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Stunning of Nile tilapia by thermonarcosis and its effect on frozen fillets

Abstract – The objective of this work was to evaluate thermonarcosis as a stunning method for Nile tilapia (*Oreochromis niloticus*) and its effect on the pH and total volatile basic nitrogen in the frozen fillets. For evaluation, 60 animals were divided into six temperature ranges: 0–1ºC, 1–2ºC, 2–3ºC, 3–4ºC, 4–5ºC, and 5–6ºC. A stunning tank with a mixture of water and ice (1:1) was used. The temperature was monitored constantly, and ice was added as needed to maintain the different temperature ranges. The fish subjected to 0–3ºC were desensitized more quickly, in 270.3 s. There was no change in the characteristics of the fillets regarding the values of pH (6.86 to 7.00) and total volatile nitrogen bases (6.36 to 8.61 mg 100 g^{-1} N), both in accordance with the stipulated by the current Brazilian legislation. As a stunning method, thermonarcosis is efficient at a temperature from 0–3ºC and does not affect the quality of the frozen fillets of Nile tilapia.

Index terms: fish, desensitization, slaughter, stunning, TVB-N.

Insensibilização de tilápia-do-nilo por termonarcose e seu efeito no filé congelado

Resumo – O objetivo deste trabalho foi avaliar a termonarcose como método de insensibilização em tilápia-do-nilo (*Oreochromis niloticus*) e seu efeito sobre o pH e as bases nitrogenadas voláteis totais no filé congelado. Para avaliação, 60 animais foram divididos em seis faixas de temperatura: 0–1ºC, 1–2ºC, 2–3ºC, 3–4ºC, 4–5ºC e 5–6ºC. Utilizou-se um tanque de insensibilização com uma mistura de água e gelo (1:1). A temperatura foi monitorada constantemente, e gelo foi adicionado sempre que necessário para manter as diferentes faixas de temperatura. Os peixes submetidos à 0–3ºC perderam os sinais de sensibilidade mais rapidamente, em 270,3 s. Não houve alteração das características dos filés quanto aos valores de pH (6,86 a 7,00) e das bases nitrogenadas voláteis totais (6,36 a 8,61 mg 100 g^{-1} de N), ambos de acordo com o estipulado pela legislação vigente no Brasil. Como método de insensibilização, a termonarcose é eficaz à temperatura de 0–3ºC e não afeta a qualidade do filé congelado de tilápia-do-nilo.

Termos para indexação: peixe, insensibilização, abate, atordoamento, BVN-T.

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Introduction

Aquaculture has been a viable alternative to meet the growing demand for animal protein worldwide (Schulter & Vieira Filho, 2018). In this context, fish farming, which has been going through successive transformations over the years, presents a great potential for growth, especially in Brazil, a country with water availability, a favorable climate, and a productive sector in constant search for improvement in management systems and production technologies (Siqueira et al., 2021).

According to de la Rosa et al. (2021), with the increase of the aquaculture sector, the concerns with the welfare of the fish in the industry have also grown, aiming a humanized slaughter. Venturini et al. (2018) added that there is the need to develop methods that can minimize stressful situations for the animals, considering that handling at the time of slaughter negatively affects the quality of the final product.

Although stunning or slaughter should be precise, quick, and effective, causing minimal suffering to the animal (Aghwan & Regenstein, 2019), there is no specific law regarding the slaughter of fish in Brazil (Coelho et al., 2022). Even the new legislation, Portaria nº 365, de 16 de julho de 2021 of Ministério da Agricultura e Pecuária (Brasil, 2021), which approves the technical regulations for pre-slaughter management, humane slaughter, and stunning methods, only covers mammals, domestic birds, wild animals raised in captivity, amphibians, and reptiles. For this reason, different methods for stunning fish have been researched for a humanized and effective slaughter. General principles to be applied are recommended by World Organization for Animal Health (WOAH) through the Aquatic Animal Health Code (OIE, 2018). However, according to Oliveira Filho et al. (2017), the information about the effect of stunning methods on fish welfare and meat quality are still scarce.

In Brazil, most industries use the immersion of fish in ice water as a form of pre-slaughter stunning (Pedrazzani et al., 2020). However, this technique has been questioned due to the possibility of causing animal suffering because of the time required to achieve stunning (Ferreira et al., 2018). Considering this, Zampacavallo et al. (2015) highlighted that the efficiency of the technique seems to depend on water temperature and the species subjected to the process, which should be further researched.

In this scenario, it is fundamental to standardize the method for different species, mainly the most cultivated in production systems, among which tilapia (*Oreochromis* spp.) stands out as a hardy species that adapts to a variety of farming systems, being easy to produce (Prabu et al., 2019). In addition, it is well accepted by consumers since its meat is white and firm, has a light flavor, does not have an unpleasant odor, and does not have Y-shaped spines (Costa et al., 2019). In Brazil, Nile tilapia (*Oreochromis niloticus*) is the species most produced (Valenti et al., 2021), requiring the standardization of thermonarcosis.

To do this, it is necessary to determine fish quality, for which freshness is an important parameter. According to Galvão & Oetterer (2014), fish freshness can be evaluated through sensory, microbiological, or physico-chemical methods. However, due to the subjectivity of sensory methods and to the time and high cost of microbiological tests, chemical methods are more frequently used, quantifying the products derived from endogenous and exogenous enzymatic action. Moreover, physico-chemical parameters are the most commonly used to evaluate tilapia fillets, particularly the values of pH and total volatile basic nitrogen (TVB-N) to determine the degree of freshness of a fish (Kim et al., 2023).

The objective of this work was to evaluate thermonarcosis as a stunning method for Nile tilapia and its effect on the pH and TVB-N in the frozen fillets.

Materials and Methods

The study was approved by the ethics committee for animal experimentation of Universidade Federal de Pelotas, under process number 23110.044571/2019-22.

The experiment was carried out in a commercial slaughterhouse, with a slaughter capacity of 6,000 kg per month, located in the municipality of Rolante, in the state of Rio Grande do Sul, Brazil. A total of 60 Nile tilapia, with an average weight of 650 g, were used. The fish were housed in two tanks, with 5,000 L capacity and constant water renewal (depuration tanks), where they remained fasted for 48 hours for the depuration process. The temperature of the depuration tank was monitored and kept at 22ºC throughout the experiment.

To evaluate and standardize the method of thermonarcosis, a stunning tank was used, in which a mixture of water and ice was placed at an initial ratio of 1:1. The temperature in the tank was monitored constantly with the AK16L water-resistant stem-type thermometer (Akso Produtos Eletrônicos LTDA., São Leopoldo, RS, Brazil), and ice was added as needed to maintain the evaluated temperatures. The treatments consisted of the six following stunning temperature ranges, each tested on ten animals: 0–1ºC, 1–2ºC, 2–3ºC, 3–4ºC, 4–5ºC, and 5–6ºC.

Before the fish were placed in the stunning tank, water temperature was allowed to stabilize at the desired range. After the temperature range was reached, a batch of ten randomly chosen Nile tilapia was removed from the depuration tank and placed in the stunning tank using a net, initiating the stunning procedure. From that moment on, the stunning times of the animals were recorded until desensitization.

Stunning was evaluated according to the Aquatic Animal Health Code of WOAH (OIE, 2018), being considered adequate when the absence of body movements (swimming and balance), the absence of respiratory movements (opercular), and the loss of the vestibulo-ocular reflex (eye rotation), i.e., inability to stabilize the gaze during head movements, were observed. After verifying that an adequate stunning was reached, the fish were placed in plastic boxes and transferred to the slaughter room, where the bleeding operation was performed by cutting their gill.

After the stunned animals were removed from the tank and transferred to the slaughter room, the temperature in the stunning tank was adjusted to the next temperature range (treatment) and the aforementioned procedure was repeated.

After being bled, the fish were placed in the washing and scaling machine, followed by fin cutting and evisceration. After that, internal washing was performed with a brush and all skin was removed. Then, the carcasses were transferred to the filleting room. There, the fillets from each group of evaluated fish were placed in identified plastic bags and taken to the freezing tunnel at -25ºC for approximately 12 hours. After the minimum temperature of -18ºC was reached inside the fillets, they were transferred to the frozen storage chamber, at -18 to -20ºC, being kept there for approximately 24 hours, before being sent to the laboratory.

After this period, the plastic bags were stored in thermal boxes with ice and transported to the laboratory for the physico-chemical analysis, using the methodologies proposed by Ministério da Agricultura e Pecuária in Instrução Normativa no. 30, de 26 de junho de 2018 (Brasil, 2018). In addition, pH and TVB-N were evaluated according to Instrução Normativa no. 21, de 31 de maio de 2017 (Brasil, 2017), based on the lowest and highest stunning temperatures, which covered the two following sets of temperature ranges: 0–3ºC and 3–6ºC.

The data were subjected to the analysis of variance, followed by Fischer's least significant difference test (α=0.05), using the Bioestat, version 5.0, software (Ayres et al., 2007).

Results and Discussion

During thermonarcosis, the fish showed different times for loss of signals and immobility due to the difference in water temperature. The time taken to reach stunning was the shortest (229.3 s) at 0–1ºC and the longest (577.9 s) at 4–5ºC (Table 1). Therefore, the tested temperature ranges significantly influenced the time of desensitization.

Based on the mean stunning times (Figure 1), there were no significant differences between the fish subjected to 0–3ºC, indicating that this temperature range did not affect the time to achieve animal stunning. However, the fish subjected to 0–3ºC lost consciousness signals faster (mean of 229.3–270.3 s) than those under 3–6ºC. Moreover, the longest stunning time was 577.9 s for the fish subjected to 5–6ºC.

This difference observed in stunning time may be related to the fact that tilapia is a species whose water thermal comfort varies from 27 to 32ºC (Costa et al., 2017). Therefore, changes in water temperature can

Table 1. Stunning times (in seconds) of Nile tilapia (*Oreochromis niloticus*) subjected to thermonarcosis under different temperature ranges (0–1ºC, 1–2ºC, 2–3ºC, 3–4ºC, 4–5ºC, and 5–6ºC)(1).

Fish	Stunning times (in seconds)					
	0–1ºC	1–2ºC	2–3ºC	3–4ºC	4–5ºC	5–6ºC
1	90	128	152	270	270	396
2	138	153	168	340	438	438
3	145	209	175	340	471	470
4	150	212	192	340	494	530
5	265	246	205	433	505	555
6	270	261	276	486	515	637
7	285	280	295	500	520	650
8	300	342	300	540	520	693
9	320	347	460	550	560	700
10	330	350	480	550	565	710
Average	229.3a	252.8a	270.3a	434.9b	485.8b	577.9c

(1)Means followed by equal letters, in the rows, do not differ by Fischer's least significant difference test, at 5% probability.

affect fish metabolism, leading to a rapid interruption of animal movement (Volkoff & Rønnestad, 2020). According to Mitra et al. (2023), fish, being poikilothermic animals, cannot regulate their body temperature, depending on the external environment for this. Therefore, when subjected to an extreme and rapid change in temperature, fish can suffer the effect of paralysis by cold, having their metabolism slowed down, which would cause them to reach stunning more quickly.

The stunning times observed in the present study are shorter than those reported in the literature. Mergen & Geraldo Junior (2021) highlighted that thermonarcosis is one of the most used methods, in which different fish species are kept in a stunning tank for 15 min before bleeding. Similarly, for mackerel (*Trachurus murphyi*), Lyu et al. (2015) observed a stunning time of 15 min. Pedrazzani et al. (2009), when comparing the effect of two stunning methods (marrow section and thermonarcosis) on the well-being of Nile tilapia, found that the animals subjected to thermonarcosis were stunned at an average time of 750 s, which was 2 min and 52 s longer than the longest time recorded in the present study. This difference could be related to the possible non-control of the temperature of the stunning water, since this procedure was not mentioned by the cited authors. Also for Nile tilapia, Santos (2013) verified an even higher stunning time of 840 s, which was approximately 4 min and 22 s longer that the one found here. This difference could be attributed to the used water and ice ratio (2:1), as well as to the fact that there was no control and monitoring of the temperature of the water in the tank. Although Oliveira Filho et al. (2015) also used a 1:1 water:ice ratio, it took 20 min for Nile tilapia to be stunned, without maintaining tank temperature. In comparison, in the present study, the lower the water temperature, the shorter the time for the animal to be stunned, making it essential to control and maintain the temperature in the stunning tank. Therefore, the results obtained here and by these authors are an indicative that the water:ice ratio and the water temperature maintained in the tank can alter the time required to achieve stunning, showing the need of standardizing the thermonarcosis method.

Regarding the effect of thermonarcosis on Nile tilapia fillets, pH was 6.95 for the 0–3ºC and 3–6ºC temperature ranges (Figure 2), a value in accordance with Instrução Normativa no. 21, de 31 de maio de 2017

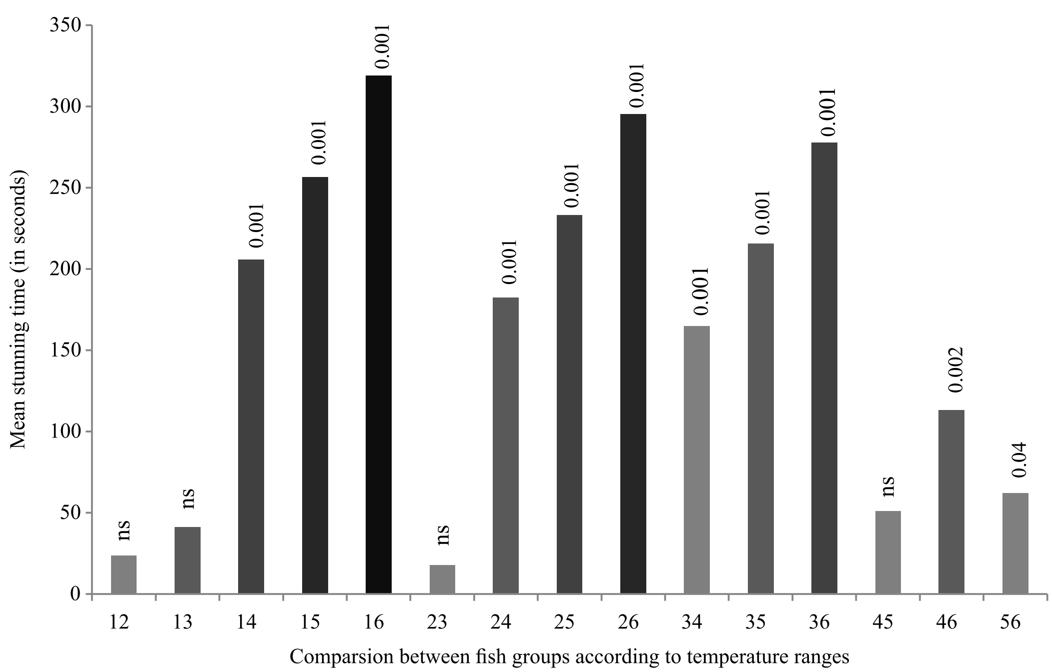

Figure 1. Difference between the average stunning times (in seconds) observed in groups of Nile tilapia (*Oreochromis niloticus*) subjected to thermonarcosis under different temperature ranges by Fischer's least significant difference test. [ns]Nonsignificant. Fish groups: 1, 0–1ºC; 2, 1–2ºC; 3, 2–3ºC; 4, 3–4ºC; 5, 4–5ºC; and 6, 5–6ºC.

(Brasil, 2017). This result shows that the variation in stunning temperature did not cause stress at the time of slaughter, as long as the glycogen reserves were not depleted, delaying and prolonging *rigor mortis* (Daskalova, 2019), which makes the fillets suitable for consumption.

According to Daskalova (2019), pH analysis is a commonly used parameter to evaluate the freshness attributes of frozen fillets because the deterioration process almost always changes the hydrogen ion concentration of the food. In this line, Tavares et al. (2021) concluded that fish can lose quality quickly due to their biochemical composition and greater susceptibility to microbial deterioration and lipid oxidation caused by pH near neutrality, as well as by handling and storage conditions. Furthermore, protein denaturation, the formation of low molecular weight volatile bases, and pH changes can occur even under refrigerated or frozen conditions (Wang et al., 2022).

To maintain fish quality, it is essential to control the pre-*rigor* and *rigor mortis* stages, because fish with a high muscle glycogen content subjected to pre-slaughter stress can develop fillets with a lower pH due to the higher glycolysis activity under anaerobic conditions (Fantini et al., 2020). Therefore, the lower the slaughter stress, the higher the amount of glycogen and adenosine triphosphate and the longer the pre-stress period, i.e., the longer the product will be free from the action of enzymes, considering that, in the *rigor mortis* stage, there is an anaerobic breakdown of

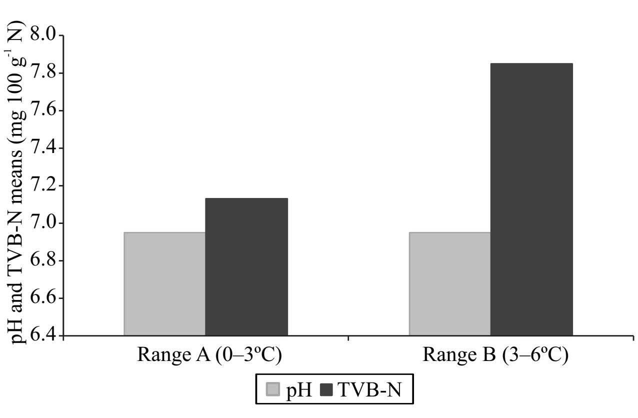

Figure 2. Means obtained for pH and total volatile basic nitrogen (TVB-N) of frozen fillets of Nile tilapia (*Oreochromis niloticus*) stunned by thermonarcosis, based on the lowest and highest stunning temperatures, covering two ranges (0–3ºC and 3–6ºC).

glycogen into lactic acid, causing a reduction in pH values, which helps to delay the degradation of the fish (Concollato et al., 2014; Lerfall et al., 2015).

Castro et al. (2017) concluded that, at the onset of *rigor mortis*, the pH of the fish flesh drops and, then, rises rapidly to levels of 6.6 to 6.8. A similar pH value of 6.79±0.02 was found by Montoya Camacho et al. (2020). According to Instrução Normativa no. 21, de 31 de maio de 2017 (Brasil, 2017), the technical regulation that establishes the standards for frozen fish, a pH of 7.0 is the maximum limit in the internal part of the muscle.

Another indicator of fish meat quality is the content of TVB-N (Hosseini et al., 2016). In the present study, the values found for TVB-N were 7.13 and 7.85 mg 100 g^{-1} N meat, respectively, for the 0–3ºC and 3–6ºC temperature ranges (Figure 2). This small accumulation of volatile substances is possibly explained by a prolonged *rigor mortis* due to the absence of stress in the animals, delaying the decomposition of proteins (autolysis) due to bacterial action as a result of the reactions of endogenous enzymes, such as cathepsin, and of exogenous enzymes.

According to Chen & Feng (2020), there is a correlation between the pH and TVB-N variables in tilapia fillets. In fish, TVB-N are produced by enzymes endogenous to meat and have bacterial origin, acting mainly on peptides, free amino acids, trimethylamine oxide, creatine, constituents of the non-protein nitrogen fraction, and proteins (Kontominas et al., 2021). Deepitha et al. (2021) added that TVB-N values correspond to the volatile molecules ammonia, trimethylamine, and dimethylamine, resulting from fish spoilage, and tend to increase as meat storage time passes.

The obtained results show that the frozen fillets maintained an excellent state of freshness. According to Kuroda et al. (2020), for fish to be considered fresh, the TVB-N content should range from 5 to 10 mg 100 g^{-1} N flesh, whereas a satisfactory fish freshness is characterized by TVB-N contents of 15 to 25 mg 100 g^{-1} N. When TVB-N reaches values of 30 to 40 mg 100 g^{-1} N, the first changes are observed, and, when values are above 50 mg 100 g^{-1} N muscle, the fish is deteriorated (Ribeiro et al., 2014).

The values found for pH and TVB-N were in accordance with those stipulated by the current Brazilian legislation, under Instrução Normativa no.

21, de 31 de maio de 2017 (Brasil, 2017), showing that the thermonarcosis method did not interfere with the quality of the final product, since the freshness of the frozen fillets was maintained from the physico-chemical point of view. The obtained results also suggest that the fillets can be stored for a longer period of time.

Conclusion

Thermonarcosis is efficient as a stunning method at a temperature from 0–3ºC and does not affect the quality of the frozen fillets of Nile tilapia (*Oreochromis niloticus*).

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