Optimum dietary crude protein level for Nile tilapia, *Oreochromis niloticus*, cultured in saline environment

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Abstract. Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured fish globally due to its fast growth, its tolerance to a wide range of environmental conditions and its relatively low cost production process. The study aimed to determine the dietary crude protein level required for growing *O. niloticus*, cultured in a saline environment with salinity levels ranging from 6.14±0.78 g L^{-1} to 6.37±0.31 g L^{-1}. A total of 270 tilapia were then stocked randomly into 24 aquaria tanks with the size of 75 × 40 × 40 cm^3 using a density of 15 fish per tank and fed with a target crude protein level of 28, 30, 32, 34, 36 and 38%. Each group was run with triplicates. Observations were carried out for 56 days, and parameters measured included final body weight (FBW), feed conversion ratio (FCR), thermal growth coefficient (TGC), percentage weight gain (PWG), survival rate (SR), biomass and proximate analysis of the whole body of fish. A completely randomized design was used for the study. All growth rates and feed efficiency had significant differences between treatments, with *O. niloticus* fed 34% CP having higher levels of FBW, TGC, PWG, and biomass compared to other treatments ($P<0.05$). For feed efficiency, the tilapia group fed 32, 34, and 36% had better FCR levels than other dietary treatments ($P=0.0022$). However, SR did not show a significant difference among the dietary treatments ($P=0.7653$). For nutrient deposition in the fish body, *O. niloticus* group fed 34% CP had a higher protein deposition level than other treatments, while the highest level of fat deposition was noticed in *O. niloticus* fed 32% CP. It was concluded that *O. niloticus* cultured in a salinity range from 6.14±0.78 g L^{-1} to 6.37±0.31 g L^{-1} required 34% CP for optimum growth and better protein deposition.
Key Words: crude protein, growth performance, proximate.

Introduction. Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured fish globally due to several advantages: its fast growth, it can be grown under high densities, it feeds on a low trophic level, it tolerates a wide range of salinity, it has a relatively low cost production process, and it is widely accepted as food fish without any restriction by any religion (Arumugam et al 2023; Gu et al 2017; Prabu et al 2019; Stickney 1986; Watanabe et al 2002; Xu & Ming 2018). Its production is a cornerstone of aquaculture, particularly in tropical and subtropical regions (da Silva et al 2021; DeMaeseneer 1984; El-Sayed & Fitzsimmons 2023). Currently, over 135 countries are involved in *O. niloticus* culture with China, Egypt, Indonesia, Brazil, and Thailand as the main producers (Munguti et al 2022;

Sunarto et al 2022). The global export value of *O. niloticus* products is estimated as exceeding USD 2 billion annually (Fitzsimmons 2024), with the United States becoming the main importer, representing 35% of total tilapia imports (El-Sayed & Fitzsimmons 2023). Regarding the production system, *O. niloticus* can be produced using various systems, including open ponds, plastic ponds, concrete tanks, and floating net cages installed in rivers, reservoirs, and lakes (Lebel et al 2015; Mallasen et al 2012; Musa et al 2022; Yi 1999). Alternatively, production can also be carried out in locations with seawater intrusion, considering *O. niloticus*'s wide salinity tolerance character (Aththar et al 2020).

The ability of *O. niloticus* to adapt to saline environments is generally supported by the capacity of the integrated osmoregulatory function of numerous organs, especially the gills, digestive tract, and kidney (Cioni et al 1991). Moreover, the gills are vital structures in osmoregulation and ion exchange processes. Some functional changes, such as the gill and Na^+K^+ATPase activity, were observed during the adaptation of tilapia to saline water (Güner et al 2005). This process involves active transport, which uses adenosine triphosphate (ATP) energy to pump ions against gradients (Verbost et al 1994). Energy metabolism for osmoregulation is highly energy consuming (Tseng & Hwang 2008), and this process will considerably affect the energy needs to support the optimum growth of tilapia. Thus, increases in salinity will likely increase the energy demand of tilapia throughout the culture period.

Studies have been conducted on optimal protein requirements to support tilapia growth in the grow-out phase (Furuya et al 2023; Konnert et al 2022; Van Trung et al 2011). On average, it has been concluded that tilapia kept in freshwater environments require 26–30% protein (Meurer et al 2024). By using saline environment as the media to culture *O. niloticus*, it is necessary to evaluate the effect of the salinity on fish growth. Thus, the aim of this research was to determine the optimum protein level for *O. niloticus* to maintain an optimum growth rate in the juvenile phase in a saline environment. Apart from growth rate, research was also carried out to see the nutritional composition in the fish's body by providing feed with different protein levels. This fundamental study's results certainly have significant benefits for developing *O. niloticus* production in saline environments.

Material and Method

Experimental diets. The experimental feeds were designed to have different protein levels with target crude protein (CP) levels of 28, 30, 32, 34, 36, and 38%. The feed formulation is based on the commonly used formulation for *O. niloticus*. Feed with label of 28% CP was designed using 9.5% poultry by-product meal (PBM), 35.5% soybean meal (SBM), 10% corn distiller's dried grains with solubles (DDGS), and 10% cassava meal (CM) as protein sources. Feed with a crude protein level of 30, 32, 34, 36, and 38% were formulated by increasing the inclusion level of PBM by 12.5, 15, 18, 20.8, and 23.5%, respectively, obtained feed was labeled with 30, 32, 34, 36, and 38% CP. Apart from PBM, all raw materials have been used with the same inclusion level to all experimental feed, except for corn starch, which is reduced due to the addition of PBM into the diet formulation. All dry ingredients were carefully weighed and mixed in a paddle mixer (Marion Mixers, Inc., Marion, IA, USA) in a 100 kg batch, followed by grinding to a particle size of <200 μm using a disk mill (Jinan Shengrun China). Fish oil was then gradually added and mixed constantly. A twin extruder (Jinan Shengrun, China) was used to extrude the feed through a 2 mm die at a temperature gradient of 62, 80, and 110ºC in three zones of the extruder barrel and the die head, respectively. All diets were oven-dried at 50–70ºC in a pulse bed dryer (Jinan Shengrun, China). All finished diets were bagged and stored in a temperature-controlled room until further use. Proximate and amino acid profile of the diets were analyzed at Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia and summarized in Table 2.

Table 1
Formulation of experimental diets (%, as is) used to evaluate the effects of various protein levels to the growth of juvenile *Oreochromis niloticus* cultured in saline environment

Ingredients	*Experimental feed*					
	28% CP	*30% CP*	*32% CP*	*34% CP*	*36% CP*	*38% CP*
Poultry by-product meal	9.50	12.50	15.00	18.00	20.80	23.50
Soybean meal	35.50	35.50	35.50	35.50	35.50	35.50
DDGS-Flint Hills	10.00	10.00	10.00	10.00	10.00	10.00
Cassava meal	10.00	10.00	10.00	10.00	10.00	10.00
Menhaden fish oil	2.00	2.00	2.00	2.00	2.00	2.00
Soy oil	1.00	1.00	1.00	1.00	1.00	1.00
Corn starch	16.90	13.90	11.40	8.40	5.60	2.90
Wheat mids	15.00	15.00	15.00	15.00	15.00	15.00
Mineral premix	2,50	2,50	2,50	2,50	2,50	2,50
Vitamin premix	2,50	2,50	2,50	2,50	2,50	2,50
Rovimix Stay-C 35%	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00

Experimental fish and feeding program. Larvae of *O. niloticus* were obtained from PT. Sinta Prima breeding center (Pasuruan, East Java) and then transported to the nursery facility at the Installation for Marine and Fisheries Field Practices, Jakarta Technical University of Fisheries located in Serang, Banten, Indonesia. The fish were acclimatized to the culture environment and fed with commercial feed for one month until reaching the suitable size. The acclimatized fish (4.00±0.01 g initial mean weight) were then randomly distributed into 18 aquaria tanks with the size of 75 × 40 × 40 cm^3 using density of 15 fish per tank. Three replicate groups of fish were administered different types of experimental diets using nutrition research standard protocol for 56 days and fed by hand four times daily, at 07:00, 11:00, 15:00, and 20:00. The amount of feed given to fish each day during the 56-day experimental period was based on historical data on *O. niloticus* growth and a feed conversion ratio of 1.5. Changes in the feed quantity ratio are then made if mortality occurs, or if there are changes in the water quality conditions of the culture media. Water quality observations are then carried out for physical parameters, which include pH, salinity, temperature, and dissolved oxygen; then chemical parameters, which include ammonium (NH_4) and nitrite nitrogen (NO_2-N).

Growth sampling. At the end of the feeding trial, the fish in each aquaria tank were counted, individually weighed to calculate the final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR), percentage survival (SR), and thermal unit growth coefficient (TGC) as follows:

$$\text{PWG} = \frac{(\text{average individual final weight} - \text{average individual initial weight})}{(\text{average individual initial weight})} \times 100$$

$$\text{FCR} = \frac{\text{feed given (g)}}{\text{alive weigh gain (g)}}$$

$$\text{SR} = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100$$

$$\text{TGC} = \frac{\text{FBW}^{1/3} - \text{IBW}^{1/3}}{\sum \text{TD}} \times 100$$

Where:
FBW - final body weight;
IBW - initial body weight;
T - water temperature (ºC);
D - number of trial days.

Analysis of proximate and amino acid profile of shrimp. At harvest time or on the 56th day of the observation period, twenty fish per treatment, or five shrimp from each aquaria tank, were randomly sampled and stored at -80ºC for body composition analysis. Prior to proximate, energy, and amino acid analyses, dried whole shrimp were rigorously blended and chopped in a mixer according to methods described by Helrich (1990). The proximate composition and amino acid profile of the whole shrimp body were analyzed at the Saraswati Indo Genetech Laboratory (Bogor, West Java, Indonesia).

Statistical analysis. The Shapiro-Wilk test assessed the normality of data distribution, and Brown-Forsythe's tests tested the homogeneity of variance before data analysis. Growth parameters were analyzed using regression and one-way analysis of variance (ANOVA) to determine significant differences among treatments, followed by Tukey's multiple comparison tests to determine the difference between treatment means among the treatments. All statistical analyses were conducted using the SAS system (V9.4. SAS Institute, Cary, NC, USA).

Results. The data in Table 2 shows that the protein level in the feed is determined by the design of the protein target, which is 28.44, 30.58, 32.37, 34.40, 35.99, and 38.29%, respectively. As for fat content, in general, it is the same, except that the fat content for 36% CP and 38% CP is slightly higher than that of other experimental diets, reflected in the energy content of the fat in the feed. For total calories, there is a tendency for the number of calories to increase as the protein level in the feed increases. For amino acid profiles, the levels are generally not significantly different. For threonine, the difference only occurred at CP 38% with a level of 1.27%, while other feeds had the same level, namely 1.24%. For lysine, there was an increasing trend when the protein level was increased, with each lysine level for 28, 30, 32, 34, 36, and 38% CP being at levels 2.04, 2.10, 2.08, 2.09, 2.03 and 2.03% in feed, respectively. Meanwhile, the same protein level sequence for methionine has concentrations of 0.48, 0.49, 0.50, 0.52, 0.53, and 0.52% in feed, respectively.

Table 2

Proximate and amino acid (AA) composition (% as is, dry matter basis) of experimental diets

Parameter	*Unit*	*Nutritional profile of the experimental diet*					
		28% CP	*30% CP*	*32% CP*	*34% CP*	*36% CP*	*38% CP*
Proximate analysis							
Protein content	%	28.44	30.58	32.37	34.40	35.99	38.29
Total fat	%	6.48	6.73	6.82	6.93	7.33	7.16
Ash content	%	8.11	8.70	9.59	9.76	10.35	10.65
Calorie from fat	Kcal 100 g^{-1}	58.28	51.57	52.34	57.83	65.93	65.90
Total calories	Kcal 100 g^{-1}	354.52	363.73	370.50	372.87	375.87	379.34
Moisture content	%	11.36	10.54	12.56	13.43	12.34	12.22
Carbohydrate	%	45.12	45.97	41.17	37.99	36.65	34.70
Amino acid profile							
L-Alanine	%	1.35	1.65	1.64	1.95	1.96	1.98
L-Arginine	%	1.89	2.19	2.23	2.28	2.31	2.39
L-Aspartic acid	%	2.64	3.08	3.11	3.44	3.47	3.54
Glycine	%	1.33	1.43	1.40	1.86	1.87	1.86

Parameter	Unit	Nutritional profile of the experimental diet					
		28% CP	30% CP	32% CP	34% CP	36% CP	38% CP
L-Glutamic acid	%	5.20	6.32	6.89	6.98	6.93	6.97
L-Histidine	%	0.80	0.91	0.87	0.86	0.95	1.02
L-Isoleucine	%	1.01	1.36	1.38	1.44	1.52	1.60
L-Cystine	%	0.77	0.76	0.83	0.87	0.91	0.92
L-Leucine	%	2.15	2.81	2.17	2.58	2.65	2.69
L-lysine	%	2.04	2.10	2.08	2.09	2.03	2.03
L-Methionine	%	0.48	0.49	0.50	0.52	0.53	0.52
L-Tryptophan	%	0.36	0.38	0.36	0.39	0.39	0.39
L-Valine	%	1.14	1.50	1.49	1.54	1.53	1.53
L-Phenylalanine	%	1.41	1.75	1.73	1.78	1.86	1.87
L-Proline	%	1.66	2.06	2.12	2.25	2.26	2.31
L-Serine	%	0.99	1.09	1.12	1.33	1.35	1.39
L-Threonine	%	1.22	1.24	1.24	1.24	1.24	1.27
L-Tyrosine	%	0.99	1.14	1.14	1.23	1.35	1.35

Water quality. During the 56 days of the observation period, the level of salinity, dissolved oxygen, temperature, and pH in the morning are in the range of 6.14±0.78 g L^{-1}, 6.85±0.42 mg L^{-1}, 28.61±0.43ºC, and 7.41±0.19, respectively. Meanwhile in the afternoon, the levels of salinity, dissolved oxygen, temperature, and pH were in the range of 6.37±0.31 g L^{-1}, 6.97±0.40 mg L^{-1}, 29.05±0.59ºC, and 7.45±0.21, respectively. For total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2-N) were in the range of 0.154±0.030 and 0.369±0.031 mg L^{-1}, respectively.

Growth performance of fish. Feed with different crude protein (CP) levels had a significant effect to the final body weight, feed conversion ratio, thermal growth coefficient, percentage weight gain, and biomass of tilapia *O. niloticus* cultured in saline condition (P<0.05), but not on the survival rate. For growth parameters, it can be seen that tilapia fed with 34% CP had the most optimum growth rate, including final body weight (Figure 1), feed conversion ratio, thermal growth coefficient, percentage weight gain, and biomass, compared to other dietary treatments (P<0.05). In general, fish had better growth rates as protein levels increased from 28 to 34% CP, but the growth rates decreased when fish were fed with 36 and 38% CP compared to the growth performance of fish fed with 34% CP.

Proximate composition of the whole fish. Table 3 shows the proximate composition of *O. niloticus* at the end of the experiment.

Table 3

Proximate composition of tilapia whole body

Parameter	Unit	Experimental diet					
		28% CP	30% CP	32% CP	34% CP	36% CP	38% CP
Ash content	%	3.70	2.86	1.51	2.80	3.96	2.95
Calorie from fat	Kcal 100 g^{-1}	14.18	21.74	27.69	12.69	14.22	20.75
Total fat	%	1.58	2.42	3.08	1.41	1.58	2.31
Moisture content	%	74.36	73.12	75.99	74.94	75.52	76.20
Total calories	Kcal 100 g^{-1}	95.64	108.18	105.41	96.09	89.98	94.95
Carbohydrate	%	3.94	3.99	1.20	0.31	0.40	0.35
Protein content	%	16.43	17.62	18.23	20.04	18.54	18.20

Higher levels of protein deposited in the fish's body were found in fish fed 34% CP compared to treatments with protein levels in other feeds. However, for total fat, the highest level was in fish fed 32% CP, and the lowest was in fish fed 34% CP. Moisture content is in the same range, while the total energy deposited in the fish body is highest in fish fed at 30% CP, followed by 32 and 34% CP. Fish with the lowest total energy were found in the 36% CP treatment.

Discussion. Many species of *O. niloticus* are euryhaline, but their adaptability to salinity differs (Angadi 2024; Prunet & Bornancin 1989; Suresh & Lin 1992). *O. niloticus* is less tolerant compared to other species, such as Mozambique tilapia *O. mossambicus* (Velan et al 2011). Fish that live in brackish water conditions with fluctuation in salinity are required to regulate water and ions in their body through osmoregulation (Norstog et al 2022), and this process involves active transport of ions across cell membranes and requires energy (Norstog et al 2022; Tseng & Hwang 2008). This energy can mainly be obtained through feed that has a significant nutrient composition, such as proteins, carbohydrates, and lipids as the source of energy (Craig et al 2017; Machiels & Henken 1985; Saravanan et al 2012). These can then be metabolized to produce energy that is needed for several physiological processes and physical activities, including digestion, absorption, growth, reproduction, and other life processes (Craig et al 2017; Gatlin 2010). In terms of energy density, the average caloric values that proteins, carbohydrates, and lipids can provide are approximately 5.65, 4.15, and 9.45 kcal g^{-1}, respectively (Gatlin 2010). However, among these major nutrient groups, protein is the component most widely used for energy reserves by fish (Radhakrishnan et al 2020). Since protein also as the most expensive component in feed formulations compared to fat and carbohydrates, it is important to accurately determine the optimum protein level in feed (Ahmad et al 2004; Craig et al 2017).

Feeding with protein levels that cannot meet the specific nutritional and energy requirements will negatively impact the growth of aquatic organisms (Abdel-Tawwab et al 2010; Ali & Rawal 2022; Prabu et al 2020). Providing excessive amounts of protein will only increase the energy needed to metabolize protein into amino acids (Halver & Hardy 2003), and increased ammonium and unionized ammonia level in the culture environment (Abdel-Tawwab et al 2010; Lloyd et al 1978). Research conducted by Abdel-Tawwab et al (2010) showed an optimum growth of tilapia (initial body weight of 17–22 g) fed with 35% CP diet compared to 45% CP diet. Similarly in largemouth bass, fish tended to have a decreasing trend in daily weight gain when fed with 54% protein compared to 43.5% protein in the feed formulation (Portz et al 2001). Results of the current study also showed that the growth performance of *O. niloticus* decreased as dietary crude protein increased. In this study, culturing tilapia in salinity ranged from 6.14±0.78 g L^{-1} in the morning and 6.37±0.31 g L^{-1} in the afternoon required 34% CP in the diet formulation (Figure 1). The requirement on crude protein level is slightly higher compared to the CP level required by *O. niloticus* cultured in the freshwater environment, range from 25 to 30% dietary protein (El-Saidy & Gaber 2005; Hafedh 1999).

The difference in protein requirements for *O. niloticus* kept in a saline environment compared to fresh water can be related to the energy needed for osmotic regulation (Zhu et al 2022). Previous studies concluded that 10 to 50% of the energy consumption will be used for osmotic regulation in fish during the salinity adaptation (Islam et al 2020; Mozanzadeh et al 2021; Singha et al 2021; Wu et al 2021). Thus, appropriate amount of protein and energy in the diet will optimize the growth of tilapia. Regarding feed efficiency, in the present study, the lowest FCR levels were noticed in group of tilapias fed with 32, 34, and 36% CP, followed by 38 and 30% CP. The low FCR in tilapia fed 38% CP may be closely related to low feed intake compared to other feeds. According to Lazo et al (1998) fish fed with high protein diet have a tendency to eating less on a per weight basis, possibly due to the presence of nutrients, especially protein, and energy in feed already meets the needs of *O. niloticus* even though it is consumed less than feed with appropriate or lower level of protein.

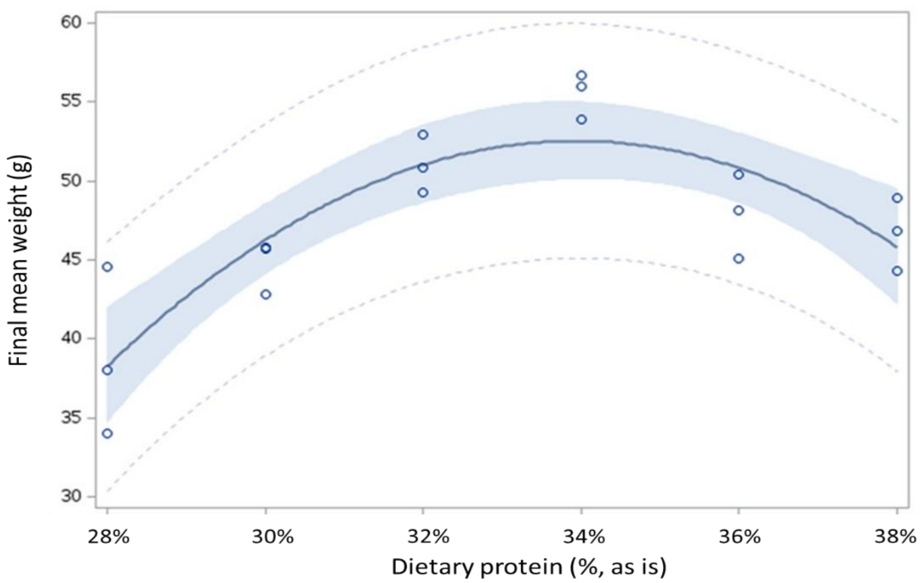

Figure 1. Response between final mean weight and dietary protein level (n=3, P-value=<0.0001, 0.95% confidence interval, R^2 = 0.719651).

Protein and fat deposition are the principal components required to evaluate the quality of fish (Caulton & Bursell 1977). In the present study, whole body protein was higher in fish fed 34% CP compared to other dietary treatment. The balance of the available amino acids in the dietary protein, and also appropriate amount of protein may promote greater protein deposition in fish (Bureau et al 2000). However, higher fat deposition was noticed in fish fed 32% CP, while fish fed 34% CP had the lowest fat deposition. Lower level of fat deposition in the whole body of *O. niloticus* fed with 34% CP could be due to the efficient use of fat as the supplemental energy to support the growth.

Conclusions. Six experimental feeds with different crude protein compositions were used to determine the optimum protein requirements for *O. niloticus* to maintain an optimum growth rate and the nutrient deposition during the juvenile phase in a saline environment, namely: 28, 30, 32, 34, 36, and 38% crude protein levels. In conclusion, the optimal crude protein level for tilapia *O. niloticus* cultured in a saline environment ranged from 6.14±0.78 g L^{-1} to 6.37±0.31 g L^{-1} was 34% CP based on the results of final body weight (FBW) and regression analyses of FBW.

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Conflict of interest. The authors declare that there is no conflict of interest.

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