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Practical diet with total replacement of fishmeal by soybean meal for Nile tilapia: growth performance and health effects

Dieta prática com substituição total da farinha de peixe por farelo de soja para tilápia-do-nilo: desempenho de crescimento e efeitos na saúde

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Abstract
This study aimed to evaluate the growth performance and animal health in juveniles of Nile tilapia (*Oreochromis niloticus*) fed a practical diet with total replacement of fishmeal (FM) by soybean meal, as well the effect on survival, following pathogenic challenge with *Aeromonas hydrophila*. Two hundred juveniles of Nile tilapia were stored in 8 tanks (800 L). The experiment consisted of two treatments: a commercial diet formulated with FM (control) and a practical diet with total replacement of FM by soybean meal (SM). The variables of water quality, zootechnical and hematological parameters were measured. In addition, at the end of the experiment, the fish were submitted to a challenge with *A. hydrophila*. Higher cost per kg of fish was obtained in the control treatment (with FM). N retention was higher in fish fed a diet without FM, while hematological, immunological parameters and survival after the experimental challenge did not differ between treatments. It is possible to reduce dietary costs by replacing FM with SM without affecting growth performance or animal health, in addition to benefiting the environment by reducing the excretion of N in water.
Keywords: plant protein; *Aeromonas hydrophila*; nitrogen retention; immunology.

Resumo
O objetivo deste estudo foi avaliar o desempenho de crescimento e saúde em juvenis de tilápia-do-nilo (*Oreochromis niloticus*) alimentados com dieta prática com substituição total da farinha de peixe (FP) por farelo de soja (FS), bem como o efeito na sobrevivência, após desafio patogênico com *Aeromonas hydrophila*. Duzentos juvenis de tilápia-do-nilo foram alojados em 8 tanques (800 L). O experimento consistiu de dois tratamentos: dieta comercial formulada com FP (controle) e dieta prática com substituição total do FP pelo FS. Foram mensuradas as variáveis de qualidade da água, parâmetros zootécnicos e hematológicos. Além disso, ao final do experimento, os peixes foram submetidos a um desafio com *A. hydrophila*. Maior custo por kg de peixe foi obtido no tratamento controle (com FP). A retenção de N foi maior nos peixes alimentados com dieta sem FP, enquanto os parâmetros hematológicos, imunológicos e a sobrevivência após o desafio experimental não diferiram entre os tratamentos. É possível reduzir os custos dietéticos substituindo a FP pelo FS sem afetar o desempenho do crescimento e a saúde do animal, além de beneficiar o meio ambiente ao reduzir a excreção de N na água.
Palavras-chave: proteína vegetal; *Aeromonas hydrophila*; retenção de nitrogênio; imunologia.

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Introduction

Nile tilapia (*Oreochromis niloticus*), as one of the most important cultivated species in the world, represents more than 8% of aquaculture production. Brazilian fish production has increased 5.9%, totaling 802.930 tons, being tilapia the main species, representing 63.5% of the national freshwater fish production in 2021, ranking Brazil as the fourth largest producer in the world [1,2].

Feed corresponds to about 50% of total production costs and inadequate diets compromise the absorption of nutrients. Among them, phosphorus is considered an essential nutrient for the formation of bone structure, mineralization, and lipid metabolism. While nitrogen is a necessary element related to the use of dietary protein and carcass yield. However, excessive concentrations of both phosphorus and nitrogen in the aquatic environment can lead to eutrophication of the environment and water quality compromise [3,4]. Thus, if there is an imbalance of the diet, it compromises the environment, increasing the risk of disease outbreaks, in addition to affecting cultivation profits [5,6,7].

Therefore, adequate nutrition plays a fundamental role in promoting the maintenance of fish health, and improving immune response. It follows that the evaluation of new ingredients, diets and formulations requires an understanding of their effects on animal health and metabolism. Deficient diets, for example, can affect the immune system of animals, favoring infection with opportunistic bacteria, such as *Aeromonas hydrophila*, causing, in turn, economic losses [6,7].

Protein sources are the most expensive ingredient in the diet, mainly in intensive aquaculture systems. Additionally, the constant growth of fish farming, has resulted in greater demand for dietary FM, although the supply did not meet the need, generating fluctuations in supply and price [1,8]. Thus, to reduce costs, the use of alternative ingredients to fishmeal (FM) has been studied, such as the use of soybean and its derivate. Despite the fact that soybean has antinutritional factors, involving protease inhibitors, phytohemagglutinin, and antivitamins, both soybean and its derivate appear to be viable alternatives as a replacement for fishmeal in the aquaculture production chain, owing to its nutritional quality, availability in the marketplace, and benefit-cost ratio [8,9].

Importantly, the availability of studies reporting on soybean as a source of protein and its influence on the immune response of tilapia is limited. When FM was replaced with soybean protein concentrate for the white shrimp *Litopenaeus vannamei*, no changes in animal susceptibility or immunocompetence were noted, either before or after bacterial infection (*Vibrio* sp.) [10], demonstrating that well-balanced diets with soybean derivatives can be used without compromising animal health, in addition to providing good growth performance [11].

Despite the importance of tilapia in aquaculture worldwide and the use of soybean and its derivatives in feed formulations, it is necessary that further studies describe the relationship between the use of soybean in diets and the health of the animals. Thus, this study aimed to evaluate the growth performance, cost (value per kg of fish) and animal health in juveniles of Nile tilapia (*Oreochromis niloticus*) fed a practical diet with total replacement of FM by soybean meal, as well the effect on survival, following pathogenic challenge against *Aeromonas hydrophila*.

Material and methods

The study was carried out according to the approval of the Ethics Committee on the Use of Animals under the protocol number "263/2018".

Experimental diets

The diets were produced by NUTRICOL®, located in São Ludgero, Santa Catarina, Brazil. To prepare the diets, some of the dry ingredients (corn, soybeans and wheat bran) were previously ground (2000 µm) in order to obtain particles with a diameter of less than 0.42 mm. Later, they were mixed with the other macro and micro ingredients and homogenized in a horizontal paddle mixer for 4 minutes. The resulting mixture went through a second grinding step (800 µm), followed by extrusion at 105 ° C in an extruder (FERRAZ®, Ribeirão Preto, SP, Brazil) with a capacity of 3,000 $kg.h^{-1}$ to obtain extrudates 3 mm in diameter.

The experiment consisted of two treatments, as shown in Table 1: control, consisting of commercial diet formulated with FM and practical ingredients, and a practical diet consisting of total replacement of FM by soybean meal. Both were isoproteic and isoenergetic and calculated in order to meet the nutritional requirements of tilapia [7]. Samples of the diets were sent to CBO ANÁLISES LABORATORIAIS to perform the aminogram using high-performance liquid chromatography (HPLC) and to evaluate the proximal composition according to the AOAC methodology [12].

Table 1. Composition of control and experimental diet without fish meal (FM)

Ingredients ($g.kg^{-1}$)	Control	Without FM
Soybean meal	350.0	466.0
Fishmeal (FM)	150.0	0.0
Corn meal	120.0	120.0
Broad bean	103.8	120.0
Wheat flour	70.0	42.2
Meat and bone meal	135.0	135.0
Blood meal	50.0	64.6
Fish oil	0.0	8.6
Soybean oil	10.0	10.3
NaCl	3.0	3.0
Calcitic Limestone	0.0	22.1
Premix[1]	4.0	4.0
DL-Methionine	0.9	0.9
Essential (Functional Oil)[2]	1.0	1.0
Antifungal[3]	1.0	1.0
Adsorber[4]	1.0	1.0
Antioxidant[5]	0.3	0.3

[1]Premix = Vitamin A (min.) 800,000 IU; Vitamin D3 (min.) 410,000 IU; Vitamin E (min.) 15,000 IU; Vitamin K3 (min.) 505 mg; Vitamin B1 (min.) 1,395.9 mg; Vitamin B2 (min.) 2,000 mg; Vitamin B6 (min.) 1,862 mg; Vitamin B12 (min.) 2,500 mg; Vitamin C (min.) 125 g; Niacin (min.) 3,781 mg; Pantothenic acid (min.) 4,018 mg; Folic Acid (min.) 198 mg; Biotin (min.) 100 mg; Choline (min.) 86.68 g; Copper (min.) 750 mg; Iron (min.) 8,310 mg; Manganese (min.) 1,320 mg; Cobalt (min.) 24 mg; Iodine (min.) 264.8 mg; Zinc (min.) 15.05 g; Selenium (min.) 47.55 mg; Inositol (min.) 25 g. [2]Castor oil and Canola oil. [3]Propionic acid. [4]Bentonite, Sepiolite, Calcium Propionate, Sodium Chloride and other ingredients. [5]B.H.T. (Butylhydroxytoluene), Propyl gallate. All other ingredients were supplied by Nutricol (São Ludgero, SC, Brazil).

Experimental design

Two hundred Nile tilapia (*O. niloticus*) juveniles with an average weight of 13.3 g, were distributed in eight polyethylene tanks (800 L useful), 25 fish per experimental unit, equipped with a water recirculation system (water renewal of 150% per day) and biological filter. The experimental units were divided into two groups, completely at random in quadruplicate: 1º) control, consisting of a commercial diet formulated with FM and practical ingredients; and 2ª) a practical diet consisting of total replacement of FM by soybean meal. Rearing time lasted eight weeks.

Physicochemical parameters of water quality and food management

The animals were fed 3 times a day (09:00; 11:00 and 15:30 h) with an offer of 3 to 6% of the total biomass. When necessary, the experimental units were cleaned to remove organic matter excess. During the experiment, dissolved oxygen and temperature were monitored twice daily, while toxic ammonia, pH, nitrite, nitrate, and alkalinity (Alfakit photocolorimeter) were weekly measured.

The water quality parameters were measured during the entire experimental period, namely: DO above 3.95 ± 0.83 mg L^{-1} and temperature 26.59 ± 1.59 ° C (YSI PRO20 Oximeter); ammonia of 0.08 ± 0.08 NH_3 mg L^{-1}, nitrite below 1.76 ± 1.15 mg L^{-1}, nitrate below 0.62 ± 0.95 mg L^{-1}, alkalinity above 97.84 ± 15.07 mg $CaCO_2$ L^{-1} and pH of 6.97 ± 0.09.

Growth performance and N and P retention

Survival, final weight, weekly weight gain, specific growth rate, food conversion, yield, protein efficiency ratio (PER), and cost per kilogram of fish (considering only the dietary cost, represented by the sum of all the ingredients, according to the equation below) were all determined at the end of the experiments.

$$Cost\ per\ kilogram\ of\ fish\ (\text{R\$/kg}) = \frac{(diet\ offered\ x\ price\ per\ kg)}{produced\ biomass}$$

Twelve fish samples (four before starting the experiment and four from each treatment) were euthanized, frozen and lyophilized in preparation for analysis of N and P concentration, according to the AOAC methodology [12], by CBO Laboratories. The retention of N and P was calculated according to DA SILVA et al. [13].

Animal health, including hematology, immunology, and pathogenic challenge by Aeromonas hydrophila

For hematological analysis, five fish per experimental unit (20 per treatment) were anesthetized with Eugenol (50mg.L^{-1}), and aliquots of blood were removed from the caudal vessel with EDTA anticoagulant. After preparing blood smears, in duplicate, the following hematological analyses were carried out: hematocrit by the standard microhematocrit method, total hemocyte count by Neubauer hemocytometer, and hemoglobin concentration. Hematimetric absolute rates of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also obtained. Blood smear slides were stained with Giemsa and May Grünwald stain [14] for total and differential leukocyte count.

For immunological analysis, 0.5 mL of blood from five animals per experimental unit was collected by puncturing the caudal vessel without anticoagulant to obtain blood serum. Subsequently, the blood was centrifuged at 1400 x g for 10 min to separate the serum, which was collected and stored at -20 ° C for subsequent immunological analysis. Total plasma protein concentration was measured with the Total Protein Kit (Biochemical Reagent, Total Proteins, LabTest, Brazil), using bovine albumin to make the standard curve. The total immunoglobulin concentration was measured according to the method described by AMAR et al. [15]. Briefly, 100 µL of blood serum were mixed with 100 µL of 12% polyethylene glycol (PEG) solution (Sigma-Aldrich) for subsequent incubation at room temperature for two hours in order to precipitate immunoglobulin molecules. The precipitate was removed by centrifugation at 5000 x g at 6º C for 10 min. After removing the supernatant, the amount of total protein was measured with the help of the Total Protein kit (Lab Test®). The total immunoglobulin concentration is expressed in mg mL^{-1} and is calculated as Total immunoglobulin = (total plasma protein - total protein treated with PEG)/Volume (mL).

For the pathogenic challenge experiment performed at the end of the experimental period, the bacterium *Aeromonas hydrophila* (ATCC 7966) was cultured in a brain-heart infusion medium (BHI) at 30°C for 24 hours, followed by centrifugation at 1000 x g for 15min. The supernatant was discarded, and the bacterial pellet was resuspended in 0.65% NaCl sterile saline to adjust the concentration of the bacterium, as defined by DL 50 (unpublished data). In this step, five fish from each unit were transferred to aquariums (45 L), all equipped with biological filters and constant temperature (24 - 25°C). All fish were inoculated intraperitoneally with 100µL of *A. hydrophila* (ATCC 7966) at concentration of $2.5x10^6$CFUmL^{-1}; Fish survival was evaluated after 96 hours.

Statistical analyses

All data were first subjected to Bartlett's analysis to verify the homogeneity of variance. Subsequently, they were submitted to the *t* test (Stata® Statistical Software). All analyses were conducted with a 5% significance level.

Results

Experimental diets

Amino acid profile and proximate composition were similar in both experimental diets (Table 2).

Table 2. Aminogram and composition proximal of control and experimental diet without fish meal (FM)

Nutrients (%)	Treatments	
	Control	Without FM
Moisture	8.99	9.32
Crude Protein	36.42	36.36
Crude Lipid	5.80	5.66
Crude Fiber	3.63	3.35
Material Mineral	10.88	11.28
Calcium	2.49	2.69
Phosphorus	1.45	1.27
Arginine	2.37	2.36
Lysine	1.96	2.24
Methionine	0.54	0.50
Cysteine	0.64	0.71
Threonine	1.44	1.41
Tryptophan	0.37	0.35
Leucine	2.81	2.93
Isoleucine	1.33	1.32
Valine	1.84	1.89
Histidine	0.95	1.04
Phenylalanine	1.66	1.79
Serine	1.82	1.76
Glycine	2.63	2.47
Taurine	0.04	0.04
Alanine	2.21	2.21
Proline	2.17	2.05
Tyrosine	1.14	1.14
Aspartic Acid	3.25	3.11
Glutamic Acid	5.40	5.47

Growth performance

Fish fed a diet without fish meal showed higher mean final weight, mean daily gain, specific growth rate, as well as yield and protein efficiency rate, than the control (FM) group. The dietary cost per kg of fish was higher in the control. N retention was higher in fish fed the soybean replacement diet, while P retention did not differ between treatments (Table 3).

Animal health (hematology, immunology and pathogenic challenge by Aeromonas hydrophila)

Neither bloodwork variables nor immunological parameters (Table 4) presented significant differences between the treatments. After 96 hours, fish survival after experimental challenge against *A. hydrophila* was 62.5± 7.5 % and 65.7 ± 10.7 % for control (FM) and soybean replacement, respectively.

Table 3. Growth performance, nitrogen (N) and phosphorus (P) retention of Nile tilapia (200 fish) reared in RAS, fed with a practical diet with total replacement of fishmeal (FM) by soybean meal (SB)

Zootechnical variables	Treatment	
	Control	Without FM
Mean final weight (g)	72.67 ± 2.66	77.75 ± 3.95*
Daily gain ($g.day^{-1}$)	1.06 ± 0.05	1.16 ± 0.06*
Specific growth rate ($\%.day^{-1}$)	1.30 ± 0.03	1.37 ± 0.04*
Feed Conversion	1.13 ±0.03	1.13 ±0.05
Protein efficiency rate	0.38 ±0.01	0.41 ±0.02*
Survival (%)	100.00 ± 0.00	100.00 ± 0.00
Yield ($Kg.m^{-3}$)	1.86 ± 0.08	2.01 ± 0.12*
Dietary cost per kg of fish ($R\$.kg^{-1}$)	1.20 ± 0.03*	1.05 ± 0.04
N retention (%)	28.42 ± 1.50	31.19 ± 2.18*
P retention (%)	27.05 ± 5.14	26.69 ± 2.04

*Statistical difference by *t*-test (P<0.05).

Table 4. Blood parameters (mean ± standard deviation) of Nile tilapia (40 fish) reared in RAS, fed with a practical diet with total replacement of fishmeal (FM) by soybean meal (SB)

Total and differential leukocyte count	Control	Without FM
Thrombocytes (x $10^4 \mu L^{-1}$)	6.67 ± 1.14	5.74 ± 1.19
Total Leukocytes (x 10^3 μL^{-1})	88.06 ± 20.92	75.69 ± 10.40
Lymphocytes (x $10^3 \mu L^{-1}$)	83.21 ± 19.94	71.15 ± 9.90
Eosinophils (x $10^3 \mu L^{-1}$)	0.21 ± 0.17	0.16 ± 0.08
Monocytes (x $10^3 \mu L^{-1}$)	2.73 ± 0.82	2.74 ± 0.42
Neutrophils (x $10^3 \mu L^{-1}$)	1.90 ± 0.17	1.64 ± 0.57
Hematimetric indexes		
Erythrocytes (x $10^6 \mu L^{-1}$)	2.67± 0.38	2.67± 0.38
Hematocrit (%)	27.80 ± 0.85	27.73 ± 1.16
Hemoglobin concentration ($g.dL^{-1}$)	8.24 ± 0.30	7.67 ± 0.37
Mean corpuscular Volume (10^{-5}.pg)	10.11 ±1.97	10.75±1.41
Mean corpuscular Hemoglobin (10^{-5}.pg)	3.12 ± 0.64	2.98 ± 0.46
Mean corpuscular Hemoglobin Concentration ($g.dL^{-1}$)	3.01 ± 0.22	2.78 ± 0.07
Immunological parameters		
Total Plasma immunoglobulin ($mg.L^{-1}$)	28.51 ± 2.76	27.94 ± 3.05
Total Plasma protein ($cg.L^{-1}$)	104.95 ± 0.27	104.78 ± 0.36

*Statistical difference by *t*-test (P<0.05)

Discussion

In this study, the diets used presented a similar proximal composition and amino acid profile with only small differences between treatments (Table 2). Among the amino acids evaluated, methionine was the only one below the recommended 7.0 $g.kg^{-1}$ [(7)]. Reduction in this amino acid is a limiting factor in diets formulated based on soybean or soybean derivatives [7]. Other studies with methionine below the recommended, showed lower growth rate and feed efficiency in rainbow trout (*Oncorhynchus mykiss*) [16] and yellowtail (*Seriola dorsalis*) [17]. However, the amount of methionine + cystine might have compensated for these levels, because 50% of the requirement for methionine can be provided by this combination [18] justifying in what way the methionine levels do not compromise the growth performance for tilapia in this work.

The use of soybean did not compromise growth performance. In fact, this substitute improved the growth performance of fish. These results corroborate the findings of other studies carried out with the same species and protein source with supplementation of synthetic amino acids, in which the daily weight gain was higher in fish that did not receive FM in the diet [19]. However, when Silva Neto et al. [20] used a diet of 30.86% of digestible protein, together with soybean protein concentrate in replacement of FM, for juveniles of 5.1 g for 61 days, they observed a decrease in the final weight, specific growth rate, and protein efficiency rate; however, the feed conversion was lower in this work. In addition, this study reduced the cost of feed per fish kg by replacing FM with soybean protein concentrate in diets for Nile tilapia fingerlings, corroborating with the data on this research. It is possible that the improved performance in the Do Espírito Santo et al. [19] study resulted from the adequate balance of amino acids, allowing a corresponding adequacy in species development.

The lower fish growth rate in the control (FM) group could be attributed to lower N retention, consequently lower retention of amino acids. This tendency leads to less protein deposition, as well as increased production costs and nitrogen excretion. It is likely that the diet containing soybean meal provides a better supply (balance and/or digestibility) of nutrients, consequently allowing higher use and N retention, which reduces the excessive excretion of N in the environment [5,21].

In this study, no significant difference was observed between treatments in blood variables and immunological parameters. These analyses can also be used to evaluate the effects of diets on animals, nutrients and feed additives. Most of the work on FM replacement is generally limited to assessments of growth performance, dietary efficiency, and production costs [22,23], ignoring its effect on an organism's health, such as immune response and resistance to pathogenic challenge. Today, it is a well-accepted concept in fish farming that the supply of feed containing adequate ingredients can positively affect the maintenance of an animal's health [24].

Nile Tilapia fed diets based on organic soybean showed no hematological variations [25]. In contrast, Dallagnol et al. [26] observed that the supply of diets based on white soybean meal for Nile tilapia did show higher values in hemoglobin than fish fed a diet with FM. In our work, the values of hematological parameters did not differ between treatments and were similar to those found by Martins et al. [27] who reported on tilapia reared in a recirculation aquaculture system with diets containing soybean meal and derivatives.

Fish survival during the experimentation and after infection was not affected by offering the diet by replacing fish meal with soybean meal; because even with the replacement it was possible to offer the nutrients in the adequate and necessary quantity for the good development of the fish immunity. When providing a diet with 32.9% protein per kg of feed, using FM or soybean protein concentrate as the main protein source in the diet to *L. vannamei*, Jatobá et al. [28] obtained good growth performance of animals. The same diets were used by Schleder et al. [10], who did not observe changes in hematological and immunological variables, as well as the replacement of the main protein source did not interfere in the susceptibility of *L. vannamei* to the challenge against Vibrio sp.

The use of soybean peptide, replacing 50% FM, had a positive influence on the immune responses for yellow catfish (*Pelteobagrus fulvidraco*) [29]. Also, for Nile tilapia fingerlings fed with a diet containing 66% replacement of FM for protein isolation of methylated soybean for 10 weeks, an improvement was observed in fish resistance against *A. hydrophila* [30]. These studies reported the benefits of substituting fishmeal with soybean; even though these studies used only partial substitution, unlike the present study which evaluated the effect of total replacement of FM by soybean. Finally, the absence of hematological changes between treatments, when added to the absence of mortality during the experiment and after the experimental infection, suggests that the replacement of fish meal with soybean meal does not impair the health of fish or alter homeostasis. Hence, this fact suggests that the changes in N retention and cost are exclusively related to the protein sources used.

Conclusions

Based on our results, the total replacement of diets with 15% fishmeal does not compromise growth performance, making it possible to reduce dietary costs by using soybean meal as a substitute. Besides it improved the use of the diet with increased nitrogen retention by Nile tilapia juveniles, despite the replacement of ingredients, the nutrient balance was proportionally maintained between the diets. After experimental infection by *Aeromonas hydrophila*, the health status of the fish was not compromised by the replacement.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: M. P. Vilvert, A. Jatobá; *Data curation*: L. Stockhausen, M. P. Vilvert, M. da Silva, A. Dartora, R. Krainz, G. B. Ferreira, L. R. da Silva; *Formal Analysis*: L. Stockhausen, M. P. Vilvert, A. Jatobá; *Funding acquisition*: M. P. Vilvert; *Investigation*: L. Stockhausen, M. da Silva, A. Dartora, R. Krainz, G. B. Ferreira, L. R. da Silva; *Methodology*: L. Stockhausen, M. P. Vilvert, A. Jatobá; *Project administration*: L. Stockhausen, M. P. Vilvert; *Resources*: M. P. Vilvert; *Supervision*: A. Jatobá; *Validation and Visualization*: L. Stockhausen, M. P. Vilvert; *Writing* (original draft, proofreading and editing): L. Stockhausen, A. Jatobá.

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