium than alternative food sources. Furthermore, selenium in fish is primarily found in an organic form, which is effectively absorbed and retained by humans (Wang et al., 2022). Administering suitable forms of selenium at optimal concentrations in fish feed not only promotes growth and health but also improves the efficacy of aquaculture products by yielding selenium-enriched, health-beneficial seafood (Cotter et al., 2008). Supplementation of organic selenium at 2 and 4 mg Se/kg of feed has shown remarkable growth performance in red tilapia (Pattipeiluhu et al., 2023). The inclusion of selenium in feed serves as a method to save protein, hence improving the utilization of non-protein energy when provided at appropriate dosages (Zhang et al., 2022).

METHODS

Time and Place of Research

This research was conducted from November 2024 to January 2025 at the Cultivation Laboratory, Aquaculture Study Program, Faculty of Fisheries and Marine Science, Pattimura University, Ambon.

Tools and Materials

The tools and materials used in this study were aquarium, aerator hose, aerator stone, aerator pump, digital scale, ruler, water pump, spoons, red tilapia, selenomethionine, Fish meal, MBM, Soy flour, Wheat flour, Tapioca flour, Pollard flour, Fish oil, Corn oil Premix (without Se), and Binder (PMC).

Experimental Diets

Se supplementation in accordance with the optimal Se level in fish feed has an impact on the growth, health condition of fish and provides aquaculture products enriched with Selenium content (Wang *et al*., 2022). The fish diet was formulated to provide 26% protein, supplemented with two levels of selenomethionine: 3 and 6 mg Se/kg feed. A study was undertaken to evaluate the effectiveness of selenium in protein utilization compared to a 30% protein diet lacking selenomethionine supplementation. (Pattipeilohy *et al*., 2020) showed that a feed protein level of 28% supplemented with selenium 3 mg/kg feed was able to increase tilapia growth compared to a feed protein level of 28% with selenium supplementation of 6 mg/kg feed.

The diets were formulated to provide an energy content of 3,700–4,000 kcal GE/kg and included powdered selenomethionine. The selenomethionine was carefully combined with other feed ingredients using a mixer, homogenized, and subsequently pelletized. The pellets were dehydrated in an oven at 35°C for 24 hours. A proximate analysis was performed on the created meal to determine its nutritional composition, and a selenium assay was executed to quantify selenium levels. Table 1 delineates the composition of raw materials, the proximate analysis of the experimental meals on a dry weight basis, and the selenium concentration of the diets. The study's test subjects were Nile tilapia (Oreochromis niloticus), measuring 10–12 cm in length, with an initial average weight of 8.05 ± 0.25 g.

Experimental Design

The study employed a Completely Randomized Design (CRD) with three treatments and three replications. The treatments consisted of P26S3 (26% protein + 3 mg selenium), P26S6 (26% protein + 6 mg selenium), and P30S0 (30% protein + 0 mg selenium).

Table 1. Composition of Test Feed Formulations and Proximate Results of Test Feeds

Raw Material	Selenomethionine Addition (mg/kg)		
	P26S3	P26S6	P30S0
Raw Material Composition (%)			
Fish meal	3.00	3.00	6.00
MBM	16.00	16.00	10.00
Soy flour	16.36	16.60	30.00
Wheat flour	14.60	14.60	16.50
Tapioca flour	2.80	2.80	2.80
Pollard flour	40.00	40.00	30.00
Fish oil	2.00	2.00	1.50
Corn oil	3.00	3.00	2.00
Premix (without Se)	1.20	1.20	1.00

Raw Material	Selenomethionine Addition (mg/kg)		
	P26S3	P26S6	P30S0
Binder (PMC)	0.20	0.20	0.20
Se (mg/kg feed)	0.30	0.60	0.00
Proximate analysis (0% Dry Weight)			
Protein	26.76	26.40	30.23
Lipid	5.63	5.80	6.37
Ash	7.72	7.90	8.55
Crude Fiber	4.19	4.65	4.72
BETN	41.36	42.23	47.73
GE (kkal/kg feed)	3843	3762	4082
C/P	14.36	14.25	13.50
Selenium feed (mg/kg)	2.61	5.33	0.19

Description: 1. MBM: Meat bone meal, 2. PMC: Polymethylolcarbamide, Se: selenomethionine, 4. BETN: Extract material without nitrogen, 5. GE: Gross energy (Watanabe 1988)

Maintenance and Data Collection

The test subjects in this study were red tilapia (Oreochromis niloticus), averaging 5±7 cm in size and an initial weight of 8.05±0.25 g. Nine aquaria, each sized 100×50×50 cm^3, were utilized to house the fish, with each aquarium containing 175 liters of water. The stocking density was 15 fish per 175 liters of water, utilizing a total of 270 red tilapia fingerlings. Feeding occurred ad libitum three times daily at 08:00, 12:00, and 16:00 WIB. Fish biomass was measured at the commencement and conclusion of the maintenance period, alongside proximate analysis of fish body composition and selenium content assessment. Biomass measurements were conducted following a 24-hour fasting interval. Upon conclusion of the maintenance phase, blood samples were obtained from a subset of fish within each treatment group for biochemical analysis.

Total of Feed Consumption

The calculation of feed consumption was determined by calculating the amount of feed given during the experiment minus the amount of uneaten and dried leftover feed.

Final Body Weight

Final Boddy weight can be calculated based on the NRC (1977) formula as follows:

$$\Delta W = Wt - Wo$$

Description:
ΔW : Final Boddy Weight
Wt : Weight of test animals at the end of the study (g)
Wo : Weight of test animals at the beginning of the study (g)

Feed Conversion Ratio

Feed conversion is the ratio of the amount of feed in dry conditions given during the maintenance process minus the weight of dead fish and the initial weight of fish at the end of maintenance (NRC,1977) With the following equation:

$$FCR = \frac{F}{(Wt + D) - Wo}$$

Description:
FCR : Feed Conversion Ratio
Wt : Weight of test animals at the end of the study (g)
Wo : Weight of test animals at the beginning of the study (g)
D : Weight of dead fish (g); and
F : Amount of feed consumed (g)

Feed Efficiency

The feed utilization efficiency (FE) value is calculated using the formula (NRC,1977) as follows:

$$\text{FE} = \frac{\text{Wt} - \text{Wo}}{\text{F}} \text{X } 100\%$$

Description:
FE : Feed utilization efficiency (%)
Wt : Weight of test animals at the end of the study (g)
Wo : Weight of test animals at the beginning of the study (g)
F : Amount of feed consumed during the study (g).

Protein Efficiency Ratio

Protein efficiency ratio value is calculated using the formula (NRC,1977):

$$\text{PER} = \frac{Wt - Wo}{Pi} X \ 100\%$$

Description:
PER : Protein efficiency ratio (%)
Wt : Weight of test animals at the end of the study (g)
Wo : Weight of test animals at the beginning of the study (g)
Pi : The consumed feed protein weight (g).

Growth Rate

The daily relative growth rate of tilapia seeds observed in the study was calculated using the formula**:**

$$\text{SGR} = \frac{\text{LnWt} - \text{LnWo}}{\text{t}} \text{X } 100\%$$

Description:
SGR : Daily weight growth rate (%/day)
Wt : Weight of test animals at the end of the study (g)
Wo : Weight of test animals at the beginning of the study (g)
t : Maintenance time (days)

Survival Rate

The percentage of survival rate was calculated using the formula from (NRC,1977) as follows:

$$\text{SR} = \frac{\text{Nt}}{\text{No}} \text{x } 100\%$$

Description:
SR : Survival (%)
Nt : Number of fish alive at the end of maintenance (tail)
No : Number of fish at the beginning of maintenance

Blood Glucose

Analysis of blood glucose levels using a glucose test device, Gluco Dr AGM-2100 with a kit in the form of Gluco Dr strip Code 8.

Selenium Retention

Se retention can be calculated based on the formula of Rider et al. (2009) as follows:

$$RSe = \frac{F - I}{Se} x\ 100\%$$

Description:
RSe : Se retention (%)
F : Total body Se at the end of the experiment (mg)
I : Total body Se at the beginning of the experiment (mg)
Se : Total amount of Se consumed (mg)

Blood Proteins

This analysis was performed using the principle of the biuret test. The absorbance reading value was analyzed based on the formula in Mushawwir et al. (2012) as follows:

$$BP = \frac{Au - Ab}{As - Ab} x\ 6g.dL\ 10^{-1}$$

Description:
BP : Blood proteins
Au : Absorbance of sample tested
Ab : Absorbance of blank
As : Absorbance of standard

Total Red Blood Cells

Total erythrocyte examination aims to determine the health condition of fish by counting total erythrocytes in the blood. The method of taking blood samples is sucked with a pipette with a scale of up to 0.5, then Hayem's solution is sucked up to a scale of 101, the pipette is shaken to form a figure eight for 3-5 minutes so that it mixes homogeneously. The first drop was discarded; the next drop was dripped into the hemocytometer and covered with a glass cover (Blaxhall & Daisley 1973). Counts were made on 5 small squares of the hemocytometer and the number was calculated using the following formula.

$$\text{Total erythrocyte} = \frac{\Sigma \text{Calculated cell x 1x dilution factor}}{\text{small box volume}}$$

Total White Blood Cells

The method of calculating total leukocytes is the same as calculating total erythrocytes, which distinguishes the solution used, namely Turk's solution. The total leukocyte count is expressed as n x $10^5/mm^3$.

$$\text{Total leukocytes} = \frac{\Sigma \text{Calculated cell x 1x dilution factor}}{\text{small box volume}}$$

Hematocrit Levels

Hematocrit measurement was performed using a hematocrit micro-tube in the form of a heparin-coated capillary tube. Blood samples were drawn using a capillary tube up to 3⁄4 of the capillary, then covered with a covering material (wax). The capillary tube containing the blood was centrifuged at 3500 rpm for 15 minutes. The reading was done by comparing the part of blood that settled with the whole part of blood in the micro hematocrit tube, using a micro hematocrit. The scale and results are expressed in percent (%), (Anderson & Siwicki 1993).

Chemical Analysis

The chemical analysis encompassed the quantification of selenium levels and the proximate makeup. The ICP-OES method was employed to assess selenium levels in the feed and fish tissues. Proximate analysis was performed on the raw feed ingredients, test feed, and fish bodies at both the commencement and conclusion of the study, adhering to the technique outlined by Takeuchi (1988). The analysis entailed quantifying moisture content via oven-drying at 105-110°C, protein content employing the Kjeldahl method, fat content utilizing the Soxhlet method for feed and the Folch method for fish bodies, ash content through incineration in a furnace at 400-600°C, and crude fiber content by dissolution in strong acids and bases followed by heating.

Data Analysis

This research utilized a Completely Randomized Design (CRD) with three treatments and three replications. The parameters analyzed statistically encompassed growth performance measures and chemical composition data. Data were organized using MS Office Excel 2013, and normality and homogeneity assessments were performed prior to ANOVA. Statistical analysis was conducted with SPSS 16.0 software. Variations among treatments were assessed using analysis of variance (ANOVA) at a 95% confidence level. Should the F-test reveal substantial differences, the study was subsequently augmented with Duncan's multiple range test.

RESULTS

The supplementation of selenomethionine significantly affected FCR, FBW, FE, PER, SGR, and SR ($p<0.05$). Table 2 indicates that selenium supplementation at 3 mg Se/kg meal with 26% protein content generally improved hunger and yielded better growth performance than alternative treatments. This was demonstrated by elevated values in feed intake, protein retention, feed efficiency, specific growth rate, and total biomass compared to the other treatments.

Table 2. Total of Feed Consumption (TFC), Final Body Weight (FBW), Feed Conversion Ratio (FCR), Feed Efficiency (FE), Protein Efficiency Ratio (PER), Specific Growth Rate (SGR), and Survival Rate (SR) in Tilapia

Parameters	Treatment		
	P26S3	**P26S6**	**P30S0**
TFC (g)	441±0^{c}	347±2^{a}	381±1^{b}
FWB (g)	441±1^{c}	234±1^{a}	269±1^{b}
FCR	1.38±0.004^{a}	3.09±0.02^{c}	2.65±0.01^{b}
FE (%)	72.37±0.19^{c}	32.32±0.25^{a}	37.63±0.19^{b}
PER (%)	30.07±2.48^{c}	13.78±3.28^{a}	16.61±0.56^{b}
SGR (%)	3.27±0.02^{c}	1.64±0.01^{a}	1.93±0.02^{b}
SR (%)	100±0^{a}	100±0^{a}	100±0^{a}

The blood glucose analysis results in tilapia demonstrated considerable variation in blood glucose levels across treatments ($p<0.05$). The minimum blood glucose level was recorded in the P30S0 treatment at 55 mg/L, but the P26S3 and P26S6 treatments exhibited blood glucose values of 65 mg/L and 72 mg/L, respectively.

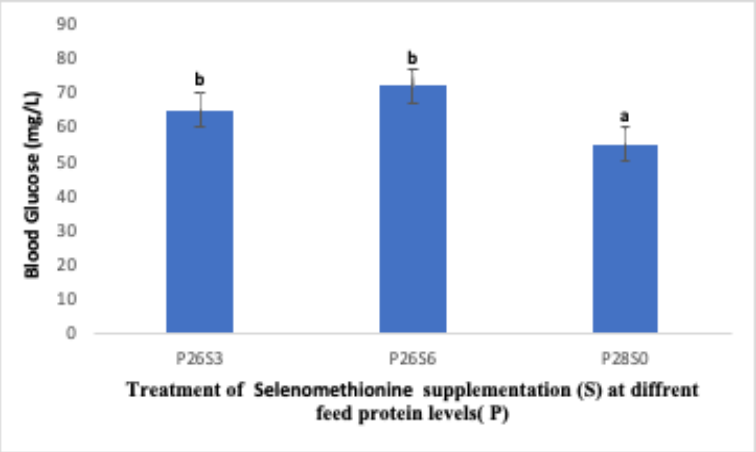

Figure 1. Blood Glucose Levels in Tilapia Subjected to Meals with Varying Protein Concentrations and Selenomethionine Supplementation (Distinct Letters on the Bar Chart Denote Significant Differences Across Treatments ($p<0.05$)

The results of the selenium retention study demonstrated that selenomethionine supplementation in diets with differing protein levels significantly influenced body selenium retention ($p<0.05$). The highest selenium retention was observed in the 26% dietary protein treatment supplemented with 3 mg Se/kg feed, followed by 6 mg Se/kg. The therapy without selenomethionine supplementation had the lowest selenium retention compared to the other therapies.

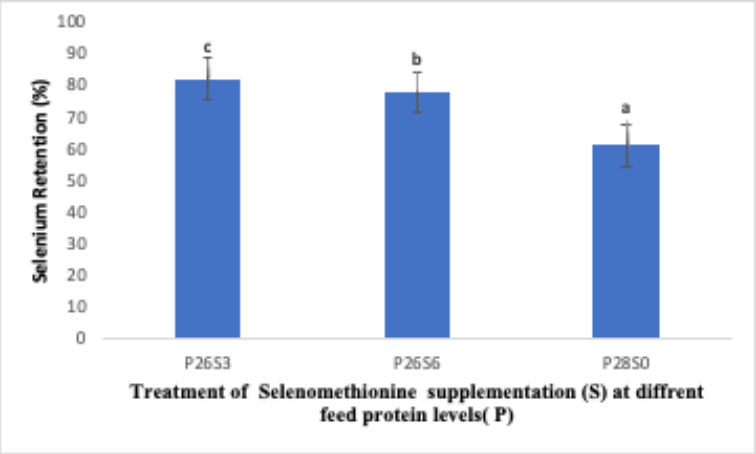

Figure 2. Retention of Selenium in Nile Tilapia Subjected to Diets with Varying Protein Concentrations and Selenomethionine Supplementation Levels (Distinct Letters on the Bar Chart Denote Significant Differences Between Treatments ($p<0.05$)

Figure 3 illustrates the blood protein concentration across three treatments, revealing the lowest concentration in the 26% protein diet supplemented with 6 mg Se/kg feed, followed by the 30% protein diet without Se supplementation. The 26% protein diet supplemented with 3 mg Se/kg meal demonstrated the highest blood protein content of 6.4 mg/L among all treatments.

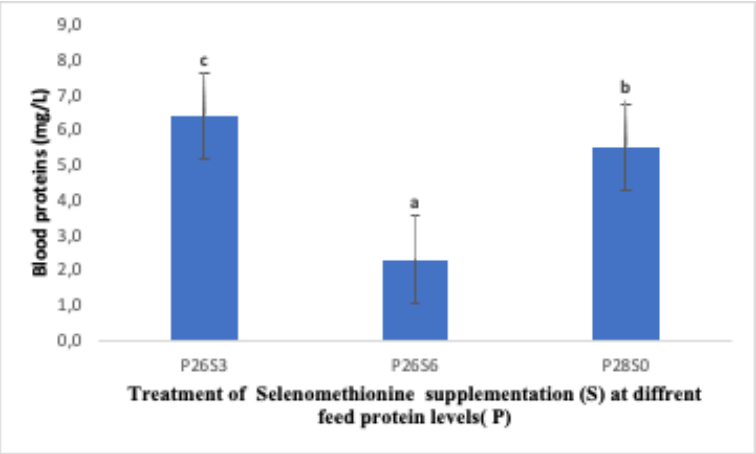

Figure 3. Blood protein Concentrations in Nile Tilapia Subjected to Diets with Varying Protein Levels and Selenomethionine Supplementation (Distinct Letters on the Bar Chart Denote Significant Differences Between Treatments ($p<0.05$)

Table 3 demonstrates that the lowest red blood cell count was observed in the 26% protein diet supplemented with 6 mg Se/kg of feed. The lowest white blood cell count occurred in the 26% protein diet supplemented with 3 mg Se/kg feed, while the highest white blood cell count was recorded in the 30% protein diet without selenomethionine supplementation. The highest hematocrit value was seen in the 26% protein diet supplemented with 3 mg Se/kg of feed.

Table 3. Red Blood Cell (HR), White Blood Cell (WBC), and Hematocrit Counts of Tilapia

Parameters	Treatments		
	P26S3	**P26S6**	**P30S0**
HR (sel/mm^{3}x10^6)	1.39±0.04^b	1.13±0.10^a	1.42±0.22^b
WBC (sel/mm^{3}x10^4)	4.98±3.66^c	7.93±1.05^{ab}	8.95±0.28^b
Hematocrit (%)	18.33±8.25^c	11.3±1.95^a	15.43±1.03^b

DISCUSSION

The supplementation of selenium (Se) not only stimulates insulin secretion but also elevates blood glucose levels, so triggering glycogenesis, which leads to the storage of energy as glycogen in the body. Similar findings have been reported in tilapia by Pattipeilohy et al. (2020) and in red tilapia by Pattipeiluhu et al. (2023). These data suggest that organic selenium supplementation in feed, at a given dosage, can influence feed palatability. The survival rate for all therapies reached 100% and was not influenced by selenium supplementation. Marked changes were noted, with elevated blood glucose levels in tilapia receiving 3 and 6 mg Se/kg feed at a 26% dietary protein level, in contrast to the 30% dietary protein treatment devoid of Se supplementation. Selenium supplementation in the diet resulted in heightened blood glucose levels, along with increased insulin release in the bloodstream. The augmented insulin secretion facilitated the accumulation of energy reserves as body glycogen, a process termed glycogenesis (Suprayudi et al. 2013).

The incorporation of 3 and 6 mg Se/kg into the meal with a 26% protein content elevated the body Se concentration relative to the 30% protein diet treatment. The elevation in body selenium levels signifies that selenium has a biological function, especially as selenoproteins. Se retention plays a crucial role in growth rates in relation to protein storage and the utilization of non-protein energy as the primary energy source (Pattipeilohy et al., 2020). Plasma proteins consist of 60% albumin, 35% globulin, and 4% fibrinogen. Albumin aids in the transport of

ions, chemicals, minerals, hormones, and metabolic waste; fibrinogen is crucial for blood clotting following damage; and globulin is involved in immune system activities (Ainsworth, 1994). These components signify the extent of the immune reaction or the health status of the test fish.

The red blood cell (RBC) count of Nile tilapia in this study varied from 1.13 to 1.42 × 10^6 cells/mm³. The erythrocyte count in healthy fish generally varies from 1.05 to 3.00 × 10^6 cells/mm³ (Robert, 1978). Erythrocytes, or red blood cells, are the most prevalent form of blood cells relative to other cellular types. Under typical circumstances, erythrocytes comprise around fifty percent of the blood volume. The typical erythrocyte count in Nile tilapia varies from 20,000 to 3,000,000 cells/mm³ (Hartika et al., 2014). White blood cells have a crucial role in the immune system, particularly in the production of antibodies in response to foreign substances (Purwanti et al., 2014). The typical hematocrit percentage in healthy Nile tilapia ranges from 27.3% to 37.8% (Hardi et al., 2011). The hematocrit levels during the three treatments in this study remained within the normal range, varying from 11.3% to 18.33%, presumably influenced by selenium's function as glutathione peroxidase (GPx). GPx is essential for cellular defense against oxidative damage in cytoplasmic structures by reducing hydrogen peroxide and lipid peroxides (Watanabe et al., 1997).

CONCLUSION

Selenomethionine supplementation in fish feed markedly affects feed intake (FI), final biomass weight (FBW), feed conversion ratio (FCR), feed efficiency (FE), protein retention (PR), and daily growth rate (DGR) in Nile tilapia (Oreochromis niloticus). This addition also affects the protein efficiency of Nile tilapia feed. This study's results indicate that a 3 mg/kg selenomethionine supplementation in a 26% protein diet produces superior growth performance relative to a 6 mg/kg selenomethionine supplementation or a 30% protein diet lacking selenomethionine supplementation.

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