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Effects of salinity and plant-based diet or animal-plant combination diet on the performance and metabolic status of juvenile Nile tilapia

Efeitos da salinidade e de dietas contendo ingredientes de origem vegetal ou de origem animal e vegetal no desempenho e na condição metabólica de juvenis de tilápia do Nilo

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Highlights

Salinity up to 30 g L^{-1} does not affect growth of fish fed animal-plant-based diet.
Salinity increases hepatosomatic index (HSI) by 30.7%.
Plasma total protein reaches a peak at 10 g L^{-1} salinity.
An animal-plant-based diet increases HSI and plasma cholesterol and triglycerides.
A plant-based diet leads to low performance and reduces HSI in juvenile Nile tilapia.

Abstract

The purpose of this study was to evaluate the effects of salinity and plant-based diet or animal-plant combination diet on the performance and metabolic status of juvenile Nile tilapia (*Oreochromis niloticus*). The experimental design was completely randomized in a 4 × 2 factorial scheme with four replicates. The treatments were established by the combination of salinities of 0, 10, 20, and 30 g L^{-1} with an animal-plant combination diet (AP) or plant-based diet (P). The replicates were 60 L tanks with 12 fish per tank. Diets were provided for 32 days, and the fish were fed three times a day (8, 12, and 17 h) until apparent satiety. Daily feed intake (DFI) was measured, body weight (BW) was recorded at the beginning and end of the trial, and total length (TL) and standard length (SL) were measured at the end of the trial. Average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate were calculated. After the

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biometric measurements were made at the end of the trial, blood samples were collected to determine the plasma concentrations of total protein (TP), glucose, cholesterol, and triglycerides (TG). The fish were euthanized, and the hepatopancreas was collected and weighed; thereafter, the hepatosomatic index (HSI) was calculated. An interaction was detected between salinity and diet type for final BW, ADG, TL, and SL. These traits were not influenced by salinity when it was associated with the AP diet, but reduced linearly with salinity in the P diet. DFI and survival rate were independently affected by salinity: DFI reduced linearly with salinity levels and survival rate was higher at a salinity of 10 g L^{-1}. HSI increased linearly with salinity levels and was lower in the P diet than in the AP diet. Salinity had a quadratic effect on plasma TP, and the maximum value for this metabolite (2.96 g dL^{-1}) is attained at a salinity of 10.26 g L^{-1}. There was an independent effect of diet on the plasma concentrations of cholesterol and TG, which were lower in the P diet than in the AP diet. The salinity of 10 g L^{-1} associated with diet composed of animal and plant ingredients led to a better performance, higher survival rate, and less stressful environmental conditions for juvenile Nile tilapia.
Key words: Average daily gain. Hepatosomatic index. Osmoregulation. Total protein. Triglycerides.

Resumo

O objetivo deste estudo foi avaliar os efeitos da salinidade e de dietas compostas por ingredientes de origem vegetal, ou por ingredientes de origem animal e vegetal no desempenho e na condição metabólica de juvenis de tilápia do Nilo (*Oreochromis niloticus*). O delineamento foi inteiramente casualizado em esquema fatorial 4 × 2 com quatro repetições. Os tratamentos foram estabelecidos pela combinação das salinidades 0, 10, 20 e 30 g L^{-1} com dietas contendo apenas ingredientes de origem vegetal (V) ou ingredientes de origem animal e vegetal (AV). As repetições foram caixas de 60 L contendo 12 peixes por caixa. As dietas foram fornecidas durante 32 dias e os peixes foram alimentados três vezes ao dia (8, 12 e 17 h) até saciedade aparente. O consumo de ração foi mensurado diariamente (CRD), o peso corporal (PC) foi registrado no início e no final do experimento, e o comprimento total (CT) e comprimento padrão (CP) foram mensurados no final do experimento. O ganho médio diário (GMD), a taxa de crescimento específico (TCE), a conversão alimentar (CA) e a taxa de sobrevivência também foram calculadas. Após a obtenção das medidas biométricas ao final do experimento, amostras de sangue foram coletadas para determinar as concentrações plasmáticas de proteínas totais (PT), glicose, colesterol e triglicerídeos (TG). Posteriormente, os peixes foram eutanasiados e o hepatopâncreas foi coletado, pesado e o índice hepatossomático (IHS) foi calculado. Houve interação entre salinidade e tipo de dieta para PC final, GMD, CT e CP. Essas variáveis não foram influenciadas pela salinidade quando associada com a dieta AV, mas reduziram linearmente com a salinidade na dieta V. O CRD e a taxa de sobrevivência foram influenciados exclusivamente pela salinidade, onde o CRD reduziu linearmente com a salinidade, e a taxa de sobrevivência foi maior na salinidade de 10 g L^{-1}. O IHS aumentou linearmente com a salinidade, e foi menor da dieta V comparado com a dieta AV. Houve efeito quadrático da salinidade na concentração plasmática de PT, em que o máximo valor para este metabólito (2.96 g dL^{-1}) pode ser alcançado com a salinidade de 10.26 g L^{-1}. As concentrações plasmáticas de colesterol e TG foram exclusivamente influenciadas pelo tipo de dieta, apresentando menores valores na dieta V comparados com a dieta AV. A salinidade de 10 g L^{-1} associada com dieta composta por ingredientes de origem animal e vegetal proporcionou melhor desempenho, maior taxa de sobrevivência e condições ambientais menos estressantes aos juvenis de tilápia do Nilo.
Palavras-chave*:* Ganho médio diário. Índice hepatossomático. Osmorregulação. Proteínas totais. Triglicerídeos.

Introduction

Owing to characteristics such as rusticity, easy adaptation to different environments, resistance to diseases, high performance and capacity to support high stocking densities, the Nile tilapia (*Oreochromis niloticus*) is the main species cultivated in the Brazilian freshwater fish farming (Vieira et al., 2005). Most species of tilapia are euryhaline and are capable of supporting a wide range of salinity levels; therefore, they can be cultivated in fresh, brackish, or saltwater. Euryhaline characteristic is of great relevance to fish farming, because climate change and habitat degradation determined by anthropogenic activities have led to an increase in the global sea level, which in the short term will result in increased salinization of coastal areas due to flooding and seawater invasion into freshwater aquifers (Kültz, 2015). Given this scenario and the great Brazilian coastal area, alternatives should be found to promote Nile tilapia farming in a sustainable way.

The development of sustainable alternatives to tilapia farming in brackish and saltwater depends on the understanding of metabolic processes that occur under different salinity levels. Studies have shown that salinity affects the number and morphology of gill chloride cells (Wang et al., 2009; Jumah et al., 2016; Pereira, Guerra-Santos, Moreira, Albinati, & Ayres, 2016), some ions (sodium and chloride) and metabolites on blood (Küçük, Karul, Yildirim, & Gamsiz, 2013; Jumah et al., 2016; Pereira et al., 2016), diet digestibility (Tran-Ngoc et al., 2017), and ion and amino acid transport through the gastrointestinal tract (Ronkin, Seroussi, Nitzan, Doron-Faigenboim, & Cnaani, 2015; Nitzan, Rozenberg, & Cnaani, 2017) in different tilapia species. However, the response of blood biochemical parameters to changes in salinity levels in juvenile Nile tilapia is not well understood. Furthermore, the performance and feed efficiency of this species have presented great variability to salinity (Alvarenga et al., 2018; Herath, Haga, & Satoh, 2018; Malik et al., 2018), and a better level of cultivation has not yet been identified.

The use of plant-based diets for Nile tilapia farming is another alternative that can contribute to environmental and economic sustainability, as well as to human health. The addition or formulation of fish diets with plant ingredients reduces feeding costs (Novelli et al., 2017; Cazcarro, López-Morales, & Duchin, 2019) and may be a strategy to improve the fatty acid profile of carcasses from freshwater fish, as these ingredients are sources of polyunsaturated fatty acids (PUFA) (Araújo-Dairiki, Chaves, & Dairiki, 2018; Lima et al., 2019). Moreover, plant-based diets may potentially increase the PUFA deposition on carcass when provided in a saline environment, where the activity of fatty acid desaturase and elongase enzymes seems to increase (Hunt, Özkan, Engin, & Tekelioglu, 2011; Sarker et al., 2011). Thus, if plant-based diets provide similar performance to diets composed of animal ingredients or a combination of animal and plant ingredients, the first one presents more advantages for use in Nile tilapia farming.

The purpose of this study was to evaluate the effects of salinity, associated with a plant-based diet or an animal-plant combination diet, on the performance and metabolic status of juvenile Nile tilapia.

Materials and Methods

All procedures involved in this research were performed in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (*Colégio Brasileiro de Experimentação Animal - COBEA*) and were approved by the Animal Care and Use Committee (*Comitê de Ética no Uso de Animais - CEUA*) of the Federal University of Paraná (*Universidade Federal do Paraná - UFPR*), Palotina Campus, under protocol number 08/2017-CEUA.

Experimental protocol

The trial was conducted from March to April and lasted for 32 days. Juvenile Nile tilapia (*Oreochromis niloticus*) from the GIFT strain (n = 384) were obtained from a commercial fish farm located in Palotina, in the western region of Paraná state, Brazil.

The experimental units were 60 L tanks that received artificial aeration constantly and were equipped with external hang on filters (500 L h^{-1} of water flow rate, Sun® model HBL-601), containing approximately 220 g of biological ceramic rings (Boyu®) and a filter plate with activated carbon. The temperature was maintained at approximately 28 °C using a thermostat heater (200 W, Sobo® model HG 200). All tanks (n = 32) were prepared 30 days before the beginning of the trial to promote the colonization of biological filters by nitrifying bacteria and system stabilization.

Artificial seawater was prepared using potable water, previously dechlorinated with the addition of 5 mg L^{-1} of sodium thiosulfate, and a commercial salt mixture (Red Sea Salt®, Houston, TX, USA). The salinity was adjusted according to the treatment defined for each tank (see below).

Fish were adapted to different salinities in 180 L tanks prepared with artificial seawater containing 5, 10, 15, 20, and 25 g L^{-1} of salt. Fish were grouped in batches of 100 units and placed in cages to facilitate changes between tanks. The acclimation to the salinity was gradually performed for three to five days to reduce the osmotic stress imposed on the fish during transfer to high levels of salinity (Al-Amoudi, 1987). Thus, the cages were changed at 24 h intervals until the salinity defined for each batch. At the end of acclimation, fish were divided into 12-unit batches, which were weighed and uniformly distributed into the tanks according to each treatment. The average body weight (BW) of fish at the beginning of the trial was 26.2 ± 1.2 g.

The experimental design was completely randomized in a 4 × 2 factorial scheme with four replicates. The treatments were established by the combination of salinities of 0, 10, 20, and 30 g L^{-1} with diets containing animal and plant ingredients (AP), or only plant ingredients (P) (Table 1). Diets were formulated to meet the minimum requirements of omega-6 and omega-3 fatty acids, and to meet the nutritional requirements of the species based on the National Research Council [NRC] (2011). The ingredient composition, and nutrient and energy contents of the AP diet were defined based on a commercial formulation, whereas the P diet was formulated to achieve the same nutrient and energy contents of the AP diet. Amino acid profiles of both diets were adjusted following the concept of ideal protein (Furuya et al.,

2005), and the balance of lysine, methionine, and threonine was made up with synthetic sources of these amino acids. To prepare the experimental rations, the ingredients were ground in a knife mill equipped with a 0.5 mm sieve. Then, the ingredients were mixed according to the formulation of each treatment (Table 1), pelleted, and dried overnight in a forced-air ventilation oven at 55 °C. The rations were stored in plastic containers at 4 °C until further use. Fish were fed three times a day (8, 12, and 17 h) until apparent satiety. A photoperiod of 12 h light and 12 h dark was maintained from the adaptation period until the end of the trial.

Water renewal occurred at 72 h intervals by siphoning 40% of the water volume from each tank, followed by the replacement of artificial seawater previously prepared for each treatment. For the treatment with freshwater, potable dechlorinated water was replaced in the tanks. Salinity was measured in each tank on the day after water renewal and was adjusted when necessary. The ceramic rings and filter plates of the external filters were washed weekly to remove excess biological residues. This was performed in a separate container using the same water from the tanks to avoid destabilization of the biological system.

Water quality parameters were monitored weekly in water samples collected from each tank (Table 2). The pH, concentrations of total ammonia, nitrate and nitrite, total hardness, and alkalinity were measured according to the standard methods of American Public Health Association [APHA] (2005) for the examination of water and waste. Salinity, conductivity, dissolved oxygen, and temperature were measured daily in tanks with a multiparameter water quality meter (AKSO® model AK-88).

Growth performance measurements

Apparent daily feed intake (DFI) was calculated for each tank using the formula DFI = (AD/SDens)/EP, where AD = amount of diet provided during the trial, on a dry matter (DM) basis, SDens = average stocking density of fish during the trial, and EP = experimental period (days).

Biometric measurements were performed at the end of the trial. All fish were fasted for 24 h prior to evaluation and then anesthetized in a solution of water with 100 mg L^{-1} of clove oil (Taylor & Roberts, 1999); body weight (BW), total length (TL), and standard length (SL) were recorded. Average individual BW calculated from each tank at the beginning and at the end of the trial were used in the calculation of average daily gain (ADG), obtained by the difference between the two measurements divided by the experimental period (days); specific growth rate (SGR), calculated by the formula SGR = [(logFBW - logIBW)/EP] × 100, where FBW = final body weight, IBW = initial body weight, and EP = experimental period (days). The feed conversion ratio (FCR) was calculated as the ratio of DFI to ADG. The survival rate was calculated using the formula SR = $[(n_I - n_F)/n_I] \times 100$, where n_I = number of fish at the beginning of the trial, and n_F = number of fish at the end of the trial.

Table 1
Ingredients and chemical composition of diets provided to juvenile Nile tilapia (*Oreochromis niloticus*) during trial

Component[I]	Diet[II]	
	AP	P
Ingredients (g kg DM^{-1})		
Ground corn	277.0	308.8
Wheat meal	100.0	0.0
Soybean meal	226.0	651.7
Meat and bone meal	37.0	0.0
Feather meal	80.0	0.0
Poultry by-product meal	200.0	0.0
Soybean oil	67.4	10.0
DL-Methionine	1.85	0.76
L-Lysine	3.95	0.00
L-Threonine	0.10	0.00
Choline chloride	1.45	0.00
Vitamin C	0.55	0.00
Dicalcium phosphate	0.00	18.54
Sodium chloride	1.60	0.00
Vitamin and mineral premix[III]	3.00	10.00
BHT	0.10	0.20
Chemical composition (analyzed)		
Dry matter (g kg^{-1} as fed)	954.0	948.8
Crude protein (g kg^{-1} DM)	353.4	335.5
Ether extract (g kg^{-1} DM)	122.8	9.0
Crude fiber (g kg^{-1} DM)	62.5	53.1
Ash g (g kg^{-1} DM)	64.5	66.8
Gross energy (Mcal kg^{-1} DM)	5.10	4.50

[I] DM: dry matter; BHT: butylated hydroxytoluene (antioxidant).
[II] AP: diet composed of animal and plant ingredients; P: diet composed of plant ingredients.
[III] Guaranteed analysis: 1000000 UI kg^{-1} vitamin A, 500000 UI kg^{-1} vitamin D3, 20000 UI kg^{-1} vitamin E, 500 mg kg^{-1} vitamin K3, 1900 mg kg^{-1} vitamin B1, 2000 mg kg^{-1} vitamin B2, 2400 mg kg^{-1} vitamin B6, 3500 mg kg^{-1} vitamin B12, 25 mg kg^{-1} vitamin C, 5000 mg kg^{-1} niacin, 4000 mg kg^{-1} pantothenic acid, 200 mg kg^{-1} folic acid, 40 mg kg^{-1} biotin, 7500 mg kg^{-1} manganese, 25 mg kg^{-1} zinc, 12.5 mg kg^{-1} iron, 2000 mg kg^{-1} copper, 200 mg kg^{-1} iodine, 70 mg kg^{-1} selenium, 300 mg kg^{-1} BHT.

Table 2
Water quality parameters recorded in each treatment during trial[I]

Parameter	Diet[III]	Salinity (g L^{-1})			
		0	10	20	30
SM[II] (g L^{-1})	AP	0.54 ± 0.07	10.16 ± 0.52	19.91 ± 0.41	29.40 ± 0.52
	P	0.49 ± 0.06	10.39 ± 0.46	19.82 ± 0.28	29.40 ± 0.37
Conductivity (mS cm^{-1})	AP	1.00 ± 0.13	16.80 ± 0.83	30.33 ± 0.79	44.08 ± 0.68
	P	0.92 ± 0.07	17.08 ± 0.72	30.30 ± 0.70	44.07 ± 0.46
Temperature (ºC)	AP	27.5 ± 0.4	27.1 ± 1.6	27.8 ± 0.4	26.7 ± 1.2
	P	27.8 ± 0.2	26.7 ± 1.4	25.9 ± 1.5	26.5 ± 1.5
DO[II] (mg L^{-1})	AP	5.73 ± 0.69	5.25 ± 0.53	5.58 ± 0.13	6.20 ± 0.27
	P	5.35 ± 0.51	5.03 ± 0.22	6.28 ± 0.80	6.28 ± 0.95
Ammonia (mg L^{-1})	AP	0.23 ± 0.14	0.17 ± 0.07	0.35 ± 0.06	0.60 ± 0.13
	P	0.22 ± 0.08	0.24 ± 0.10	0.37 ± 0.20	0.61 ± 0.17
Nitrite (mg L^{-1})	AP	0.59 ± 0.08	2.29 ± 0.13	2.08 ± 0.40	1.98 ± 0.37
	P	0.67 ± 0.12	2.15 ± 0.07	2.22 ± 0.19	2.10 ± 0.30
Nitrate (mg L^{-1})	AP	7.23 ± 1.57	5.19 ± 1.85	3.39 ± 3.04	4.19 ± 2.35
	P	6.77 ± 2.51	5.41 ± 1.90	3.67 ± 2.08	2.60 ± 1.46
WH[II] (mg L^{-1})	AP	372 ± 68	1425 ± 293	1993 ± 254	1973 ± 297
	P	445 ± 178	1561 ± 203	1930 ± 28	2148 ± 42
Alkalinity (mg L^{-1})	AP	71 ± 3	124 ± 13	182 ± 16	244 ± 11
	P	81 ± 20	140 ± 13	190 ± 23	242 ± 28
pH (1 - 14)	AP	7.75 ± 0.35	8.35 ± 0.07	8.73 ± 0.07	9.06 ± 0.04
	P	7.57 ± 0.14	8.50 ± 0.10	8.79 ± 0.05	9.07 ± 0.07

[I] Values expressed as mean ± standard deviation (M ± SD).
[II] SM: salinity measured; DO: dissolved oxygen; WH: water hardness.
[III] AP: diet composed of animal and plant ingredients; P: diet composed of plant ingredients.

Assessment of metabolic status

After the biometric measurements were made at the end of the trial, three fish were randomly selected from each tank for blood sampling. The samples were collected by caudal venipuncture with hypodermic syringes containing anticoagulant (0.1 mL of 4% sodium citrate solution/1 mL blood). They were then transferred to Eppendorf microtubes (1.5 mL) and centrifuged (600 × g for 10 min) for plasma separation. Plasma was collected with a micropipette, transferred to new Eppendorf microtubes, and stored in a freezer (-20 °C) until the time of biochemical analysis. Using commercial kits (Ebram®) and an automated biochemical analyzer (BS-200 Mindray®), the plasma concentrations of total protein (TP, colorimetric method of biuret; Ref nº 3006), glucose (kinetic method of glucose oxidase; Ref nº 3034), cholesterol (enzymatic method of esterase peroxidase; Ref nº 3012), and triglycerides (TG, enzymatic method of glycerol phosphate oxidase-peroxidase; Ref nº 3014) were determined.

The fish used for blood sampling were euthanized in a solution of water with 300 mg L^{-1} of clove oil (Taylor & Roberts, 1999) and eviscerated for the collection of hepatopancreas. Thereafter, its weight was recorded and the hepatosomatic index (HSI) was calculated using the formula HSI = (HPW/FBW) × 100, where HPW = hepatopancreas weight and FBW = final body weight.

Statistical analysis

Data were analyzed for normality of distribution using the Shapiro-Wilk test and homogeneity of variance between treatments using the Levene's test. After both conditions were confirmed, a two-way analysis of variance (ANOVA) in a 4 × 2 factorial scheme was performed to determine the independent effects and interaction effects between the levels of salinity and types of diets tested. When the effect of salinity was significant, the means were compared using Tukey's test and a simple regression analysis was performed until the second order (quadratic), with the salinity level as the independent variable. When the effect of diet was significant, the means were compared using the F-test. All analyses were performed using Statistical Analysis System software, version 9.0 (Statistical Analysis System [SAS Institute], 2002), and significance level was set at $p < 0.05$.

Results and Discussion

Growth performance

An interaction was detected between salinity and diet type for FBW, ADG, TL, and SL (Table 3). These traits were not influenced by salinity when it was associated with the AP diet, but reduced linearly with salinity in the P diet (Table 4). In this condition, an increase in salinity from 0 to 30 g L^{-1} led to a reduction of 23% in FBW, 56.2% in ADG, 7.9% in TL, and 9.7% in SL.

The DFI and survival rate were independently affected by salinity (Table 3). The DFI reduced linearly with salinity levels and the increase in salinity from 0 to 30 g L^{-1} led to a reduction of 41.5% in this trait. Although the relationship between survival rate and salinity did not fit into a regression equation, mean comparison test indicated that this trait was higher at a salinity of 10 g L^{-1} (64.6%) than at other salinities, which presented similar values (32.8% on average).

Table 3
Performance traits and survival rate of juvenile Nile tilapia (*Oreochromis niloticus*) cultivated for 32 days under increasing levels of salinity and fed diets containing animal and plant ingredients (AP) or only plant ingredients (P)

Variable[I]	Salinity (g L^{-1})				Diet		Mean	SEM[II]	*p*-value[III]		
	0	10	20	30	AP	P			Salinity	Diet	S × D
FBW (g)	45.1	44.5	43.6	40.2	43.7	42.9	43.3	1.2	0.4744	0.7297	0.0379
DFI (g DM day^{-1})[IV]	0.65 a	0.65 a	0.53 ab	0.38 b	0.53	0.58	0.55	0.03	0.0035	0.3202	0.8109
ADG (g BW day^{-1})	0.60	0.58	0.55	0.40	0.55	0.52	0.53	0.04	0.2573	0.6975	0.0436
SGR (% day^{-1})	0.74	0.72	0.68	0.50	0.67	0.65	0.66	0.04	0.1663	0.7228	0.0674
FCR (g DM g $gain^{-1}$)	1.18	1.27	1.07	1.32	1.14	1.27	1.21	0.09	0.7829	0.4861	0.1390
TL (cm)	13.4	13.2	13.2	12.9	13.2	13.2	13.2	0.1	0.5601	0.7540	0.0391
SL (cm)	10.9	10.8	10.8	10.4	10.7	10.7	10.7	0.1	0.4118	0.9645	0.0154
SR (%)	38.5 b	64.6 a	30.0 b	29.8 b	37.4	44.1	40.7	3.8	0.0010	0.2738	0.3580

[I] DM: dry matter; BW: body weight; FBW: final body weight; DFI: daily feed intake; ADG: average daily gain; SGR: specific growth rate; FCR: feed conversion ratio; TL: total length; SL: standard length; SR: survival rate.
[II] SEM: standard error of the mean.
[III] Probability values obtained from the two-way analysis of variance (ANOVA); S × D: interaction effect between salinity and diet.
[IV] Regression equation: DFI = 0.690 - 0.0090S (R^2 = 0.36; p = 0.0004); S = salinity (g L^{-1}).
Means for salinity followed by different lowercase letters in the same row differ by Tukey test ($p < 0.05$).

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Table 4
Interaction effect between salinity and diet on final body weight (FBW), average daily gain (ADG), total length (TL), and standard length (SL) of juvenile Nile tilapia (*Oreochromis niloticus*) cultivated for 32 days under increasing levels of salinity and fed diets containing animal and plant ingredients (AP) or only plant ingredients (P)

Variable	Diet	Salinity (g L^{-1})			
		0	10	20	30
FBW (g)	AP	41.1 aA	42.6 aA	48.7 aA	42.5 aA
	P[I]	49.1 aA	46.3 abA	38.5 bB	37.8 bA
ADG (g BW day^{-1})	AP	0.48 aA	0.53 aA	0.70 aA	0.49 aA
	P[I]	0.73 aA	0.62 abA	0.40 bB	0.32 bA
TL (cm)	AP	12.9 aA	12.9 aA	13.7 aA	13.0 aA
	P[I]	13.9 aA	13.4 abA	12.8 bB	12.8 bA
SL (cm)	AP	10.5 aA	10.5 aA	11.3 aA	10.6 aA
	P[I]	11.3 aA	11.1 abA	10.3 bB	10.2 bA

[I] Regression equation: $FBW_P = 49.192 - 0.4172S$ ($R^2 = 0.48$; $p = 0.0031$); $ADG_P = 0.735 - 0.0145S$ ($R^2 = 0.54$; $p = 0.0011$); $TL_P = 13.806 - 0.0384S$ ($R^2 = 0.41$; $p = 0.0075$); $SL_P = 11.312 - 0.0391S$ ($R^2 = 0.49$; $p = 0.0024$); S = salinity (g L^{-1}).
Means followed by different lowercase letters in the same row differ by Tukey test ($p < 0.05$), and means followed by different uppercase letters in the same column differ by F test ($p < 0.05$).

The lower energy content of P diet compared to AP diet (4.50 *vs.* 5.10 kcal g DM^{-1}; Table 1) and the high energy expenditure owing to osmoregulation process and adaptation to saline environment may explain the decrease in the performance of juvenile Nile tilapia fed P diet under high salinity level (Tables 3 and 4). The inhibition of growth in freshwater fish adapted to saline environments is mainly attributed to the high energy cost of osmoregulation (Prunet & Bornancin, 1989). When fish are kept in an isotonic environment, where there is a small difference between the ionic gradients of water and blood, the energy expenditure owing to osmoregulation is reduced, thus, more energy is available for growth (Boeuf & Payan, 2001). Moreover, some physiological changes, such as increased passage rate of feed through the gastrointestinal tract (Ferraris, Catacutan, Mabelin, & Jazul, 1986) and interactions between the absorption mechanisms of salt and nutrients in the intestine (Moutou, Panagiotaki, & Mamuris, 2004; Hallali et al., 2018) can affect the diet digestibility in fish subjected to different salinities. A possible reason for changes in the values of diet digestibility in a saline environment is the energy expenditure owing to osmoregulation, since the demand for energy to maintain osmotic homeostasis is lower under low salinities (12-15 g L^{-1}), resulting in increased energy availability for digestion and absorption of nutrients (Tran-Ngoc et al., 2017).

The use of soybean meal as a protein source in the P diet (which contained 652 g kg DM^{-1} of this ingredient; Table 1) can also explain the reduced performance of fish

under different salinity levels (Tables 3 and 4). Soybean meal contains anti-nutritional factors, such as proteases inhibitors and phytic acid, which reduce the bioavailability of nutrients and minerals (Francis, Makkar, & Becker, 2001; Hardy, 2010). In this case, the addition of phytase and proteases can improve the utilization of this protein source by increasing the digestibility of nutrients and energy availability (Novelli et al., 2017). Animal protein sources such as meat and bone meal, and poultry by-product meal (which represented 237 g kg DM^{-1} of AP diet) have amino acid profiles closer to the dietary requirements of Nile tilapia and greater bioavailability of nutrients than soybean meal (Bicudo, Pinto, & Cyrino, 2010). This may explain the similar performance of fish fed the AP diet under different salinity levels (Tables 3 and 4).

The negative effect of salinity on the performance of Nile tilapia has also been observed in other studies (Alvarenga et al., 2018; Herath et al., 2018; Malik et al., 2018), which reinforces the great impact of changes in salinity on the productive response of this species. In the present study, the highest survival rate (64.6%) was obtained at a salinity of 10 g L^{-1} (Table 3). Conversely, a low survival rate (38.5%) was observed in freshwater. During feeding and water renewal management, it was noted that fish cultivated in freshwater exhibited agonistic behavior with higher intensity than those cultivated in saline environments. This resulted in a higher frequency of aggression, chasing, and fleeing among fish, and may have contributed to the low survival rate in freshwater. Moreover, all the performance traits recorded at a salinity of 10 g L^{-1} were similar to those observed in the treatments with freshwater, independent of the diet provided to the fish. These results indicate that the osmoregulatory and metabolic adaptations of juvenile Nile tilapia were less stressful, and probably, demand less energy at a salinity of 10 g L^{-1} than in freshwater and at salinities of 20 and 30 g L^{-1}. Indeed, the best performance results have been reported within a range of 10-12 g L^{-1} for this species (Malik et al., 2018; You, Lu, Wang, Chen, & Li, 2019). This is justified by the fact that teleost fish maintain the osmolality of their extracellular body fluids relatively constant at approximately 9 g L^{-1} of salinity (Kültz, 2015). Thus, at salinities higher than 9 to 10 g L^{-1}, the diet must have energy content capable of supporting the energy expenditure to adapt to saline environment and guarantee a satisfactory growth rate for juvenile Nile tilapia.

Metabolic status

The hepatopancreas weight was not affected by salinity or diet; however, both factors had independent effects on HSI (Table 5). This trait increased linearly with salinity levels (Figure 1), with an increase of 30.7% between the salinities of 0 and 30 g L^{-1}. The HSI was lower in the P diet than in the AP diet, with a reduction of 16.8% in the first diet.

The linear increase in HSI with salinity levels (Figure 1) is probably related to energy and osmotic metabolism. Due to its direct effect on osmoregulation, salinity does not only lead to physiological changes in the gastrointestinal tract (as previously described) but is also involved with the nutrient supply to other organs and tissues that adjust osmolality (Ronkin et al., 2015; Nitzan et al., 2017), such as the hepatopancreas (Xu et al.,

2015). As this is the major organ responsible for lipid metabolism in fish (Tocher, 2003), the increase in salinity may have overloaded it and increased the synthesis of nutrients, especially fatty acids (Xu et al., 2015). Fatty acid is an important energy source, and its biosynthesis, mainly of long-chain PUFA, is also necessary to alter the fatty acid composition and membrane fluidity of cellular membrane during variations in water salinity (Fonseca-Madrigal, Pineda-Delgado, Martínez-Palacios, Rodríguez, & Tocher, 2012; You et al., 2019).

Salinity did not influence the plasma concentrations of glucose, cholesterol, and TG (Table 5), but had a quadratic effect on plasma TP (Figure 2). According to the regression equation, the maximum estimated value for this metabolite was 2.96 g dL^{-1} at a salinity of 10.26 g L^{-1}. In addition, there was an independent effect of diet on plasma concentrations of cholesterol and TG, which were lower in the P diet than in the AP diet. These metabolites were reduced by 20.6% and 25.3%, respectively in the P diet.

Table 5
Characteristics of hepatopancreas and plasma concentration of metabolites in juvenile Nile tilapia (*Oreochromis niloticus*) cultivated for 32 days under increasing levels of salinity and fed diets containing animal and plant ingredients (AP) or only plant ingredients (P)

Variable[I]	Salinity (g L^{-1})				Diet		Mean	SEM[II]	*p*-value[III]		
	0	10	20	30	AP	P			Salinity	Diet	S × D
HPW (g)	0.89	0.97	1.09	0.93	1.05	0.89	0.97	0.05	0.5894	0.1508	0.6470
HSI (% BW)	1.92 b	2.06 ab	2.45 a	2.51 a	2.44 A	2.03 B	2.23	0.09	0.0319	0.0144	0.9939
TP (g dL^{-1})	2.71 ab	2.89 a	2.84 a	1.95 b	2.62	2.57	2.59	0.12	0.0198	0.8140	0.2308
Glucose (mg dL^{-1})	57.6	63.5	54.7	50.5	56.4	56.7	56.6	2.3	0.2957	0.9532	0.7264
Cholesterol (mg dL^{-1})	129.1	131.6	135.0	125.5	145.3 A	115.3 B	130.3	5.2	0.9114	0.0047	0.2409
TG (mg dL^{-1})	63.1	80.3	71.6	46.8	74.9 A	56.0 B	65.5	5.1	0.0994	0.0519	0.4346

[I] HPW: hepatopancreas weight; HSI: hepatosomatic index; TP: total proteins; TG: triglycerides.
[II] SEM: standard error of the mean.
[III] Probability values obtained from the two-way analysis of variance (ANOVA); S × D: interaction effect between salinity and diet.
Means for salinity followed by different lowercase letters in the same row differ by Tukey test ($p < 0.05$), and means for diet followed by different uppercase letters in the same row differ by F test ($p < 0.05$).

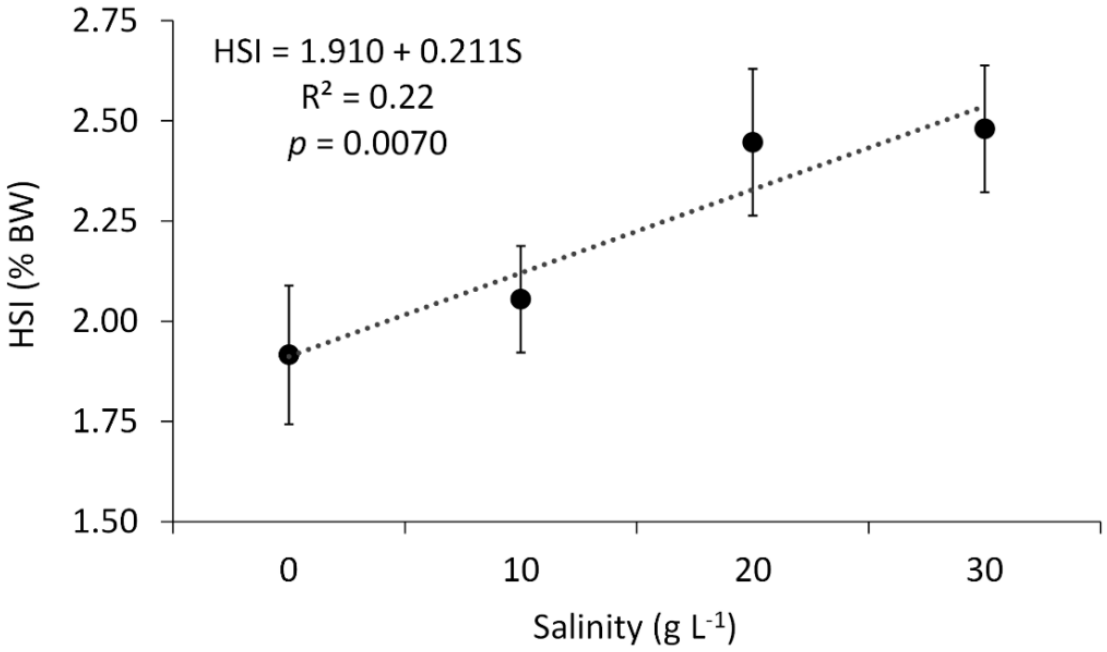

Figure 1. Effect of salinity on the hepatosomatic index (HSI) of juvenile Nile tilapia (*Oreochromis niloticus*).
Values expressed as mean (dot) ± standard error (error bar).

The quadratic response of plasma TP to salinity levels (Figure 2) is related to the adaptation of juvenile Nile tilapia to a saline environment. Under this condition, plasma osmolality allostasis is supported by elevated concentrations of myo-inositol and higher activities of the enzymes involved in its synthesis (myoinositol phosphate synthase and inositol monophosphatase); as direct ionic activation of these enzymes greatly increases their catalytic efficiency and enzymatic activity, they may accumulate damage more rapidly during repeated cycles of structural changes associated with catalysis; this would then accelerate their degradation and turnover, explaining the need for increased rates of de novo synthesis (Kalujnaia et al., 2013; Kültz, 2015). This may explain why plasma TP remained relatively constant at 0 to 20 g L^{-1} of salinity (2.81 g dL^{-1} on average) and decreased at 30 g L^{-1} of salinity (1.95 g dL^{-1}; Table 5; Figure 2). Furthermore, the highest level of plasma TP (2.96 g dL^{-1}) estimated for the salinity of 10.26 g L^{-1} suggests that a balance between synthesis and degradation of the enzymes, owing to osmoregulation, is reached in the plasma of juvenile Nile tilapia at this salinity level. When evaluating the hematological parameters of this species in response to salinity levels (0 to 25 g L^{-1}), Pereira et al. (2016) observed that plasma TP presented higher values at 10 g L^{-1} salinity, corroborating the results obtained in the present study.

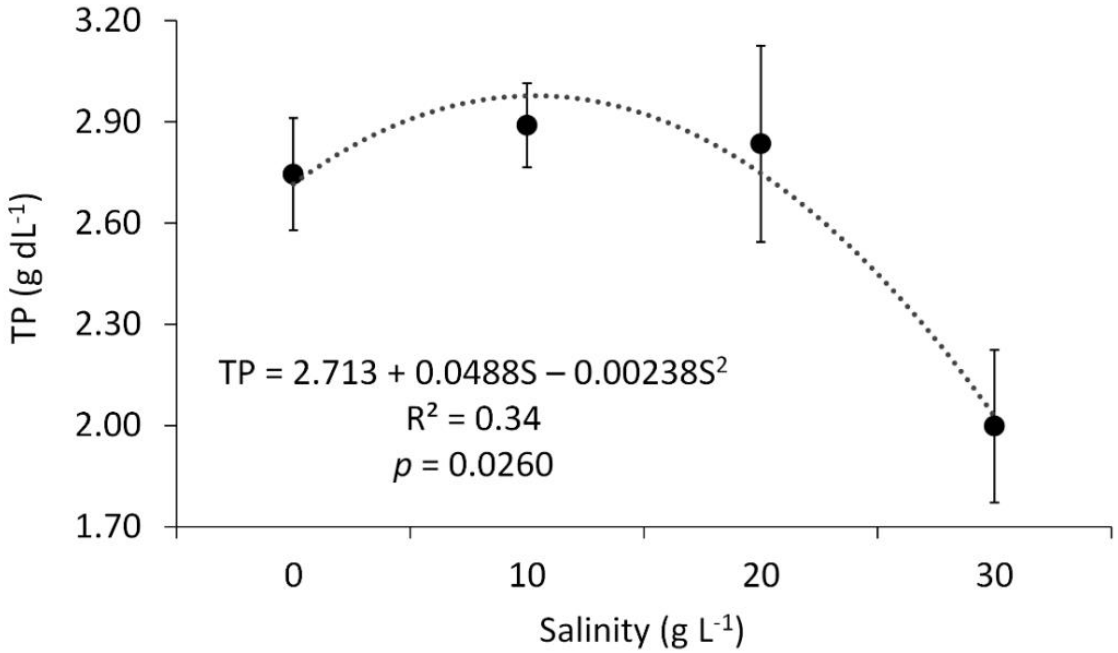

Figure 2. Effect of salinity on the plasma concentration of total proteins (TP) of juvenile Nile tilapia (*Oreochromis niloticus*).
Values expressed as mean (dot) ± standard error (error bar).

The higher HSI and increased plasma concentrations of cholesterol and TG in the fish fed the AP diet compared to those fed the P diet (Table 5) are clear evidence of the effect of diet composition on the lipid metabolism of juvenile Nile tilapia. To meet the minimum requirements of omega-6 and omega-3 fatty acids in both diets, the amount of soybean oil added to the AP diet was almost seven times higher than that of the P diet (67 *vs.* 10 g kg DM^{-1}; Table 1). This resulted in a higher content of ether extract (123 *vs.* 9 g kg DM^{-1}; Table 1) and justifies the higher energy content of the AP diet. Fish and vegetable oils are non-protein energy sources that can improve the efficiency of protein utilization and are readily available for metabolization after intake (Li et al., 2014; Lima et al., 2019). Particularly, the intake of soybean oil increases the carbohydrate content and the abundance of proteins related to cell division process, oxidative stress, and immune and inflammatory responses in the hepatopancreas of Nile tilapia (Boonanuntanasarn, Nakharuthai, Schrama, Duangkaew, & Rodrigues, 2019). Thus, the energy obtained from soybean oil was sufficient to supply the maintenance energy requirements and support a higher growth rate in the fish fed the AP diet. In contrast, due to the lower content of soybean oil, the fish fed the P diet used most of the energy for maintenance (mainly for osmoregulation), corroborating their lower HSI, decreased plasma concentrations of cholesterol and TG, and lower performance.

Conclusions

The salinity of 10 g L^{-1} associated with diet composed of animal and plant ingredients led to a better performance, higher survival rate, and less stressful environmental conditions for juvenile Nile tilapia. This salinity level is the limit for rearing this species without affecting growth performance and is optimum for improving their survival rate. Finally, the plant-based diet did not promote satisfactory performance for juvenile Nile tilapia cultivated in freshwater and saline environments.

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