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PROTEIN LEVEL REDUCTION IN CORN AND SOYBEAN MEAL-BASED DIETS SUPPLEMENTED WITH ESSENTIAL AMINO ACIDS DURING THE FATTENING PHASE OF NILE TILAPIA

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Abstract

This study evaluated the effect of reducing dietary protein levels with essential amino acid (EAA) supplementation in soybean meal and corn-based diets on the performance of Nile tilapia (*Oreochromis niloticus*) during the fattening phase. A total of 195 GIFT-lineage tilapia (initial weight 297±5.06 g) were randomly assigned to five treatments, with three replicates per treatment. The fish in each treatment were fed for 70 days with a diet containing one of five crude protein (CP) levels (300, 275, 249, 222, or 197 g kg^{-1}). All diets were isoenergetic and isoaminoacidic for EAA. Fish fed higher CP levels (249, 275, and 300 g kg^{-1}) had significantly better performance in final weight (553.66–560.37 g; P = 1.44e−05), weight gain (256.35–260.35 g; P = 6.06e−06), specific growth rate (0.88–0.90; P = 2.68e−05), and feed conversion ratio (1.56–1.58; P = 9.69e−06) compared to fish fed 197 and 222 g kg^{-1} CP. Protein efficiency ratio was highest (2.78; P = 0.0132) with CP level 249 g kg^{-1}, while carcass protein content was significantly higher with CP levels 275 and 300 g kg^{-1} CP (17.35 and 17.08 g 100 g^{-1}, respectively; P = 0.0118). Regression analysis using the broken-line model estimated dietary requirements of 229 g kg^{-1} digestible protein (DP) and 248 g kg^{-1} CP. This study demonstrates that reducing CP from 300 to 248 g kg^{-1} (269 to 228 g kg^{-1} DP), with adequate EAA supplementation in soybean meal and corn-based diets, maintains growth performance in Nile tilapia weighing 300–530 g, whereas further reductions impair performance.

Key words: nutritional requirements, feed formulation, fish meal-free diets, fish nutrition

Nile tilapia (*Oreochromis niloticus*) is widely recognized for its numerous advantages in commercial aquaculture systems. This fish stands out for its remarkable ability to adapt to different cultivation systems, wide tolerance to various water environmental and physicochemical parameters, ease of reproduction in captivity, and acceptance of commercial diets (Engdaw and Geremew, 2024; Mugwanya et al., 2022; Nunes et al., 2024; Tesfaye et al., 2021). Additionally, Nile tilapia has effective physiological and immunological mechanisms to deal with different types of environmental stressors, in both heat and cold stress conditions (Mashhadizadeh et al., 2024; Yilmaz et al., 2021). For these reasons, it has become one of the most commercially important freshwater fish species in the world (Food and Agriculture Organization, 2024).

In view of the increasing intensification of aquaculture systems, it is essential to develop more sustainable and efficient nutritional strategies, focusing on reducing the protein content, without compromising the productive performance of fish (Meurer et al., 2025). Protein is the most expensive component in feed formulation (Deck et al., 2023; Ramakrishnan et al., 2023), and its optimization through adequate amino acid profiles is essential to maximize fish growth and health, in addition to contributing to the reduction of production costs and nutrient excretion in the aquatic environment (Furuya et al., 2023; Teodósio et al., 2020).

Challenges related to the cost of protein sources have led to a growing demand for alternative plant-based ingredients in aquaculture (Ma and Hu, 2024; Matos et al., 2024). Several studies have shown that diets formulated with corn gluten concentrate, soy protein concentrate, and soybean meal, among others, provide good zootechnical performance for tilapia, in addition to improving the cost-benefit ratio of production (Magbanua and Ragaza, 2024; Moyo and Rapatsa-Malatji, 2023).

The use of soybean meal in fish feed is already consolidated in aquaculture (Al-Dubakel and Taher, 2021; Ayyat et al., 2021; Glencross et al., 2020; Ragab et al., 2024). Among its advantages are good protein content, low cost, and stable supply (Hussain et al., 2024). However, soybeans contain several antinutritional factors that, for the most part, are denatured during thermal processing (Ansia and Drackley, 2020; Kohli and Singha, 2024). After oil extraction, the resulting solid residue, called "soybean meal" (SM), is destined for numerous markets, including the aquaculture feed industry. The SM has an apparent protein digestibility coefficient of 90.26±1.19%

for Nile tilapia (Boscolo et al., 2002; Pezzato et al., 2002; Yimer and Tadesse, 2022).

Although it is possible to find several studies in the early growth phases evaluating the protein requirement of Nile tilapia with plant-based diets (Bomfim et al., 2008 a; Botaro et al., 2007; Carneiro et al., 2017; El-Saidy and Gaber, 2005), studies that address this requirement in the final phase of growth are still scarce (Meurer et al., 2025).

The reduction of protein levels by the supplementation of essential amino acids (EAA) can be an interesting tool to increase the protein efficiency of diets and decrease nitrogen excretion in the aquatic environment. According to Novodworski et al. (2024), Nile tilapia in the fattening phase requires 298 g kg^{-1} crude protein (CP) (267 g kg^{-1} digestible protein (DP)) when fed diets based on soybean meal and corn without amino acid supplementation. Thus, the present study investigates the feasibility of reducing the protein content in corn- and soybean meal-based diets in the final growth phase of Nile tilapia, maintaining the adequacy of the essential amino acid profile.

Material and methods

The study was carried out at the Aquaculture Technology Laboratory (LATAq) of the Federal University of Paraná (UFPR), Advanced Campus of Jandaia do Sul (PR, Brazil). The protocol of the experimental procedures was approved by the Ethics Committee on the Use of Animals in the Palotina Sector of UFPR (Protocol No. 05/2021 – CEUA/Palotina).

Experimental design and infrastructure

After a 30-day acclimatization period, 195 adult Nile tilapia of Genetically Improved Farmed Tilapia lineage (GIFT), with an initial average weight of 297±5.1 g, were anesthetized (immersion in eugenol solution at 50 mg L^{-1}) (Santos et al., 2022), weighed, and randomly distributed among five treatments with three replicates each. The experiment lasted 70 days from November 2023 to January 2024.

The experimental structure was composed of 15 circular cages, with an individual volume of 300 L and 13 fish per experimental unit. Each cage was considered an experimental unit. These tanks were installed in a 30,000-L circular tank, connected to a 2,000-L mechanical filtration tank and a 30,000-L biofiltration tank, all interconnected in circulation, forming a recirculation aquaculture system. The recirculation rate in the system was 14 times a day, and aeration was performed by means of microporous hoses installed in the 30,000-L tank, connected to a 2.25 hp radial blower, ensuring homogeneous oxygenation in all tanks. The biofiltration tank had part of its surface covered by the aquatic plant *Eichhornia crassipes*, which was removed as necessary, maintaining a coverage between 50% and 80%.

Experimental feeding and diets

The diets were formulated and processed at the LATAq/UFPR. Protein levels were selected based on the requirement value of 298 g kg^{-1} CP (267 g kg^{-1} DP) determined by Novodworski et al. (2024), with feeds based on soybean meal and corn and without amino acid supplementation. Thus, five experimental treatments (T300, T275, T249, T222, and T197) each consisted of a diet formulated with one of five levels, 300, 275, 249, 222, or 197 g kg^{-1}, of CP. All diets were isoenergetic, isophosphoric, isocaloric, and isoaminoacidic only for EAA, with supplementation from crystalline sources (Tables 1 and 2). The values of DP and digestible energy (DE) were determined based on the digestibility coefficients of soybean meal and corn proposed by Boscolo et al. (2002). The experimental diets were weighed daily and fed in equal amounts to the fish in all experimental units (1.5% of the average fish biomass) in two daily feedings, one at 8:00 am and one at 5:00 pm.

Table 1. Ingredient composition of the experimental diets (natural basis)

Ingredients	T197	T222	T249	T275	T300
			Quantity (g kg^{-1})		
Corn	749.41	653.18	556.71	460.25	363.81
Soybean meal	102.68	217.60	332.53	447.46	562.35
Soy oil	23.52	27.46	31.39	35.33	39.27
$CaHPO_4$	32.27	29.59	26.92	24.25	21.57
$CaCO_3$	3.87	4.60	5.33	6.06	6.79
Vitamin-mineral premix[1]	5.00	5.00	5.00	5.00	5.00
Salt	1.00	1.00	1.00	1.00	1.00
BHT[2]	0.10	0.10	0.10	0.10	0.10
Adsorbent[3]	0.10	0.10	0.10	0.10	0.10
Arginine	13.76	10.32	6.88	3.43	–
Histidine	4.72	3.54	2.36	1.18	–
Isoleucine	9.43	7.07	4.71	2.36	–
Leucine	13.25	9.93	6.62	3.31	–
Lysine	10.44	7.83	5.22	2.61	–
Methionine	2.23	1.67	1.12	0.56	–
Phenylalanine	9.73	7.29	4.86	2.43	–
Threonine	7.03	5.27	3.51	1.76	–
Tryptophan	2.20	1.16	1.10	0.55	–
Valine	9.02	6.79	4.51	2.26	–

[1]Guaranteed vitamin and mineral supplement levels per kilogram of product: vitamin A = 1,200,000 IU; D_3 = 200,000 IU; E = 12,000 mg; K_3 = 2400 mg; B_1 = 4800 mg; B_2 = 4800 mg; B_6 = 4000 mg; B_{12} = 4800 mg; folic acid = 1200 mg; calcium pantothenate = 12,000 mg; C = 48,000 mg; biotin = 48 mg; choline = 65,000 mg; niacin = 24,000 mg; Fe = 10,000 mg; Cu = 6000 mg; Mn = 4000 mg; Zn = 6000 mg; I = 20 mg; Co = 2 mg; Se = 20 mg; [2]Butylated hydroxytoluene; [3]Mycotoxin adsorbent.

Table 2. Proximate analysis of the experimental diets (natural basis)

Nutrients	Corn[1]	SM[1]	T197[2]	T222[2]	T249[2]	T275[2]	T300[2]
				Quantity (g kg^{-1})			
Dry matter[3]	927.50	908.00	933.58	930.41	927.23	925.28	920.89
Crude protein[3]	91.00	470.02	196.88	221.53	249.21	275.30	299.67
Digestible protein[4]	85.00	419.80	187.65	207.31	229.87	250.83	269.01
Gross energy (MJ kg^{-1})[3]	16.48	17.38	16.57	16.74	16.90	17.06	17.28
Digestible energy (MJ kg^{-1})[4]	12.67	12.40	12.98	12.98	12.98	12.98	12.98
Lipids[3]	34.50	29.40	52.17	56.12	60.00	62.30	67.95
Ash[3]	14.00	74.50	18.14	25.36	32.57	37.12	46.98
Calcium	0.20	3.50	10.00	10.00	10.00	10.00	10.00
Phosphorus	1.90	5.90	8.00	8.00	8.00	8.00	8.00
				Essential amino acids			
Arginine	4.00	32.70	19.84	19.84	19.84	19.84	19.84
Histidine	2.90	12.50	8.08	8.08	8.08	8.08	8.08
Isoleucine	3.10	22.70	13.89	13.89	13.89	13.89	13.89
Leucine	10.80	37.30	24.90	24.90	24.90	24.90	24.90
Lysine	2.30	24.30	14.50	14.50	14.50	14.50	14.50
Methionine	1.00	5.60	3.51	3.51	3.51	3.51	3.51
Methionine + Cystine	1.90	10.36	4.66	5.12	5.57	6.03	6.49
Phenylalanine	4.00	24.10	15.01	15.01	15.01	15.01	15.01
Threonine	2.90	17.50	10.90	10.90	10.90	10.90	10.90
Tryptophan	0.60	5.20	3.14	3.14	3.14	3.14	3.14
Valine	0.40	22.60	14.16	14.16	14.16	14.16	14.16
				Non-essential amino acids			
Aspartic acid	5.30	53.30	5.47	11.59	17.72	23.85	29.98
Glutamic acid	15.80	83.60	20.42	28.51	36.59	44.68	52.76
Alanine	6.00	20.70	2.12	4.50	6.88	9.26	11.64
Cystine	0.90	4.70	1.15	1.61	2.06	2.52	2.98
Glycine	3.20	20.30	4.48	5.52	8.53	10.55	12.58
Proline	8.10	23.90	8.53	10.49	12.46	14.42	16.38
Serine	4.20	25.60	5.78	8.31	10.85	13.39	15.92
Tyrosine	2.60	17.50	3.75	5.50	7.27	9.03	10.78

SM: soybean meal; [1]Amino acid composition analyzed for corn and soybean meal; [2]Amino acids calculated based on the analyzed composition of corn and soybean meal. [3]Proximate compositions analyzed for corn, soybean meal, and experimental diets; [4]Calculated value using the digestibility coefficient determined by Boscolo et al. (2002).

For the processing of the feed, corn and soybean meal were finely ground and homogenized to the microingredients. Then, 175 mL kg^{-1} of water were added, and the experimental diets were processed using an extruder (Ex Laboratory, Exteec® Máquinas, Ribeirão Preto, Brazil) with a 3-mm die at a temperature of 60°C. This resulted in extruded pellets with a 5.5-mm diameter, which showed 100% buoyancy in tests carried out over a period of 0–60 minutes (floatability and integrity test of extruded pellets).

Water quality

During the experimental period, a multiparameter probe (Akso®, model Ak88, São Leopoldo, RS, Brazil) was used to monitor temperature (25.95±2.88ºC) and dissolved oxygen (8.15±1.05 mg L^{-1}) and pH (7.22±0.43) weekly, at 9:00 a.m. and 5:00 p.m. Water samples were collected every 21 days to analyze the concentrations of total ammonia (0.1014±0.003 mg^{-1}L), nitrite (0.0162±0.0.002 mg L^{-1}), nitrate (0.1453±0.142 mg L^{-1}) (method No. 4500-B), dissolved orthophosphate

(PO_4^{3-}), alkalinity (223.50±12.02 mg L^{-1}), and hardness (255±35.35 mg L^{-1}) according to APHA (2017).

Growth performance

At the end of the experiment, the fish fasted for 24 hours, then they were anesthetized (immersion in eugenol solution at 120 mg L^{-1}) (Moreira et al., 2010) and counted, measured and weighed. From the final weight (FW) data, weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and protein efficiency rate (PER) were calculated (Sakomura and Rostagno, 2016). Twelve fish per treatment, i.e., four individuals per experimental unit, were randomly chosen and dissected to determine carcass yield (CY), fillet yield (FY), visceral fat (VF), and viscerosomatic index (VSI).

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

$$FCR = \left(\frac{\textit{(feed intake (g)}}{\textit{(weight gain (g)}}\right)$$

$$SGR = \left(\frac{\textit{(ln final average weight} - \textit{ln initial average weight)}}{\textit{(experimental period (days)}}\right) \times 100$$

$$PER = \left(\frac{\textit{(weight gain (g)}}{\textit{(protein intake (g)}}\right)$$

$$CY = \left(\frac{\textit{(carcass weight(g)}}{\textit{(whole fish weight(g)}}\right) \times 100$$

$$FY = \left(\frac{\textit{(fillet weight (g)}}{\textit{(whole fish weight(g)}}\right) \times 100$$

$$VF = \left(\frac{\textit{(visceral fat weight (g)}}{\textit{(body weight (g)}}\right) \times 100$$

$$VSI = \left(\frac{\textit{(visceral weight (g)}}{\textit{(body weight (g)}}\right) \times 100$$

Bromatology

Soybean meal, corn, and experimental diets were analyzed for dry matter (DM, method no. 930.15), crude protein (CP, method no. 984.13), ether extract (EE, method no. 920.39), and mineral matter (MM, method no. 924.05), according to the international procedures of the Association of Official Analytical Chemists (AOAC, 2005). The gross energy of soybean meal and corn was determined by means of a calorimetric pump, and the amino acid composition was measured in a specialized laboratory (CBO Análises Laboratoriais Ltda).

After collecting the performance data, a whole fish, two carcasses, and the fillets of two other fish were randomly chosen from each experimental unit to determine the centesimal composition for DM, CP, EE, and MM contents (AOAC, 2005).

Statistical analysis

The data were subjected to an analysis of variance (ANOVA), following a completely randomized design. When a significant effect was identified ($P \leq 0.05$), Tukey's test was applied to compare means. Additionally, regression analysis was conducted to identify the model that best fit the data. Among the models tested, the segmented linear model (Broken-line) was selected because it presents the best statistical fit, with the lowest estimated error and the highest coefficient of determination (R^2), according to the evaluation criteria adopted. This model describes a linear increment to a breakpoint, followed by a plateau, which represents the point at which nutritional requirements are met (Sakomura and Rostagno, 2016). Statistical analyses were performed with RStudio 4.3.1 software (R Core Team, 2023).

Results

Growth performance

The performance parameters of the Nile tilapia (Table 3) show that the treatments T249, T275, and T300 resulted in FW of 553.66 g, 559.06 g, and 560.37 g, respectively, and WG of 256.35 g, 261.36 g, and 260.50 g, respectively, significantly higher than the FW and WG in the T197 and T222 treatments. The PER showed a quadratic behavior as a function of the reduction in the CP content of the diet ($y = 2.58 - 0.13x - 0.455x^2$ $R^2 = 0.62$), indicating greater efficiency in the T249 treatment, a value close to the maximum point of the curve.

The SGR and FCR of the Nile tilapia exhibited linear responses to increasing dietary protein levels up to their respective breakpoints. For SGR, the estimated requirements were 228.73 g kg^{-1} of DP and 248.26 g kg^{-1} of CP, with an R^2 of 0.8696 (Figure 1 a–b). For FCR, the breakpoints were 228.63 g kg^{-1} DP and 248.14 g kg^{-1} CP, with an R^2 of 0.8861 (Figure 2 a–b).

Table 3. Performance parameters of Nile tilapia in the fattening phase fed diets with decreasing levels of protein with amino acid supplementation

Variable	Treatment					CV	P value
	T197	T222	T249	T275	T300		
IW (g)	297.00	291.79	297.31	297.68	299.87	1.69	0.4236
FW (g	487.64 b	511.56 b	553.66 a	559.06 a	560.37 a	1.94	1.44e-05
WG (g)	190.64 c	219.76 b	256.35 a	261.36 a	260.5 a	3.79	6.06e-06
FI (g)	406.76	406.77	406.18	407.27	408.26	0.46	0.7158
PER	2.50 ab	2.61 ab	2.78 a	2.6 ab	2.39 b	4.14	0.0132
Survival (%)	89.74	89.74	94.87	92.31	95.21	9.34	0.8824

CV: coefficient of variation; IW: initial weight; FW: final weight; WG: weight gain; FI: feed intake; PER: protein efficiency ratio ($y = 2.58 - 0.13x - 0.455x^2$ $R^2 = 0.62$); a – c – means followed by different letters in the lines differ statistically by Tukey's test ($P<0.05$).

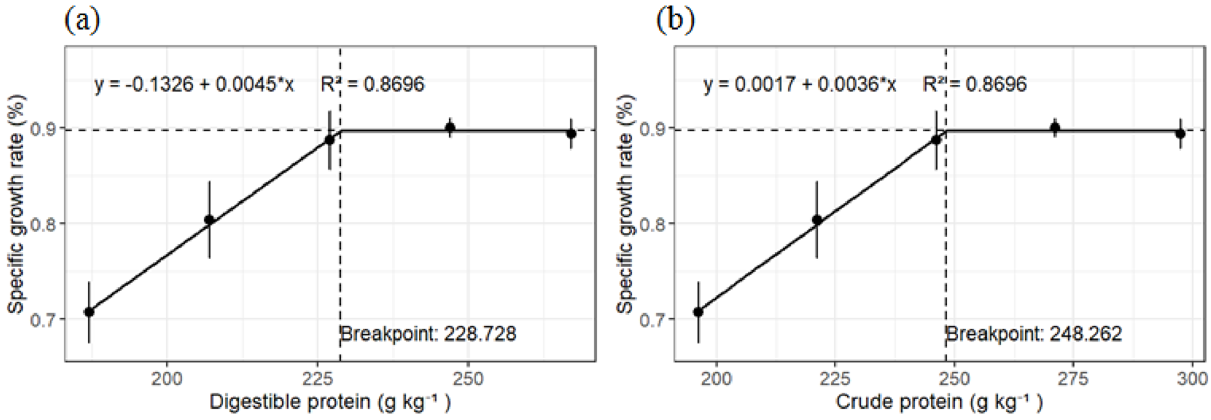

Figure 1. Specific growth rate of Nile tilapia fed with different concentrations of DP (a) and CP (b) during the fattening phase, estimated by linear-plateau regression (*broken-line*)

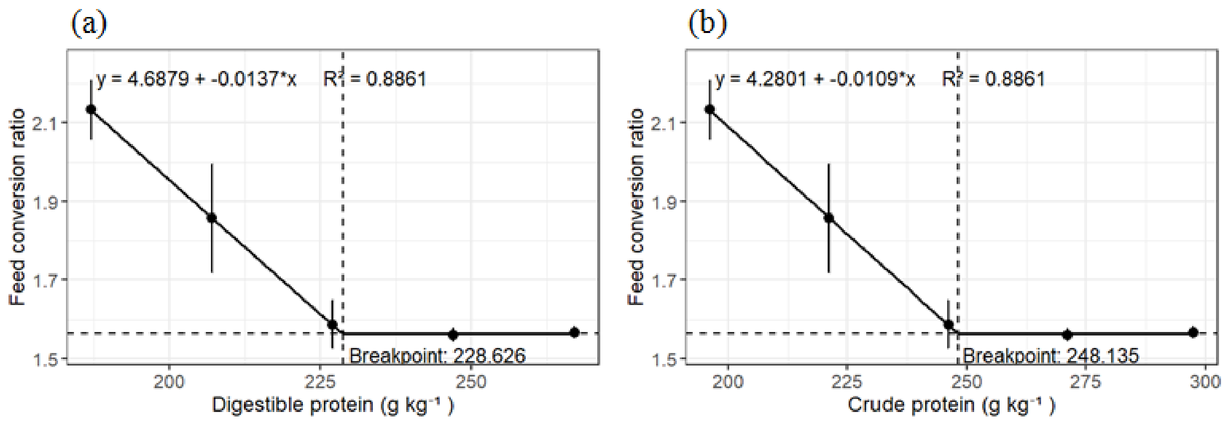

Figure 2. Feed conversion of Nile tilapia fed with different concentrations of DP (a) and CP (b) during the fattening phase, estimated by linear-plateau regression (*broken-line*)

The body performance variables VF, FY, and TL were significantly influenced by the level of CP (Table 4). The T197 treatment had the highest VF (3.02 g 100 g^{-1}) and the lowest FY (32.68 g 100 g^{-1}), while T300 had the lowest VF (1.56 g 100 g^{-1}) and the highest fillet yield (34.59 g 100 g^{-1}). The intermediate treatments did not differ significantly from each other in relation to VF and FY. The TL varied significantly, with T275 having on average the longest fish (30.32 cm) and T197 and T222 having on average the short fish (28.46 cm and 28.81 cm, respectively). There were no significant differences ($P>0.05$) for the VSI, CY, SL, and TH.

Table 4. Body performance parameters of Nile tilapia fed different levels of protein in the fattening phase

Variable	Treatment					CV	P value
	T197	T222	T249	T275	T300		
VF (g 100 g^{-1})	3.02 a	2.71 ab	2.73 ab	2.28 ab	1.56 b	17.77	0.0171
VSI (g 100 g^{-1})	11.20	9.05	9.34	9.87	10.73	10.79	0.1519
CY (g 100 g^{-1})	85.66	87.46	87.85	87.85	87.75	1.08	0.0741
FY (g 100 g^{-1})	32.68 b	33.43 ab	34.08 ab	34.15 ab	34.59 a	1.78	0.0234
TL (cm)	28.46 c	28.81 bc	30.11 ab	30.32 a	30.01 ab	1.78	0.0042
SL (cm)	23.92	24.22	25.25	25.28	25.22	2.45	0.0488
TH (cm)	10.00	10.11	10.50	10.56	10.53	3.41	0.2320

CV: coefficient of variation; VF: visceral fat; VSI: viscerosomatic index; CY: carcass yield; FY: fillet yield; TL: total length; SL: standard length; TH: trunk height; a–c – means within row with different letters differ (P<0.05).

Table 5. Body and fillet chemical compositions of Nile tilapia fed different levels of protein in the fattening phase

Variable (g 100 g^{-1})	Treatment					CV[1]	P value
	T197	T222	T249	T275	T300		
Moisture							
fish	67.27	66.40	67.19	68.74	68.43	2.36	0.4145
carcass	69.10	69.16	69.79	67.64	69.14	1.37	0.1549
fillet	76.05	75.73	76.00	75.63	76.73	1.39	0.7404
Protein							
fish	15.22	15.35	15.45	15.43	15.26	2.44	0.9225
carcass	16.34 b	16.59 b	16.34 b	17.35 a	17.08 a	1.97	0.0118
fillet	19.84	19.72	19.32	19.14	19.23	3.64	0.6805
Mineral matter							
fish	4.14	4.12	3.87	4.14	4.42	6.91	0.3156
carcass	4.72	4.55	4.30	4.43	4.69	6.46	0.4080
fillet	1.24	1.24	1.25	1.25	1.12	7.68	0.6648
Ether extract							
fish	13.17	14.81	13.37	11.20	12.36	20.20	0.5671
carcass	11.27	11.31	11.45	12.25	10.32	8.58	0.2762
fillet	3.82	4.00	4.72	5.09	4.57	17.39	0.3105

[1]CV: coefficient of variation. a–b – means within row with different letters differ (P<0.05).

Bromatology

The chemical composition parameters of the whole fish, carcass, and fillet of Nile tilapia in the fattening phase (Table 5) indicate that, despite some variations, most of the chemical composition parameters were not significantly influenced by the CP level in the fish diets. There were no significant differences among treatments for moisture in fish, carcasses, and fillets and for protein in fish and fillets. Treatments T275 and T300 had significantly higher protein values in carcasses (17.35 g 100 g^{-1} and 17.08 g 100 g^{-1}, respectively) compared to the carcass protein levels in treatments T197, T222, and T249. The MM and EE in the whole fish, carcasses, and fillets were not significantly different among the treatments.

Discussion

The determination of the protein requirement of Nile tilapia can be impacted by several methodological and biological factors, leading to divergences in results in the various stages of development (Meurer et al., 2025). This can also apply to the requirement of amino acids, where the differences between the methodological approaches and statistical models used provided inconsistency among the amino acid requirements reported in the literature (Furuya et al., 2023; Xing et al., 2024). As with the requirement for protein (Meurer et al., 2025; NRC, 2011), the amino acid requirements are also influenced by the age and weight of the fish (Furuya and Furuya, 2010), as well as by the genetic differences of the lineages. In this sense, Fatan et al. (2023) demonstrated that genetic improvement promoted higher performance and may have altered the need for lysine in GIFT tilapia, when comparing the 16th and 17th generations during the fattening phase. Although Fatan et al. (2023) did not estimate an optimal level of lysine, they observed a positive linear response to the increase in this amino acid, suggesting greater demand in the genetically enhanced lineage.

Novodworski et al. (2024) determined that the protein requirement for Nile tilapia with an average weight between 400 and 700 g and fed diets based on soybean meal and corn without amino acid supplementation was 298 g kg^{-1} CP (267 g kg^{-1} DP). In our study, considering this CP diet as ideal, the effects of reducing the CP were investigated, maintaining isoaminoacidic diets for EAA through supplementation. The results showed that the reduction of CP in the diet from 300 g kg^{-1} to 249 g kg^{-1} did not significantly compromise the zootechnical performance of Nile tilapia. The treatments T249, T275, and T300 promoted higher gains in FW and WG compared to the treatments with lower levels of protein. These findings corroborate those of previous studies (Khan et al., 2019; Konnert et al., 2022; Meurer et al., 2025; Schrama et al., 2018), which emphasize the importance of adequate nutrition, especially in terms of protein and adequate amino acid balance, to optimize growth and yield efficiency in tilapia.

Our findings also corroborate studies that evaluated the protein requirement of tilapia by using the concept of ideal protein, but in younger stages. It was possible to reduce the requirement from 320 to 280 g kg^{-1} CP in fish weighing 0.8–13.4 g (Bomfim et al., 2008 b), from 300 to 275 g kg^{-1} DP in fish weighing 5–125 g (Furuya et al., 2005), from 340 to 300 g kg^{-1} DP in fish weighing 30–165 g (Gonçalves et al., 2009), and from 270 to 243 g kg^{-1} DP and 267 to 245 g kg^{-1} DP in fish weighing 100–500 g (Botaro et al., 2007; Righetti et al., 2011, respectively). However, evaluating the effect of the protein-energy relationship on the tilapia's growth phase (190–450 g) by feeding the fish diets based on soybean meal and corn, without supplementation of synthetic amino acids, the ideal level of 240 g kg^{-1} of CP with 12.56 MJ kg^{-1} of DE was identified (Carneiro et al., 2020), a result similar to that observed in some of the studies cited above. On the other hand, it is important to highlight that there is a reduction in protein requirements due to fish growth, with lower requirements in the growth phase (final weight of 200–500 g), increasing again in larger fish (Meurer et al., 2025). Thus, our findings disagree with those of studies, carried out on fish with similar final average weight, that present average requirements of 307±6.36 g kg^{-1} of CP (Costa et al., 2009; Liu et al., 2018) and 275±5.13 g kg^{-1} DP (Fernandes Junior et al., 2016; Green et al., 2019; Koch et al., 2017).

Different from what was observed by Novodworski et al. (2024), where protein levels above or below 298 g kg^{-1} CP (267 g kg^{-1} DP) had a negative effect on fish performance, in our study, through supplementation with synthetic amino acids, it was possible to reduce the protein level from 300 g kg^{-1} CP (269 g kg^{-1} DP) to 248 g kg^{-1} CP (230 g kg^{-1} DP) without negatively impacting the growth performance results (Figures 1 and 2). However, even by maintaining the isoaminoacidic diets for the EAA, there was a worsening in the performance results in the T197 and T222 treatments.

The difference in performance in the treatments with lower CP may be related to multiple factors. In addition to the lower total nitrogen supply, the highest DE:DP ratio observed in these treatments stands out at 16.14 and 14.30 kcal g^{-1} for treatments T197 and T222, respectively. This unbalanced ratio may have resulted in an accumulation of energy not used for protein synthesis, reducing feed efficiency, as already reported by Carneiro et al. (2020). In addition, the greater inclusion of crystalline amino acids in these treatments may have negatively influenced the efficiency of amino acid utilization. As discussed by da Cruz et al. (2021), the rapid absorption of synthetic amino acids, especially when supplemented in excess, can cause imbalances in the availability of amino acids in the blood, resulting in lower efficiency in protein synthesis and leading to a possible imbalance between essential and non-essential amino acids in the plasma.

The lower performance observed in treatments T197 and T222, even with the supplementation of EAA, may also be associated with the reduction of non-essential amino acids (NEAA), due to the increase in the proportion of corn and less inclusion of soybean meal in the formulation of these diets. This imbalance can compromise the synthesis of proteins and nitrogenous compounds. According to Furuya et al. (2023), recent evidence indicates that NEAA supplementation can have beneficial and significant effects on performance. Although fish can synthesize these amino acids, limiting NEAA in the diet can cause utilization rates to exceed synthesis rates, making them conditional EAA. Moreover, antagonistic interactions between amino acids, caused by the excess of certain amino acids, can increase the demand for others and compromise the growth performance of the fish (Ahmad et al., 2021; Peres and Oliva, 2017; Wang et al., 2021; Xing et al., 2024).

The PER was higher in the T249 treatment, promoting an efficient use of nutrients for protein synthesis, essential for muscle growth (Liu et al., 2018). On the other hand, the T300 treatment showed a reduced PER, possibly indicating an excess of protein in the diet that was not fully used by the fish. Similarly, the T197 and T222 treatments, with lower protein levels, also had lower PER, indicating protein insufficiency even with amino acid supplementation. These results suggest that there is a specific range in which the protein is most effectively utilized. This corroborates the observations of Righetti et al. (2011), where the best PER was found in intermediate treatments, coinciding with the best results of the other performance variables.

Treatment T197, with lowest protein content, resulted in higher VF, which may be indicative of lipid reserve synthesis. Reduced CP results in increased carbohydrates in the diet, which can lead to greater hepatic glycogen deposition. Excess carbohydrates can favor lipogenesis and consequently contribute to the accumulation of VF in fish, as observed by Coutinho et al. (2018) who reported an increase in the hepatosomatic index in diets with higher proportions of carbohydrates, mainly due to the accumulation of lipids in the liver. Liu et al. (2017) also observed that the VI was influenced by protein levels in the diet, suggesting a possible relationship between lower CP and higher deposition of reserves in the viscera but without directly evaluating VF deposition, which was not observed in our study.

The FY and TL were also significantly impacted by the levels of CP in the diet. The treatment with the lowest CP level resulted in a lower FY and a lower TL, which suggests that a diet with insufficient protein can limit muscle growth and structural development of fish, even when supplemented with EAA. In contrast, the treatments with higher protein levels (T222, T249, T275, and T300 for FY, and T249, T275, and T300 for TL) were not significantly different for these variables, suggesting that there is an optimal protein level that maximizes growth without the negative effects associated with extreme dietary protein variations. Our findings corroborate those of Furuya et al. (2005), Oliveira et al. (2014), and Youssef et al. (2023), indicating that the reduction of protein below the requirement of fish promotes less muscle growth and, consequently, reduction in FY. This effect can be attributed to a more adequate balance of EAA and NEAA in the diets, which optimizes the utilization of the nitrogen fraction by fish and increases the efficiency of body protein synthesis (Furuya et al., 2005; Li et al., 2021), thus promoting better muscle growth and impacting both FY and TL.

The body and fillet chemical compositions of Nile tilapia varied, although not all of differences were statistically significant. Carcass protein in treatments T275 and T300 was significantly higher than in T197, T222, and T249 treatments. This suggests that higher levels of protein in the diet may improve protein deposition in the carcass. On the other hand, the protein levels in the whole fish and in the fillet were not significantly different among the treatments, which agrees with similar findings in previous studies (Carneiro et al., 2020; Green et al., 2019; Peres et al., 2022; Zeng et al., 2021).

The possibility of reducing the CP in the feed for Nile tilapia from 300 to 248 g kg^{-1} CP, while maintaining zootechnical performance, represents a promising strategy for the formulation of diets based on plant ingredients. Considering the high cost of protein sources and the need for sustainable alternatives to fishmeal, our results demonstrate the nutritional feasibility of using diets based on corn and soybean meal supplemented with EAA. Future studies with an economic approach that compare the cost of production per kg of weight gain with diets with or without crystalline amino acid supplementation may provide valuable information for commercial-scale adoption.

Conclusion

It is possible to reduce the CP level from 300 g kg^{-1} (269 g kg^{-1} DP) to 248 g kg^{-1} CP (228 g kg^{-1} DP) in the feed for Nile tilapia weighing 300–530 g when the food is based on corn and soybean meal supplemented with EAA.

Authorship contribution statement

J.N.: conceptualization, methodology, data curation, formal analysis, investigation, writing – original draft, writing – review and editing. É.J.A.M.: formal analysis, writing – original draft. R.M.G.: formal analysis. R.A.B.: writing review and editing. F.M.: conceptualization, methodology, validation, resources, supervision, writing – review and editing.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statement

This study is in accordance with the Ethical Principles of Animal Experimentation, adopted by the National Council for the Control of Animal Experimentation (CONCEA) and was approved by the Ethics Committee on the Use of Animals in the Palotina Sector of UFPR (CEUA/Palotina) on 05/02/2021 (Protocol no. 05/2021).

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