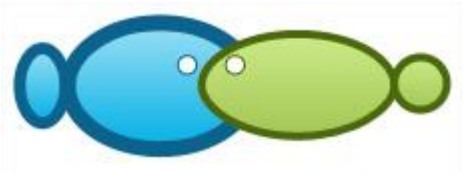

Dietary protein levels affected on the growth and body composition of tilapia (*Oreochromis niloticus*)

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Abstract. The experiment was carried out for 40 days in order to determine the effect of dietary protein levels on growth performance and body composition of tilapia (*Oreochromis niloticus*). Four isolipidic test diets, containing protein concentrations of 28, 32, 36 and 40%, each one with three replications, were used. Fifty fish with the average body weight of 5.0±0.5 g were reared in a 120 L aquarium. The specimens were fed three times daily. The results showed that the best *O. niloticus* growth performance was gained on the diet with the protein level of 40.06% (diet 4), with the weight gain (WG) and specific growth rate (SGR) values of 388.05±3.50% and 3.90±0.21% day^{-1}, respectively. The WG and SGR values corresponding to the consumed diets containing protein at concentrations of 40.06% (diet 4) and 36.05% (diet 3) were not significantly different ($p>0.05$). Significant differences were shown for the body composition, including crude protein and amino acids profiles, as the effects of different dietary protein levels. The WG and SGR polynomial regression analysis showed that dietary protein requirements for the *O. niloticus* were 37.85 and 37.43%, while the dietary lipid level was 10.05%.
Key Words: growth performance, body composition, dietary protein requirements, isolipidic test diets.

Introduction. *Oreochromis niloticus* is a typical freshwater fish species and its cultivation was known as one of the food-producing sectors with the capacity to provide nutrition and safe food for humans. The species was one of the most important aquacultural commodities in the world (Kushayadi et al 2020). Data from FAO (2017) showed an increasing trend of its production in the cultivation industries. It is expected that in 2030, *O. niloticus* from cultivation will reach 128 million tons of the global production. The *O. niloticus* production will increase the feed requirements. Thus, better feed formulations have to be developed in order to meet the feeding needs of *O. niloticus* at various stages of growth.

Protein is the main organic material in fish tissue. The protein of about 65 to 75% dry basis was a major constituent of the fish total body weight (Wilson 2002). Dietary protein level plays an important role in the feed formulas. Feed protein is broken down into essential and non-essential amino acids in fish's body (Millamena et al 2002). Therefore, the feed protein was the most important component that affected the fish growth performance, and feed costs (Lee & Kim 2005). Feed quality can be measured from the protein levels of the feed. Low protein level in feeds caused decreasing the fish growth. However, high protein level in feeds could result in a negative effect on the fish growth, due to their conversion into energy, also leading to increased nitrogen excretion into waters (McGoogan & Gatlin 1999; Sá et al 2006). Therefore, optimal utilization of feed protein is a priority in artificial feed formulations, in order to achieve the best fish growth performances at the lowest cost (Tu et al 2015).

Some of the feed nutrition experiments on *O. niloticus* included the role of the microalgae *Nannochloropsis gaditana* in nutrient digestion (Teuling et al 2019), the use of rubber seed oil as a source of lipids in fish feed (Kushayadi et al 2020), the substitution of fish meal with 33% isoprotein-based earthworm flour (Reynaldy et al 2019) and the utilization of *Lemna minor* fermentation in diets (Pinandoyo et al 2019). However, the

optimal feed protein requirement for juvenile tilapia with a weight size of 5 g has not been determined, although protein plays an important role in growth performance and feed costs, therefore the current experiment has a determinant role.

This research was carried out to determine the optimal dietary protein requirements for *O. niloticus* with body weight of 5.0±0.5 g. Four types of diets with different protein contents were applied to evaluate growth performance, proximate composition and amino acid profile of the fish body.

Material and Method

Experiment design. This research was conducted at the Laboratory of the Fish Seeds Center (FSC), Siwarak, Semarang, Central Java, Indonesia, from January to April 2020. The experimental design used was a completely randomized design (CRD). Four levels of dietary protein, namely 28% (diet 1), 32% (diet 2), 36% (diet 3), and 40% (diet 4) were tested on *O. niloticus* (weight 5.0±0.5 g). Each experiment was repeated three times. The experimental fish were cultured in an aquarium container with the dimension of 60×40×50 cm^3. The aquarium was equipped with an aerator and a thermostat. The aerator is used to increase the dissolved oxygen content, while the thermostat is used to regulate water temperature values.

Experiment diets. The ingredients and composition of the test diets for *O. niloticus* are listed in Table 1. Four types of isoenergetic and isolipidic diets (diets 1-4) were formulated with crude protein (CP) levels of 28, 32, 36, and 40%, respectively.

Table 1

Formulation and composition of test diets

Feed ingredients (g 100 g^{-1})	*Diets (Protein %)*			
	Diet 1 (28)	*Diet 2 (32)*	*Diet 3 (36)*	*Diet 4 (40)*
Fish meal	40.50	40.50	40.50	40.50
Casein	0.00	4.50	9.15	11.55
Fish oil	7.15	7.15	7.15	7.15
Wheat powder	11.15	11.15	11.15	11.15
Starch	32.70	27.20	21.65	16.05
Vitamin premix*	1.00	1.00	1.00	1.00
Mineral premix**	2.00	2.00	2.00	2.00
Choline chloride	0.50	0.50	0.50	0.50
$Ca(H_2PO_4)_2$	1.00	1.00	1.00	1.00
Cellulose	1.00	2.00	2.90	6.10
Sodium carboxymethyl cellulose	2.00	2.00	2.00	2.00
Phagostimulant	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Proximate composition (%)				
Ash	7.96	8.05	7.96	7.78
Lipid	10.05	10.05	10.05	10.05
Crude protein (CP)	27.90	32.03	36.05	40.06
Gross energy (kJg^{-1})	15.58	15.55	15.61	15.65

*Vitamin (mg kg^{-1} of feed): VB1 50 mg, VB2 200 mg, VB6 50 mg, VB12 20 mg, folic acid 15 mg, VC (30%) 325 mg, pantothenate 400 mg, inositol 1,500 mg, D-biotin (2%) 5 mg, niacin 750 mg, VA 2.5 mg, VE (50%) 100 mg, VD3 2 mg, VK 20 mg; **Mineral (mg kg^{-1} of feed): $Ca(H_2PO_4)_2$ 1,800 mg, KH2PO4 1,350 mg, NaCl 500 mg, $MgSO_4 \cdot 7H_2O$ 750 mg, $NaH_2PO_4 \cdot 2H_2O$ 650 mg, KI 1.5 mg, $COSO_4 \cdot 6H_2O$ 2.5 mg, $CuSO_4 \cdot 5H_2O$ 15 mg, $ZnSO_4 \cdot 7H_2O$ 350 mg, $FeSO_4 \cdot 7H_2O$ 1,250 mg, $MnSO_4 \cdot 4H_2O$ 80 mg, Na_2SeO_3 6.00 mg.

Fish meal and casein were used as the main source of protein. Fish oil was the main source of lipid. All ingredients were sieved using a 160 µm-mesh size and then mixed using a blender. Micro ingredients consisted of premix vitamins, premix minerals, choline chloride, and calcium carbonate. The micro ingredients were mixed in a plastic container before being added to the macro materials. Fish oil was homogenized before adding to

the mixed ingredients. The final mixture was then transformed in 2 mm diameter pellets, using a meat grinder. Furthermore, the pellet feed was dried in an oven at a temperature of 60°C. Then, the dry pellets were packaged in plastic bags and stored at 0°C.

Experimental fish and feeding management. *O. niloticus* (weight 5.0±0.5 g) was used as experimental fish. A total of six hundred *O. niloticus* were transported from the Center of Fish Seeds (CFS), Siwarak, Semarang. After a 2 weeks acclimatization period, the fish were randomly placed in twelve aquariums of (60×40×50) cm^3 with a density of 50 fish per aquarium. For the acclimatization to feeds, the fish were fed on commercial feed, while test diets were gradually added up until 20, 40, 60, 80, and 100%. The trial fish were fed three times daily by applying the ad satiation method, for 40 days. During the experiment, feed consumption was recorded and fish feces were removed from the aquarium every day. Temperature was maintained at 28.92±2.55°C, with dissolved oxygen >3 mg L^{-1} and ammonia nitrogen levels <0.05 mg L^{-1}.

Sample collection and analysis. At the end of the experiment, the fish was fasted for 24 hours. Then, all fish in the aquarium were anesthetized, counted and weighed. The results were used to calculate the growth performance and feed utilization, including the survival rate (SR), body weight gain (WG), feed conversion ratio (FCR), and specific growth rate (SGR).

The *O. niloticus* body composition was measured by the AOAC (1990) method. Six fish samples in each treatment (n=24) were collected and stored at 0°C for measurement of its body composition. The fish and diet samples were dried at 100°C for determination of their water content. The concentration of crude protein and body lipids were detected using the Kjeldahl and the Soxhlet method, respectively. The ash concentration was measured by burning the sample in a muffle furnace at 550°C. The gross energy level of the four diets was measured using a calorimeter bomb. The three other fish at each treatment (n=12) were taken as samples for the determination of the body's amino acid profiles, using the L-8900 amino acid analyzer (Hitachi, Japan) (Unnikrishnan & Paulraj 2010).

Calculation of variables and analysis statistics. The response variables were calculated as follows (Subandiyono & Hastuti 2016):

$$\text{Weight gain (WG \%)} = 100 \times (W_t - W_0) \times W_0^{-1}$$
$$\text{Specific growth rate (SGR \% day}^{-1}\text{)} = 100 \times (\text{Ln } W_t - \text{Ln } W_0) \times T^{-1}$$
$$\text{Feed conversion ratio (FCR)} = \text{Feed consumption (g)} \times \text{weight gain}^{-1}\text{ (g)}$$

Where:
W_t and W_0 - the final fish weight and initial fish weight;
T - the time (days) of fish maintenance, which is 40 days.

The results of the calculated variables were presented as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's test with SPSS 22.0 software. The significance test was performed with a probability level of $p<0.05$. Furthermore, WG and SGR values were calculated using a polynomial regression model (Robbins et al 1979) to estimate the optimal dietary protein requirements.

Results

Growth performance. After 40 days feeding period, the growth performances of *O. niloticus* except for the survival rates (SR), were affected by the level of protein in the diet ($p>0.05$). The SR values ranged between 91.69 to 95.18% (Table 2).

Table 2

Growth performance of *Oreochromis niloticus* fed on different dietary protein levels

Growth performance	*Dietary protein levels*			
	Diet 1 (28%)	*Diet 2 (32%)*	*Diet 3 (36%)*	*Diet 4 (40%)*
Initial weight (g)	5.02±0.17	5.25±0.14	5.42±0.62	5.48±0.59
Final weight (g)	18.90±1.37^{c}	23.40±1.71^{b}	25.52±2.16^{a}	26.10±1.57^{a}
Survival rate (%)	91.69±3.40^{a}	95.18±3.30^{a}	94.07±4.46^{a}	94.96±5.58^{a}
FCR	1.39±022^{a}	1.25±0.31^{b}	1. 15±0.87^{b}	1.20±0.58^{b}
WG (%)	276.49±7.53^{c}	345.70±4.20^{b}	370.85±18.20^{a}	388.05±3.50^{a}
SGR (% day^{-1})	3.31±1.26^{c}	3.74±0.27^{b}	3.87±0.32^{a}	3.90±0.21^{a}

Data were expressed as mean±SD of three replications. Values in the same line with different superscripts are significantly different ($p<0.05$).

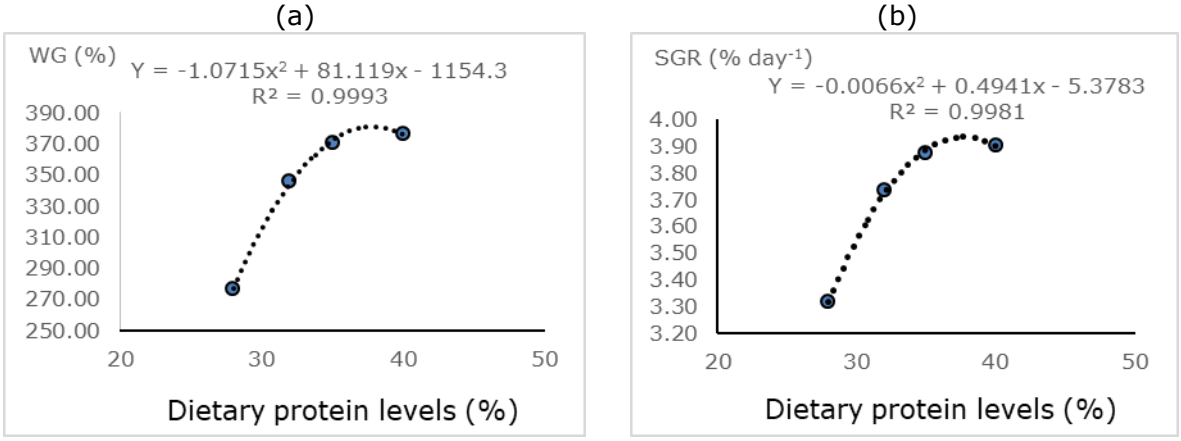


Figure 1. Effects of dietary protein levels on the body weight gain (WG, a) and specific growth rate (SGR, b) of *Oreochromis niloticus.*

Significant increase in WG and SGR were observed with an increase in dietary protein from 27.90% day^{-1} (diet 1) to 40.06% day^{-1} (diet 4). The best growth performance, namely the highest WG (388.05±3.50%) and SGR (3.90±0.21% day^{-1}) values were found on the experiment diet 4 (i.e. protein level of 40.06%), although not significantly different from the experimental values of diet 3. Second order polynomial regression curves of the WG and SGR in relation to the dietary protein levels followed the equations from Figure 1a and Figure 1b. Therefore, the optimal dietary protein levels calculated from the equations were 37.85% and 37.43% day^{-1}, respectively, with the feed lipidic level being 10.05%. The highest FCR value was observed in the experimental diet 1, which was significantly different from diets 2, 3, and 4 (Table 2).

Body composition and amino acid profiles. The body's lipid content seems to decrease with the increase of the dietary protein levels ($p<0.05$). Conversely, the body's protein content seems to increase, under the same conditions (Table 3). The highest ash content was found in the *O. niloticus* that were fed on test diet containing protein of 40.06% (diet 4). However, it was significantly different from the other test diets containing protein levels of 27.90, 32.23 and 35.95% (diets 1-3) ($p<0.05$). The ash content of *O. niloticus* fed on the diet 1, 2 and 3 were similar ($p>0.05$). Water contents of the *O. niloticus*'s body was not affected by the dietary protein levels ($p>0.05$).

Both glutamine and aspartate were the most concentrated amino acids present in the fish tissues (Figure 2, Table 4). The lysine content in the fish tissues was also high. Similar results were found in the Mozambique *O. niloticus* (Herawati at el 2019). Generally, the body's amino acid patterns of the *O. niloticus* were not affected by the concentration of dietary protein (Figure 2). However, dietary protein affected the amino acid profiles, except for the glycine and proline (Table 4).

Table 3

Effects of dietary protein levels on the *Oreochromis niloticus* body composition (% dry basis)

Body composition (%)	*Dietary protein levels*			
	Diet 1 (28%)	*Diet 2 (32%)*	*Diet 3 (36%)*	*Diet 4 (40%)*
Moisture	70.35±0.34^{a}	70.56±0.34^{a}	71.27±0.83^{a}	70.56±0.48^{a}
Crude protein	51.60±0.44^{d}	53.09±0.99^{c}	55.55±0.56^{b}	58.90±2.28^{a}
Lipid	26.58±1.05^{a}	27.82±1.26^{a}	24.33±0.97^{b}	19.33±1.32^{c}
Ash	9.75±0.37^{a}	9.41±0.88^{a}	9.95±0.59^{a}	10.87±0.99^{a}

Data are expressed as mean±SD of three replications. Values in the same row as different superscripts are significantly different ($p<0.05$).

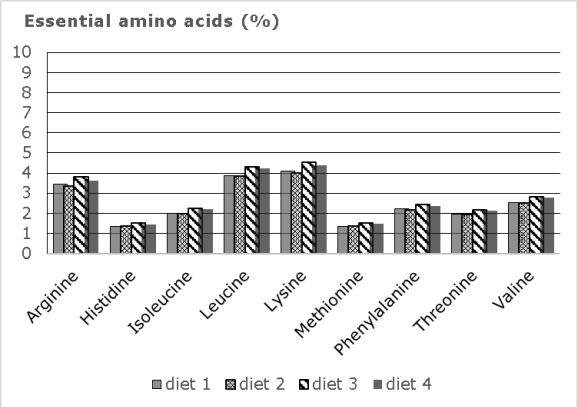
Essential and non-essential amino acids have been examined in the body of the experimental fish. The amino acid contents were significantly changed, except for the proline and glycine (Table 4). In addition, the total amino acids (TAA) and essential amino acids (EAA) were also affected ($p<0.05$). Dietary protein levels have no effect on the EAA/NEAA ratio ($p>0.05$) of essential to non-essential (NEAA) amino-acids. The *O. niloticus* that consumed a diet containing 36.05 and 40.06% of protein (diet 3 and diet 4) resulted in significantly higher EAA and NEAA compared to those of the *O. niloticus* fed on the other diets (diet 1, 2), while EAA/NEAA and EAA/TAA ratios of the *O. niloticus* fed on experimental diets 1, 2, 3 and 4 remained similar ($p>0.05$).

Table 4

Effect of dietary protein levels on the body's amino acid profiles of *Oreochromis niloticus*

Essential amino acids (%)	*Dietary protein levels*			
	Diet 1 (28%)	*Diet 2 (32%)*	*Diet 3 (36%)*	*Diet 4 (40%)*
Arginine	3.44±0.09^{b}	3.36±0.13^{b}	3.80±0.19^{a}	3.62±0.10^{a}
Histidine	1.36±0.08^{b}	1.37±0.06^{b}	1.51±0.11^{a}	1.43±0.05^{ab}
Isoleucine	2.01±0.10^{b}	1.99±0.08^{b}	2.24±0.06^{a}	2.22±0.07^{a}
Leucine	3.86±0.13^{b}	3.83±0.13^{b}	4.31±0.14^{a}	4.22±0.12^{a}
Lysine	4.11±0.12^{b}	4.01±0.15^{b}	4.53±0.13^{a}	4.39±0.11^{c}
Methionine	1.35±0.05^{b}	1.35±0.04^{b}	1.52±0.08^{a}	1.49±0.05^{ab}
Phenylalanine	2.21±0.10^{b}	2.18±0.07^{b}	2.44±0.05^{a}	2.37±0.08^{a}
Threonine	1.96±0.05^{b}	1.93±0.06^{b}	2.17±0.08^{a}	2.13±0.07^{a}
Valine	2.51±0.09^{b}	2.51±0.10^{b}	2.82±0.08^{a}	2.77±0.09^{a}
Non-essential amino acids (%)				
Alanine	3.95±0.08^{b}	3.84±0.19^{b}	4.25±0.15^{a}	4.06±0.11^{a}
Aspartate	5.06±0.13^{b}	4.95±0.18^{b}	5.51±0.14^{a}	5.46±0.18^{a}
Cysteine	0.18±0.02^{a}	0.16±0.01^{a}	0.19±0.03^{a}	0.19±0.03^{a}
Glutamine	8.21±0.34^{b}	7.94±0.36^{b}	8.87±0.26^{a}	8.78±0.26^{a}
Glycine	5.11±0.05^{a}	5.03±0.44^{a}	5.55±0.24^{a}	5.29±0.12^{a}
Proline	2.85±0.27^{a}	2.80±0.19^{a}	2.97±0.21^{a}	2.85±0.28^{a}
Total amino acids (TAA)	52.03±1.39^{b}	51.27±2.05^{b}	57.14±2.11^{a}	55.58±1.30^{a}
Essential amino acids (EAA)	22.81±0.76^{b}	22.53±0.67^{b}	25.34±0.91^{a}	24.64±0.74^{a}
Non-essential amino acids (NEAA)	29.22±1.12^{ab}	28.74±0.71^{b}	31.80±1.03^{a}	30.94±0.74^{a}
EAA/TAA ratio (%)	43.84±0.47^{a}	43.94±0.80^{a}	44.35±0.13^{a}	44.33±0.40^{a}
EAA/NEAA ratio (%)	78.06±1.02^{a}	78.39±1.21^{a}	79.69±1.23^{a}	79.64±1.04^{a}

Data are expressed as mean±SD of three replications. Values in the same row as different superscripts are significantly different ($p<0.05$).



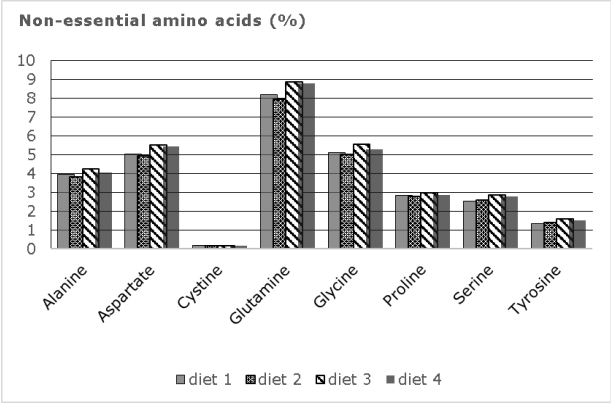


Figure 2. The body's amino acid patterns of *Oreochromis niloticus*.

Discussion. The results of this study indicated that dietary protein levels significantly affected the growth performances of *O. niloticus*. Furthermore, the optimal dietary protein requirement for maximum growth of the *O. niloticus* ranged between 37.85 to 37.43% (Figure 1). These results were lower than that of the optimum dietary protein needed by tropical catfish, *Horabagrus brachysoma* (which was 39.1%) (Giri et al 2011). Basically, dietary protein requirements for the fish decreased with the increase of the fish size. According to Wilson (2002), dietary protein requirement of fish, from juveniles to adults, will decrease with the maturation stage. However, it was reported in *O. niloticus* that the optimal dietary protein requirement was about 30% in the immature gonadal growth stage and about 40% in the reproductive stage (Al Hafedh 1999; El-Sayed et al 2003). Similar results were found in carp, *Cyprinus carpio* (NRC 1993), *Carassius auratus* (Ye et al 2017) and Atlantic cod, *Gadus morhua* (Arnason et al 2010). In another study on goldfish, *C. auratus*, the need for dietary protein at the size of subadults was lower than that of adults or broodstock (Tu et al 2015).

This study found that the FCR values for *O. niloticus* ranged among 1.15 to 1.39 (Table 2), less than the range of 1.2 to 1.8 reported in *O. niloticus* with body weight of 116-117 g (Kushayadi et al 2020) and higher than the values reported by Pinandoyo et al (2019) and Teuling et al (2019). Dietary protein levels have a significant effect on the FCR value ($p<0.05$). An adaptation procedure by gradual changes of the test diet given in this experiment might lead to be better fish survival rates (Table 2). The WG and SGR values increased significantly in *O. niloticus* which was fed with a higher protein content (Table 2). Specimens that consumed a diet containing 28% protein r had lower WG and SGR values and those that consumed a diet containing 32% protein had higher WG and SGR values. The increase continued in dietary protein levels of 36 and 40%. However, the WG and SGR values were not significantly different ($p>0.05$) in fish consuming diets containing 36 and 40% protein. It was thought that a higher amount of protein was converted to energy (Jauncey 1982; Kim et al 1991), explaining the previous observation. The maximum WG and SGR values were obtained in *O. niloticus* which consumed diets containing protein levels ranging from 37.43 to 37.85% (Figure 1). This was thought to be caused by casein in the diet (Table 1). The optimum casein requirement in fish diets was 10% (Subandiyah et al 2014).

The main sources of protein and lipids in the test diets applied were fish meal and fish oil (Table 1), which were known as being rich in n-3 polyunsaturated fatty acids (PUFA). High levels of unsaturated fatty acids n-3 PUFA caused the fish oil sensitiveness to lipid peroxidation (Huang et al 1998; Lin & Huang 2007) and excessive PUFA induces a negative impact on fish growth (Ng et al 2001; Ng et al 2003; Chen et al 2011). Therefore, other lipid sources such as soybean oil can be included for the future fish feed developments to reduce the probability of growth restriction by high levels of PUFA n-3.

The *O. niloticus* body protein content increased significantly by increasing the protein feed levels, while the body fat content decreased, with a peak for diet 2 (Table 3). Similar results were found in mud crabs, *Scylla serrata* (Unnikrishnan & Paulraj 2010) and in white shrimp, *Litopenaeus vannamei* (Hu et al 2008). The results showed that *O. niloticus* prefers using protein than lipid as an energy source, in relation to the

treatments of high dietary proteins. On the other hand, these patterns were shown in crab, *Portunus* sp. (Jin et al 2013) and crabs (Unnikrishnan & Paulraj 2010). Both fish and shrimp protein requirements were determined by their needs for essential amino acids (EAA) and the need for non-essential amino acids (NEAA) (NRC 2011). In the current study, the total amino acid content was significantly affected by the addition of dietary protein levels. Meanwhile, the EAA profile was also changed significantly (Table 4, Figure 2). The increase in total amino acids strongly correlates with the growth performance. This was an indication of better balance of the amino acids in the feeds.

Conclusions. The results of this study indicated that the optimal protein requirement for *O. niloticus* ranged from 37.43 to 37.85%, with a feed lipidic level of 10.05%. Protein content significantly affected the growth performances, nutritional compositions of the fish body and amino acid profiles.

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