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RESEARCH ARTICLE

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Dietary sodium chloride effect in Nile tilapia fed with fish meal-free diets

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Abstract

Aim of study: To evaluate the effect of the dietary NaCl level for Nile tilapia fingerlings.

Area of study: Palotina sector of Federal University of Paraná, in southern Brazil.

Material and methods: An experiment was carried using 750 Nile tilapia fingerlings (4.61 ± 0.09 g) distributed in 30 1,000-L circular tanks in a completely randomized design with six treatments and five replicates. NaCl was added to extruded fish meal-free diets at 0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 (g/kg). After the experimental period, fish were submitted to a 24 h-fast and weighed. All data were submitted to an analysis of variance and Tukey's test followed by Levene's test and regression test, using the Statistica 7.0® software package.

Main results: Fish fed the NaCl-free diet presented better growth performance (p<0.05) compared to the other diets. Linear decrease effects on final weight (y = 50.1754 – 0.1672x, r^2 = 0.6984), specific growth rate (y = 45.5418 – 0.1688x, r^2 = 0.6966), weight gain (y = 4.9465 – 0.008x; r^2 = 0.6886) and apparent feed conversion (y = 0.7113 + 0.0043x; r^2 = 0.7655) were noted. Efficiency and apparent protein retention were better in the NaCl-free diet (p<0.05). Significant differences (p<0.05) were observed for fillet fat and crude protein content. Increasing NaCl levels led to significant decreases (p<0.05) in Nile tilapia fingerling protein efficiency rates (y = 3.7804 - 0.0116x; r^2 = 0.6549).

Research highlights: Thus, NaCl should not be included in fish meal-free diets for Nile tilapia fingerlings.

Additional keywords: aquaculture; fish nutrition; growth; salt.

Abbreviations used: AFC (apparent feed conversion); AFI (average feed intake); APR (apparent protein retention); CP (crude protein); CYH (carcass yield with head); CYWH (carcass yield without head); DM (dry matter); FW (final weight); FY (fillet yield); HSI (hepatossomatic index); MM (mineral matter); MO (moisture); PER (protein efficiency rate); SGR (specific growth rate); SR (survival rates); UFPR (Universidade Federal do Paraná); VFI (visceral fat index); WG (weight gain)

Authors' contributions: All the authors contributed on the design, sampling, data analysis and wrote and approved the final manuscript.

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Introduction

Nile tilapia is one of the widely cultivated fish species (Hallali *et al.,* 2018), as it displays adequate zootechnical characteristics and adaptability, is easy to reproduce and presents excellent growth rates when fed both natural and artificial diets, in addition to being resistant to handling and to disease-causing agents (Webster & Lim, 2006). Thus, this species forms the basis of aquaculture-based economy worldwide.

One of the most important requirements in the production chain is nutrition, which impacts three factors of paramount importance for sustainable aquaculture: feed cost, fish growth and nutrient excretion into the water. Consequently, studies in this regard are of great scientific and practical importance. However, even with the high number of studies concerning this species little is known about the effect of dietary sodium chloride (NaCl) on the productive performance of these animals. Sodium (Na^+) and chlorine (Cl^-) are widely available and cheap macrominerals, essential to animal metabolism (Silva *et al.,* 2009). They can be easily administered in the form of NaCl, improving dietary palatability and be used as a source of both Na^+ (39.74%) and Cl^- (60.23%) (Maynard & Loosli, 1974; Borges *et al.,* 1998).

As essential elements, Na^+ and Cl^- play a role in maintaining osmotic pressure, basic acid balance and controlling nutrient transport to cells, water metabolism and the composition of gastric juice (Evans *et al.,* 2005). NaCl is widely used in aquatic animals, applied to the water to prevent and control parasitic diseases caused by fungi and bacteria (Marchioro & Baldisserotto, 1999) and in transport processes (Schalch *et al.,* 2009; Silva *et al.,* 2009), with the purpose of raising the salinity of the external environment to values close to the internal salinity of the animal, thus reducing metabolic and hormonal stress responses and the ionic gradient of the medium (Tomasso *et al.,* 1980), reducing mortality rates.

However, fish dietary NaCl growth effects are not yet clear, although some studies have shown a positive effect when this compound is added to fish diets in higher amounts (Fontaínhas-Fernandes *et al.,* 2000a,b; Nandeesha *et al.,* 2000; Gangadhara *et al.,* 2004; Eroldogan *et al.,* 2005; Harpaz *et al.,* 2005).

Studies assessing fish dietary NaCl inclusion have been carried out with euryhaline species cultivated in low salinity environments, such as *Sparus aurata* and *Lates calcarifer,* and NaCl levels between 8% and 12% were reported as the best range for adequate growth and survival rates (Appelbaum & Arockiaraj, 2009; Arockiaraj & Appelbaum, 2010). Concerning freshwater species, such as the jundiá *Rhamdia quelen,* high dietary NaCl levels were shown to influence final fish weights and biomasses (Garcia *et al.,* 2007), while hybrid tilapia displayed improved performance following dietary NaCl supplementation (Cnaani *et al.,* 2010). Dietary salt addition at 1.5% is common in freshwater carp cultivation in India (Nandeesha *et al.,* 2000).

Dietary salt has many nutritional effects on growth and feeding efficiency in fish, nutritional effect are not fully understood, with difference assessments resulting from differences in diet preparation methods, salt contents, nutrients balance, feeding levels and other practices (Salman, 2009). Na^+ and Cl^- deficiencies lead to deleterious fish growth effects (NRC, 2011). In this context, the aim of the present study was to assess the effects of increasing dietary NaCl levels in Nile tilapia fingerlings (*Oreochromis niloticus*) on growth performance and body chemical composition.

Material and methods

This study was carried out at the Aquatic Organism Production and Reproduction Systems Laboratory, belonging to the Animal Science Department of the Federal University of Paraná (UFPR), for 48 days. All procedures were carried out in accordance with the Ethical Principles in Animal Experimentation and were approved by the UFPR Palotina Sector Ethics and Animal Use Committee (protocol no. 21/2017).

They were used 750 Nile GIFT line male tilapia fingerlings (weight = 4.61 ± 0.09 g) distributed in 30 1,000-L circular tanks (25 fis per tank) in a completely randomized design consisting of six treatments and five replicates.

A water recirculation system was mounted in the experimental units, with daily water volume renewal of approximately five times the total water volume. This system comprised a 2,000-L tank used as a mechanical filter and a 30,000-L tank containing *Eichornia crassipes* for phosphorus and ammoniacal nitrogen biofiltration. The recirculation system was completed with water and received macrophytes (*E. crassipes*) in order to cover about 10% of the area of the biofiltration tank. Subsequently, the system was kept recirculating for 15 days, until the time of placing the fish. The macrophytes started to be removed when they came to cover more than 70% of the biofilter area,

The aeration system consisted of a 1 HP air blower connected to PVC pipes and silicone hoses with porous stones at the ends for oxygen distribution to each experimental unit. Approximately 100 L of water was siphoned from each experimental unit to remove possible feed leftovers, feces and organic matter prior to the last daily feeding event.

Isopropic and isoenergetic feed treatments containing increasing NaCl levels (0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 g/kg) were investigated (Table 1). The feed was initially milled in a hammer-type grinder coupled to a 0.7 mm sieve. After grinding, the ingredients were weighed, mixed and extruded using a 15 kg/h-capacity experimental extruder. After extrusion, each feed was oven dried at 55 °C for 24 h, packed in plastic bags and stored under refrigeration. Feeding was offered to the fish three times a day (at 8 am, 1 pm and 6 pm) until apparent satiety.

Water physico-chemical variables, pH (6.99 ± 0.14), ammonia (0.037 ± 0.03 mg/L), nitrite (0.165 ± 0.19 mg $N\text{-}NH_4/L$), conductivity (329.74 ± 132.52 µS/cm) and dissolved oxygen (5.45 ± 0.06 mg/L) were monitored weekly, while the minimum (27.2 ± 2.15 °C) and maximum (28.4 ± 1.88 °C) temperatures were measured daily. The chemical variable values were within the recommended range for freshwater fish rearing (Sá, 2012), and temperature values were within the recommended range for the thermal comfort of the species (Lund & Figueira, 1989).

Table 1. Composition and nutrient levels of experimental diets

	Level of NaCl in diet (g/kg)					
	0.0	**2.5**	**5.0**	**7.5**	**10.0**	**12.5**
Ingredient (g/kg)						
Soybean meal	719.7	719.7	719.7	719.7	719.7	719.7
Maize	212.7	212.7	212.7	212.7	212.7	212.7
Soybean oil	18.1	18.1	18.1	18.1	18.1	18.1
Dicalcium phosphate	30.0	30.0	30.0	30.0	30.0	30.0
Limestone	1.9	1.9	1.9	1.9	1.9	1.9
Vitamin and mineral mixture[1]	5.0	5.0	5.0	5.0	5.0	5.0
Common salt (NaCl)	0.0	2.5	5.0	7.5	10.0	12.5
Inert[2]	12.5	10.0	7.5	5.0	2.5	0.0
BHT[3]	0.1	0.1	0.1	0.1	0.1	0.1
Nutrient levels						
Digestible energy (kcal/kg)	4,096.5	4,096.5	4,096.5	4,096.5	4,096.5	4,096.5
Gross energy (kcal/kg)	3,000.0	3,000.0	3,000.0	3,000.0	3,000.0	3,000.0
Digestible protein (g/kg)	312.0	312.0	312.0	312.0	312.0	312.0
Crude protein (g/kg)	385.0	385.0	385.0	385.0	385.0	385.0
Crude lipid (g/kg)	16.9	16.9	16.9	16.9	16.9	16.9
Crude ash (g/kg)	85.6	85.6	85.6	85.6	85.6	85.6

[1]Vitamin and mineral supplement, basic composition: folic acid, 200 mg; pantothenic acid, 4000 mg; biotin, 40 mg; copper, 2000 mg; iron, 12500 mg; iodine, 200 mg; manganese, 7500 mg; niacin, 5000 mg; selenium, 70 mg; vitamin A, 1;000;000 IU; vitamin B1, 1900 mg; vitamin B12, 3500 mg; vitamin B2, 2000 mg; vitamin B6, 2400 mg; vitamin C, 50000 mg; vitamin D, 500000 IU; vitamin E, 20;000 IU; vitamin K3, 500 mg; zinc, 25;000 mg. [2]Red clay. [3]BHT: butil hydroxy toluene.

At the end of the experimental period, fish were fasted for 24 hours, anesthetized with benzocaine hydrochloride (100 mg/L) and individually weighed. Three specimens from each experimental unit were euthanized to obtain their eviscerated weight both with and without heads, as well as fillet, liver and viscera weights. Final weight (FW), weight gain (WG), specific growth rate (SGR), apparent feed conversion (AFC), survival rates (SR), carcass yield with and without the head (CYH, CYWH), fillet yield (FY), visceral fat index (VFI) and the hepatossomatic index (HSI) were determined.

At the beginning of the experimental period, three batches containing 25 fish were weighed, measured, anesthetized and euthanized for further analysis of initial proximal body composition. Likewise, at the end of the experimental period, six randomly selected fish from each experimental unit were weighed, measured and euthanized for evaluation of final body and fillet composition.

Carcasses and fillets were milled until a homogeneous mixture was obtained and the analyses were carried out according to the Association of Official Analytical Chemists (AOAC, 2000). Moisture (MO), crude protein (CP), fat and mineral matter (MM) of both the carcasses and fillets were determined.

Statistical analyses were performed using the Statistica 7.0 for Windows, Statsoft Inc. Performance data and body chemical composition data were submitted to a normal distribution homoscedastic analysis (Levene's test) and an analysis of variance (one-way ANOVA). Tukey's test and a regression analysis were applied when significant differences were found between variable means ($p<0.05$).

Results

The results of the performance variables of Nile tilapia fingerlings fed diets containing increasing NaCl levels are presented in Table 2. Dietary NaCl levels influenced FW, WG, SGR, AFC, FY, average feed intake (AFI), SR, and HSI ($p<0.05$). The best means for the assessed variables were observed for the NaCl-free diet. The diet containing 2.5 g/kg NaCl presented lower parameter values than the NaCl-free diet, but higher when compared to the other diets. NaCl level did not influence CYH and CYWH. FY reached its best value in the 5.0 g/kg NaCl diet and its worst value at 12.5 g/kg NaCl, while the other diets presented intermediate values and were not statistically different compared to each other.

Dietary NaCl led a significant effect ($p<0.05$) on AFI, where the highest intake was observed in the NaCl-free diet, followed by fish fed 2.5 g/kg NaCl, while the lowest ingestion was observed in fish fed 5.0 and 7.5 g/kg NaCl feed. However, fish that received 10.0 and 12.5 g/kg NaCl presented similar AFI to those fed 5.0 and 7.5 g/kg NaCl.

No mortality was observed for the fingerling groups that received 0.0 and 2.5 g/kg NaCl, whereas the higher level of NaCl resulted in high mortality rates. NaCl at 5.0, 7.5 and 10.0 g/kg led to intermediate fat, but statistically

Table 2. Effect of diets with growing levels of NaCl on performance of Nile tilapia fingerlings (means ± standard deviation)

Variable[1]	Level of NaCl in diet (g/kg)					
	0.0	2.5	5.0	7.5	10.0	12.5
IW (g)	4.63±0.06	4.60±0.12	4.61±0.09	4.69±0.04	4.55±0.09	4.57±0.04
FW (g)	55.02^{a}±3.46	46.33^{b}±3.33	35.72^{c}±2.30	34.54^{c}±1.51	33.46^{c}±2.08	33.25^{c}±1.39
AFC	0.66^{c}±0.08	0.79^{c}±0.13	1.08^{ab}±0.09	1.00^{a}±0.04	1.13^{ab}±0.05	1.23^{b}±0.07
SGR (%)	5.15^{c}±0.12	4.81^{b}±0.18	4.19^{a}±0.14	4.23^{a}±±0.10	4.15^{a}±0.13	4.13^{a}±0.07
WG (g)	50.40^{a}±3.42	41.74^{b}±3.38	31.03^{c}±2.29	29.93^{c}±1.54	28.91^{c}±2.06	28.68^{c}±1.09
CYH (%)	79.51±6.41	79.69±1.39	82.01±1.42	82.91±1.64	82.91±3.13	81.62±6.15
CYWH (%)	57.99±5.25	56.07±2.51	56.06±2.04	57.06±2.91	58.78±2.91	55.70±4.34
FY (%)	25.54^{ab}±3.20	25.73^{ab}±1.51	29.46^{b}±3.30	26.73^{ab}±2.30	26.73^{ab}±2.14	23.92^{a}±1.87
AFY (g)	791.92^{a}±10.58	749.02^{b}±16.81	700.69^{c}±16.85	710.25^{c}±15.73	721.70^{bc}±6.56	721.34^{bc}±12.75
SR (%)	100.00^{a}±0.00	100.00^{a}±0.00	96.80^{ab}±8.00	96.00^{ab}±3.92	94.40^{ab}±3.20	88.80^{b}±7.76
VFI	0.40±0.26	0.43±0.15	0.47±0.16	0.45±0.13	0.37±0.11	0.48±0.16
HSI	1.66b±0.28	1.67^{b}±0.21	1.21^{a}±0.13	1.30^{ab}±0.13	1.30^{ab}±0.14	1.26^{a}±0.17

[1]IW, initial weight; FW, final weight (g); AFC, apparent feed conversion; SGR, specific growth rate (calculated according Baoshan *et al.,* 2019); WG, weight gain; CYH, carcass yield with head; CYWH, carcass yield without head; FY, fillet yield; AFY, average feed intake (g per tank); SR, survival rate; VFI, visceral fat index; HSI, hepatossomatic index. Means followed by different letters on the same line differ by Tukey's test ($p<0.05$).

close to the 12.5 g/kg NaCl feed results. NaCl levels did not influence the VFI. The HSI was lower in the 5.0 and 12.5 g/kg NaCl diets. Linear effects on FW, WG, SGR and AFC were observed. Increasing NaCl inclusion levels led to decreased FW ($y = 50.1754 – 0.16472x$; $r^2 = 0.6984$), SGR ($y = 45.5418 – 0.16868x$; $r^2= 0.6966$) and WG ($y = 4.9465 – 0.008x$; $r^2 = 0.6886$) values and increasing AFC ($y = 0.7113 + 0.0043x$; $r^2 = 0.7655$) values.

Body chemical composition variable results of Nile tilapia fingerlings fed feeds containing increasing NaCl levels are displayed in Table 3. Only fat and the BMI were influenced by NaCl levels ($p<0.05$). Decreased fat values were observed at 2.5 g/kg NaCl and the highest fat value was found for 5.0 g/kg NaCl, while the other treatments resulted in intermediate values. The highest dry matter (DM) level was observed in the 7.5 g/kg NaCl treatment and the lowest at 12.5 g/kg, while the other treatments resulted in intermediate values.

The NaCl-free diet resulted in a higher protein efficiency rate (PER) and apparent protein retention (APR) ($p<0.05$) values. The 10.0 g/kg NaCl inclusion treatment, on the other hand, led to the lowest values of these variables. A decreasing linear effect on PER ($y = 3.7804 - 0.0116x$; $r^2 = 0.6549$) was noted with increasing NaCl levels.

The chemical composition results of Nile tilapia fingerlings fed increasing NaCl levels are displayed in Table 4. Fat and CP fillet values were influenced by NaCl levels ($p<0.05$), contrary to what was observed for MM and DM ($p> 0.05$). The highest fat deposition in fillets was observed in the NaCl-free treatment, and the lowest, in the 5.0 and 7.5 g/kg NaCl treatments, while the other treatments presented intermediate values. Fillet CP values were higher in the 5.0, 7.5 and 12.5 g/kg NaCl treatments, the worst value was observed in the 5.0 g/kg NaCl treatment and the NaCl-free treatment resulted in an intermediate value. The best fillet protein retention values ($p<0.05$) were observed in the NaCl-free treatment, and the lowest value, in the 12.5 g/kg NaCl treatment, while the other treatments also presented intermediate values.

Discussion

No consensus has been reached among researchers regarding the amount of NaCl to be included in fish diets.

Table 3. Body chemical composition of the Nile tilapia fingerlings fed with diets containing increasing levels of sodium chloride (means ± standard deviation).

Variable[1]	Initial fingerling	Level of NaCl in diet (g/kg)					
		0.0	2.5	5.0	7.5	10.0	12.5
Fat	7.5	15.4^{ab}±0.45	9.6^{a}±0.13	17.4^{b}±0.37	14.5^{ab}±0.29	14.6^{ab}±0.48	14.9^{ab}±0.36
MM	35.9	33.5±0.48	33.0±0.30	30.6±0.61	31.8±0.51	34.6±0,78	24.3±0.92
DM	209.6	223.8^{ab}±0.63	220.8^{ab}±0.87	218.1^{ab}±0.74	228.5^{b}±1.63	218.9^{ab}±1.37	202.1^{a}±0.20
CP	142.1	163.2±1.67	158.2±1.31	154.5±1.47	154.5±1.54	152.3±1.21	162.1±2,36
PER	-	4.13^{d}±0.14	3.44^{c}±0.22	2.64^{ab}±1.62	3.00^{bc}±0.96	2.54^{a}±0.13	2.57^{ab}±0.045
APR (%)	-	74.21^{c}±8.51	72.96^{bc}±15.22	47.40^{a}±10.31	53.58^{ab}±4.18	44.82^{a}±7.00	48.21^{a}±11.60

[1]MM, mineral matter; DM, dry matter; CP, crude protein, PER, protein efficiency rate; APR, apparent protein retention. Means followed by different letters on the same line differ by Tukey's test ($p<0.05$).

Table 4. Chemical composition of fillet of Nile tilapia fingerlings fed diets containing increasing levels of sodium chloride (means ± standard deviation)

	Level of NaCl in diet (g/kg)					
	0.0	**2.5**	**5.0**	**7.5**	**10.0**	**12.5**
Fat	10.1^{d}±0.43	7.6^{bc}±0.12	5.5^{a}±0.046	5.4^{a}±0.046	5.8^{ab}±0.80	9.2^{cd}±0.17
MM	17.9±0.081	13.3±0.041	12.0±0.14	12.1±0.14	13.1±0.081	12.6±0.047
DM	219.1±0.30	221.7±0.58	219.8±0.61	213.0±0.61	217.1±1.66	211.1±0.70
CP	194.1^{ab}±1.20	182.8^{b}±0.86	204.8^{a}±0.63	205.9^{a}±0.63	193.2^{ab}±0.78	204.3^{a}±1.04
APR (%)	18.70^{c}±3.06	18.00^{bc}±5.32	15.12^{abc}±4.50	15.26^{abc}±2.03	11.52^{ab}±3.19	10.34^{a}±1.99

MM, mineral matter; DM, dry matter; CP, crude protein; APR, apparent protein retention. Means followed by different letters on the same line differ by Tukey's test ($p<0.05$).

Cl^- and Na^+ deficiencies are difficult to demonstrate in fish (NRC, 1993) as these ions are abundant in both food and water. Because of this, metabolic deficiencies are rarely observed (NRC, 2011). Thus, although the possibility of deficiency is remote, the excess can be detrimental to the performance of the fish.

NaCl is an abundant and cheap mineral, and its bioavailability in ingredients should also be observed taken into account since ingredients of animal origin, such as fish meal contain high levels (5.0 to 10.0 g/kg), while concentrates of vegetable origin contain relatively low levels. In some cases, Na^+ concentrations in plant foods are so low that it is impossible to determine the bioavailability for this ion (Sugiura *et al.,* 1998). For example, soybean meal contains around 0.1 g/kg and corn, from 0.2 g/kg to 0.5 g/kg (Rostagno *et al.,* 2011).

Commercial fish diets contain about 2.4 g/kg of Na^+. The recommended NaCl level in purified feeds for the best weight gain for hybrid tilapia (*O. niloticus* × *Oreochromis aureus*) is of 1.5 g/kg (Shiau & Lu, 2004). According to Lall (2002), fish easily absorb these elements from the aquatic environment. Diets containing NaCl sources for freshwater-cultured fish can satisfy osmoregulatory requirements, and the energy used for osmoregulation is then free to be used for growth (Zaugg *et al.,* 1983; Gatlin *et al.,* 1992).

The results of the present study demonstrated that the inclusion of NaCl at increasing levels decreases Nile tilapia fingerling performance and increases mortality rates. Mzengereza & Kang'ombe (2015) concluded that the inclusion of NaCl at 20.0 g/kg in tilapia (*Oreochromis shiranus*) diets led to deleterious growth effects, as AFC, SGR and WG, were negatively affected. However, Shiua & Lu (2004) and Cnaani *et al.* (2010) stated the opposite in a study carried out on hybrid tilapia (*O. niloticus* × *O. aureus*), as well as Nandeesha *et al.* (2000) and Nasir & Hamed (2016) in a study carried out on carp (*Cyprinus carpio*).

Conflicting results to the present study were also reported in studies that demonstrated the benefit of dietary NaCl supplementation at higher levels, especially in euryhaline species raised in low salinity environments. Eroldogan *et al.* (2005), for example, found good performance for freshwater-cultivated sea bass (*Dicentrarchus labrax*) after 50.0 g/kg NaCl supplementation. Harpaz *et al.* (2005) found that 40.0 g/kg NaCl supplementation in *L. calcarifer* significantly improved feed conversion and enzymatic activity in the intestinal *villi* of these animals.

In contrast, other studies suggest that salt supplementation has no influence on fish growth and food use efficiency. Salman & Eddy (1988) found that acclimatization with a supplemented diet containing up to 120.0 g/kg NaCl decreased mortality rates in *Oncorhynchus mykiss*, but that this diet interfered with fish growth rates and food use efficiency during the first two weeks after transfer to salt water. On the other hand, Murray & Andrews (1979) indicate that salt supplementation had no effect on the growth of the catfish *Ictalurus punctatus*.

The same occurs with teleost, through osmoregulation, which consists in avoiding ion losses to a maximum (or presenting efficient mechanisms to capture ions from the media) and eliminating all excess water (or avoid its entry into the body) (Baldisserotto *et al.,* 2014). NaCl intake has a strong influence on Na^+, K^+-ATPase activity, which is accompanied by increased chloride cells in gills.

Another fact that may have impaired Nile tilapia fingerling performance was the amount of Cl- contained in common salt (602.3 g/kg), which is much higher than sodium 397.4 g/kg (Maynard & Loosli, 1974; Borges *et al.,* 1998).

Excess NaCl may have exceeded the ability of Nile tilapia fingerlings to absorb ions through their gills and ion losses through urine, leading to metabolic disturbances that could be responsible for growth.

Barros *et al.* (2004) suggest that Na^+ increases or decreases lead to higher water consumption, in order to maintain an ideal relation between electrolytes, or that less water is excreted via urine, in order to maintain body homeostasis. Therefore, dietary NaCl inclusion may have caused toxicity and/or increased energy expenditure by the Na-K pump for body homeostasis control, decreasing Nile tilapia fingerling growth performance and increasing mortality rates.

Excess NaCl may cause adverse effects on food intake and absorption, due to changes in intestinal gastric juices, inducing pathology development and decreased animal growth

(MacLeod, 1978). No pathologies were observed in Nile tilapia fingerlings during the study period, although weight, feed conversion, growth rate, feed intake and survival were influenced by NaCl inclusion. Dietary NaCl increases may have been a stress factor, interfering in the dietary balance of other essential components, leading to growth effects.

Nile tilapia fingerling CYH presented values close to those reported by Boscolo *et al.* (2010). The same authors found lower values for clean carcass yields compared to the present study, considered satisfactory and corroborating the results obtained by Souza *et al.* (1999), who reported that the tilapia clean carcass yields can vary according to the processing method.

The FY values observed in the present study were lower than those reported by Souza *et al.* (1999, 2000), Boscolo *et al.* (2001) and Souza (2002). According to these authors, FY depends both on the head removal methodology and filleting method.

No significant differences in VFI ($p> 0.05$) were observed between fish fed increasing dietary NaCl levels. According to Boscolo *et al.* (2010) the main fat deposit area in lean fish, such as tilapia, are the viscera. However, the different feeds did not lead to differences in visceral fat deposition, which can be explained by the fact that the feeds display balanced energy and protein levels. Excess carcass fat is an undesirable characteristic, although an amount that does not affect the meat quality must be maintained. Excess carcass fat is mainly noted in the adipose tissue of the abdominal cavity, which decreases fillet yields and, consequently, the commercial value of the fish (Meurer *et al.,* 2002).

The highest HSI observed in the 0.0 and 2.5 g/kg NaCl diets are related to the higher growth values observed in these treatments, as this index is directly related to energy reserve accumulation. Thus, higher amounts of salt may have negatively influenced Nile tilapia fingerling metabolism. In general, fish have the ability to store glycogen and fat in the liver, and energetic variations are significantly perceived in the weight of this organ through the hepatosomatic relationship (Hoar & Randall, 1971).

The chemical composition of the Nile tilapia fingerlings changed due to alterations in carcass fat and MO values. NaCl feed inclusion led to decreased feed intake, and, therefore, the metabolism of fewer nutrients. However, as the requirements were adequate, a decrease in feed consumption was noted due to increased NaCl values, leading to decreased growth, with an impact on carcass fat and MO values.

Fish chemical composition is directly related to dietary nutrient concentrations (Signor *et al.,* 2007; Ribeiro, 2014). Fingerlings submitted to the NaCl-free diet ingested more feed amounts, leading to slightly fatter carcasses without, however, significantly affecting visceral fat content. Similar results have been reported by Sales & Sales (1990) and Simões *et al.,* (2007).

The chemical fillet composition results were similar to that reported for Nile tilapia juveniles (Boscolo *et al.,* 2010; Carneiro *et al.,* 2017). For fish fillets, the highest fat value was observed for the NaCl-free and 12.5 g/kg NaCl diets, most likely due to the aforementioned reasons. On the other hand, the lowest values observed in the 5.0 to 10.0 g/kg NaCl feed treatments may be related to low nutrient and energy availability, due to low feed intake, resulting in smaller and leaner fish.

The NaCl-free diet led to the best PER, which indicates how much of dietary CP was converted to body weight (Sá & Fracalossi, 2002). In terms of absolute PER values, similar results were reported by Furuya *et al.* (2005), while lower values were reported by Righetti *et al.* (2011) and Furuya *et al.* (2013). Although Hallali *et al.* (2018) stated that evidence exists of increased protein digestibility, lipids, ashes and diet dry matter due to NaCl inclusion in Nile tilapia feed.

As for APR and APR of fillet, the NaCl-free diet provided the best protein retention values. This demonstrates that the feed consumption decreases affected Nile tilapia fingerling growth, and that part of this protein was used to maintain fish metabolism and not for body and fillet protein deposition. This can be corroborated by the lower AFC values, which may be related to the use of protein as a source of energy for fish maintenance.

Despite the very clear effect on Nile tilapia fingerling performance and survival due to the inclusion of dietary NaCl, it is quite clear that further studies in this regard are required, as it acts as a Nile tilapia fingerling feed ingestion regulator. The inclusion of dietary NaCl affects Nile tilapia fingerling feed intake, performance and survival and is not recommended.

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