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Effect of different stocking densities on pre-slaughter stress based on respiratory parameters in Nile tilapia (*Oreochromis niloticus*)

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Abstract: This study evaluated whether the resting density after transport in Nile tilapia *Oreochromis niloticus* shows responses in the respiratory and ionic dynamics related to stress parameters. Forty specimens were submitted to four experimental treatments consisted of control (minimum stress/after removal from water), after transport and one hour of rest, in two stocking densities (50 and 300 kg of live weight)/m³), with 10 repetitions per treatment. Each fish was considered an experimental unit. Serum stress was evaluated by measuring the glucose and lactate levels, and respiratory dynamics were determined by measuring ionic concentration. The fish in the group after transportation remained in hypoventilation due to high PCO_2 values, demonstrating compensatory respiratory acidosis. The stress level was high, indicating increased glycemia. The group of fish in the 300 kg/m³ density remained in respiratory acidosis. No change in lactate was observed in any of the resting densities. It is concluded that the 50 kg/m^3 density is suitable for pre-slaughter rest because it results in fish with reduced stress responses and with reestablished respiratory responses.

Key words: alkalosis, homeostasis, stress, transporting.

Resumo: Efeito de diferentes densidades de estocagem no descanso pré abate sobre esforço respiratório e muscular de tilápia do Nilo (*Oreochromis nilticus*). Este trabalho teve como objetivo avaliar se a densidade de repouso pós transporte de tilápia-do-Nilo apresenta respostas na dinâmica respiratória e iônica relacionadas com parâmetros de estresse. Foram submetidos ao experimento 40 espécimes distribuídas em quatro tratamentos que foram compostos por: controle (mínimo estresse/após a retirada na água), após o transporte e após o descanso de uma hora em duas densidades de estocagem (50 e 300 kg de peso vivo/m³), com 10 repetições por tratamento. Cada peixe foi considerado uma unidade experimental. Para mensuração do estresse sérico foram mensurados níveis de glicose e lactato, e a concentração iônicas para determinação da dinâmica respiratória. Os peixes do grupo após o transporte mantiveram-se em hipoventilação devido aos elevados valores de PCO_2, demonstrando acidose respiratória compensatória. O nível de estresse foi elevado, demonstrado pelo aumento da glicemia. O grupo de peixes da densidade de 300 kg/m³ manteve- se em acidose respiratória. Não houve alteração na concentração de lactato plasmático. Conclui-se que a densidade de 50 kg/m^3 é a adequada para o descanso pré-abate, pois resulta em peixes com diminuição das respostas de estresse e reestabelecimento das respostas respiratórias.

Palavras-Chave: alcalose, homeostasia, estrese, transporte.

Introduction

The handling care of fish, from removal from the tank to transport, and during the period before slaughter, directly affects the well-being that can reflect the fillet's sensory quality (Fantini *et al.* 2020). If pre-slaughter procedures are not carried out with technical criteria, stressing conditions can be increased, reducing the product's shelf life (Goes *et al*. 2019). Concern for well-being is an aspect that requires attention due to the impact on the quality of fish for the final consumer. Some studies have been looking for biochemical (Venturini *et al.* 2018) and molecular markers (Zuanazzi *et al*. 2019) to prematurely identify the quality of the fish that will be slaughtered.

The timeline of the management of fish farming before the slaughter begins with capture in the tank, transport, arrival at the slaughterhouse, unloading, resting before slaughter, and finally, the stress of the slaughtering process. In these steps, the resting period can be crucial for restoring fish homeostasis (Fantini *et al.* 2020); however, densification can promote changes in the stress responses in Nile tilapia, which, consequently, can change the quality of the fillet (Hong *et al*. 2019). The increase in density causes changes due to stress mainly for Nile tilapia, fish of importance in aquaculture worldwide (Goes *et al*. 2018). Neotropical fish species also show undesirable meat quality responses when subjected to improper handling such as tambaqui *Colossoma macropomum* (Mendes *et al*. 2015) and matrinxã *Brycon amazonicus* (Venturini *et al*. 2018).

These pre-slaughter management stages are known as stress promoters in fish (Poli *et al*. 2005). However, the physiological and biochemical characteristics, such as mobilization of energy reserves in muscles and liver and changes in blood acid-base balance (Gatica *et al*. 2010) are not yet described in each of these stages. The physiological responses to stress depend on the nature of the stress (Ferreira & Barcellos 2008); changes in heart rate, oxygen absorption, mobilization of energy substrates, and disturbance of the hydromineral balance (Fantini *et al*. 2020) can lead to exhaustion (Bagni *et al*. 2007; Goes *et al*. 2019). The changes promoted by the pre-slaughter are measured by the blood indirect indicators and reveal to us if there are conditions to obtain quality of meat of great quality (Anders *et al*. 2020; Venturini *et al*. 2018).

Furthermore, the depuration process can also be stressful to fish. This procedure is commonly performed after arrival at the slaughter unit to decrease the microbial load and remove the excess of specific compounds or unwanted contaminants from the fish's organism (Fontenele *et al*. 2013).

The *Oreochromis niloticus* (Nile tilapia) is a widespread species in aquaculture. Some studies report the effect of stress on the quality of fish (Goes *et al*. 2019; Venturini *et al*. 2018; Zuanazzi *et al*. 2019); however, some parameters have not yet been elucidated, mainly those associated with respiratory metabolism responses and their implications in pre-slaughter management.

In management situations that promote fish stress, intracellular oxygen supply is inadequate, with low ATP production, due to the lower energy yield of anaerobic respiration (Honorato *et al*. 2014). The concomitant production of lactate, electrolytic imbalance may be the result of inadequate management in the pre-slaughter of fish (Anders *et al*. 2020). Therefore, the use of blood variables may be indicative for rational management and adequacy of clearance time for reestablishment of fish homeostasis

Therefore, this study evaluated whether the resting density after Nile tilapia's transport shows responses in the respiratory and ionic dynamics related to stress parameters.

Material and methods

The fish are from a semi intensive production system, produced in excavated tanks. The fish were fed species-specific commercial feed during the production cycle. The experiment was conducted in a linear process scheme consisting of four steps, totaling four treatments with 10 repetitions per treatment (each fish was considered an experimental unit); 40 fish were sampled, with an average weight of 762 ± 105 g.

Steps:

I Control Group was exposed to minimal stress after removal from the water.

II After transportation.

III After transportation and 1-hour rest at the stocking densities of 50 kg of live weight/m³.

IV After transportation and 1-hour rest at the stocking densities of 300 kg of live weight/m³.

The fish were initially removed from nurseries with the aid of a net and dip net and placed in a transporting box with constant aeration at a density of 200 kg/m³. The transport water was added with 6 mg/L sodium chloride, and the water temperature was lowered to 21 °C with ice. The fish were transported for one hour until arriving at the Laboratory of Analysis of Agricultural Products of the College of Agricultural Sciences of the Federal University of Grande Dourados.

In the laboratory, the fish were placed in polyethylene boxes with a capacity of 500 L and supplied with an artificial aeration system. Each experimental density (50 and 300 kg of live weight/m³) was placed in separate boxes. The fish remained resting for one hour; 10 fish per density was sampled afterward.

Hemogasometric analyzes: Blood samples were collected by caudal puncture in 3 mL syringes (Ishikawa *et al*. 2010) and analyzed in a blood gas analysis device (Cobas HB121 - Roche Diagnostica Brasil, São Paulo, SP, Brazil). Sodium (Na^+), Potassium (K^+), and Chloride (Cl^-) ions were measured. Respiratory blood parameters, such as pH, H^+, bicarbonate (HCO^-3), partial oxygen pressure (PO_2), partial carbon dioxide pressure (PCO_2), and functional oxygen saturation (SO_2), were also determined (Fantini *et al*. 2020)

Evaluation of biochemical parameters: Glucose and creatinine analyses were conducted in blood samples collected by vena caudal puncture using 3 mL heparinized syringes. These analyses were performed using commercial *kits* (Gold Analisa Diagnóstica®), according to the manufacturer's instructions, and readings of results were conducted on a semi-automatic spectrophotometer (BIOPLUS S200). The enzymatic parameters related to lactate (Lactate Kit; Katal Biotecnológica Ind. Com. Ltda. Minas Gerais, MG, Brazil) were measured at 546 nm (RA-XT; Technicon Instrument Corp, Tarrytown, NY).

Statistical analysis: The results were subjected to analysis of variance (ANOVA) using the General Linear Models procedure in the STATISTICA 7.1® software (Statsoft Inc., Tulsa, OK, USA) at a 5% level of significance. The Tukey test was applied to verify differences between means when significant differences ($p<0.05$) were observed. Data were expressed as mean ± standard error.

Results

The fish had sanitary conditions that guaranteed their quality for transportation, slaughter and commercialization. After transport, the fish remained in hypoventilation due to high PCO_2 values, demonstrating compensatory respiratory acidosis because the pH maintained the homeostatic control with its buffer function, along with $CHCO_3$ stability (Table I).

Hyperkalaemia and excessive blood nitrogen metabolite are showed on Table II. The fish in the group kept at rest in the density of 50 Kg/m³ showed respiratory acidosis, high pH compensation (Table I), hypercalcemia, increased glycemia, and decreased creatinine compared to the control group. The fish group with the density of 300 Kg/m³ remained in respiratory acidosis and increased lactate values compared to the control group (Table I). No lactate alterations were observed in any of the resting densities.

Discussion

The stocking density during the resting process may have an influence on the well-being of fish, restoring physiological characteristics to those before the handling of transport. Management studies are critical to optimize the fish production chain (Fantini *et al*. 2020; Zuanazzi *et al*. 2019). In this experiment, we demonstrate that the stocking density can influence respiratory function and affect homeostasis.

Changes in respiratory rates that result in differences in oxygen saturation can be considered a response to environmental changes (Honorato & Nascimento, 2016) and fish management (Fantini *et al*. 2020). In this study, the ventilatory responses were insufficient to maintain the oxygen dynamics immediately after transport in fish subjected to the resting density of 300 kg/m³, resulting in compensatory respiratory acidosis due to the increase of blood HCO_3. The decrease in blood pH is compensated by the rise in blood regulation by the level of HCO_3 (Foss *et al*. 2007; Jia *et al*. 2021).

The fish at the resting density of 50 Kg/m³ remained at baseline values after transport, suggesting that the acid-base balance was restored. It is noteworthy that the resting density effectively restores homeostasis, demonstrating blood pH values close to neutrality.

Table I. Mean ± SE respiratory parameters of Nile tilapia *Oreochromis niloticus* subjected to transport capture management and pre-slaughter rest density.

Parameters	**Control Group removal from the water**	**After transportation**	**Stocking densities**		**P-value**
			50 kg/m³	**300 kg/m³**	
pH	7,26 ± 0,03**b**	7,19 ± 0,02**b**	7,42 ± 0,04**a**	7,22 ± 0,08**b**	**0,0033**
[1]PCO_2(mmHg)	25,34 ± 1,37**a**	32,45 ± 0,96**b**	32,36 ± 7,94**abc**	41,77 ± 6,42**c**	0,0872
[2]PO_2(mmHg)	98,56 ± 32,03**ab**	131,54 ± 24,91**a**	36,24 ± 7,87**c**	37,01 ± 3,09**c**	**0,0221**
[3]SO_2 (%)	60,90 ± 7,41**a**	79,56 ± 4,35**b**	59,04 ± 5,31**a**	45,96 ± 1,81**c**	**0,0021**
[4]HCO_3 (mmol/L)	11,17±0,82**b**	12,15±0,45**b**	11,26±0,64**b**	18,16±1,40**a**	**0,0000**

Means in the same row with different letters indicate statistical difference by the Tukey test ($p<0.05$). 1partial carbon dioxide pressure (PCO_2), 2 partial oxygen pressure (PO_2), 3 functional oxygen saturation (SO_2) and 4 bicarbonate (HCO^-_3).

Table II. Mean ± SE blood electrolytes of Nile tilapia *Oreochromis niloticus* subjected to transport capture management and pre-slaughter rest density.

Parameters	**Control Group removal from the water**	**After transportation**	**Stocking densities**		**P-value**
			50 kg/m³	**300 kg/m³**	
Plasmatic ions (nmol.L^{-1})					
Na^+	151,14±4,59	150,19±2,76	143,97±4,76	136,71±1,83	0,0510
K^+	3,72±0,19	3,05±0,19	3,29±0,29	3,04±0,30	0,1287
Ca^+	7,13±0,10c	7,74±0,05a	7,57±0,13ab	7,34±0,11bc	0,0002
Biochemical parameters (mg/dL)					
Glucose	91,16±8,12a	128,86±13,76b	159,04±12,76c	122,13±5,88b	0,0020
Lactate	1,13±0,11	1,36±0,27	1,42±0,23	1,26±0,12	0,7488
Creatinine	0,22±0,03b	0,40±0,05a	0,17±0,01b	0,41±0,04a	0,0000

Means in the same row with different letters indicate statistical difference by the Tukey test ($p<0.05$).

Among the most pronounced changes in management associated with transport is plasma glucose (Hong *et al*. 2019). Stress-related hyperglycemia is described in tilápia (*Oreochromis niloticus*) and surubim (*Pseudoplatystoma* sp) (Fantini *et al*. 2020; Goes *et al*. 2019; Venturini *et al*. 2018) because increased serum glucose in fish is a common reaction to transport (Acerete *et al*. 2004; Hong *et al*. 2019). However, the restoration of glucose levels to baseline values is required because it can interfere with the quality of fish to be marketed (Fantini *et al*. 2020; Sampaio & Freire 2016). Acute hyperglycemic response has been reported for fish submitted to exhaustive swimming (Olsen *et al*. 2008). The energy demand for maintaining respiratory rates is high, so it is observed that circulating glucose rates are higher in fish subjected to stress (Honorato *et al.* 2014) to maintain energy production.

The sodium and potassium balance did not show a difference in the electrolyte balance. Although these parameters are not reported in the transport stress assessment, they reflect homeostasis in fish (Sampaio & Freire 2016). The fish submitted to transport showed increased plasmatic calcium and creatinine as the result of muscle contraction. The group in the stocking density of 300 Kg/m³ showed an increase in creatinine compared to the control and the 50 Kg/m³ storage density groups. Creatinine is a metabolite of the muscle tissue and indicates muscle protein catabolism (Yousefi *et al*. 2016). Fish subjected to stressing conditions tend to mobilize energy sources by increasing the concentrations of lactate (Inoue *et al*. 2019) and creatinine (Hong *et al*. 2019) in the muscles. Creatinine is an exclusive metabolite of muscles, there is evidence that this measurement estimates body mass (Toledo *et al*. 2015; Akçakaya *et al*., 2016) However, for fish creatinine would be not used as a biochemical marker (Yousefi *et al*. 2016; Hong *et al*. 2019). Although it is not a specific marker for determining muscle changes or damage it can along with the other analyses comparing an indicative picture of muscle injury.

Conclusion, the resting density of 50 Kg/m³ restores respiratory functions and homeostasis in fish, being recommended for Nile tilapia to reduce pre-slaughter stress and increase marketed fillets' quality.

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