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Zootechnical parameters and hepatosomatic index of two genetic strains of Nile tilapia fed with β-Glucan + Mannan oligosaccharide (βG+MOS)

Parâmetros zootécnicos e índice hepatossomático de duas linhagens genéticas de tilápia do Nilo alimentadas com β-Glucano + Mananoligossacarídeo (βG+MOS)

Cindy Namie Seino Leal[1]*; Ed Christian Suzuki de Lima[1]; Carlos Antônio Lopes de Oliveira[2]; Ricardo Pereira Ribeiro[2]; Angela Rocio Poveda-Parra[3]; Eduardo Keiti Machida[4]; Vinícius Alves Prestes[4]; Alesi Cristian Lopes[4]; Ulisses de Pádua Pereira[5]; Nelson Mauricio Lopera-Barrero[6]

Highlights

Condition factor showed no differences, but adequate health and growth were observed.
Hepatosomatic index showed no differences, but proportional size was observed.
There were no differences in the other zootechnical parameters evaluated.
The results were positive for the use of the test strain in breeding programs.
The results serve as the starting point for further research.

Abstract

The increasing expansion and the rising demand for food require a greater intensification in the production of Nile tilapia (*Oreochromis niloticus*), and genetic improvement programs are essential to achieve this goal. In managing these programs, new genetic material is introduced after several generations of selections to reduce inbreeding and enhance genetic diversity. In this sense, analyzing

[1] Students of the Doctorate Course of the Postgraduate Program in Animal Science, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: cindynamie.seino@uel.br; edchris7@hotmail.com
[2] Profs. Drs., Department of Animal Science and Postgraduate Program in Animal Science, Universidade Estadual de Maringá, UEM, Maringá, PR, Brazil. E-mail: caloliveira@uem.br; rpribeiro@uem.br
[3] Profª Drª, Department of Animal Science, UEL, Londrina, PR, Brazil. E-mail: angelapoveda@uel.br
[4] Students of the Graduate Course in Animal Science, UEL, Londrina, PR, Brazil. E-mail: eduardo.keiti.machida@uel.br; viniciusalvesprestes@outlook.com; alesi.cristian@uel.br
[5] Prof. Dr., Department of Preventive Veterinary Medicine and Postgraduate Program in Animal Science, UEL, Londrina, PR, Brazil. E-mail: upaduapereira@uel.br
[6] Prof. Dr., Department of Animal Science and Postgraduate Program in Animal Science, UEL, Londrina, PR, Brazil. E-mail: nmlopera@uel.br
* Author for correspondence

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the zootechnical parameters of these introductions, with an emphasis on the use of nutritional additives, is of great importance for understanding their impact on breeding programs. Thus, the aim of this study was to evaluate the zootechnical parameters and hepatosomatic index of two genetic strains of Nile tilapia from the genetic improvement program fed with β-Glucan + Mannan oligosaccharide (βG+MOS). This study utilized a 2 × 2 factorial design containing two genetic groups and two treatments with βG+MOS (Control, 0.2%). The groups were formed from the 12th generation of the Tilamax genetic improvement program (TILAMAX) and by crossing Tilamax with an introduced test strain (CBTILAMAX). In total, 96 tilapias (31.1 ± 5.73 g) were distributed into 16 aquariums, with 6 fish per aquarium, 24 per group, and four repetitions. The experimental period lasted for 41 days, and the water parameters were measured daily and maintained under ideal conditions. The condition factor and hepatosomatic index were analyzed. Final weight, total length, and standard length were measured, and other zootechnical parameters were calculated based on these data. The condition factor showed that the fish in both groups exhibited adequate health and growth during the experiment, with no differences in strain or use of additives. Similarly, the hepatosomatic index did not differ between the groups, indicating that liver size was proportional to fish body size. Similarly, no significant differences (P>0.05) were observed for the other zootechnical parameters evaluated (initial and final total and standard lengths, final weight, total weight gain, condition factor using total and standard lengths, and specific growth rate). Based on these results, it is concluded that there were no differences between TILAMAX and CBTILAMAX related to performance and health under the effect of feeding with βG+MOS. This result is positive regarding the introduction of the test strain into the Tilamax genetic improvement program and establishes a starting point for further research that may provide greater clarity on its future effects.
Key words: Genetic breeding program. Strain differentiation. Nutritional additive. Prebiotic. Immunomodulators.

Resumo

A crescente expansão e a demanda crescente por alimentos exigem uma maior intensificação na produção de tilápia-do-Nilo (*Oreochromis niloticus*), e programas de melhoramento genético são essenciais para alcançar esse objetivo. Na gestão desses programas, novos materiais genéticos são introduzidos após várias gerações de seleções para reduzir a consanguinidade e aumentar a diversidade genética. Nesse sentido, analisar os parâmetros zootécnicos dessas introduções, com ênfase no uso de aditivos nutricionais, é de grande importância para entender seu impacto nos programas de melhoramento. Assim, o objetivo deste estudo foi avaliar os parâmetros zootécnicos e o índice hepatossomático de duas linhagens genéticas de tilápia-do-Nilo de um programa de melhoramento genético alimentadas com β-Glucano + Mananoligossacarídeo (βG+MOS). Este estudo utilizou um delineamento fatorial 2 × 2 contendo dois grupos genéticos e dois tratamentos com βG+MOS (Controle, 0,2%). Os grupos foram formados a partir da 12ª geração do programa de melhoramento genético Tilamax (TILAMAX) e pelo cruzamento do Tilamax com uma linhagem de teste introduzida (CBTILAMAX). No total, 96 tilápias (31,1 ± 5,73 g) foram distribuídas em 16 aquários, com 6 peixes por aquário, 24 por grupo, e quatro repetições. O período experimental durou 41 dias, e os parâmetros da água foram medidos diariamente, mantidos em condições ideais. O fator de condição e o índice hepatossomático também foram analisados. Peso final, comprimento total e comprimento padrão foram medidos, e outros parâmetros zootécnicos foram

calculados com base nesses dados. O fator de condição mostrou que os peixes em ambos os grupos exibiram saúde e crescimento adequados durante o experimento, sem diferenças entre as linhagens ou uso de aditivos. Da mesma forma, o índice hepatossomático não diferiu entre os grupos, indicando que o tamanho do fígado era proporcional ao tamanho do corpo dos peixes. Da mesma forma, não foram observadas diferenças significativas (P>0,05) para os outros parâmetros zootécnicos avaliados (comprimentos total e padrão iniciais e finais, peso final, ganho de peso total, fator de condição usando comprimentos total e padrão, e taxa de crescimento específica). Com base nesses resultados, conclui-se que não houve diferenças entre TILAMAX e CBTILAMAX relacionadas ao desempenho e saúde sob o efeito da alimentação com βG+MOS. Este resultado é positivo em relação à introdução da linhagem de teste no programa de melhoramento genético Tilamax e estabelece um ponto de partida para futuras pesquisas que possam fornecer maior clareza sobre seus efeitos futuros.
Palavras-chave: Programa de melhoramento genético. Diferenciação de linhagens. Aditivo nutricional. Prebiótico. Imunomoduladores.

In recent years, aquaculture has been gaining ground in the animal protein production sector, reaching a record 122.6 million tons, with Nile tilapia (*Oreochromis niloticus*) being the third most-produced fish in the world (Food and Agriculture Organization [FAO], 2022). Tilapia is the primary species produced in Brazil, accounting for 65.3% of the national freshwater fish production in 2023. This scenario positions Brazil as the fourth largest global producer, significantly influencing the country's economy, primarily due to socioeconomic impacts and the growing focus on sustainable production (Peixe BR, 2024).

Genetic breeding programs aiming to identify animals with accelerated growth and superior genetic value for economically important traits have been prominent to enhance the productive efficiency of tilapia farming. In Brazil, the first Nile tilapia breeding program conducted in a rearing environment, currently in its 15th generation of selection, was Tilamax/UEM, which was initiated in 2005 at the State University of Maringá (Oliveira et al., 2012).

However, fixation or loss of genes of zootechnical importance can occur in genetic improvement programs conducted over many generations. Through the crossbreeding of new individuals with the main lineage of the breeding program, efforts are made to maintain genetic variability, reduce inbreeding, and improve the availability of genes of interest, which are crucial for ensuring adequate genetic gain per generation and satisfactory expression of performance traits. In this regard, an initial assessment of the potential of these introductions is through the evaluation of performance parameters, with an emphasis on the use of nutritional additives, which indicates how the new strain behaves from a zootechnical perspective.

Among the prebiotic nutritional additives used in aquaculture, glucans, which are macromolecules found in yeast and fungal cells, have been highlighted for their role in the animal's innate immunity (Vijayaram et al., 2022). Studies also show that the inclusion of β-Glucan can improve growth and an increase in survival rate after bacterial infection (Dou

et al., 2023). Mannan oligosaccharide (MOS) is also a representative of this category, which promotes binding to the cell wall of pathogenic microorganisms, preventing colony formation, and may also improve growth performance, immunity, antioxidant capacity, and intestinal health (Xue et al., 2022).

Based on the presented information, the aim of this study was to evaluate the zootechnical parameters and hepatosomatic index of two genetic strains of Nile tilapia from the genetic improvement program fed with βG+MOS. All animal procedures were approved by the Ethics Committee on the Use of Animals of the State University of Londrina (CEUA Approval no. 040.2021) and the Ethics Committee on the Use of Animals of the State University of Maringá (CEUA Approval no. 5121251021).

The animals used were specimens of Nile tilapia provided by the Tilamax/UEM genetic breeding program at the UEM/CODAPAR fish farming station located in the district of Floriano (Latitude 23°25'11"S and longitude 51°56'23"W) and animals from a test strain (confidential strain which is currently under study) with no genetic link to the Tilamax/UEM strain. Mating to obtain the first group (referred to as TILAMAX in this study) was conducted exclusively using Tilamax/UEM strain fish from the 12th generation of genetic breeding. Animals from the second group (CBTILAMAX) were produced by directed mating of four crossbred males (1/2 Tilamax/UEM - 1/2 test strain) and four females Tilamax/UEM.

Commercial feed for juvenile Nile tilapia was used (460 g kg^{-1} crude protein, 80 g kg^{-1} ether extract, 30 g kg^{-1} crude fiber, and 140 g kg^{-1} ash). The βG + MOS additive was incorporated into the feed by diluting it in 40 mL of distilled water with 40 mL of a fixing binder containing carboxymethylcellulose (Universal Vansil, Descalvado, SP, Brazil), which was subsequently homogenized and evenly distributed by spraying throughout the feed. The incorporation was performed at a dose of 2 g kg^{-1} (0.2%). The control sample did not contain any additives.

A total of 96 fish were transported in plastic bags to the laboratory of the Center for Study and Research in Aquaculture and Genetics (NEPAG) at the State University of Londrina (UEL; latitude 23°19'31"S and longitude 51°11'59"W). Upon arrival, the bags were acclimatized to the water temperature of the 16 available aquariums (42 L) for 20 minutes and the fish were subsequently released. The temperature was maintained at a constant value using a space heater. Renewal of 50% of the aquarium water using dechlorinated water and assessment of water quality parameters (temperature (°C), pH, dissolved oxygen (mL L^{-1}), and ammonia (mg L^{-1})) were conducted. The fish were acclimatized to the experimental conditions for 10 days before starting the experiment. After this, they were anesthetized with 0.1 g L^{-1} benzocaine (0.1 g mL^{-1} of 96° alcohol in 10 L of water) (Wedemeyer, 1970), immobilized with damp towels to reduce stress, weighed, measured, and returned to the aquariums.

A completely randomized design was used in a 2 × 2 factorial arrangement, with the inclusion and non-inclusion of βG+MOS (Control and 0.2%) and two genetic groups (TILAMAX and CBTILAMAX). Four replicates were used, with 6 fish per aquarium (16) and 24 fish per group, totaling 96 juveniles with an average initial weight of 31.1 ± 5.73 g. During

the experimental period (41 days), the fish were manually fed twice a day (at 10:00 a.m. and 3:00 p.m.) until they appeared satiated, and the temperature, pH, and dissolved oxygen were measured daily. Ammonia levels (mg L^{-1}) were measured thrice per week. The water parameters were measured using a multiparameter device (Hanna Instruments, Barueri, SP, Brazil), pH meter (Akso, São Leopoldo, RS, Brazil), and commercial kit for colorimetric assay (Labcon Test Amônia Tóxica Água Doce, Alcon Pet, Camboriú, SC, Brazil). The parameters of temperature (26.0 ± 3.15 °C), pH (7.2 ± 0.27), dissolved oxygen (5.02 ± 2.50 mg L^{-1}), and ammonia (1.10 ± 0.54 mg L^{-1}) remained within the requirements suitable for the species (Chakraborty & Banerjee, 2010; Dwiardani & Rahardja, 2021). As the experiment progressed, the aquarium water was renewed at a rate of 50–90% twice a day to ensure better control of the water parameters, especially ammonia levels.

At the end of the experimental trial and after fasting for 15 h, biometrics were performed on all 96 fish. The fish were anesthetized with 0.1 g L^{-1} benzocaine (0.1 g mL^{-1} of 96° alcohol in 10 L of water) and then immobilized with damp towels to minimize stress during measurements and for subsequent procedures. Final weight (g), total length (cm), and standard length (cm) were measured. Based on these data, the following parameters were calculated: Condition factor using total length (CF_{TL}, %) [CF_{TL} = (final weight / final total length $(cm)^3$) × 100], Condition factor using standard length (CF_{SL}, %) [CF_{SL} = (final weight / final standard length $(cm)^3$) × 100], Final total length (cm), Final standard length (cm), Final weight (g), Total weight gain (g) [final weight − initial weight], and Specific growth rate (% g day^{-1}) [(Ln final weight (g) − Ln initial weight (g)) / Experimental period in days] × 100]. All parameters were analyzed using the same methods as those described by Lopera-Barrero et al. (2024). After anaesthesia, all fish were euthanized through spinal sectioning. After confirming death, a ventral incision was made in the animals, and the weight of each specimen's liver was determined. This weight was used to calculate the hepatosomatic index (HSI (%)) (HSI = [liver weight / fish weight] × 100).

The data were subjected to two-way analysis of variance with a significance level of 0.05. The statistical analyzes were carried out using the R studio program (version 3.1.4).

There was no statistical effect ($P>0.05$) of strain, additives, or their interaction on the final weight, standard, and total length (Table 1). Analysis of CF_{TL} and CF_{SL} (Table 1) revealed no differences ($P>0.05$) between the tested strains (TILAMAX × CBTILAMAX), with no influence of the treatments. Values higher than 1.0 were observed (CF_{TL}: 2.07–2.20; CF_{SL}: 3.57–3.74), suggesting good health conditions, adequate feeding conditions, and isometric growth, desirable situations in farmed fish (Kwikiriza et al., 2023). Similarly, no differences were observed in the hepatosomatic index with respect to strain or additive use (Table 1), indicating that liver size was proportional to fish body size. This suggested that there was no retention or expenditure of the liver lipid source, indicating that digestion and nutrient absorption processes were equal between the two strains during the experiment.

Table 1
Effects of β-Glucan + Mannan oligosaccharide (βG+MOS; additive) and genetic strain on condition factor, hepatosomatic index and zootechnical parameters (mean ± standard error) in Nile tilapia juveniles

Variable [I]	Additive [II]	Strain		Mean	*P*-value		
		TILAMAX	CBTILAMAX		Additive	Strain	A x S
CFTL (%)	Control	2.20 ± 0.12	2.12 ± 0.03	2.16 ± 0.06	0.338	0.915	0.288
	0.2%	2.07 ± 0.03	2.15 ± 0.02	2.11 ± 0.02			
	Mean	2.14 ± 0.23	2.13 ± 0.02				
CFSL (%)	Control	3.72 ± 0.21	3.63 ± 0.09	3.68 ± 0.11	0.845	0.757	0.276
	0.2%	3.57 ± 0.05	3.74 ± 0.05	3.66 ± 0.04			
	Mean	3.65 ± 0.11	3.69 ± 0.05				
FTL (cm)	Control	15.9 ± 0.31	16.5 ± 0.27	16.2 ± 0.21	0.855	0.491	0.154
	0.2%	16.4 ± 0.28	16.2 ± 0.26	16.3 ± 0.17			
	Mean	16.21 ± 0.21	16.4 ± 0.19				
FSL (cm)	Control	13.4 ± 0.27	13.9 ± 0.25	13.6 ± 0.19	0.795	0.573	0.158
	0.2%	13.7 ± 0.27	13.5 ± 0.23	13.6 ± 0.17			
	Mean	13.5 ± 0.19	13.7± 0.17				
FW (g)	Control	89.1 ± 4.01	99.1 ± 4.82	94.10 ± 3.44	0.942	0.373	0.219
	0.2%	94.5 ± 5.72	92.9 ± 3.94	93.76 ± 3.44			
	Mean	91.84 ± 3.48	96.02 ± 3.11				
TWG (g)	Control	58.1 ± 3.01	64.28 ± 4.35	61.91 ± 2.65	0.743	0.847	0.217
	0.2%	64.8 ± 5.70	60.3 ± 3.69	62.60 ± 3.37			
	Mean	61.4 ± 3.22	62.3 ± 2.84				
SGR (% g day^{-1})	Control	2.64 ± 0.07	2.59 ± 0.11	2.61 ± 0.06	0.308	0.244	0.439
	0.2%	2.86 ±0.18	2.62 ± 0.12	2.74 ± 0.11			
	Mean	2.75 ± 0.10	2.60 ± 0.08				
HSI (%)	Control	2.45 ± 0.10	2.36 ± 0.16	2.41 ± 0.09	0.932	0.472	0.913
	0.2%	2.48 ± 0.15	2.35 ± 0.20	2.42 ± 0.12			
	Mean	2.47 ± 0.09	2.36 ± 0.13				

[I]CF_{TL}: condition factor using total length; CF_{SL}: condition factor using standard length; FTL: final total length; FSL: final standard length; FW: final weight; TWG: total weight gain; SGR: specific growth rate; HSI: hepatosomatic index
[II]Control: Commercial feed for juvenile Nile tilapia; 0.2%: inclusion of βG + MOS in the diet
Note: The initial weight, initial total length, and initial standard length were 31.1 ± 5.73 g, 11.40 ± 0.83 cm and 9.42 ± 0.69 cm respectively.

The condition factor is an assessment measure that analyzes the physical condition and well-being of fish based on the relationship between body weight and organism length and reflects recent feeding conditions and/or expenditure of reserves in cyclical and growth activities (Vazzoler, 1996). When a fish of a certain length has a higher weight, it signifies better condition (Ameh et al., 2020). In contrast, the hepatosomatic index is a parameter used to assess the health and physiological condition of fish liver, widely employed as a bioindicator (Baghel & Reddy, 2022) in reproductive characteristics (Melillo et al., 2020) and in evaluating environmental and nutritional effects (Gonçalves et al., 2022).

The absence of differences observed between the genetic strains for the condition factor, hepatosomatic index, and zootechnical parameters suggested that the introduction of the test strain into the Tilamax/UEM genetic improvement program did not result in differences in growth, performance, and health. This initial indication demonstrates its potential for use. These results form the basis for the use of the test strain in the improvement program for the upcoming selection processes.

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