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Evaluation of protein sparing effect in Nile tilapia *Oreochromis niloticus* fed with organic selenium supplemented diet

Evaluasi efek protein sparing pada ikan nila *Oreochromis niloticus* yang diberi pakan dengan suplementasi selenium organik

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ABSTRACT

The objective of this research was to determine the optimum level of organic selenium supplementation in improving the growth performance of Nile tilapia *Oreochromis niloticus* fed with diet containing low protein level. Basal diet was formulated to contain 28% protein with three different levels of organic selenium supplementation, namely 0 (control), 3, and 6 mg Se/kg feed. Furthermore, to evaluate selenium function on protein utilization and sparring effect, a diet with 30% of protein content was also used as a comparison. In this regard, this study was conducted using a completely randomized experimental design with four treatments and three replications. Nile tilapia with an average body weight of 8.05 ± 0.25 g were reared in the 100×50×50 cm^3 aquarium at a density of 15 fish/aquarium. The experimental fish were reared for 60 days and fed three times daily to apparent satiation levels. Dietary supplementation of organic Se resulted in higher fish biomass, lower feed conversion ratio, higher protein retention and daily growth rate compared to the control diets with 28% and 30% protein levels. In conclusion, dietary supplementation of organic Se at 3 mg organic Se/kg feed could significantly increase protein utilization and improve the growth performance of Nile tilapia.

Keywords: tilapia, protein sparing effect, organic selenium, feed

ABSTRAK

Tujuan dari penelitian ini adalah menentukan tingkat suplementasi selenium organik pada pakan untuk meningkatkan kinerja pertumbuhan ikan nila *Oreochromis niloticus*. Pakan basal diformulasikan mengandung 28% protein dengan tiga tingkat selenium organik yaitu 0 (kontrol), 3, dan 6 mg Se/kg pakan. Selanjutnya untuk mengevaluasi penggunaan selenium pada pemanfaatan protein dan efek sparring, pakan dengan 30% protein juga ditambahkan sebagai perlakuan pembanding. Penelitian ini menggunakan rancangan acak lengkap dengan empat perlakuan dan tiga ulangan. Ikan nila dengan bobot rata-rata adalah 8.05 ± 0.25 g dipelihara dalam akuarium 100×50×50 cm^3 dengan kepadatan 15 ikan/akuarium. Ikan dipelihara selama 60 hari dan diberi pakan tiga kali sehari secara at satiation. Pemberian pakan dengan suplementasi Se organic menghasilkan biomassa ikan yang lebih tinggi, rasio konversi pakan yang lebih rendah, retensi protein dan laju pertumbuhan harian yang lebih tinggi daripada perlakuan control dengan kadar protein 28% dan 30%. Berdasarkan hasil penelitian ini, dapat disimpulkan bahwa suplemtasi pakan ikan nila dengan 3 mg Se organik/kg pakan dapat meningkatkan pemanfaatan protein pakan dan kinerja pertumbuhan ikan nila.

Kata kunci: ikan nila, *protein sparing effect*, selenium organik, pakan.

INTRODUCTION

Nile tilapia *Oreochromis niloticus* is an economically important fish and is an important commodity in the global aquaculture business (Sudiarto *et al*., 2014; Prabu *et al.,* 2019). Tilapia growth performance is strongly influenced by quality and quantity of the feed. Feed costs represent 75–90% of the total operating costs of the fish farms (El-Sayeda *et al.*, 2015; Baki & Yücel, 2017). Fish requires complete nutrients such as protein, fat, carbohydrates, minerals, or vitamins to support maximum fish growth (Suprayudi *et al*., 2013; Prabu *et al.*, 2017). Fish fed with a proper protein content, but not balance in the term of energy, potentially causes lower growth performance and protein retention. The relationship between energy and protein is known as the protein sparing effect (Welengane *et al.*, 2019; Kim *et al.,* 2012).

Protein is the most important nutrient in aquafeed, which has a higher price per unit compared to that of lipid or carbohydrate (Hua *et al.*, 2019; El-Wahab *et al.*, 2020). The quantity and quality of feed protein will affect the growth rate, survival rate, and the cost of production in a rearing process (Daniel, 2017; Syahailatua *et al.*, 2017). If proteins in the feed are less, the proteins in the body tissues will be utilized to maintain vital tissues function (Carneiro *et al*., 2017). Furthermore, if the protein feed is excessive and not being used in the protein synthesis, it will be excreted as nitrogen discharges, mainly in the form of ammonia (Suprayudi *et al*., 2013; Handajani *et al.*, 2018; Xia *et al.*, 2015).

Selenium is one of the microminerals that plays a role in fish growth and health (Prabhu *et al.*, 2016; Nazari *et al.*, 2017). One way that can be explored to improve the efficiency of protein utilization in the aquafeed is supplementation with organic selenium (Handique *et al.*, 2020). This mineral is found to be an integral part of the enzyme glutathione peroxidase (Zoidis *et al*., 2018). Glutathione peroxidase (GPx) plays a role in the cellular defense against oxidative damage to cytoplasmic structures by catalyzing the reduction of hydrogen peroxide and lipid peroxide (Biller & Takahashi, 2018). Baidya and Murthy (2015) added the reactions necessary that this enzyme catalyzes for the conversion of hydrogen peroxide and fatty acids into water and alcoholic fatty acids by the use of reduced glutathione, thus protecting the cell membrane from oxidative damage. Besides that, another important function of Se mineral is its role in thyroid hormone metabolism (Pacitti *et al.*, 2015). In addition, selenium is also an essential trace element that serves as a component of the iodothironin deiodinase enzyme (Suprayudi *et al*., 2013)

Dietary selenium is required for optimum growth and physiological function of fish (Khan *et al.*, 2017; Iqbal *et al.*, 2020). The content of selenium in fish feed should be in accordance with the nutrient needs of the fish. Excessive amounts of selenium can be toxic to fish causing death and disruption of growth, whereas selenium deficiency can lead to disruption of growth, low feed efficiency, and decreased glutathione peroxidase (Biller & Takahashi, 2018; Odedeyi & Odo, 2017). This mineral is found to be an integral part of the enzyme peroxidase (Zoidis *et al*., 2018).

The relationship between selenium and protein sparing effect can be seen indirectly from the role of selenium as an iodotironin deiodinase enzyme. Iodothyronine deiodinase (ID) is a selenoprotein (enzyme containing Se) that catalyzes the production of active forms of thyroid hormone (3,5,3 "-triiodtironin, T3) from thyroxine (T4) (Brown & Arthur, 2001). The concentration of thyroxine controls the production of insulin. When thyroxine concentration increases, the splitting of insulin will increase. As a consequence, the production of insulin by the pancreas is also increased (Ismail *et al*., 2017).

The supplementation of selenium to increase the growth rate and survival rate of the fish has been investigated by Hamzah *et al*. (2012a) and Suprayudi *et al*. (2013). Hamzah *et al*. (2012a) reported that the supplementation of organic selenium to juvenile humpback grouper at 4 g Se/kg of feed resulted greater protein retention compared to other treatments. Furthermore, another study also showed increasing selenium supplementation to a certain level resulted a positive correlation with protein retention (Ilham *et al.*, 2017). Suprayudi *et al*. (2013) also reported a similar case with the supplementation of organic selenium of 4 g Se/kg of feed gave the best growth performance in red tilapia when compared to other treatments ie 0, 1, and 2 g Se/kg of feed. Selenium supplementation in the feed was done as an attempt to sparing effect on proteins, thus the supplementation of selenium in the right dose feed could increase the use of non-protein energy Suprayudi *et al*. (2014). Thus, the objective of this research was to determine the optimum amount of organic selenium supplementation and how it perform compared to different protein content in relation to growth performance and protein sparring effect in Nile tilapia.

MATERIALS AND METHODS

Dietary treatment and rearing method

All the diet in this study was formulated using fish meal, meat and bone meal, and soybean meal as main protein source. Protein requires of tilapia was 34–56% (Jauncey, 1982) while hybrid tilapia was 30–32%. Protein basal diet was formulated to have 28% of protein thus three level of organic selenium namely 0 (control), 3, and 6 mg Se/kg feed were added to simulate increase level of selenium. Furthermore to evaluate selenium use on protein utilization and sparring effect, diet with 30% of protein was also used as comparison. All the diet was formulated to have 3.700–4.000 kcal GE/kg/g. Organic selenium used in the form of powder. Organic selenium is mixed with other feed ingredients then homogenized using a mixer and then formed into pellet shape. The pelleted feed is dried in an oven at 35°C for 24 hours. After that, a proximate analysis was done on the feed that was made to determine the nutrient levels and selenium analysis to determine the levels of selenium in the feed. The composition of feedstock and test feed proximate in dry weight and feed selenium levels are presented in Table 1. The test animals used in this study were tilapia *Oreochromis niloticus* with size 10–12 cm and initial weight 8.05 ± 0.25 g.

Experimental design

The experimental design of this study used completely randomized design with 4 treatments and 3 replications. The treatment consisted of treatment 1 (without selenium + 28% of protein content), treatment 2 (3 mg of selenium + 28% of protein content), treatment 3 (6 mg of selenium + 28% of protein content), and treatment 4 (without selenium + 30% of protein content).

Table 1. The composition of the test feed formulation and the proximate result of the test feed

Raw materails	Treatment of organic selenium supplementation (mg/kg)			
	0	3	6	0
Composition of raw materials (%)				
Fish meal	3.00	3.00	3.00	6.00
MBM[1]	9.00	9.00	9.00	10.00
Soybean meal	30.30	30.30	30.30	30.00
Wheat flour	17.80	17.80	17.80	16.50
Tapioca flour	2.80	2.80	2.80	2.80
Pollard flour	30.00	30.00	30.00	30.00
Fish oil	2.00	2.00	2.00	1.50
Corn oil	2.70	2.70	2.70	2.00
Premix (without Se)	1.20	1.20	1.20	1.00
Binder (PMC)[2]	0.20	0.20	0.20	0.20
Se[3] (mg/kg feed)	0.00	0.30	0.60	0.00
Proximate analysis results (0% dry weight)				
Protein	28.56	28.84	28.32	30.23
Fat	5.96	5.80	5.84	6.37
Ash	8.64	8.38	8.41	8.55
Crude fiber	4.46	4.50	4.12	4.72
BETN[4]	45.46	44.78	45.27	47.73
GE[5] (kcal/100 g feed)	3786	4034	3697	4082
C/P (kcal/ 100 g feed)	14.56	14.41	13.20	13.60
Feed selenium (mg/kg)	0.103	0.115	0.119	0.103

Note: 1. MBM= Meat bone meal, 2. PMC = Polymethylolcarbamide, 3. Se= Organic selenium, 4. BETN =non-nitrogen extract, and 5. GE= Gross energy (Watanabe, 1988), 1 g protein= 5.6 kcal GE, 1 g fat= 9.4 kcal GE, 1 g carbohydrate/BETN= 4.1 kcal GE.

Fish maintenance and data collection

Fish was reared using 18 aquarium containers with the size of 100×50×50 cm^3, the volume of water as much as 175 L/aquarium, and stocking density of 15 fish per aquaria. Feeding on tilapia was conducted by until apparent satiation three times a day at 08.00, 12.00, and 16.00 WIB.

During rearing period, water quality was maintained within the optimum temperature range at 28−31°C, 4.9−6.6 mg/L for dissolved oxygen content, pH 6.72−7.44, and TAN 0.48−0.7 mg/L. Water quality was maintained by conducting 30% water exchange every three day a times Each aquarium was equipped with a thermostat to keep the temperature stable, an aeration to maintain oxygen solubility, and a top filters to reduce the turbidity of organic matter. Water temperature measurements were performed daily in the morning and afternoon, while the measurements of pH, dissolved oxygen, and total ammonia nitrogen (TAN) were performed three times during maintenance i.e. at the beginning of maintenance, day 40, and day 60. At the beginning and the end of rearing period, fish biomass, proximate analysis of fish body and, analysis of selenium body content of the tested fish were conducted. Biomass weighing was done after the fish was fasted for 24 hours. At the end of rearing period, after the biomass weighing, several tested fish from each treatment were taken for blood biochemical test.

Chemical analysis

Chemical analysis included analysis of selenium and proximate levels. Analysis of selenium levels in the feed and fish body used inductively coupled plasma optical emission spectrometry (ICP-OES) method. Proximate analysis of the whole body of tested fish were performed at beginning and at the end of study. The proximate analysis were performed according to Takeuchi (1998).

The parameters measured in the study included initial biomass of tested fish, final biomass of tested fish, total feed consumption, specific growth rate, feed efficiency (NRC, 1977), survival rate (Opasola *et al.*, 2013), protein retention (Watanabe, 1988), selenium retention (Rider *et al.*, 2009), blood glucose, and blood profile which consisted of erythrocyte, leukocyte, hemoglobin (Hb), and hematocrit levels. Sampling was carried out at the beginning of the rearing period and on the 60^{th} day, with the number of fish sampled was three individuals for each treatment.

The calculation of total feed consumption was determined by calculating the amount of feed given during the experiment minus uneaten feed and has been dried (Suprayudi *et al.*, 2013). Analysis of blood glucose level was conducted using glucose test apparatus Gluco Dr. AGM-2100 with kit in the form of Gluco Dr strip Code 8. Observation of blood profile was done to determine the response of fish towards organic selenium supplementation.

Total erythrocyte examination aimed to determine the health status by calculating total erythrocytes (Dal'Bó *et al.*, 2015). The calculation of total leukocytes was equal to the total calculation of erythrocytes, which distinguished the solution used i.e. Turk's solution. The number of total leucocytes was expressed by n × 10^5/mm^3. Hematocrit measurements were performed using a hematocrit-shaped capillary heparin capillary tube (Anderson & Siwicki, 1993). The concentration of hemoglobin was measured using the Sahli method with Sahlinometer, the result was expressed in g% (Wedemeyer & Yasutake, 1977).

Data analysis

This study used completely randomized design (CRD) with four treatments and three replications. The statistically tested parameters were growth measurement and chemical analysis parameters. The data obtained was tabulated with the Microsoft Office Office Excel 2013 and the ANOVA test were analyzed using the SPSS 16.0. Differences between treatments could be discovered through the test results using analysis of variance with 95% confidence interval and Duncan test as post-hoc if significant difference was found at 95% confidence interval.

RESULTS AND DISCUSSION

Results

Growth performance

Organic selenium supplementation had significant effect on total of feed consumption (TFC), final biomass weight (Bf), feed conversion ratio (FCR), feed efficiency (FE), protein retention (PR), daily growth rate (DGR), and survival rate (SR) on feed protein 28% and 30%. Table 2 shows that organic selenium supplementation of 3 mg Se/kg of feed at 28% protein content gave significant effect for all parameters compared to organic selenium supplementation 3 mg/kg of feed at feed protein content 28%, and other treatments. This

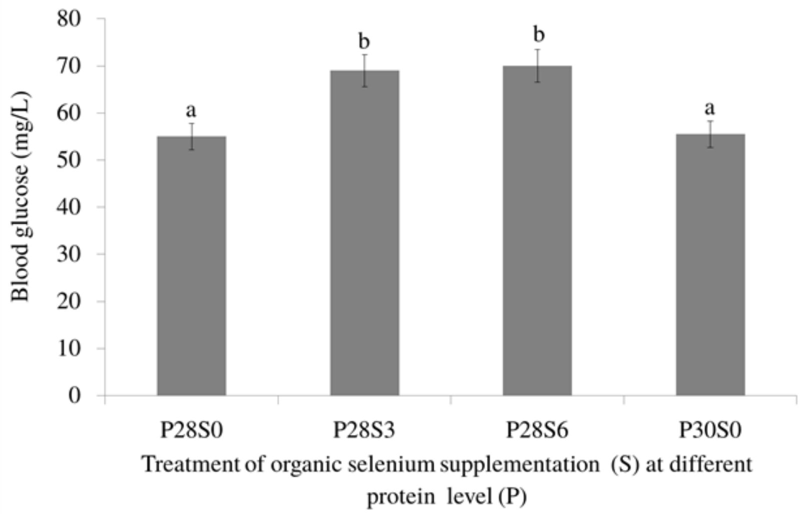

Figure 1. Blood glucose of tilapia supplemented by organic selenium dose. Different letters on the same line showed significantly different treatment effect (P<0.05). The value shown is the average value and standard deviation. P28S0 = Without selenium at protein content 28%; P2836= Selenium treatment 3 mg/kg at protein content 28%; P28S6 = Selenium treatment 6 mg/kg at protein content 28%; and P30S0 = Without selenium treatment at protein content 30%.

Table 2. Total of feed consumption (TFC), final biomass weight (Bf), feed conversion ratio (FCR), feed efficiency (FE), protein retention (PR), daily growth rate (DGR), and survival rate (SR) at tilapia by different selenium supplemented

Parameters	Treatments			
	Protein 28 % Selenium 0	Protein 28 % Selenium 3 mg	Protein 28 % Selenium 6 mg	Protein 30 % Selenium 0
TFC (g)	362.50 ± 1.49[a]	390.90 ± 1.31[e]	362.98 ± 1.65[c]	381.28 ± 0.93[d]
Bf (g)	261.85 ± 0.85[a]	333.78 ± 1.33[d]	284.17 ± 1.14[c]	268.54 ± 0.75[b]
FCR	2.62 ± 0.03[c]	1.85 ± 0.02[a]	2.25 ± 0.01[b]	2.65 ± 0.01[d]
FE (%)	38.05 ± 0.41[a]	53.99 ± 0.51[c]	44.44 ± 0.27[b]	37.63 ± 0.19[a]
PR (%)	16.18 ± 1.96[a]	22.53 ± 2.33[b]	21.45 ± 2..25[b]	16.61 ± 0.56[a]
DGR (%)	1.89 ± 0.04[a]	2.53 ± 0.04[c]	2.12 ± 0.01[b]	1.93 ± 0.02[a]
SR (%)	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]

can be seen with the high protein retention, feed efficiency, daily growth rate and total biomass for feeding treatment of 28% with supplementation of 3 mg Se/kg of feed, although the survival rate for all parameters is the same i.e. 100%. This is certainly different for the treatment without the supplementation of organic selenium either in the feed protein treatment of 28% and 30% both did not have a significantly different effect on protein retention, feed efficiency and daily growth rate. The table also shows that, with increasing organic selenium supplementation in feed, will affect the decrease of feed intake, final biomass, feed efficiency, protein retention and daily growth rate.

Blood glucose

The analysis of blood glucose level of tilapia fish during 60 days of maintenance was presented in Figure 1. The result of blood glucose level analysis showed that blood glucose level for each treatment gave significant different effect (P>0.05).

Shows differences in blood glucose levels in fish supplemented with organic selenium and without organic selenium supplementation. Treatment without selenium supplementation showed the lowest blood glucose levels at both feed protein levels of 28% and 30% at 55 mg/L, whereas in the treatment of feed with organic selenium supplementation the blood glucose levels were in the range of 65–72 mg/L.

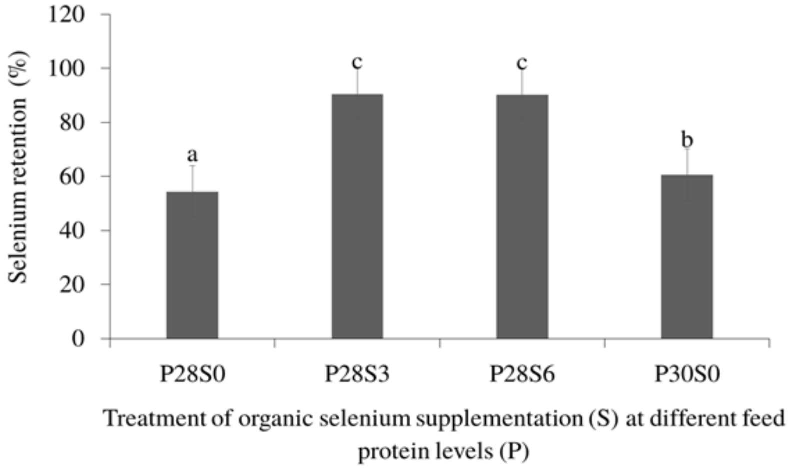

Figure 2. Selenium retention of fish supplemented by different organic selenium doses. Different letters on the same line showed significantly different treatment effect (P<0.05). The value shown is the average value and standard deviation. P28S0 = Without selenium at protein content 28%; P2836= Selenium treatment 3 mg/kg at protein content 28%; P28S6= Selenium treatment 6 mg/kg at protein content 28%; and P30S0= Without selenium treatment at protein content 30%.

Table 3. Number of red blood cells, white blood cells, hemoglobin, and hematocrit of tilapia

Tested parameters	Level of selenium feed (mg/kg)			
	Protein 28 % Selenium 0 mg	Protein 28 % Selenium 3 mg	Protein 28 % selenium 6 mg	Protein 30 % Selenium 0
Red blood cells ($\times10^6$ cell/ mm^3)	0.73 ± 0.07^{a}	1.07 ± 0.28^{ab}	1.24 ± 0.36^{ab}	1.42 ± 0.22^{b}
White blood cells ($\times10^4$ cell/ mm^3)	6.67 ± 0.58^{ab}	6.14 ± 0.58^{ab}	8.13 ± 0.11^{b}	8.95 ± 0.28^{b}
Hemoglobin (g%)	3.10 ± 0.1^{a}	3.9 ± 0.62^{ab}	5.27 ± 0.94^{bc}	4.36 ± 0.25^{abc}
Hematocrit (%)	13.13 ± 2.90^{a}	15.3 ± 3.32^{a}	15.7 ± 1.02^{a}	15.43 ± 1.03^{a}

Note: Different letters on the same line showed significantly different treatment effect (P<0.05). The value shown is the average value and standard deviation.

Selenium retention

Selenium retention analysis results show that organic selenium supplementation in different protein feeds has a significant effect on selenium retention of the body. Figure 2 shows that selenium retention will increase with increasing organic selenium supplementation in the feed. However, in the 28% feed protein with selenium supplementation 6 mg/kg the selenium retention feed was highest compared to other treatments. Figure 2 also shows that treatment without organic selenium supplementation gives the lowest yield on selenium retention.

Blood analysis

The result of tilapia fish blood analysis showed that the supplementation of organic selenium to the tilapia feed gave the same effect to the number of red blood cells and blood hematocrit level of tilapia for all treatments. The number of red blood cells showed the same range in all treatment ranged from 0.73–1.42×10^6 cells/mm^3, white blood cells ranged from 6.14–8.95×10^4 cells/mm, hematocrit levels ranged from 13.13–15.7% and hemoglobin levels ranged from 3.9–5.27 g%.

Discussion

Based on this research, fish with selenium supplementation treatment of 3 mg/kg of feed at 28% protein content have total feed consumption, final biomass weight, feed conversion ratio, feed efficiency, protein retention, and daily growth rate higher than other treatment while survival rate live the same for all treatments. This is thought to be related to the function of selenium itself in the iodotironin deiodinase enzyme. Iodothyronine deiodinase (ID) is a selenoprotein (enzyme containing Se) that catalyzes the production of active forms of thyroid hormone (3,5,3 "-triiodtironin, T3) from thyroxine (T4) (Brown & Arthur, 2001), thereby causing growth rate Increased. Similar results were reported by Tawwab *et al*. (2007) that the supplementation of Sel-Plex® equivalent to 3.67 mg Se/kg of feed can increase the specific growth rate in juvenile African catfish.

The amount of feed consumption increases with the presence of organic selenium supplementation in the feed of 3 mg/kg of feed, but decreases with increasing selenium dose up to 6 mg/kg in feed. Based on this it can be seen that organic selenium supplementation Se/kg of feed up to a certain dose can give effect to the palatability of feed. Organic selenium supplementation in tilapia feed showed the highest value of feed efficiency, blood protein and protein retention obtained in tilapia fed with organic selenium supplementation of 3 mg/kg of feed, and the lowest selenium 6 mg/kg of feed.

The value of feed efficiency, blood protein, and protein retention is increasing with the presence of organic selenium supplementation in the feed. Protein retention shows the amount of protein stored in the fish body from the protein eaten. The protein retention rate obtained was different for each treatment, i.e. organic selenium supplementation of 3 mg Se/kg of feed at 28% protein content showed the highest protein retention value of 22.53% but decreased with increasing selenium dose up to 6 mg Se/kg. This indicates that the supplementation of organic selenium in the feed can increase the energy utilization of non-proteins i.e. from carbohydrates and fats (protein sparring effect). Increased selenium in supplementation to triggering insulin secretion also triggers an increase in blood glucose followed by glycogenesis that causes the storage of energy reserves in the form of glycogen in the body. Similar results also occurred in tilapia studied by Suprayudi *et al*. (2013). The ability of fish to utilize non-protein energy is thought to be associated with the role of selenium, i.e., ID (Iodotironin deiodinase) role in improving thyroid hormone that will trigger insulin secretion (Brown & Arthur, 2001; Zairin, 2003). However, the efficiency of feed, blood proteins and protein retention decreases with increasing selenium supplementation in feed. This is thought to be caused by excessive levels of selenium in the body of tilapia. Excess levels of selenium can cause pro-oxidants. Pro-oxidants produce free radicals (superoxides, hydroperoxides, and others) that can react with surrounding molecules such as proteins, fats, carbohydrates and DNA. The formation of free radicals will be neutralized by normal endogenous antioxidants, but if excessive it will cause oxidative stress that ultimately leads to local damage, organ dysfunction and death in fish (Hamzah *et al*., 2012a).

Blood glucose levels did not show any significant differences between treatments, either without organic selenium supplementation or supplementation of 3 and 6 mg Se/kg of feed. This suggests that an increase in organic selenium supplementation to a dose of 6 mg Se/kg of feed does not have a different effect on the blood glucose levels of tilapia.

The results of the fish selenium retention analysis positively correlate with the increase in organic selenium supplementation in the feed. The supplementation of organic selenium to 6 mg Se/kg of feed increased body selenium levels compared to selenium supplementation of 3 mg Se/kg of feed or in the absence of selenium supplementation in feed. The high value of Se retention in supplementation of 6 mg/kg of feed indicates that the amount of Se stored in the body of fish more than other treatments, and certainly will affect the rate of growth. Supplementation of Se on the feed must be in accordance with the dose needed by each type of fish, a suboptimal dose of Se can cause physiological irregularities in fish. Overdose of selenium will be toxic which will eventually cause growth abnormalities and even death in fish, while Se deficiency can result in decreased glutathione peroxidase enzyme activity, fish's immune response, causing abnormal growth and low feed efficiency (Hamzah *et al*., 2012b; Suprayudi et al., 2012). These results are associated with several other growth parameters, namely feed efficiency, protein retention and growth rates that show the lowest values as organic selenium supplementation increases in feed. This suggests that high Se retention is not always followed by high growth, as the growth of organisms is influenced by many factors, not just from Se (Suprayudi *et al*., 2012).

One way to diagnose the health status of an organism by looking at changes in hematology as an illustration of the physiological conditions of fish under stress conditions (Osman *et al*., 2010; Erhunmwunse & Ainerua, 2013).

Stress conditions affect various physiological processes such as intermediary metabolism and immune function (Hamzah *et al.*, 2013b) such as suppressing the ability of leukocyte fish to form the immune system (Zairin, 2003).

The number of red blood cells of tilapia in this study ranged from 0.73–1.42×10^6 cells/mm^3. Robert (1978) states that in normal fish, the number of red blood cells ranges from 1.05–3.00×10^6 cells/mm^3. This suggests that feeding with a protein content of 28% without the supplementation of organic selenium produces tilapia with the amount of human resources below the normal range. The number of red blood cells in animals is affected by age, environment, nutritional status, and hypoxia or oxygen deprivation, but also by gender (Witeska, 2013). Several other studies comparing the doses of Se 1, 2, and 4 mg Se/kg of feed showed that, the highest value of red blood cells was in fish treated with 4 mg Se/kg of feed, followed by treatment of 1 mg Se/kg and 2 mg Se/kg of feed and the lowest is in the treatment without supplementation of selenium. The highest hemoglobin value was obtained in the treatment of 1 mg Se, followed by 4 mg Se and 2 mg Se/kg of feed and the lowest in the treatment without supplementation of selenium. While the highest hematocrit value was obtained in the treatment of 2 mg Se/kg of feed followed by treatment of 4 mg Se/kg of feed and 1 kg Se/kg of feed. While the lowest value is in the treatment without supplementation of selenium.

The number of white blood cells ranges from 6.14–8.95×10^4 cells/mm^3. Rastogi (1977) and Affandi and Tang (2002) stated that the number of white blood cells in fish ranged from 2.0×10^4 to 1.5×10^5 cells/mm^3 (20.000–150.000 cells/mm^3) of blood. White blood cell count exceeding normal limits indicates that fish treated with the supplementation of organic selenium to the feed are able to give the fish body's response more actively to the presence of foreign matter (Hamzah *et al.*, 2012b; Suprayudi *et al.*, 2012). This is in accordance with (Suprayudi *et al.*, 2012) which states that the number of white blood cells will increase when supplementation of organic selenium to the feed until a certain dose can increase the fish's immune response. This is related to phagocytosis which is a non-specific fish defense mechanism and is the first step in forming a specific response (Hamzah *et al.*, 2012b; Suprayudi *et al.*, 2012). The number of white blood cells can also decrease when the fish body condition is stressed (Yanto *et al.*, 2015). White blood cells are blood cells that play a role in the immune system, such as the formation of antibodies as the body's response to foreign substances (Purwanti *et al.*, 2014). According to Hastuti and Subandiyono (2011), the variability of fish white blood cell is closely related to environmental factors such as temperature and season, feed input, density and maintenance system, while hemoglobin is related to its role of oxygen binding.

Tilapia hemoglobin content obtained in this study is 3.9–5.27 g%. Wedemeyer & Yasutake (1977) states that normal hemoglobin levels in tilapia range from 10–11.1 g%. This indicates that the hemoglobin level in the study was below the normal Hb level range in tilapia. Low levels of Hb is suspected because the fish experience stress. Levels of hemotocrit obtained in this study ranged from 13.13–15.7%. The results of this study indicate that the hematocrit level in the tested tilapia is still within the normal range of hematocrit in fish is 5–60% (Anderson & Siwicki, 1993).

CONCLUSION

Supplementation of organic selenium in feed, can increase the growth rate of tilapia *Oreochromis niloticus*. Excessive supplementation can decrease the growth performance, therefore based on this study recommended proper selenium organic supplementation of 3 mg/kg of feed at protein content 28%.

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