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SHORT COMMUNICATION

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Sodium chloride levels in pacu (*Piaractus mesopotamicus*) fingerling feeds free of fish meal

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Abstract

Aim of study: To evaluate the effects of increasing NaCl levels on the zootechnical performance of pacu fingerling.

Area of study: The experiments were conducted at the Aquatic Organism Production and Reproduction Systems Laboratory belonging to the Federal University of Paraná (UFPR), in the Palotina Sector, Paraná Estate, Brazil.

Material and methods: Seven hundred and fifty fingerlings with an average weight of 3.41 ± 0.09 g were distributed in circular boxes, in a completely randomized design consisting of six treatments and five replications. Treatments comprised soybean- and maize-based diets containing increasing levels of NaCl (0.00, 0.25, 0.50, 0.75, 1.00 and 1.25%). The experiments were conducted for 50 days. At the end of the experimental period the fish were fasted for 24 hours, anesthetized, weighed and measured to calculate zootechnical performances. Performance data were subjected to an analysis of variance followed by Tukey's test when significant differences were found between the means (p<0.05).

Main results: The influence (p<0.05) of dietary NaCl levels on final weight, feed intake, apparent feed conversion, specific growth rate, average weight gain, clean trunk production, head carcass yield, headless carcass yield, feed intake and survival was assessed. The results indicate that non-salt treated fingerlings along with the 0.25% salt inclusion treatment led to better feed use, as evidenced by apparent feed conversions of 1.64 and 2.02, respectively.

Research highlights: The inclusion of NaCl in pacu fingerling soybean and maize-based diets is not recommended.

Additional key words: salt; macrominerals; round fish; zootechnical performance.

Authors' contributions: FM and RAB determined the experimental design, formulated diets, and acquired the ingredients of diets. NCA, IVZ, PSD, MTM participated in the experimental execution, data collection and analysis of physic-chemical variables of water. All authors participated in the article writing and discussion of results.

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Introduction

The cultivation of native fish species from Brazil has increased significantly in the last decade, and pacu (*Piaractus mesopotamicus*) is noteworthy, as it presents several desirable characteristics, including rusticity, feed acceptability and a tasty meat with high commercial value (Jomori *et al.*, 2005). Originally from rivers that make up the La Plata Basin, this species is found through the Paraná, Paraguay and Uruguay rivers (Godoy, 1975; Saint-Paul, 1986) and is widely distributed throughout South America.

Sodium (Na) and chloride (Cl) are widely available and inexpensive macrominerals essential to animal metabolism. These elements can be easily administered in the form of dietary sodium chloride (NaCl), improving feed palatability as a source of Na^+ (39.74%) and Cl^- (60.23%) (Maynard & Loosli, 1974; Mzengereza & Kang'ombe, 2015). As essential elements, they play a role in maintaining osmotic pressure, basic acid balance and water metabolism, in the control of nutrient passage to cells and as a gastric juice constituent (Evans *et al.*, 2005).

NaCl is widely exploited in aquatic animals, specifically concerning its application in water, as a way to prevent and control parasitic diseases caused by fungi and bacteria (Marchioro & Baldisserotto, 1999). Although dietary NaCl use effects on fish growth are yet not clear, some studies have reported a positive effect when this compound is added in higher amounts to certain fish diets, such as *Oreochromis niloticus* (Fontaínhas-Fernandes *et al.*, 2000a,b), *Cyprinus carpio* and *Cirrhinus mrigala* (Nandeesha *et al.*, 2000), Labeo rohita (Gangadhara *et al.*, 2004), *Lates calcarifer* (Harpaz *et al.*, 2005), *Dicentrarchus labrax* (Eroldogan *et al.*, 2005) and *Oreochromis shiranus* (Mzengereza & Kang'ombe, 2015).

Studies involving the use of NaCl in fish diets have been reported for the euraline species Sparus aurata and *Lates calcarifer* grown in low salinity environment, indicating good growth and survival results when 8-12% of salt is added (Appelbaum & Jesuarockiaraj, 2009; Arockiaraj & Appelbaum, 2010). The final weight and biomass of other freshwater species, such as jundiá (*Rhamdia quelen*), have been reported as suffering the influence of diets containing high levels of NaCl (Garcia *et al.*, 2007), while hybrid tilapia displayed improved performance due to dietary NaCl supplementation (Cnaani *et al.*, 2010). Hallali *et al.* (2018), reported that dietary salt supplementation (5%) in Nile tilapia significantly increased protein, lipid, ash and dry matter digestibility, peptide transporter and ion pump expression activity. In addition, changes to intestinal microbial diversity were observed and associated with lipid digestibility and Na+/K+-ATPase expression.

Na^+ and Cl^- deficiency impair fish growth (NRC, 2011). Considering the results obtained for euraline species, and the lack of information on the use of NaCl in the diet of freshwater fish, this work aimed to study the inclusion of increasing levels of NaCl in pacu (*Piaractus mesopotamicus*) fingerlings diets, evaluating zootechnical performance aspects.

Material and methods

The experiments were conducted at the Aquatic Organism Production and Reproduction Systems Laboratory belonging to the Federal University of Paraná (UFPR), in the Palotina Sector. The procedures adopted in this study are in accordance with the Ethical Principles in Animal Experimentation and were approved by the UFPR Palotina Sector Ethics and Animal Use Commission (CEUA) (Protocol nº 21/2017). The experimental period was 50 days.

A total of 750 Pacu fingerlings with average weight of 3.41 ± 0.09 g and total average length of 5.31 ± 0.62 cm were distributed in thirty 1,000-L circular boxes, in a water recirculation system, with a 2,000-L box acting as a mechanical filter and a 30,000-L tank containing *Eichornia crassipes* for biofiltration, with daily renewal of approximately five times the total water volume. The aeration system consisted of a 0.9863 horsepower air blower connected to PVC pipes that delivered oxygen to each experimental unit through silicone hoses containing porous stones at the ends. Before the last daily feeding, approximately 10% of water was siphoned from each experimental unit to remove possible feed leftovers, feces and organic matter. Each 1,000-L box containing 25 fingerlings was considered an experimental unit, in a completely randomized design consisting of six treatments and five replications. Treatments (Table 1) consisted in isoproteic (24.65% digestible protein) and isoenergetic (3000 kcal kg^{-1}) soybean and maize based diets (Neves *et al.*, 2015) containing increasing levels of NaCl (0.00, 0.25, 0.50, 0.75, 1.00, and 1.25%). Red clay was used as an inert feed ingredient, although some studies indicate that certain types of clays can be used as additives aiming to improve feed quality, as they display the ability to adhere aflatoxins (Hussain *et al.*, 2017; Hussain, 2018). The ingredients were initially ground in a 0.7-mm sieve hammer type crusher. After milling, the ingredients were weighed and mixed and extruded using a 15 kg h^{-1} experimental extruder. After extrusion, the feeds were dried in a forced ventilation oven at 55 °C for 24 h and then packaged in plastic bags. Fish feeding was carried out three times a day (8 am, 1 pm and 6 pm) until apparent satiety.

Physical and chemical water variables (pH, ammonia, nitrite, conductivity and dissolved oxygen) were monitored once a week, while the minimum and maximum temperatures were measured daily. The physical and chemical water variables pH (8.1 ± 0.36), ammonia (0.074 ± 0.10 mg L^{-1}), nitrite (0.017 ± 0.005 mg L^{-1}), electrical conductivity (174 , 64 ± 17.83 µS cm-1), dissolved oxygen (6.78 ± 0.35 mg L^{-1}) and minimum (24.4 ± 1.98 °C) and maximum (28.4 ± 1.88 °C) temperatures were within the recommended range for freshwater fish farming (Sá, 2012).

Performance data were subjected to a normal homogeneity distribution analysis (Levene) and analysis of variance (one-way ANOVA) and when significant differences were found between the means, the Tukey test was applied ($p<0.05$), using the Statistica 7.0® statistical software (Statsoft, 2005).

Results and discussion

An influence ($p<0.05$) of dietary NaCl levels on final weight, apparent feed conversion, average weight gain, biomass, feed intake and survival was noted (Table 2), as well as the specific growth rate, head carcass yield and clean trunk production were also affected (Table 3).

The final average weight was lower in the treatments with inclusion of 0.75, 1.00, and 1.25% NaCl ($p<0.05$). The specific growth rate was better in the zero NaCl treatment; however, and equal to the 0.25%, 0.50% and 1.25%

Table 1. Formulation and chemical composition of experimental diets containing different levels of NaCl based on dry matter.

Ingredients	NaCl (%)					
	0.00	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**
Soybean meal	60.30	60.30	60.30	60.30	60.30	60.30
Maize	31.27	31.27	31.27	31.27	31.27	31.27
Soy oil	3.30	3.30	3.30	3.30	3.30	3.30
Dicalcium phosphate	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin and mineral supplement[1]	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	0.00	0.25	0.50	0.75	1.00	1.25
Inert[2]	1.25	1.00	0.75	0.50	0.25	0.00
BHT[3]	0.01	0.01	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00
	Nutrients and energy					
Digestible energy (kcal kg^{-1})	3000.0	3000.0	3000.0	3000.0	3000.0	3000.0
Gross energy (kcal kg^{-1})	4135.8	4135.8	4135.8	4135.8	4135.8	4135.8
Digestible protein (%)	24.65	24.65	24.65	24.65	24.65	24.65
Raw fiber (%)	3.6	3.6	3.6	3.6	3.6	3.6
Total phosphorous (%)	1.00	1.00	1.00	1.00	1.00	1.00
Ethereal extract (%)	5.28	5.28	5.28	5.28	5.28	5.28
Mineral matter (%)	8.42	8.42	8.42	8.42	8.42	8.42

[1] Vitamin and mineral supplement: product composition in milligrams per kilogram (folic acid: 200 mg; pantothenic acid: 4,000 mg; biotin: 40 mg; copper: 2,000 mg; iron: 12,500 mg; iodine: 200 mg; manganese: 7,500 mg; niacin: 5,000 mg; selenium: 70 mg; vitamin A: 1,000,000 IU; vitamin B1: 1,900 mg; vitamin B12: 3,500 mg; vitamin B2: 2,000 mg; vitamin B6: 2,400 mg; vitamin C: 50,000 mg; vitamin D3: 500,000 IU; vitamin E: 20,000 IU; vitamin K3: 500 mg; zinc: 25,000 mg). [2] Red clay. [3] Butyl hydroxy toluene (antioxidant).

NaCl treatments. Garcia *et al.* (2007) offered a basal diet supplemented by increasing NaCl levels of 1.2%, 2.5%, 5.0% and 6.0% to jundiá (*Rhamdia quelen*) fingerlings and reported an influence on the weight and final biomass of fish, indicating that the 1.2% NaCl diet led to the best results. Motlagh *et al.* (2012) found good growth results for flag acara (*Pterophyllum scalare*) fed diets containing 0.75% NaCl inclusion, while a study on tambaqui (*Colossoma macropomum*) demonstrated opposite growth results for fish meal diets with the inclusion of 1.5% NaCl (Gangadhar & Keshavanath, 2012). It is important to note that these authors used feeds with the inclusion of animal ingredients, while the present study used vegetable components (soybean meal and maize).

NaCl is an abundant and inexpensive mineral, and its bioavailability in ingredients should also be noted, as animal ingredients, such as fishmeal, are considered good sodium sources (0.5 to 1%), while plant concentrates contain relatively low sodium levels, ranging from 0.01 to 0.07%, with soybean meal sodium contents of around 0.01% and maize contents ranging from 0.02 to 0.05% (Rostagno *et al.*, 2011). In addition, sodium concentrations in plant

Table 2. Performance parameters of pacu (*Piaractus mesopotamicus*) fingerlings fed diets containing increasing levels of NaCl.

Variable	Dietary NaCl feed inclusion (%)						
	0.00	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**	***p*-value**
Average starting weight (g)	3.38±0.11	3.39±0.09	3.41±0.10	3.42±0.10	3.42±0.07	3.41±0.07	0.947
Average final weight (g)	29.95±1.41^{b}	24.81±4.03^{ab}	26.60±3.05^{ab}	24.31±1.75^{a}	23.59±3.23^{a}	25.06±0.82^{a}	0.011
Average weight gain (g)	26.57±1.45^{b}	21.42±4.02^{ab}	23.19±3.12^{ab}	20.89±1.66^{a}	20.16±3.20^{a}	21.64±0.76^{a}	0.010
Average feed consumption (g)	1089.36±14.90^{a}	1050.17±12.72^{c}	1092.48±16.83^{ab}	1103.44±17.11^{ab}	1119.12±11.61^{b}	1093.22±13.01^{ab}	0.000
Biomass (g)	748.71±35.35^{a}	620.26±100.70^{ab}	564.41±51.76^{b}	579.41±65.62^{b}	532.83±121.88^{b}	491.65±62.84^{b}	0.006
Apparent feed conversion	1.64±0.09^{b}	2.02±0.39^{ab}	2.26±0.29^{ab}	2.30±0.21^{ab}	2.67±0.72^{a}	2.72±0.49^{a}	0.005
Feed consumption (% live weight)	4.75±0.19^{c}	5.39±0.68^{bc}	6.18±0.49^{abc}	5.99±0.65^{abc}	6.75±1.44^{ab}	7.09±1.01^{a}	0.002
Survival (%)	100.00±0.00^{a}	100.00±0.00^{a}	85.60±10.43^{ab}	95.20±6.57^{a}	89.60±10.81^{ab}	78.40±8.76^{b}	0.000

Means followed by different letters on the same line differ from each other by the Tukey test (p<0.05).

Table 3. Specific growth rate, clean trunk production, carcass yield, and hepatosomatic and visceral fat indices of pacu *Piaractus mesopotamicus* fingerlings fed diets containing increasing NaCl levels.

Variable	Dietary NaCl feed inclusion (%)						
	0.00	0.25	0.50	0.75	1.00	1.25	*p*-value
Specific growth rate (% day^{-1})	4.45±0.13[b]	4.04±0.34[ab]	4.18±0.29[ab]	4.00±0.10[a]	3.92±0.25[a]	4.07±0.03[ab]	0.013
Head carcass yield (%)	83.37±5.84[b]	79.28±1.33[ab]	74.08±5.85[a]	77.86±1.97[ab]	78.59±1.00[ab]	76.89±1.87[ab]	0.014
Clean trunk production (g)	280.47±19.60[a]	243.66±46.47[ab]	221.16±37.75[ab]	232.14±27.39[ab]	213.78±50.67[ab]	190.28±26.37[b]	0.016
Clean trunk yield (%)	37.52±3.13	39.19±2.67	39.02±3.78	40.08±2.11	40.04±1.00	38.67±1.57	0.631
Visceral fat index	0.68±0.15	1.05±0.19	1.14±0.54	0.72±0.14	1.30±0.68	1.36±0.64	0.124
Hepatosomatic index	1.83±0.21	1.64±0.59	2.11±0.16	2.15±0.38	2.20±0.19	2.20±0.22	0.060

Means followed by different letters on the same line differ from each other by the Tukey test ($p<0.05$).

foods are so low in some cases that bioavailability cannot be determined (Sugiura *et al.*, 1998). Therefore, it could be necessary to include salt depending on the source of origin of the ingredients; however, each case must be studied to ascertain its effects on performance and health, which will depend on and be specific to each fish species.

Survival rates were 100% in the 0 and 0.25% sodium chloride treatments, but statistically equal to the 0.50, 0.75 and 1.00% inclusions. Aguiar *et al.* (2020), also obtained greater survival in treatments without or with lower NaCl inclusions in the Nile tilapia diet.

Feed NaCl inclusion led to a significant effect on feed intake ($p<0.05$), with a higher average intake in the 1.00% NaCl inclusion treatment, followed by 0.75% NaCl. Feed intake (as a percentage of live body weight) increased with increasing dietary NaCl. The highest consumption was observed in the 1.25% NaCl treatment, differing from the 0.00% treatment ($p<0.05$). Some authors have reported the beneficial effect of dietary salt supplementation, including increased appetite and improved digestibility (Fontainhas-Fernandes *et al.*, 2000a), increased digestive enzymatic activity (Harpaz *et al.*, 2005), and increased Na^+, K^+-ATPase activity in the branchial epithelium marked by growth (Hallali *et al.*, 2018). However, diets containing increasing salt levels did not improve pacu fingerling performance despite the increased feed intake observed in the present study.

As for the other aforementioned results, apparent feed conversion was better for the treatment without NaCl inclusion, but was equal for the 0.25%, 0.50% and 0.75% NaCl inclusion treatments. The results indicate that non-salt treated fingerlings and fingerlings fed the 0.25% salt inclusion treatment showed better feed use, as evidenced by apparent feed conversions of 1.64 and 2.02, respectively. This indicates an appropriate diet, leading to higher specific growth rates.

On the other hand, the present study differed from the study carried out by Mzengereza & Kang'ombe (2015) on tilapia (*Oreochromis shiranus*) fed diets containing animal ingredients with NaCl inclusion levels of 0.0, 1.0, 1.5 and 2.0%. The authors reported better performance parameter responses in diets containing 1.0% and 1.5% NaCl inclusion and apparent feed conversions of 1.57 and 1.44, while indicating that a 2% NaCl inclusion level negatively affected food intake, digestion and/or absorption due to changes in the gastric/intestinal environment, with possible pathological effects. The results also contrast from the study carried out by Gangadhara *et al.* (2004), which reported good results for L. rohita fed diets containing 0.5% and 1.0% NaCl inclusion, with apparent feed conversions of 1.52 and 1.44, respectively and, consequently better specific growth rates.

Increased dietary salt may have become a stress factor, interfering with the dietary balance of other essential components, leading to animal growth effects. Mzengereza & Kang'ombe (2015) concluded that the inclusion of 2.0% salt in tilapia diets impaired apparent feed conversion, specific growth rate and weight gain, causing deleterious growth effects.

Headless carcass yield was also better in zero NaCl inclusion treatment, but did not differ from the 0.25% inclusion treatment. Clean trunk production was higher in the 0% NaCl treatment when compared to the highest inclusion level (1.25%) ($p<0.05$). As for Nile tilapia fingerlings fed with increasing levels of NaCl (0 to 1.25%), the influence of salt on carcass yield with and without head was not verified (Aguiar *et al.*, 2020).

Regarding the visceral fat and hepatosomal indices, results were similar for all treatments and no significant differences were observed ($p > 0.05$) between treatments. Aguiar *et al.* (2020), also found no effects of dietary salt on the visceral fat index of Nile tilapia fingerlings, but found that the hepatosomatic index was higher in fish fed diets containing 0, 0.25, 0.75 and 1.0% of NaCl.

Intake of NaCl by freshwater fish has a strong influence on the gill activity of Na+-K+-ATPase, which is accompanied by an increase in chloride cells, for there is a need to increase the extrusion capacity of NaCl through the gills. Salt feeding imposes a salt load that exceeds the normal capacity for loss by diffusion through the gills and excretion through the urine (Mzengereza & Kang'ombe, 2015). Another fact which may have affected animal performance in the present study is the amount of chloride contained in common salt, as chloride contents (60.23%), are much higher than sodium contents (39.74%) (Maynard & Loosli, 1974). Therefore, excess salt may have caused metabolic disturbances, preventing the normal metabolic processes responsible for growth (Mzengereza & Kang'ombe, 2015).

However, no concrete studies on the quantitative requirements of NaCl are available, especially because fish easily absorb these elements from the surrounding environment (Lall, 2002). Thus, evaluations concerning diets containing salt levels between 0.00 and 0.25% are paramount in order to verify the possibility of an optimum inclusion level between these values. In summary, the inclusion of NaCl in pacu fingerling soybean and maize meal-based diets ranging from 0.0 to 1.25% common salt at 0.25% intervals is not recommended.

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