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Dry biomass of *Aspergillus niger* in commercial diets for juveniles of Nile tilapia (*Oreochromis niloticus*) in the intermediate growth phase (15 to 70g)

Biomassa seca de *Aspergillus niger* em dietas comerciais para juvenis de tilápia-do-Nilo (*Oreochromis niloticus*) na fase de pré-engorda (15 a 70g)

Biomasa seca de *Aspergillus niger* en dietas comerciales para juveniles de tilapia del Nilo (*Oreochromis niloticus*) en fase de preceba (15 a 70g)

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Abstract
The objective of the present study was to evaluate different inclusion levels of drymass of the yeast *Aspergillus niger* (0; 0.125; 0.250; 0.500; 1.00; 2.00; 4.00 and 8.00%) in commercial diets for juveniles of Nile tilapia (16.11 ± 1.74g) in a water recirculation system. The productive performance, organosomatic indexes and carcass composition of these animals were evaluated after 58 days of experiment. A total of 192 juveniles were distributed among 32 tanks made up of eight treatments with four repetitions. A significant difference was observed for the hepatosomatic index at 2.00% of inclusion, which was similar to the other treatments except for the 0.125% inclusion level. The inclusion of *A. niger* had no negative impacts on performance when compared with the control diet. Since it is a by-product, the use of *A. niger* can be an alternative in the formulation of diets for tilapia in the initial stages (15-70g).
Keywords: Animal nutrition; *Aspergillus niger*; Productive performance; Yeast.

Resumo

O objetivo do presente trabalho foi avaliar diferentes níveis de inclusão de massa seca da levedura *Aspergillus niger* (0; 0,125; 0,250; 0,500; 1,00; 2,00; 4,00 e 8,00%) em dietas comerciais para juvenis de tilápia do Nilo (16,11 ± 1,74g) em um sistema de recirculação de água. O desempenho produtivo, índices organossomáticos e composição de carcaça desses animais foram avaliados após 58 dias de experimento. Um total de 192 juvenis foram distribuídos em 32 tanques compostos por oito tratamentos com quatro repetições. Foi observada diferença significativa para o índice hepatossomático a 2,00% de inclusão, que foi semelhante aos demais tratamentos, exceto pelo nível de inclusão de 0,125%. A inclusão de *A. niger* não teve impactos negativos no desempenho quando comparada com a dieta controle. Por ser um subproduto, o uso de *A. niger* pode ser uma alternativa na formulação de dietas para tilápias em estágios iniciais (15-70g).

Palavras-chave: Nutrição animal*; Aspergillus niger*; Desempenho produtivo; Levedura.

Resumen

El objetivo del presente trabajo fue evaluar diferentes niveles de inclusión de masa seca de la levadura *Aspergillus niger* (0; 0.125; 0.250; 0.500; 1.00; 2.00; 4.00 y 8.00%) en dietas comerciales para juveniles de tilapia del Nilo (16.11 ± 1,74 g) en un sistema de recirculación de agua. El comportamiento productivo, los índices organosomáticos y la composición de la canal de estos animales se evaluaron después de 58 días de experimento. Se distribuyeron un total de 192 juveniles en 32 tanques compuestos por ocho tratamientos con cuatro repeticiones. Se observó una diferencia significativa para el índice hepatosomático al 2,00% de inclusión, que fue similar a los demás tratamientos, excepto por el nivel de inclusión de 0,125%. La inclusión de *A. niger* no tuvo un impacto negativo en el rendimiento en comparación con la dieta de control. Como subproducto, el uso de *A. niger* puede ser una alternativa en la formulación de dietas para tilapia en estadios tempranos (15-70g).

Palabras clave: Nutrición animal; *Aspergillus niger*; Desempeño productivo; Levadura.

1. Introduction

Based on the growing global demand for fish, the search for efficiency in production systems is vital. Consequently, there is a constant need for improvements in aquaculture feed formulations aiming to both maxismise economic indexes and improve the productive performance and health of the cultivated animals (Costa 2015). Alternative raw materials linked with efficient management is a strategy that may reduce production costs, mainly with feeds. Raw materials used for feeds are the most costly inputs in aquaculture and hinders its expansion (Portz et al. 2001; Guimarães et al. 2008).

In this sense, the addition of yeast in small concentrations to aquaculture feeds has shown improvements in animal health and better productive performance indexes (Meurer et al. 2000; Gonçalves et al. 2010). Filamentous yeast of the genus *Aspergillus spp*. are an important group in the biodegradation processes and naturally occur in the environment, mainly in liquid and solid media (Rodrigues et al. 2006). The *Aspergillus niger* species is composed of colonies of dark brown to black color, bisexual sterigma with prominent vesicles and unevenly rough conidia (Kozakiewicz et al. 1992; Raper et al., 1965; Nascimento et al. 2018). This yeast is used in the food, textile, cellulose, paper, ethanol and feed industries (Bailey and Ollis 1986; Couto and Sanroman 2006; Shankar et al., 2007), mainly to produce enzymes (amylase, cellulase, phytase, pectinase, among others) as it is easy to manipulate and has high fermentation and citric acid production (Yokoya 1992). The action of exogenous enzymes in fish from microorganisms can increase the digestibility of the feed and improve its utilization, decreasing feed conversion and maximizing performance (Durigon et al. 2018).

Yeast has been used in fish nutrition for years and has shown promising results, mainly for performance (Hisano et al. 2007; Gonçalves et al. 2010; Schwarz et al. 2016; Durigon et al, 2018) and the intestinal (Schwarz et al. 2011; Lima 2014) and liver (Meurer et al. 2009) health of these animals. However, most of these studies were restricted to experimental diets in the pelleted form (Meurer et al. 2000; Baccarin and Pezzato 2001; Meurer et al. 2009). The use of these microorganisms is highlighted in aquaculture diets for their immunostimulating capacity and their inclusion in small amounts in the feed formulations. In addition, they have excellent intrinsic nutritional levels and act as a pro-nutrient (Hisano et al. 2007; Schwarz et al. 2016). Diets with the inclusion of *A. niger* mycelium dry biomass were recently tested for tilapia and showed positive results for productive performance and somatic indexes (Durigon et al. 2018). However, this study was limited to a few

inclusion levels and with animals up to of 50g. Hence it is necessary to investigate the use of *A. niger* in commercial extruded diets for tilapia of higher final weight and with a greater range of inclusions. Therefore, the present study evaluated the performance, organosomatic indexes and carcass composition of juvenile Nile tilapia (*Oreochromis niloticus*) in the intermediate growth phase (15 to 70g) fed with extruded diets with or without the addition of *A. niger* dry biomass.

2. Methodology

The experiment was carried out at the Aquaculture Laboratory of the University of West Santa Catarina - UDESC Campus Chapecó (Santa Catarina, Brazil) and lasted 58 days. A total of 192 juveniles of Nile tilapia with an average initial weight of 16.11 ± 1.74 g were acquired from commercial fish farms. Each experimental unit was stocked with six animals and distributed in a completely randomized manner, totalling eight treatments with four replications of each.

Different levels of *A. niger* dry biomass using the "drum dried" method were added to the commercial diet at the following inclusion levels (treatments): T0% or control (commercial feed with 0% inclusion), T 0.125 % (commercial feed with 0.125% inclusion), T 0.250% (commercial feed with 0.250% inclusion), T 0.500% (commercial feed with 0.500% inclusion), T 1.00% (commercial feed with 1.00% inclusion), T 2.00% (commercial feed with 2.00% inclusion), T 4.00% (commercial feed with 4.00% inclusion) and T 8.00% (commercial feed with 8.00% of inclusion), extruded in 2-4mm pellets. The ingredients of the basic diet used in the experiment are contained in Table 1.

Table 1. Manufacturer guaranteed levels of nutrients in the base diet used for the inclusion of *Aspergillus niger* dry biomass in commercial feeds for Nile tilapia juveniles.

Nutrient	Concentration
Moisture (Max.)	100.00 g/kg
Crude Protein (Min.)	400.00 g/kg
Ether Extract (Min.)	80.00 g/kg
Fiber (Max.)	50.00 g/kg
Mineral Matter (Max.)	120.00 g/kg
Calcium (Min.)	10.00 g/kg
Calcium (Max.)	30.00 g/kg
Phosphorus (Min.)	10.00 g/kg
Sodium (Min.)	2000.00 mg/kg
Vitamin A (Min.)	11200.00 UI/kg
Vitamin D3 (Min.)	2240.00 UI/kg
Vitamin E (Min.)	128.00 UI/kg
Vitamin K3 (Min.)	15.00 mg/kg
Vitamin B1 (Min.)	16.00 mg/kg
Vitamin B2 (Min.)	16.00 mg/kg
Vitamin B6 (Min.)	16.00 mg/kg
Vitamin B12 (Min.)	16.00 mcg/kg
Biotine (Min.)	0.06 mg/kg
Nicotinic Acid (Min.)	80.00 mg/kg
Panthotenic Acid (Min.)	40.00 mg/kg
Folic Acid (Min.)	5.00 mg/kg
Choline (Min.)	2000.00 mg/kg
Vitamin C (Min.)	600.00 mg/kg
Iodine (Min.)	1.50 mg/kg
Selenium (Min.)	0.30 mg/kg
Iron (Min.)	85.00 mg/kg

Copper (Min.)	11.50 mg/kg
Zinc (Min.)	80.00 mg/kg
Manganese (Min.)	25.50 mg/kg
Cobalt (Min.)	0.50 mg/kg

Min. = minimum; Max. = maximum; g/kg = gram per kg; mg/kg = milligram per kg; mcg/kg = microgram per kg; UI/kg = International unit.
Source: Authors.

The fish were fed three times per day (8:00 am, 1:00 pm and 5:00 pm) at 3.00% of their biomass throughout the experimental period. Biometrics were performed every 15 days for all experimental units. The animals were kept in circular polyethylene tanks (useful volume of 70 L) with aeration from a porous air stone (1x2 cm), coupled to a radial air compressor (ASTEN® model CRC - 2 330 11 SS, 0, 80 kW, 1.10 hp, Single phase, 50/60 Hz, 4.1 A, 2.75 m³ / min, São Paulo / SP, Brazil) and connected to a water recirculation system (RAS). This system was arranged in three groups and connected to three circular tanks (useful volume: 850 L) called "macrocosms" that contained a pump (ELETROPLAS® model ICS - 50AB, 0.37 kW, 60Hz, 0.5 hp, 1, 8m³ / h, 220v, Navegantes / SC, Brazil) responsible for the distribution of water in the system. It also contained a filtration system combined with a mechanical filter (60µm - perlon mesh) and a biological particulate material composed of 0.2m³ of low density polyethylene bio-balls analog and 0.05m³ of expanded clay stone. These materials serve as a substrate for the nitrifying bacteria to carry out nitrification. Each macrocosm was equipped with a heated thermostat (NOBRE BRASIL®, 3000 W, 200v, Ribeirao Preto / SP, Brazil) and a digital temperature controller with a sensor (BY-LOX® 15 A, 2000 W, 200v, China) for maintaining the temperature (28ºC).

Organic residues suspended in the experimental units were siphoned every two days and the water was replaced. The water used for replacement was previously kept in a 500 liter container with aeration to remove residual chlorine. Water quality parameters were measured daily in the morning (8 am) and afternoon (5 pm): temperature, dissolved oxygen (ALFAKIT® oximeter, model AT170, Florianópolis-SC, Brazil) and pH (pH meter ALFAKIT®, model AT315, Florianópolis-SC, Brazil). The nitrogen compounds (ammonia, nitrite, nitrate) and alkalinity of the cultures were analyzed weekly using a photocolorimeter (ALFAKIT®, model AT100P, Florianópolis-SC, Brazil).

After the 58 days of the experiment, the animals were fasted for 12 hours and were individually weighed (precision scale 0.01 g, Mars ML 600, São Paulo, Brazil) and measured for final weight (Pf), final total length (CTf), average weight gain (GPM), specific growth rate (TBI), final biomass (Bf), apparent feed conversion (CAA), condition factor (FC), productivity (P) and survival (S). Finally, the fish were sedated with eugenol and sacrificed by spinal section upon showing no movement as described by the National Council for Animal Experimentation Control (CONCEA). Eight fish were randomly selected per treatment to weigh carcasses without viscera, digestive tract, liver, visceral fat, spleen and gonads. All of these collections were performed to measure organosomatic indexes: RC carcass yield (eviscerated weight / whole weight x 100), viscerosomatic index (digestive tract weight / total weight x 100), IHS hepatosomatic index (liver weight / total weight x 100), IGS visceral fat index (fat weight / total weight x 100), IES splenosomatic index (spleen weight / total weight x 100) and IGS gonadosomatic index (gonad weight / total weight x 100), respectively (Picoli et al., 2019).

The carcasses devoid of viscera from the eight animals were separated by treatment to perform the bromatological composition analyses. From these, the contents of dry matter (DM), crude protein (PB) and mineral matter (MM) were measured according to the AOAC (1999) with the fat content (ether extract - EE) determined according to Bligh and Dyer (1959). These analyses were performed at the Animal Nutrition Laboratory (LANA) at the State University of Santa Catarina - UDESC Campus Chapecó, Santa Catarina, Brazil.

Descriptive statistics were performed with the means and standard deviations of the water quality parameters of each treatment and each macrocosm, as well as the bromatological composition of the carcasses. The rest of the data (productive performance and organosomatic) was analyzed for normality using the Kolmogorov-Smirnov test and for the homogeneity of variances using the Levene test (Sokal and Rohlf, 1995). Then, means were compared by one-way ANOVA and for significant effects using the Tukey test ($P < 0.05$) (Sampaio, 1998).

3. Results

The mean and standard deviation of the water quality parameters evaluated during the experimental period of each treatment and macrocosm can be seen in Table 2. No fluctuations were observed for these parameters, showing consistent management of the systems throughout the experimental period.

Table 2. Water quality parameters (mean ± standard deviation) of Nile tilapia juveniles fed with feeds containing different inclusion levels of *Aspergillus niger* dry biomass.

	Treatment (% inclusion of dry biomass)										
Variable	**M1**	**M2**	**M3**	**0**	**0.125**	**0.250**	**0.500**	**1.00**	**2.00**	**4.00**	**8.00**
T^a (°C)	25.27 (±1.96)	25.27 (±2.08)	25.26 (±2.16)	25.34 (±1.97)	25.22 (±2.33)	25.22 (±2.33)	24.89 (±3.46)	25.15 (±2.22)	25.31 (±2.06)	25.16 (±2.20)	25.40 (±2.04)
DO (mg/L^{-1})	7.90 (±1.23)	7.77 (±1.10)	7.78 (±1.07)	8.04 (±1.17)	8.27 (±1.55)	8.27 (±1.55)	8.12 (±1.16)	8.00 (±0.97)	7.96 (±0.95)	7.92 (±0.95)	7.92 (±0.89)
pH	7.13 (±0.37)	7.08 (±0.40)	7.06 (±0.41)	7.08 (±0.32)	7.08 (±0.35)	7.08 (±0.35)	7.07 (±0.36)	7.06 (±0.34)	7.06 (±0.33)	7.08 (±0.37)	7.07 (±0.33)
NH_4 t (mg/L^{-1})	0.40 (±0.50)	0.45 (±0.51)	0.21 (±0.26)	0.46 (±0.79)	0.56 (±0.70)	0.56 (±0.70)	0.54 (±0.76)	0.70 (±0.69)	0.61 (±0.83)	0.56 (±0.80)	0.59 (±0.73)

M1 = Macrocosm 1; M2 = Macrocosm 2; M3 = Macrocosm 3; T^a = Temperature; DO = Dissolved Oxygen; pH = Hydrogenic Potential; NH_4 t = Total Ammonia. Source: Authors.

There were no significant changes in the zootechnical performance variables assessed during the experiment, as shown in Table 3.

Table 3. Productive performance (mean ± standard deviation) of Nile tilapia juveniles fed with feeds containing different inclusion levels of *Aspergillus niger* dry biomass.

	Treatment (% inclusion of dry mass)								
Variable	**0**	**0.125**	**0.250**	**0.500**	**1.00**	**2.00**	**4.00**	**8.00**	***p***
Wi (g)	16.03 (±0.30)	16.10 (±0.27)	16.46 (±0.24)	15.92 (±0.27)	16.13 (±0.38)	15.81 (±0.27)	16.14 (±0.49)	16.19 (±0.41)	0.340
Wf (g)	59.95 (±11.31)	62.18 (±15.03)	62.78 (±17.58)	66.31 (±12.32)	62.12 (±18.23)	67.74 (±10.47)	64.04 (±13.04)	60.33 (±12.98)	0.604
TLf (cm)	15.15 (±1.08)	15.06 (±1.08)	15.07 (±1.23)	15.36 (±0.85)	15.19 (±1.31)	15.65 (±0.93)	15.32 (±1.01)	15.20 (±1.15)	0.704
MWG (g)	43.92 (±12.02)	46.84 (±15.00)	46.41 (±17.55)	50.50 (±11.70)	46.13 (±17.88)	51.92 (±11.55)	47.98 (±13.40)	44.42 (±12.93)	0.588
SGR (%day)	7.57 (±5.70)	8.90 (±18.47)	7.93 (±8.07)	9.01 (±8.36)	7.94 (±2.01)	8.95 (±7.46)	8.20 (±6.95)	7.62 (±5.42)	0.370
Bf (g)	344.71 (±33.66)	323.23 (±30.33)	361.02 (±55.65)	309.47 (±81.61)	357.23 (24.38±)	406.46 (±26.87)	352.23 (±87.24)	301.69 (±73.13)	0.357
AFC	1.18 (±0.10)	1.32 (±0.20)	1.14 (±0.10)	1.31 (±0.31)	1.17 (±0.07)	0.98 (±0.06)	1.15 (±0.18)	1.20 (±0.12)	0.174
CF	1.60 (±0.23)	1.81 (±0.02)	1.82 (±0.06)	1.91 (±0.10)	1.77 (±0.06)	1.76 (±0.10)	1.78 (±0.07)	1.72 (±0.09)	0.075
P (kg/m³)	4.92 (±0.48)	3.18 (±1.43)	5.15 (±0.79)	3.94 (±0.82)	5.10 (±0.34)	5.80 (±0.38)	5.03 (±1.24)	4.30 (±1.04)	0.113
S (%)	94.44 (±9.62)	83.33 (±8.33)	95.83 (±8.33)	91.66 (±11.78)	95.83 (±8.33)	100 (±0)	100 (±0)	88.88 (±9.62)	0.053

Wi = Initial Weight; Wf = Final Weight; TLf = Final Total Length; MWG = Mean Weight Gain; SGR = Specific Growth Rate; Bf = Final Biomass; AFC = Apparent Feed Conversion; CF = Condition Factor; P = Productivity; S = Survival. *p* = value of *p*. Source: Authors.

In the case of organosomatic indexes, there was a significant difference for the hepatosomatic index. The lowest value found for this variable was in the 2.00% treatment, which did not differ from the control (0%), 0.250%, 0.500%, 1.00%, 4.00% 8.00%. The 0.125% treatment had the highest value for IHS but only differed from the 2.00% inclusion treatment. The other variables (RC, IVS, IES, IGV, IGS) showed no significant changes over the experimental period (Table 4).

Table 4. Organosomatic indexes (mean ± standard deviation) of Nile tilapia juveniles fed with feeds containing different inclusion levels of *Aspergillus niger* dry biomass.

	Treatment (% inclusion of dry mass)								
Variable	**0**	**0.125**	**0.250**	**0.500**	**1.00**	**2.00**	**4.00**	**8.00**	***p***
CY (%)	86.22 (±6.48)	88.78 (±14.40)	87.04 (±23.57)	89.97 (±3.21)	84.09 (±15.69)	91.38 (±4.82)	77.95 (±14.23)	92.41 (±15.61)	0.859
HSI (%)	2.72^{ab} (±0.59)	3.46^{b} (±1.32)	2.82^{ab} (±0.82)	2.64^{ab} (±0.25)	2.14^{ab} (±0.42)	1.59^{a} (±0.49)	2.14^{ab} (±0.60)	2.21^{ab} (±0.60)	0.038
VSI (%)	3.37 (±1.03)	3.36 (±0.42)	3.46 (±0.23)	2.93 (±0.34)	2.79 (±0.75)	3.72 (±0.55)	2.93 (±0.71)	3.33 (±0.95)	0.513
SSI (%)	0.19 (±0.03)	0.13 (±0.05)	0.18 (±0.06)	0.13 (±0.04)	0.10 (±0.08)	0.12 (±0.03)	0.12 (±0.03)	0.15 (±0.07)	0.209
VFI (%)	1.05 (±0.38)	1.18 (±0.26)	1.19 (±0.47)	0.84 (±0.25)	0.62 (±0.33)	0.68 (±0.02)	0.92 (±0.32)	1.03 (±0.28)	0.133
GSI (%)	0.69 (±0.28)	0.98 (±0.44)	1.30 (±0.58)	1.30 (±0.22)	0.93 (±0.42)	0.87 (±0.33)	0.88 (±0.37)	0.99 (±0.77)	0.567

CY = Carcass Yield; HSI = Hepatosomatic Index; VSI = Viscerosomatic Index; SSI = Splenosomatic Index; VFI = Visceral Fat Index; GSI = Gonadosomatic Index. *p* = value of *p*. Different letters indicate significant difference according to the Tukey test (5%). Source: Authors.

The proximate composition of the carcasses in this experiment showed means of 28.07 g / 100g, 16.26 g / 100g, 0.68 g / 100g and 0.15 g / 100g for dry matter, crude protein, ether extract and mineral matter, respectively (Table 5).

Table 5. Descriptive statistics (mean ± standard deviation) of carcass compositions of Nile tilapia juveniles fed with feeds containing different inclusion levels of *Aspergillus niger* dry biomass.

	Treatment (% inclusion of dry mass)							
Variable	**0**	**0.125**	**0.250**	**0.500**	**1.00**	**2.00**	**4.00**	**8.00**
DM (%)	28.50 (±0.42)	30.13 (±0.19)	28.03 (±0.19)	26.64 (±0.40)	27.95 (±0.64)	27.65 (±0.39)	27.47 (±0.40)	28.20 (±0.42)
EE (g)	0.58 (±0.05)	0.72 (±0.07)	0.62 (±0.09)	0.71 (±0.08)	0.94 (±0.34)	0.73 (±0.28)	0.60 (±0.11)	0.54 (±0.02)
CP (%)	18.87 (±0.82)	12.67 (±6.46)	17.08 (±0.44)	14.10 (±4.97)	12.31 (±3.48)	16.81 (±5.98)	18.99 (±1.92)	19.30 (±2.50)
MM (g)	0.15 (±0.05)	0.17 (±0.07)	0.15 (±0.03)	0.14 (±0.07)	0.17 (±0.04)	0.17 (±0.04)	0.16 (±0.02)	0.16 (±0.05)

DM = Dry Matter; EE = Ether Extract; CP = Crude Protein; MM = Mineral Matter. Source: Authors.

4. Discussion

The physical-chemical parameters of the cultivation water (Table 2) remained adequate and within the expected standard for the species studied, as well as for tropical fish (Arana 2004; El-Sayed 2020). Promising results have already been reported for the inclusion of fungi in diets of different fish species (Hisano et al. 2007; Meurer et al. 2009; Gonçalves et al. 2010; Schwarz et al. 2011; Lima 2014; Schwarz et al. 2016; Durigon et al. 2018), reinforcing the possibility that *A. niger* may have improved the productive performance of animals, as well as, promoted and contributed to their health. In the present study, the different levels of *A. niger* dry biomass inclusion had no effect on the performance of tilapia when compared to the control treatment.

Gonçalves et al. (2010) used whole yeast and *Saccharomyces cerevisiae* derivatives for animals of the same species (52.1g) and observed improvements in weight gain and in the specific growth rate and with no changes in feed conversion and protein efficiency rate. Durigon et al. (2018) evaluated the inclusion of *A. niger* in commercial feed extruded for Nile tilapia of 50g and observed a quadratic effect for final weight, weight gain, feed conversion, carcass yield, specific growth rate, hepatosomatic index and visceral fat index. However, no significant effects were observed for length, feed intake, survival, condition factor, and splenosomatic and gonadosomatic indexes. The authors suggested that the optimal inclusion levels of this yeast in commercial extruded diets are between 3.7% and 4.1%. Differences between the present study and the prior study may be related to a longer evaluation period used here (58 days vs 42 days) and the genetics of the animals used. However, the performance results were similar between the two studies. It is worth noting that the apparent feed conversion of the animals in the present study presented excellent results for the species and life stage in all treatments and the control. Similar results can be observed in other studies with the supplementation of fungi in tilapia diets (Gaiotto 2005; Pezzato et al. 2006; Hisano et al. 2007; Hisano et al. 2008; Carvalho et al. 2011, Schwarz et al. 2016).

Furuya et al. (2000) suggested that the addition of 14% yeast to sex reversed Nile tilapia fingerling diets is the optimal inclusion level. The inclusion of yeasts in monogastric diets must be carried out carefully and in small concentrations (Meurer et al. 2000), since they have a high composition of nucleic acids in their cell wall and high uptake of nucleic acids may cause toxicity (Schulz and Oslage 1976). However, toxicity resulting from nucleic acids has not yet been reported in fish, perhaps due to the high uricase activity in the liver of these animals (Rumsey et al. 1991; Peres and Oliva-Teles 2003). Nevertheless, correct dosages can increase the digestibility of nutrients through exogenous enzymes, in addition to helping in the production of energy and in strengthening the immune system since nucleic acids have considerable concentrations of B vitamins (Deminicis and Martins 2013). High survival rates observed in all treatments of the present study are perhaps due to the appropriate experimental conditions for cultivation and the inclusion of *A. niger* (rich in nucleotides, oligosaccharides and

protein complexes with polysaccharides) in the diets, which may have contributed to the resistant of the fish to pathogens (Sakai et al. 2001; Ortuño et al. 2002; Li and Gatlin 2004).

Organosomatic indexes are important parameters for an accurate assessment of physiological conditions of fish, since different diets can cause changes in the metabolism and physiology of these animals (Santos et al. 2009). They can also be significant identifiers of stressors (Barton et al. 2002). In the present study, no significant changes were observed for carcass yield and the visceral, splenosomatic, viscerosomatic and gonadosomatic fat indexes (Table 4). However, the hepatosomatic index in animals supplemented with 0.125% (3.46%) was significantly higher than in those of the 2.00% (1.56%) treatment, which had the lowest indexes. The 2.00% level of supplementation may have less stress on the liver with a possible protective effect on this organ, in addition to better use of nutrients from the diet as suggested in Durigon et al. (2018).

It is possible that the lowest level *A. niger* (0.125%) was sufficient to stimulate certain metabolic pathways, promoting a greater accumulation of nutrients in the liver. However, supersaturation may have inhibited this same effect (Baldissera et al. 2019). Meurer et al. (2009) also obtained low hepatosomatic indexes in tilapia fed with *S. cerevisiae* yeast (0.1% of commercial feed). Corroborating this, Freccia et al., (2021) when supplementing tilapia with 0.1% *S. cerevisiae* yeast as a probiotic, also observed improvements in liver health and in the oxygenation of the cultured water of Nile tilapia juveniles. cultivated at stocking density of 15 to 25 fish/m³ in RAS.

On the other hand, Schwarz et al. (2010; 2011) found no differences for this index in tilapia fed mannan oligosaccharides. This variable is important because it is directly linked to animal nutrition, in addition to representing hepatic expenditure or accumulation of metabolic reserves (Yogata and Oku 2000). An increase in this index may be related to liver stress (Meurer et al. 2009) from an accumulation of energy in the cytoplasm of liver cells (hepatocytes), whether in the form of glycogen and / or lipids (Wolf et al. 2015). This usually occurs when there is a supply of high energy diets and the animals do not have excessive energy expenditure or when they have a high metabolic rate, although this ability to concentrate glycogen or lipids depends on the species and the diet (Takashima and Hibiya 1995; Wolf et al. 2015).

Most values of centesimal composition of tilapia carcasses, before and after supplementation with *A. niger* (Table 5), are near to those cited by Contreras-Guzmán (2002) for the same species. For dry matter, lipid and crude protein values, these authors cite values between 18 and 26 g / 100g-1, 0.3 and 5.5 g / 100g-1 and 14 and 19 g / 100g-1, respectively, corroborating with our findings (28.07 g / 100g-1, 0.68 g / 100g-1 and 16.26 g / 100g-1). Values between 0.7 and 3.1 g 100g-1 are cited for mineral matter (ash), which were above our findings (0.15 g / 100g-1) perhaps due to the low mineral levels present in the diets provided to animals.

It is worth noting that under natural cultivation conditions, animals are able to absorb the minerals present in the water through the gills. In experimental conditions, they need additional supplementation in feeds so that the requirements of these nutrients are met (Ribeiro et al. 2012). Baccarin and Pezzato (2001) and Gonçalves et al. (2010) observed carcasses with lower levels of crude protein than those of the present study when supplementing the same species with dehydrated alcohol yeast and whole sugarcane yeast, respectively. This result may be attributed to the differences in formulations of the diets. Gonçalves et al. (2010), Li and Gatlin (2003), Gaiotto (2005), Pezzato (2006) and Watanabe (2006) did not observe any relevant changes in the proximate composition of fillets of animals fed with yeast. Thus, they suggest that the yeasts used in the diets had no affect on fish metabolism and the deposition of nutrients in the carcass.

5. Final Considerations

Although no significant changes were observed in the present study for most of the parameters, *A. niger* dry biomass was shown to have no affect on the performance variables evaluated when compared with the control diet. Since it is a by-

product, the use of *A. niger* can be an alternative in the formulation of tilapia diets in the initial stages (15 to 70g). Future studies should be carried out to evaluate this ingredient for the final growth phase and for breeders. More research to enable the use of *A. niger* biomass for other aquatic species and in other cultivation systems (eg biofloc system).

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Conflicts of interest

The authors declare no conflicts of interest.

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