



Digestible protein requirement of Nile tilapia fingerlings fed fishmeal-free diets based on soybean meal and corn: growth performance, liver histology, and carcass characteristics

[Exigência de proteína digestível de alevinos de tilápia-do-Nilo alimentados com dietas *fishmeal-free* à base de farelo de soja e milho: Desempenho zootécnico, histologia hepática e características de carcaça]

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Abstract: This experiment aimed to determine the digestible protein (DP) requirement of Nile tilapia fingerlings fed fishmeal-free diets based on soybean meal and corn by evaluating growth performance and carcass characteristics. Nile tilapia fingerlings (0.84 ± 0.11 g; $n = 450$) were distributed in a completely randomized design with six treatments consisting of isocaloric diets based on soybean meal and corn containing increasing levels of DP (200, 240, 270, 320, 360, and 390 g kg⁻¹) and 3,000 kcal kg⁻¹ digestible energy, with five replicates per treatment. Dietary DP levels had a quadratic effect ($p < 0.05$) on final weight, weight gain, feed conversion ratio, specific growth rate, standard length, and whole-body protein, moisture, and ash contents. No significant effects ($p > 0.05$) were observed on hepatosomatic index, hepatocyte diameter, or the other carcass traits evaluated (carcass yield, headless carcass yield, clean trunk yield, fillet yield, and whole-body ether extract content). This study demonstrated that fishmeal-free diets based on soybean meal and corn can be used for Nile tilapia fingerlings, and a dietary level of 320.24 g kg⁻¹ DP is recommended to optimize final weight, weight gain, feed conversion ratio, and whole-body protein content.

Keywords: *Oreochromis niloticus*; carcass characteristics; digestible protein; zootechnical performance.

Resumo: O experimento teve como objetivo determinar a exigência de proteína digestível de alevinos de tilápia-do-Nilo alimentados com dietas isentas de farinha de peixe à base de farelo de soja e milho, avaliando o desempenho de crescimento e as características de carcaça. Alevinos de tilápia-do-Nilo ($0,84 \pm 0,11$ g; $n = 450$) foram distribuídos em delineamento inteiramente casualizado com seis tratamentos (dietas isocalóricas à base de farelo de soja e milho contendo níveis crescentes de proteína digestível (PD): 200, 240, 270, 320, 360 e 390 g kg⁻¹; 3.000 kcal kg⁻¹ de energia digestível) e cinco repetições. Os tratamentos tiveram efeito quadrático ($p < 0,05$) sobre o peso final médio, ganho de peso, conversão alimentar, taxa de crescimento específico, comprimento padrão e teores de proteína, umidade e cinzas de corpo inteiro. Não foi observado efeito significativo ($p > 0,05$) sobre o índice hepatossômático, o diâmetro dos hepatócitos e outros atributos de carcaça avaliados (rendimento de carcaça, rendimento de carcaça sem cabeça, rendimento de tronco limpo, rendimento de filé e teor de extrato etéreo de corpo inteiro). Este



estudo demonstrou que dietas sem farinha de peixe à base de farelo de soja e milho podem ser usadas para alevinos de tilápia-do-Nilo e um nível de 320,24 g kg⁻¹ de PD é recomendado para melhorar o peso final, ganho de peso, conversão alimentar e teor de proteína de corpo inteiro.

Palavras-chave: características de carcaça; desempenho zootécnico; *Oreochromis niloticus*; proteína digestível.

1. Introduction

Aquaculture has taken on a strategic role in the production of food of animal origin, especially in the face of increasing global demand for protein and the need for more sustainable production systems. In this context, Nile tilapia (*Oreochromis niloticus*) stands out as one of the main aquaculture species cultivated worldwide ⁽¹⁾. Brazil is among the world's leading producers of tilapia, with national production reaching 707,495 tons in 2025. Paraná remained the main producing state, accounting for approximately 38 % of Brazilian production of the species ⁽²⁾.

The intensification of aquaculture production has increased the need for nutritional strategies capable of reconciling zootechnical performance, cost reduction, and lower environmental impact. In this scenario, protein occupies a central position because it is the main nutrient related to fish growth and one of the most expensive components in feed formulation ^(3–5). When supplied below the requirement, protein can limit growth, body protein deposition, and feed efficiency; on the other hand, when supplied in excess, surplus amino acids can be catabolized, increasing nitrogen losses in the aquatic environment ^(6, 7).

The determination of protein requirement must be understood within a contemporary approach to precision nutrition and aquafeed sustainability, in which the objective is not simply to increase the protein content of the diet but to adjust the supply of digestible protein (DP), amino acids, and energy according to the stage of development, cultivation system, ingredient composition, and physiological capacity of the fish to utilize nutrients ^(4, 6, 8, 9). This perspective is aligned with the concept of Aquafeed 3.0, based on the formulation of more resilient and efficient diets integrated with the circular bioeconomy, with greater efficiency in resource utilization, reduced dependence on marine ingredients, minimization of nutritional waste, and lower environmental impact of aquaculture production ^(10–13). In Nile tilapia diets, this focus is particularly relevant because the adequate balance among protein, energy, and amino acids contributes to greater nutrient utilization efficiency and lower potential environmental impact ^(6, 7, 14).

The formulation of diets based on digestible nutrients is particularly relevant for species such as Nile tilapia because the nutritional requirement is not related to crude protein (CP) itself, but rather to the fraction of amino acids effectively available for absorption, metabolism, and tissue deposition. Ingredient digestibility, the protein ratio, and the balance between essential and non-essential amino acids are determining factors for the efficiency of dietary protein utilization ^(4, 8, 15).

Another important aspect of modern aquaculture is reducing dependence on ingredients of animal origin, especially fishmeal, because of its limited availability, high cost, competition with other productive sectors, and pressure on marine fisheries resources ^(11, 17). Although this ingredient has high nutritional value, high digestibility, good palatability, and a favorable amino acid profile, these limitations have stimulated the development of fishmeal-free diets based on plant ingredients and other alternative ingredients ^(16–19).

Recent studies show that the protein requirement of Nile tilapia can vary according to body weight, stage of development, lineage, cultivation system, ingredient quality, amino acid profile, and the protein ratio of the diet ⁽⁴⁾. The fingerling phase, in turn, constitutes a fundamental stage in the production chain because it directly influences batch uniformity, performance in subsequent

phases, and the efficiency of production systems ⁽²⁰⁾. During this phase, the high growth rate and intense tissue deposition make it necessary to supply adequate levels of protein to achieve optimal performance and productivity.

Thus, the objective of this study was to determine the DP requirement of Nile tilapia fingerlings fed fishmeal-free diets based on soybean meal and corn through the evaluation of zootechnical performance and carcass characteristics.

2. Material and methods

This animal study followed the Ethical Principles of Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Paraná (Protocol No. E11/2010 – CEUA/Palotina-UFPR).

The experiment was conducted for 30 days. A total of 450 Nile tilapia (*O. niloticus*) fingerlings of the GIFT strain, sexually reverted (0.84 ± 0.11 g; 3.73 ± 0.17 cm), were used. The fish were distributed in a completely randomized design among six treatments with five replicates.

Each experimental unit consisted of a plastic box (60 L) containing 15 fingerlings (66 fish m^{-2} and 55 g m^{-2}), with individual water inflow adjusted daily (5 L min^{-1}). The water outlet was directed to biofiltration tanks (two 500-L tanks in series). A third 500-L tank received the filtered water, which was heated by an electric resistance heater (3000 W) controlled by a thermostat and pumped back to the experimental units by means of two pumps (0.5 HP each).

Aeration was provided using a BOYU electric aerator (model ACQ-009; 105 W and 160 L min^{-1}), with oxygen distributed to all experimental units through silicone hoses and porous stones. Daily management consisted of three ad libitum feedings (07:00, 13:00, and 18:00) and two siphonings (10:00 and 18:30) corresponding to 10 % of the water volume, to remove possible residues from the bottom of the experimental boxes. To maintain hardness and alkalinity, 2 g of CaCO_3 and 12 g of NaHCO_3 were added for every 100 L of replenished water, when necessary. Oxygen and temperature were measured twice daily (09:30 and 17:30), before siphoning, using an LT LUTRON meter (model DO 5519).

Once a week, a water sample (500 mL) was collected from each experimental unit, before feeding and siphoning, to measure water quality variables including ammonia concentration ⁽²¹⁾, by means of photonic UV-VIS BEL spectrophotometry (model SP 2000 UV), nitrite ⁽²²⁾, and alkalinity and hardness ⁽²³⁾. The pH was measured using an MS TECNOPON meter (model mPA210).

The mean temperature values were $26.9^\circ\text{C} \pm 0.4^\circ\text{C}$ in the morning and $28.1^\circ\text{C} \pm 0.3^\circ\text{C}$ in the afternoon, while the dissolved oxygen concentration was $6.46 \pm 0.05 \text{ mg L}^{-1}$. The pH was 8.1 ± 0.01 , hardness was $50.1 \pm 0.5 \text{ mg L}^{-1}$ as CaCO_3 , alkalinity was $100.7 \pm 0.5 \text{ mg L}^{-1}$, nitrite was $0.0018 \pm 0.0 \text{ mg}$, and ammonia was $0.21 \pm 0.0 \text{ mg L}^{-1}$. All these values were within acceptable ranges for Nile tilapia culture and were consistent with those reported for recirculating systems ^(24, 25).

Six isocaloric diets with increasing levels of DP (200, 240, 270, 320, 360, and 390 g kg^{-1} feed) were formulated using soybean meal and corn as the primary ingredients (Table 1). For feed manufacture, the corn and soybean meal were ground and sieved (0.5 mm). The ingredients were weighed according to each formulation, mixed until homogeneous, and moistened with water at 75°C until a dough was formed. The dough was processed in an experimental pellet mill, and the pellets were dried in a forced-ventilation oven for 18 h at 40°C . The diets were produced in pelleted form, with pellet diameters of 1 mm during the first and second weeks and 2 mm during

the third to fifth weeks, to better accommodate the fingerlings' mouth size. A sample of each diet was collected and sent to Evonik Degussa Brasil Ltda. for amino acid quantification. The diets were stored in plastic bags and kept refrigerated until use.

Table 1. Experimental fishmeal-free diets with increasing levels of digestible protein for reverted Nile tilapia fingerlings.

Ingredients	Dietary digestible protein level (g kg ⁻¹)*					
	200	240	270	320	360	390
Soybean meal	350.96	470.00	585.30	699.42	815.14	930.72
Ground corn	554.94	453.76	344.47	235.51	123.85	14.24
Soybean oil	32.99	32.48	28.45	24.36	23.35	19.33
Dicalcium phosphate	35.99	33.49	31.50	29.43	25.38	25.43
Vit. and min. suppl. ¹	5.08	5.07	5.08	5.07	5.08	5.09
Salt	5.05	5.07	5.08	5.07	5.08	5.09
BHT	0.10	0.10	0.10	0.10	0.10	0.10
Calcitic limestone	0.00	0.00	1.01	1.01	2.03	2.03
Nutrients (g kg ⁻¹)						
Linoleic acid	31	28	26	22	19	17
Starch	393	341	289	237	185	132
Calcium	10	10	10	10	10	10
Ash	68	70	72	74	76	78
DE (kcal kg ⁻¹)*	3.000	3.000	3.000	3.000	3.000	3.000
GE (kcal kg ⁻¹)	3.963	4.001	4.039	4.077	4.114	4.152
Crude fiber	36	33	30	27	24	21
Phosphorus	10	10	10	10	10	10
Fat	53	48	44	40	36	32
Dry matter	897	899	899	900	901	902
Crude protein	217	261	305	349	393	437
Amino acids (g kg ⁻¹)						
Arginine	14.4	17.9	21.3	24.8	28.2	31.7
Cysteine	3.5	4.1	4.7	5.2	5.8	6.4
Phenylalanine	11	13.2	15.4	17.7	19.9	22.2
Histidine	5.8	6.9	8.0	9.2	10.3	11.4
Isoleucine	9.3	11.4	13.5	15.6	17.7	19.8
Leucine	19	21.8	24.6	27.5	30.3	33.1
Lysine	11.3	14.2	17.1	20.1	23.0	25.9
Met + Cis	7.0	7.9	9.0	10.1	11.2	12.3
Methionine	3.2	3.7	4.2	4.7	5.2	5.7
Threonine	8.3	9.9	11.7	13.4	15.1	16.8
Tryptophan	2.5	3.1	3.8	4.4	5.1	5.7
Valine	10.2	12.2	14.3	16.4	18.4	20.5

¹Vitamin and mineral supplement per kg of diet: pantothenic acid (4,000 mg), biotin (40 mg), copper (2,000 mg), iron (12,500 mg), iodine (200 mg), manganese (7,500 mg), niacin (5,000 mg), selenium (70 mg); vitamin A (1,000,000 IU), vitamin B1 (1,900 mg), vitamin B12 (3,500 mg), vitamin B2 (2,000 mg), vitamin B6 (2,400 mg), vitamin C (50,000 mg), vitamin D3 (500,000 IU), vitamin E (20,000 IU), vitamin K3 (500 mg), zinc (25,000 mg). BHT, butyl hydroxytoluene; DE, digestible energy; GE, gross energy; Met + Cis, methionine + cystine. *Calculated value using the digestibility coefficient determined by Boscolo et al. ⁽²⁶⁾.

At the end of the experimental period, all fingerlings were anesthetized with a 50 mg L⁻¹ clove oil solution ⁽²⁷⁾, weighed, and measured for total and standard length. Subsequently, weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and protein efficiency ratio (PER) were calculated as follows:

$$\text{WG} = \text{final body weight (g)} - \text{initial body weight (g)}$$

$$\text{FCR} = \text{feed consumed (g, dry weight)} / \text{WG (g)}$$

$$\text{SGR} = [(\ln \text{ final body weight} - \ln \text{ initial body weight}) / \text{days}] \times 100$$

$$\text{PER} = \text{WG (g)} / \text{protein intake (g)}$$

In addition, three fingerlings were randomly selected from each experimental unit for the evaluation of body yield (weights of eviscerated fish, eviscerated fish without head, skinless trunks, and fillets). The livers of these fish were removed and weighed on a semi-analytical balance (0.001 g) to calculate the hepatosomatic index. The liver samples were then fixed in a 10 % formalin solution for 8 h and stored in 70 % ethanol for subsequent histological evaluation. Carcass yield (CY), headless carcass yield (HCY), clean trunk yield (CTY), fillet yield (FY), and hepatosomatic index (HSI) were determined as follows:

$$CY = (\text{gutted carcass weight, g} / \text{body weight, g}) \times 100$$

$$HCY = (\text{headless gutted carcass weight, g} / \text{body weight, g}) \times 100$$

$$CTY = (\text{skinless and finless trunk weight, g} / \text{body weight, g}) \times 100$$

$$FY = (\text{skinless fillet weight, g} / \text{body weight, g}) \times 100$$

$$HSI = (\text{liver weight, g} / \text{body weight, g}) \times 100$$

The liver samples underwent progressive dehydration in alcohol solutions (80 %, 90 %, and 100 %), clearing in xylene, and embedding in paraffin. Microtomy was then performed using a disposable blade in an automatic microtome (LEICA model RM-2155), producing 5- μm histological sections that were stained with hematoxylin and eosin. Photographic documentation was performed using a Zeiss Primo Star optical microscope with a 40 $\times$ objective and a computerized imaging system (Image Pro Plus, version 5.2; Media Cybernetics).

The whole carcasses of the remaining fish were placed in plastic bags for subsequent processing and analysis of CP, fat, dry matter, and ash. Moisture content was determined by the direct method, in which fish from each treatment were ground, the material was homogenized, and samples were placed in an oven at 105°C for 24 h. Ash content was determined by incinerating the samples in a muffle furnace at 600°C for 4 h. The nitrogen fraction of each sample was determined using sulfuric acid digestion followed by ammonia distillation in a Kjeldahl apparatus, with quantification by titration in hydrochloric acid solution. Ether extract was determined by ether extraction for 16 h. All bromatological procedures were performed according to AOAC methods ⁽²⁸⁾.

The zootechnical and bromatological data obtained were subjected to one-way analysis of variance (ANOVA). Before ANOVA, the assumptions of residual normality and homogeneity of variances were evaluated using the Shapiro–Wilk and Bartlett tests, respectively. When significant differences were detected ($p < 0.05$), means were compared using Tukey's test. In addition, because DP levels represented a quantitative factor, quadratic regression analysis was performed to estimate the DP requirement. Statistical analyses were performed using RStudio ⁽²⁹⁾, with the ExpDes package ⁽³⁰⁾ for hypothesis and post hoc tests and the dplyr ⁽³¹⁾, ggplot2 ⁽³²⁾, and minpack.lm ⁽³³⁾ packages for nonlinear regression analysis, in which the maximum or minimum points of the quadratic equation were used to define the DP requirement.

3. Results

The mean values of the growth performance variables of Nile tilapia fingerlings fed fishmeal-free diets with increasing levels of DP are presented in Table 2. DP levels significantly affected all evaluated variables ($p < 0.05$).

Table 2. Growth performance of Nile tilapia fingerlings fed fishmeal-free diets containing increasing levels of digestible protein

Variables	Dietary digestible protein level (g kg ⁻¹)						p-value
	200	240	270	320	360	390	
FW (g)	5.09 ± 0.54 ^a	7.64 ± 0.62 ^b	7.97 ± 1.28 ^b	8.16 ± 0.38 ^b	7.41 ± 0.52 ^b	7.32 ± 1.61 ^b	3.66 × 10 ⁻⁴
WG (g)	4.15 ± 0.52 ^a	6.72 ± 0.62 ^b	7.06 ± 1.29 ^b	7.21 ± 0.39 ^b	6.23 ± 0.53 ^b	6.38 ± 1.59 ^b	3.54 × 10 ⁻⁴
FCR	2.47 ± 0.27 ^a	1.25 ± 0.11 ^b	1.50 ± 0.2 ^b	1.45 ± 0.2 ^b	1.71 ± 0.6 ^b	1.53 ± 0.3 ^b	8.20 × 10 ⁻⁴
SGR (% day ⁻¹)	5.63 ± 0.31 ^a	6.99 ± 0.30 ^b	7.13 ± 0.51 ^b	7.18 ± 0.18 ^b	6.81 ± 0.27 ^b	6.78 ± 0.64 ^b	2.7 × 10 ⁻⁵
PER	1.82 ± 0.13 ^{bc}	3.07 ± 0.33 ^a	2.21 ± 0.33 ^b	1.97 ± 0.28 ^{bc}	1.6 ± 0.45 ^{bc}	1.49 ± 0.39 ^c	1.2 × 10 ⁻⁵
TL (cm)	6.6 ± 0.17 ^a	7.66 ± 0.23 ^{ab}	7.51 ± 0.27 ^{ab}	7.68 ± 0.05 ^a	7.51 ± 0.31 ^b	7.35 ± 0.42 ^b	7.37 × 10 ⁻⁷
SL (cm)	5.30 ± 0.13 ^c	6.20 ± 0.25 ^{ab}	6.21 ± 0.24 ^{ab}	6.33 ± 0.011 ^a	6.21 ± 0.26 ^{ab}	6.02 ± 0.24 ^b	0.001

Data are expressed as mean ± standard deviation. Values in the same row with different superscripts are significantly different ($p < 0.05$). FW, final body weight ($y = -0.000231x^2 + 0.1441x - 14.154$, $R^2 = 0.84$) WG, weight gain ($y = -0.000231x^2 + 0.1439x - 15.067$, $R^2 = 0.84$) FCR, feed conversion ratio ($0.000072x^2 - 0.0452x + 8.388$, $R^2 = 0.58$) SGR, specific growth rate ($y = -0.000118x^2 + 0.0739x - 4.229$, $R^2 = 0.85$) PER, protein efficiency ratio ($y = -0.0046x + 3.398$, $R^2 = 0.34$) TL, total length ($y = -0.000081x^2 + 0.0507x - 0.143$, $R^2 = 0.81$) SL, standard length ($y = -0.000077x^2 + 0.0488x - 1.262$, $R^2 = 0.91$).

The mean final weight was highest in fingerlings fed diets containing 320 g kg⁻¹ DP, followed by those fed diets containing 270 and 240 g kg⁻¹ DP. The lowest mean final weight was observed in fish fed the diet containing 200 g kg⁻¹ DP. A quadratic effect was observed ($y = -0.000231x^2 + 0.1441x - 14.154$; $R^2 = 0.84$), with the maximum final weight occurring at 311.7 g kg⁻¹ DP (339.95 g kg⁻¹ CP) in the diet (Figure 1).

Mean WG was also affected quadratically by dietary DP ($y = -0.000231x^2 + 0.1439x - 15.067$; $R^2 = 0.84$), with the maximum point occurring at 311.7 g kg⁻¹ DP (339.94 g kg⁻¹ CP) in the diet. FCR showed a quadratic response to dietary DP concentration ($y = 0.000072x^2 - 0.0452x + 8.388$; $R^2 = 0.58$), with the lowest value estimated at 313.05 g kg⁻¹ DP (341.41 g kg⁻¹ CP) in the diet.

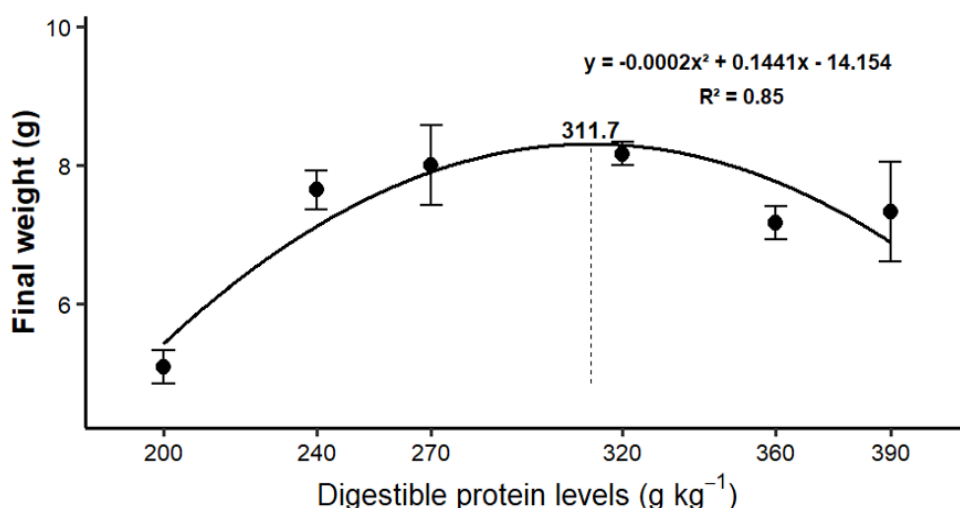


Figure 1. Observed quadratic responses for mean final weight of Nile tilapia (*Oreochromis niloticus*) fingerlings fed fishmeal-free diets containing increasing levels of digestible protein.

The SGR was influenced quadratically by dietary DP level ($y = -0.000118x^2 + 0.0739x - 4.229$; $R^2 = 0.85$), with the maximum point occurring at 313.10 g kg⁻¹ DP (340.65 g kg⁻¹ CP). The highest PER was observed in fish fed the diet containing 240 g kg⁻¹ DP, followed by those fed diets containing 270, 320, and 200 g kg⁻¹ DP, whereas fish fed diets containing 360 and 390 g kg⁻¹ DP showed the lowest values.

DP levels significantly influenced ($p < 0.05$) the total length of Nile tilapia fingerlings, with the greatest lengths observed in fish fed diets containing 240 to 390 g kg⁻¹ DP and the lowest value observed in fish fed the diet containing 200 g kg⁻¹ DP. For standard length, the highest value was found in fish fed the diet containing 320 g kg⁻¹ DP, followed by those fed diets containing 240, 270, and 360 g kg⁻¹ DP, which did not differ from one another. The next highest value was observed in fish fed the diet containing 390 g kg⁻¹ DP, whereas the lowest value was recorded in fish fed the diet containing 200 g kg⁻¹ DP. In addition, a quadratic response was observed ($y = -0.000077x^2 + 0.0488x - 1.262$; $R^2 = 0.91$) for this variable, with the maximum point occurring at 314.6 g kg⁻¹ DP (343.17 g kg⁻¹ CP) in the diet.

Table 3 presents the body yield and histological data of Nile tilapia fingerlings fed fishmeal-free diets containing increasing levels of DP. There was no influence ($p > 0.05$) of the treatments on any of the evaluated variables.

Table 3. Body performance and liver histology of Nile tilapia fingerlings fed fishmeal-free diets containing increasing levels of digestible protein.

Variable	Dietary digestible protein level (g kg ⁻¹)						p-value
	200	240	270	320	360	390	
CY (%)	83.88 ± 2.49	83.65 ± 1.31	85.02 ± 1.42	84.17 ± 0.89	84.00 ± 1.11	79.92 ± 9.45	0.958
HCY (%)	55.65 ± 3.12	55.10 ± 1.46	57.37 ± 1.10	58.62 ± 4.44	56.32 ± 2.18	54.01 ± 13.70	0.886
CTY (%)	45.15 ± 2.64	44.13 ± 2.10	46.25 ± 1.18	45.85 ± 0.66	46.04 ± 1.69	46.51 ± 1.00	0.279
FY (%)	23.00 ± 2.55	22.83 ± 1.38	24.69 ± 2.31	24.60 ± 1.91	24.37 ± 1.66	21.66 ± 5.95	0.544
HIS (%)	1.04 ± 0.34	1.45 ± 0.19	1.13 ± 0.23	0.93 ± 0.21	1.11 ± 0.15	1.35 ± 0.30	0.552
HD (µm)	170.41 ± 25.28	173.08 ± 2.76	171.10 ± 4.01	163.30 ± 14.42	171.60 ± 1.54	181.78 ± 4.24	0.788

Data are expressed as mean ± standard deviation. CY, carcass yield; HCY, headless carcass yield; CTY, clean trunk yield; FY, fillet yield; HIS, hepatosomatic index; HD, hepatocyte diameter (µm).

Table 4 presents the body chemical composition of Nile tilapia fingerlings fed fishmeal-free diets containing increasing levels of DP. Whole-body CP content (Figure 2) was influenced quadratically ($y = -0.0002x^2 + 0.1243x - 5.027$; $R^2 = 0.84$), with the maximum value estimated at 320.24 g kg⁻¹ DP (348.80 g kg⁻¹ CP) in the diet. By contrast, carcass ether extract was not influenced by the treatments ($p > 0.05$).

Table 4. Whole-body chemical composition of Nile tilapia fingerlings fed fishmeal-free diets containing increasing levels of digestible protein.

Variable	Dietary digestible protein level (g kg ⁻¹)					
	200	240	270	320	360	390
CP (%)	12.42 ± 0.49 ^c	12.97 ± 0.49 ^{bc}	14.29 ± 0.58 ^{ab}	15.51 ± 0.39 ^a	14.44 ± 0.08 ^{ab}	13.80 ± 0.11 ^{bc}
EE (%)	1.41 ± 0.27	2.03 ± 0.09	0.72 ± 0.05	0.79 ± 0.04	1.95 ± 0.17	1.88 ± 0.57
Moisture	78.55 ± 0.15 ^b	77.40 ± 0.09 ^b	77.18 ± 0.21 ^c	77.07 ± 0.02 ^c	78.84 ± 0.15 ^b	79.92 ± 0.03 ^a
Ash (%)	1.84 ± 0.03 ^c	1.78 ± 0.09 ^c	2.84 ± 0.04 ^b	3.09 ± 0.12 ^a	2.68 ± 0.01 ^b	1.72 ± 0.06 ^c

Data are expressed as mean ± standard deviation ($n = 3$). Values in the same row with different superscripts are significantly different ($p < 0.05$). CP, crude protein ($y = -0.0002x^2 + 0.1243x - 5.027$, $R^2 = 0.84$); EE, ether extract; Moisture: ($y = -0.000248x^2 - 0.1391x + 96.470$, $R^2 = 0.95$); Ash: ($y = -0.000127x^2 + 0.0773x - 8.826$, $R^2 = 0.68$).

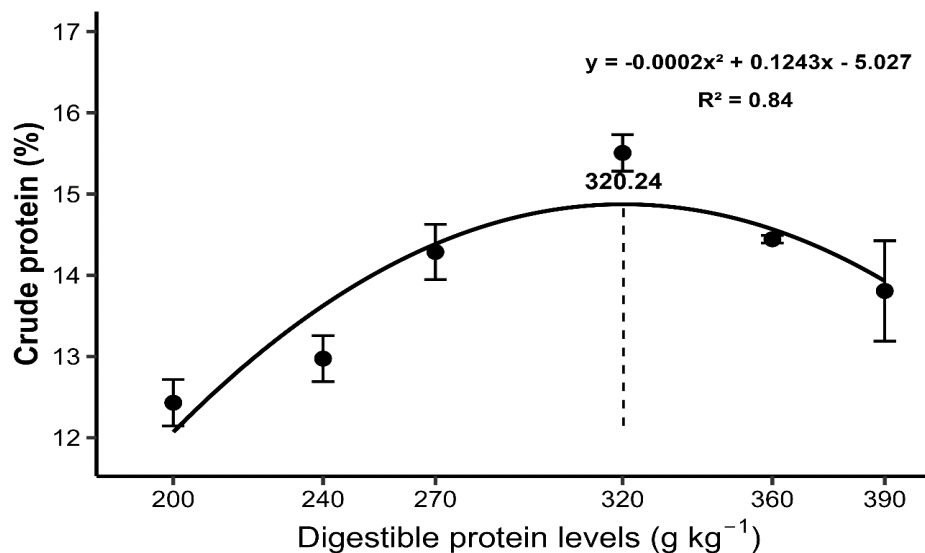


Figure 2. Observed quadratic response of whole-body crude protein content in Nile tilapia (*Oreochromis niloticus*) fingerlings fed fishmeal-free diets containing increasing levels of digestible protein.

Moisture content showed a quadratic response to increasing dietary DP levels ($y = 0.000248x^2 - 0.1391x + 96.470$; $R^2 = 0.95$), with the maximum point estimated at 279.8 g kg⁻¹ DP (304.99 g kg⁻¹ CP) in the diet. A quadratic effect was also observed for ash content ($y = -0.000127x^2 + 0.0773x - 8.826$; $R^2 = 0.68$), with the maximum point estimated at 303.27 g kg⁻¹ DP (330.75 g kg⁻¹ CP) in the diet.

4. Discussion

The quadratic response observed in the different zootechnical parameters indicates that the performance of the fingerlings was likely not determined solely by the linear increase in DP in the diet, but rather by the balance among protein, energy, and amino acid profile^(4, 6, 8). Although the diets were formulated with the same level of digestible energy, the increase in DP simultaneously altered the protein ratio, the ratio of soybean meal to corn, and the concentration of essential amino acids. At levels below the requirement, the limited availability of amino acids can restrict protein synthesis and growth because fish have a physiological requirement for essential amino acids rather than for total dietary protein alone^(6, 34). On the other hand, at levels above the optimum point, surplus amino acids may have been catabolized for energy purposes, reducing the efficiency of protein utilization and contributing to the decline in zootechnical performance^(8, 35). In addition, because of its omnivorous feeding habit, Nile tilapia has the ability to utilize carbohydrates as a non-protein energy source, which can contribute to the protein-sparing effect when the protein ratio is adequately balanced^(8, 36). Thus, the interpretation of the results should not be restricted to the classical determination of a protein requirement, but should also consider the concept of nutrient utilization efficiency, especially within the context of precision nutrition and productive sustainability.

The estimated requirement of 320 g kg⁻¹ DP to maximize final weight, WG, and whole-body protein content is consistent with the high nutritional demand expected during the fingerling phase. At this stage, fish exhibit an intense growth rate and high muscle tissue deposition, which justifies a greater demand for amino acids compared with later phases. Meurer et al.⁽⁴⁾, when evaluating studies on the protein requirement of Nile tilapia in different production phases, highlighted that the requirement is influenced by body weight, developmental stage, cultivation system, ingredient quality, and the protein ratio of the diet.

The DP requirement estimated in the present study based on final weight and WG was higher than the values reported by Durigon et al. ⁽³⁶⁾ and Sgnaulin et al. ⁽³⁷⁾ for Nile tilapia fingerlings grown in a biofloc system. This difference may be associated with the characteristics of the culture system, since the microbial biomass present in the biofloc can act as a complementary food source, providing protein, amino acids, and other nutritional compounds ^(38, 39), thereby improving protein utilization efficiency ⁽⁴⁰⁾.

When expressed as CP, the estimated requirement in the present study, corresponding to approximately 347 g kg⁻¹ CP, is within the range described in the literature for tilapia in early growth stages ⁽⁴⁾, although it differs from values reported in previous studies. This value is higher than that observed by Sweilum et al. ⁽⁴¹⁾, who reported a better response with a diet containing approximately 300 g kg⁻¹ CP, and the value of 330 g kg⁻¹ indicated by Sayed ⁽⁴²⁾ for juvenile Nile tilapia. On the other hand, it is close to the highest values reported for early growth phases, such as those observed by Chen et al. ⁽⁴³⁾, which were close to 350 g kg⁻¹ CP.

The highest SGR was obtained with 313.10 g kg⁻¹ DP (341.47 g kg⁻¹ CP) in the diet, a lower level than that described by Abdel-Tawwab et al. ⁽⁴⁴⁾ for fingerlings weighing 0.5 g, for which a requirement of 450 g kg⁻¹ CP was reported. Fish in early developmental stages tend to have higher protein requirements than larger fish because of their high relative growth rate and intense tissue deposition ^(45, 46). In addition, SGR tends to decrease as development progresses and body weight increases, which may help explain the differences among values estimated in different studies. Differences in initial fish weight, developmental stage, and dietary ingredients may also have contributed to the lower requirement estimated in the present study compared with that reported by Abdel-Tawwab et al. ⁽⁴⁴⁾.

For FCR, the minimum point was estimated at 313.05 g kg⁻¹ DP (341.41 g kg⁻¹ CP) in the diet. This was a higher DP level than that reported by Gonçalves et al. ⁽⁴⁷⁾, who estimated a requirement of 300 g kg⁻¹ DP using the ideal protein concept. Using the same concept and aiming to reduce protein use, Righetti et al. ⁽⁴⁸⁾ found better results with diets containing 230.8 and 258.2 g kg⁻¹ DP for juveniles weighing 100 to 500 g.

Based on the results obtained, it can be inferred that lower DP levels were utilized more efficiently than higher levels. A similar pattern was reported by Quadros et al. ⁽⁴⁹⁾ for Nile tilapia weighing 10.46 ± 0.12 g, in which the PER decreased with increasing dietary DP but increased with the supplementation of digestible amino acids, such as lysine. The PER values were close to those reported for diets containing approximately 300 g kg⁻¹ CP in the studies of Goda et al. ⁽⁵⁰⁾ and Mohanta et al. ⁽⁵¹⁾. The highest PER values were observed at the lowest DP levels, which may be explained by more efficient amino acid utilization and reduced amino acid catabolism.

The parameters of body yield, hepatosomatic index, and hepatocyte diameter were not affected by the treatments. The hepatocytes exhibited a cord-like arrangement, as described by Vicentini et al. ⁽⁵²⁾, and the morphology of the liver tissue was consistent with that described for teleost fish, in which cytoplasmic vacuolization indicates the probable location of lipid and glycogen reserves ⁽⁵³⁾. The absence of significant changes in the hepatosomatic index and hepatocyte diameter suggests that the different DP levels did not induce marked hepatic morphometric changes under the experimental conditions of the present study.

Increasing DP levels resulted in a quadratic response for whole-body protein, moisture, and ash contents. For whole-body protein, the values observed differed from those reported by Zuanon et al. ⁽⁵⁴⁾ but were similar to those reported by Furuya et al. ⁽⁵⁵⁾. Dietary protein level can influence fish body composition because variation in protein concentration in isoenergetic diets may alter the use of protein as an energy source when protein is supplied in excess.

On the other hand, diets containing protein levels below the requirement may not provide sufficient amino acids for protein synthesis ⁽⁵⁶⁾, potentially resulting in greater fat deposition, because excess carbohydrates may be converted into fat when the protein balance is inadequate. However, no significant difference was observed in whole-body fat content, probably because of the high energy demand associated with body growth during this life stage.

Tilapia fingerlings showed lower carcass dry matter values at dietary levels of 240 and 320 g kg⁻¹ DP. At these intermediate DP and CP levels, greater CP deposition in the carcass was also observed, differing from the results reported by Elfeki et al. ⁽⁵⁷⁾. Regarding ash content, the observed response may be related to protein deposition and WG because greater mineral incorporation is required to support tissue growth.

The detailed analysis of protein requirements for Nile tilapia at different developmental stages highlights the complexity of this issue ⁽⁴⁾. Fish have a physiological requirement for essential amino acids rather than dietary protein levels per se; however, amino acid retention follows a pattern similar to that of protein retention in the carcass ⁽⁷⁾. Knowledge of feed amino acid profiles allows greater confidence in diet formulation because amino acid levels may be lower in diets based on plant ingredients such as soybean meal and corn ⁽⁵⁸⁾. The amino acid levels present in the diets were adequate to support maximum WG and were close to those recommended by the NRC ⁽³⁴⁾. The diets used in the present study were formulated using soybean meal and corn as the sole amino acid sources and, according to Rostagno et al. ⁽⁵⁹⁾, the composition of these ingredients shows less variation than that of many animal-derived protein ingredients, allowing a reliable basis for comparison with the literature. In this context, Carneiro et al. ⁽⁶⁰⁾ also highlighted that diets based on soybean meal and corn allow greater predictability in nutritional formulation because of the relatively stable composition of these ingredients, in addition to enabling the production of tilapia feeds free of animal-derived ingredients.

Lysine is an essential amino acid closely associated with protein deposition and fish growth and is commonly used as the reference amino acid for estimating the requirements of other essential amino acids within the ideal protein concept ⁽⁶¹⁾. The optimal levels estimated by quadratic regression for final weight, WG, FCR, and carcass CP were between 313.7 and 320.24 g kg⁻¹ DP in the diet (340 and 348.80 g kg⁻¹ CP), corresponding to lysine levels between 19.5 and 20.1 g kg⁻¹ feed. These values are similar to those reported for juvenile Nile tilapia by Prabhu et al. ⁽⁶²⁾, who determined optimal lysine levels for WG between 18 and 19.5 g kg⁻¹ diet. For diets containing 350 g kg⁻¹ CP, a lysine content of 20 g kg⁻¹ is recommended ⁽⁶⁾.

Methionine is one of the principal limiting sulfur amino acids in soybean meal-based diets ⁽⁶³⁾. The amount of methionine + cystine present in the diet containing 320 g kg⁻¹ DP (349 g kg⁻¹ CP) corresponded to 10.1 g kg⁻¹ feed, a value similar to that reported by He et al. ⁽⁶⁴⁾ for juvenile Nile tilapia. For threonine, recommended values range from 9.9 to 14.3 g kg⁻¹ feed for fingerlings ⁽⁶⁵⁾. In the present study, the threonine level corresponding to the estimated DP requirements for FCR, WG, and final weight (between 311.07 and 311.7 g kg⁻¹ DP) was 13.4 g kg⁻¹ feed.

The results for carcass chemical composition showed that the maximum values were associated with the most favorable amino acid profiles at the different DP levels, supporting the use of these variables in determining protein requirements and identifying diets that maximize actual protein deposition. For CP, the values estimated by quadratic regression (320.24 g kg⁻¹ DP) differed little from the levels estimated using the zootechnical variables. The use of zootechnical parameters alone can make it difficult to estimate actual nutrient retention because WG without bromatological analysis may not accurately reflect protein deposition in the carcass, potentially

leading to misleading conclusions ⁽³⁴⁾. In the present study, the maximum points for final weight and WG occurred at 311.7 g kg⁻¹ DP, whereas the maximum carcass protein content was estimated at 320.24 g kg⁻¹ DP. Taken together, these results suggest that intermediate DP levels promoted WG with greater body protein deposition.

The results obtained in the present study reinforce that higher dietary protein levels do not necessarily result in better performance. The best results were observed at intermediate DP levels, whereas the highest levels did not provide additional gains. This finding is relevant from the perspective of Aquafeed 3.0 because it demonstrates that production efficiency depends on the balance between nutrient supply and the metabolic capacity of the animals. When protein is supplied above the requirement, surplus amino acids tend to be deaminated and used as an energy source, reducing protein retention efficiency and potentially increasing the excretion of nitrogenous compounds into the aquatic environment ⁽⁶⁾.

Although nitrogen excretion was not directly measured in the present study, the absence of productive benefits at higher DP levels suggests that supplying protein above the requirement is not nutritionally efficient. This interpretation is consistent with the concept of rational nitrogen use in aquaculture diets, whereby protein should be supplied in sufficient amounts to support growth and protein deposition without increasing costs and metabolic losses through excessive supplementation. Optimizing Nile tilapia diets may contribute to reducing the environmental impact of production, particularly when protein, energy, and amino acid levels are appropriately balanced ⁽⁷⁾.

The use of fishmeal-free diets based on soybean meal and corn should also be highlighted because replacing fishmeal with plant-based ingredients is one of the key strategies for reducing dependence on marine resources and supporting the sustainable expansion of aquaculture production ^(17, 19). Soybean meal is one of the most widely used plant protein sources in aquaculture feeds because of its availability, relatively stable composition, and good nutritional value, although limitations related to amino acid profile and antinutritional factors remain ^(16, 66). In the present study, the formulation based on soybean meal and corn allowed the evaluation of a practical diet applicable to commercial production conditions, contributing to recommendations that are more representative of production reality. Thus, the results reinforce that plant-based diets, when adequately balanced in terms of DP level and amino acid profile, can sustain the performance of Nile tilapia fingerlings and contribute to more efficient and sustainable aquaculture systems.

5. Conclusion

Based on the results of the present study, fishmeal-free diets based on soybean meal and corn can be used for Nile tilapia fingerlings, and a dietary level of 320.24 g kg⁻¹ DP (348.50 g kg⁻¹ CP) is recommended to optimize final weight, WG, FCR, and whole-body protein content.

Conflict of interest statement

The authors declare no conflicts of interest.

Data availability statement

The data of this study will be available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: Junior, G. B.; Meurer, F.; Formal analysis: Junior, G. B.; Rosa da Silva, L. C.; Funding acquisition: Meurer, F.; Methodology: Meurer, F.; Validation: Junior, G. B.; Novodvorski, J.; Investigation: Junior, G. B.; Resources: Novodvorski, J.; Data curation: Balen, R. E.; Writing – original draft: Junior, G. B.; Writing – review & editing: Balen, R. E.; Visualization: Rosa da Silva, L. C.; Supervision: Meurer, F.; Project administration: Meurer, F.

Declaration of Generative AI Use

The authors did not use generative AI tools or technologies in the creation or editing of any part of this manuscript.

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