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Evaluation of the quality characteristics of chilled tilapia fillets subjected to different levels of pre-slaughter stress

Evolução das características de qualidade de filés refrigerados de tilápias submetidas a diferentes níveis de estresse pré-abate

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Highlights

Handling at 300 kg m-³ may compromise the final product quality.
Stress alters the color and texture of fillets.
Fillets from stressed fish show higher luminosity.
Prolonged stress results in paler, softer fillets with elevated pH.

Abstract

The objective of this study was to evaluate the effect of three stress levels, minimal (MS), short-term (SS), and long-term (LS), on the quality of Nile tilapia fillets during refrigerated storage. In the MS treatment, fish were maintained at a density of 60 kg m-³ for 24 hr, whereas in the SS and LS treatments, fish were kept at a density of 300 kg m-³ for 1 hr (SS) or 24 hr (LS), respectively. Serum cortisol levels were measured, and meat pH, color, and tenderness were assessed for 10 fish per treatment 0, 1, 2, 4, and 8 post-mortem days. The highest serum cortisol level was found in LS fish, while the lowest average was observed in MS fish. The pH was higher in LS fish. Fillets from MS fish had greater tenderness at 8 days post-mortem. The color development of stressed fish showed higher lightness on days 0, 2, and 8 post-mortem, and

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red intensity was lower in MS fish. No significant differences were observed in the yellow intensity of the fillets. It is concluded that short- and long-term crowding stress results in higher stress levels, leading to alterations in the development of pH, texture, and coloration of Nile tilapia fillets.
Key words: Fillet. Fish stress. *Oreochromis niloticus.* Processing.

Resumo

O objetivo deste estudo foi avaliar o efeito de três níveis de estresse, mínimo (MS), curto (SS) e longo prazo (LS), sobre os parâmetros de qualidade dos filés de tilápia-do-Nilo durante o armazenamento refrigerado. No tratamento MS, os peixes foram mantidos a uma densidade de 60 kg m^{-3} por 24 horas, enquanto nos tratamentos SS e LS, os peixes foram mantidos a uma densidade de 300 kg m^{-3} por 1 hora (SS) ou 24 horas (LS), respectivamente. Os níveis séricos de cortisol foram medidos e o pH, a cor e a maciez da carne foram avaliados em 10 peixes por tratamento nos tempos 0, 1, 2, 4 e 8 dias após a morte. O nível de cortisol mais alto foi encontrado nos peixes LS, enquanto a média mais baixa foi observada nos peixes MS. O pH foi mais alto nos peixes LS. Os filés dos peixes MS apresentaram maior maciez 8 dias após a morte. O desenvolvimento da cor dos peixes estressados apresentou maior luminosidade nos dias 0, 2 e 8 após a morte, e a intensidade do vermelho foi menor nos peixes MS. Não foram observadas diferenças significativas na intensidade do amarelo dos filés. Conclui-se que o estresse no pré-abate pelo adensamento de curto e longo prazo resulta em níveis mais elevados de estresse, levando a alterações no desenvolvimento do pH, textura e coloração dos filés de tilápia-do-Nilo.
Palavras-chave: Estresse em peixes. Filés. *Oreochromis niloticus.* Processamento.

Introduction

Nile tilapia (*Oreochromis niloticus*) is cultivated worldwide and considered one of the most important species for freshwater aquaculture (Food and Agriculture Organization of the United Nations [FAO], 2024). Key characteristics include rapid growth, the ability to reach market size in as little as nine months, hardiness, the absence of "Y" bones, and high fillet yield (Delphino et al., 2019; Leonard & Skov, 2022; Rahman et al., 2021). Additionally, it has a relatively low production cost (Yousefi et al., 2022).

Tilapia farming is practiced in various production systems, generating profitability when properly managed (Kumar & Engle, 2016; Manduca et al., 2020; Valenti et al., 2021). Additionally, the industry contributes to improving livelihoods, particularly in developing countries (Yue et al., 2016). In Brazil, tilapia production in 2024 reached approximately 662,230 tons, representing about 68.4% of the total species cultivated in the aquaculture sector (PeixeBR, 2025).

The welfare of Nile tilapia in production systems should be a priority, particularly in intensive farming systems that involve large numbers of animals (Cheng et al., 2015). Procedures and strategies to detect, prevent, or reduce stress are crucial, especially during transport, pre-slaughter handling, and stunning (Daskalova, 2019). Pre-slaughter conditions directly influence meat quality, in addition to affecting other factors widely discussed in the literature (Anders et al., 2020; Goes et al., 2019; Kayan et al., 2015; Mohamed et al., 2016). After slaughter, biochemical processes occur in fish muscle, leading to a series of physical changes, such as lactic acid production, ATP depletion, pH decline, reduced water retention capacity, and early onset of rigor mortis, which results in a rapid loss of freshness (Daskalova, 2019).

To prolong product freshness, it is essential to apply proper handling techniques (Pongsetkul et al., 2022). Maintaining optimal stocking densities before slaughter allows the animals to maintain their baseline stress levels and re-establish homeostasis, resulting in better product quality (Banhara et al., 2021). Many changes are observed immediately or one day after slaughter. However, it is hypothesized that pre-slaughter handling can also affect meat quality during refrigerated storage. The aim of the present study was to evaluate the effect of three levels of stress (minimal, short-term, and long-term) on the quality of tilapia fillets during refrigerated storage.

Material and Methods

Experimental fish were obtained from cage farming in the Corvo River, located in the municipality of Diamante do Norte, Paraná State, Brazil (22°39’ S, 52°46’ W). A total of 150 Nile tilapia of the Tilamax variety, with an average body weight of approximately 800 g, were transported to the Aquaculture Station of UEM/CODAPAR, in Maringá, Paraná State, Brazil. Upon arrival, the fish were stocked in 10-m^3 concrete tanks at a density of 5 kg m^{-3} and maintained for 40 days to allow recovery from transport stress and acclimatization to the experimental facilities.

The fish were subjected to one of three pre-slaughter handling procedures, minimal stress (MS), short-term stress through densification (SS), and long-term stress through a longer period of densification (LS) (Bahuaud et al., 2010) and fasted for 24 hr before slaughter. There were 50 replicates (fish) per treatment.

The fish were removed from the tanks and placed in three polyethylene boxes with a capacity of 1,000 L supplied with water and an artificial aeration system. The stocking densities were 60 kg of fish m^{-3} for MS and 300 kg of fish m^{-3} for SS and LS. In the SS treatment, the fish were kept at high density for 60 minutes, while in the LS treatment, the fish were kept at high density for 24 hr. In the MS treatment, the fish were kept at low density for 24 hours to allow them to recover from the stress associated with handling during capture. At the end of each treatment, the fish were euthanized by spinal cord section and immediately placed in ice water to promote bleeding until opercular movements ceased. Filleting was then performed in a laboratory adapted for fish processing, under hygienic conditions and in compliance with good manufacturing practices to ensure sample quality and safety. The skinless fillets were washed in 5 ppm of chlorinated water, vacuum-packed, and transported on ice for 1 hr to the laboratory for meat quality analysis.

For cortisol analysis, blood was collected from five fish per treatment by caudal puncture using disposable syringes. Serum was obtained by immersing the blood samples in a water bath at 37 °C for 10 min, followed by centrifugation at 1,000 g for 10 min and collection of the supernatant (serum). The concentration of serum cortisol was analyzed with a chemiluminescence microparticle immunoassay with Architect Ci8200 equipment (Abbott Laboratories, Abbott Park, IL, USA) and a reagent kit from the same brand. The analyses of pH, color, and tenderness were conducted at time points 0 (immediately after slaughter) and after 1, 2, 4, and 8 days, using ten fillets per treatment at each experimental time.

The pH was measured at three points (dorsal, central, and ventral regions) of each fillet with a portable digital potentiometer (Mettler Toledo, model 1140) equipped with a puncture electrode for direct measurement in meat. Color was evaluated on the ventral surface of the fillets with a colorimeter (Minolta, model CR-10) according to the CIELAB system. Six readings were recorded per sample, and the following variables were measured: lightness ($L^* = 0$ = black; $L^* = 100$ = white); redness (a^*; red–green axis), and yellowness (b^*; blue–yellow axis).

Tenderness was determined by measuring the shear force with a Brookfield CT3 texture analyzer (Brookfield Engineering, Middleboro, MA, USA), equipped with a Warner-Bratzler blade (3 mm thickness, 70 mm length, 60° angle). Fillets were cut transversely to the muscle fibers into cubes of approximately 20 × 25 × 20 mm after being equilibrated to room temperature for 1 hr. Three measurements were taken per fillet, and shear force was expressed in gram-force (gf), corresponding to the maximum peak force required to shear through the sample.

The data were subjected to analysis of variance. In cases of significant differences ($p < 0.05$), Tukey's test was applied to compare the means with STATISTICA 7.1 software (StatSoft Inc., Tulsa, OK, USA). All data are expressed as mean ± standard error.

The experiment was approved by the Animal Care Committee of the Universidade Federal da Grande Dourados (CEUA; protocol No. 43/2016 - UFGD/Brazil) and conducted following the guidelines of the Brazilian College of Animal Experimentation (COBEA; http://www.cobea.org.br).

Results and Discussion

The highest level of serum cortisol was in fish subjected to LS, while the lowest level was observed in animals subjected to MS (Figure 1). Cortisol is widely recognized as a reliable indicator of stress in fish (Sadoul & Geffroy, 2019). Cortisol and catecholamines are released into the bloodstream, stimulating the production of additional metabolic energy to facilitate escape or adaptation to rearing conditions (Yarahmadi et al., 2015). Furthermore, depending on the degree and duration of stress, these mechanisms can have positive and negative effects on fillet quality post-slaughter (Lerfall et al., 2015). Mild or minimal stress allows fish to maintain homeostasis, preventing excessive glycogen depletion and contributing to more stable post-mortem pH and texture. In contrast, short-term and prolonged stress accelerate glycogen consumption and lactic acid accumulation, leading to undesirable changes such as rapid pH decline, decreased water-holding capacity, paler coloration, and reduced tenderness (Goes et al., 2019; Lerfall et al., 2015).

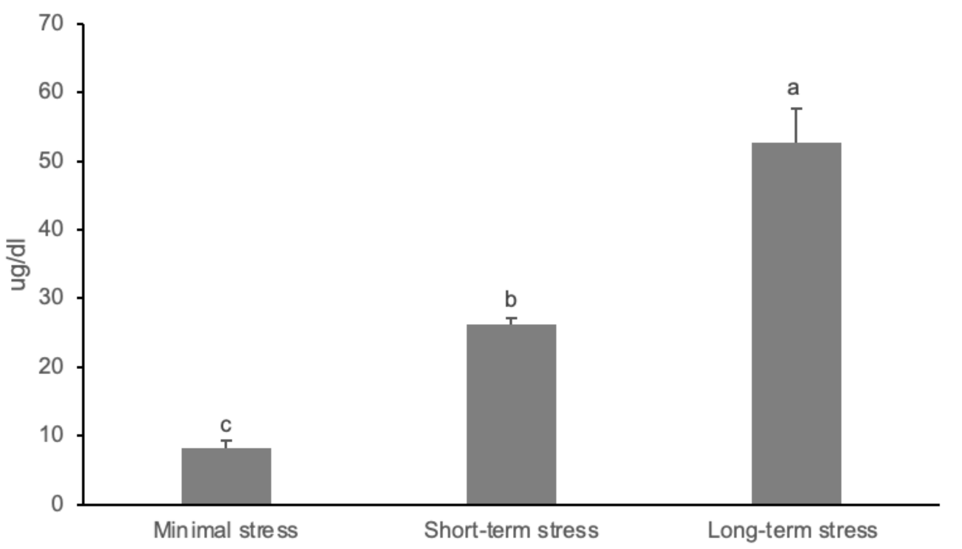


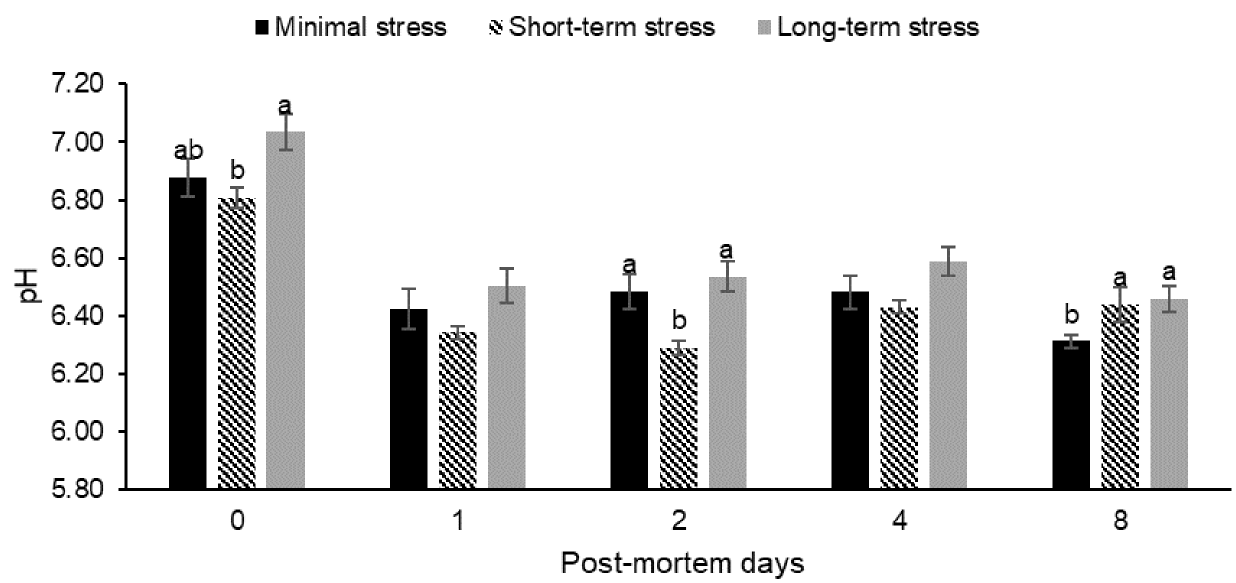
Figure 1. Serum cortisol levels of Nile tilapia subjected to minimal stress, short-term stress, and long-term stress during pre-slaughter are shown. Different letters denote significant differences ($p < 0.05$) between treatments.

Research indicates that animals subjected to high-density crowding stress for a short period can adapt to their environment by adjusting their metabolism (Bai et al., 2024; Banhara et al., 2021). In contrast, prolonged exposure to high densities disrupts homeostasis, triggering stress and increasing cortisol levels (Li et al., 2023). This suggests that the increase in crowding before slaughter is directly related to the duration of exposure and the effort exerted by the fish to maintain a normal swimming pattern.

The Nile tilapia that exhibited higher cortisol levels during the stocking period were likely making an adaptive effort to cope with the high density by mobilizing more energy. In contrast, animals subjected to MS acclimatized to the densities to which they were exposed. Although fish subjected to SS also showed a significant increase in cortisol levels compared to the MS group, the values were lower than those observed under LS, suggesting a milder physiological response to crowding.

Higher pH was observed in fillets of fish subjected to LS, while lower pH was noted in fillets of fish subjected to SS at 0 and 2 days after slaughter (Figure 2A). The LS treatment led to a higher pH possibly due to the depletion of glycogen reserves from intense locomotor activity (Magalhães et al., 2020). A significant reduction in pH was observed between days 0 and 2, attributed to lactic acid formation, followed by a slight increase on day 8, likely caused by ammonia production. These temporal variations highlight that fillet quality may be variably affected by the post-mortem period. In studies with salmon (*Salmo salar*), 24-hr stress resulted in meat with a higher pH and firmer texture, which was linked to the severe depletion of glycogen stores (Skjervold et al., 2001). In our study, although MS was associated with lower pH values, particularly at day 8, the fillets maintained greater tenderness compared to LS fish. This indicates that reduced stress levels may allow better energy balance and recovery of homeostasis, thereby favoring texture. However, it is important to note that a lower pH may negatively affect certain technological aspects of processing, such as water-holding capacity.

(A)



(B)

Figure 2. Changes in pH (A) and texture (B) of Nile tilapia fillets subjected to minimal stress, short-term stress, and long-term pre-slaughter stress during storage. Different letters denote significant differences ($p < 0.05$) between treatments at the same time.

In the SS treatment, there was a faster consumption of glycogen, resulting in greater lactic acid production and a subsequent reduction in meat pH. Vigorous swimming during periods of stress leads to intense use of the white muscle, increases anaerobic glycolysis, and results in higher lactic acid production (Rahmanifarah et al., 2011). On day 8 post-mortem, fillets of fish subjected to MS exhibited a lower pH compared to those subjected to LS. This result may be explained by the fact that MS fish, despite experiencing some stress, were not exhausted and had more time to re-establish homeostasis and greater space to swim (Banhara et al., 2021).

In the texture analysis (Figure 2B), a significant effect ($p < 0.05$) was observed only after 8 days of storage, when the fillets of fish subjected to MS exhibited softer meat. It is known that the physical stress on myofibrils and connective tissues caused by muscle activity before slaughter increases the activation of muscle proteases, leading to meat softening (Daskalova, 2019). In our study, this effect was more evident in MS fish, possibly due to a more balanced energy metabolism and greater recovery of homeostasis. Conversely, in LS fish, the severe glycogen depletion did not lead to the same degree of softening, likely because the higher ultimate pH contributed to firmer texture (Skjervold et al., 2001). Thus, the relationship between stress, glycogen depletion, and pH should be interpreted as multifactorial since the extent and duration of stress can modulate post-mortem proteolysis and meat tenderness (Zhang et al., 2023).

On the other hand, the texture of fillets of fish exposed to SS and LS did not differ significantly from that of fillets of MS fish. Overall, the tenderness of the fillets increased over storage time in all treatments, likely due to the enzymatic degradation of proteins.

Greater luminosity was observed in the fillets of stressed fish on days 0, 2, and 8 post-mortem (Figure 3). These findings are consistent with studies indicating that pre-slaughter stress can promote muscle protein denaturation and fluid loss, altering light reflection on the flesh surface and resulting in a paler appearance (Daskalova, 2019). No significant difference in redness was detected between MS and SS fish fillets at day 0, while at day 4 the SS fish fillets showed the higher red intensity than MS and LS fish fillets. This result may be related to the accumulation of residual blood — especially hemoglobin — in the fillet, resulting from acute stress and exhaustive muscular activity during pre-slaughter; this increases redness (a) and is positively correlated with muscle hemoglobin levels (Svalheim et al., 2020). No significant differences ($p > 0.05$) were observed in yellow intensity among treatments during storage.

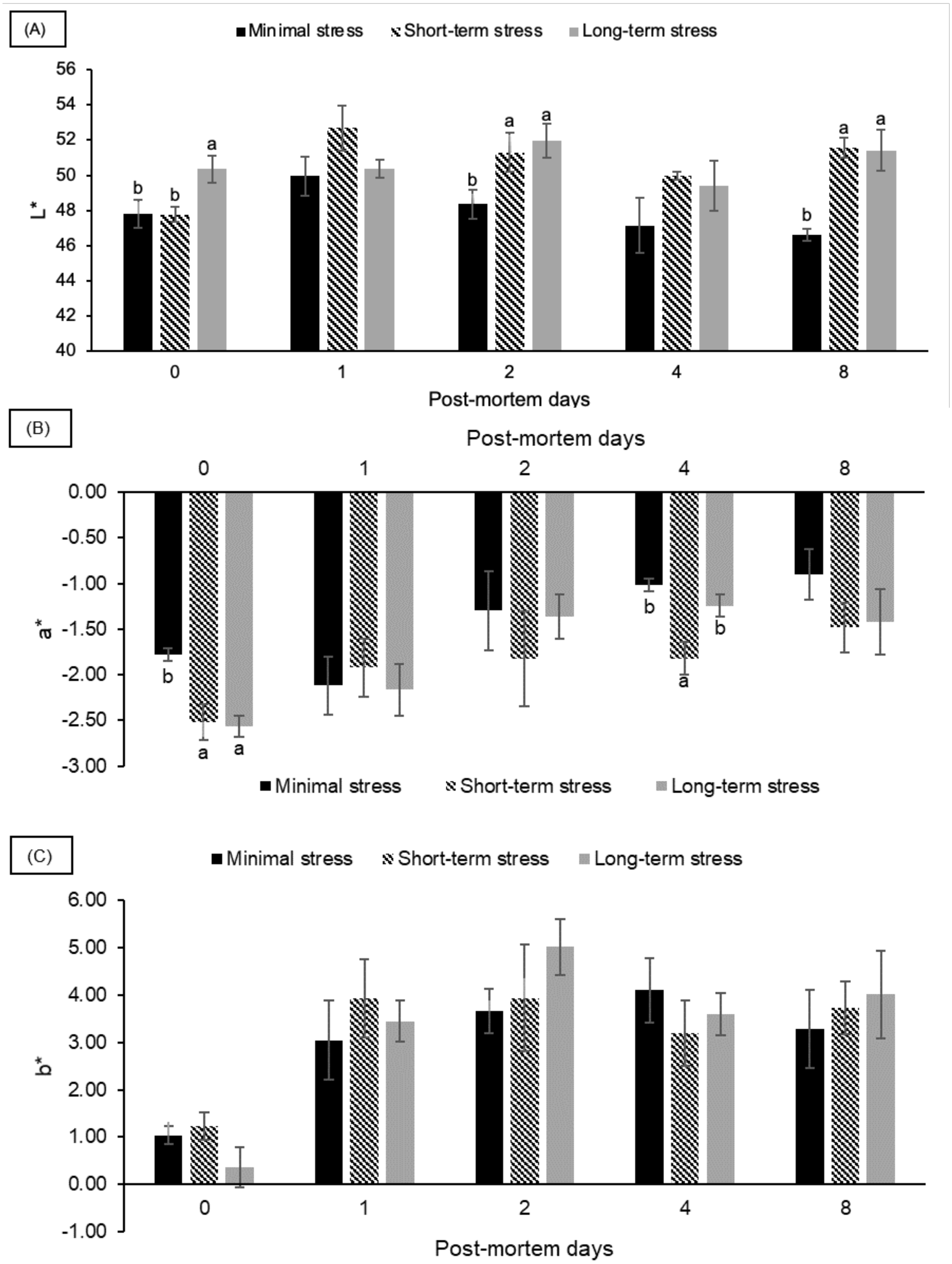


Figure 3. Changes during the storage period (0 to 8 days *post-mortem*) in luminosity L* (A), red intensity a* (B), and yellow intensity b* (C) of Nile tilapia fillets subjected to minimal stress, short-term stress, and long-term pre-slaughter stress. Different letters denote significant differences ($p < 0.05$) between treatments at the same time.

Previous studies indicate that stress caused by high density can affect the color of tilapia fillets (Almeida et al., 2022; Goes et al., 2019; Santos et al., 2024). In general, there was a decrease in red intensity and an increase in yellow intensity over the post-mortem days, related to the reduction in pH. As storage time progresses, a greater intensity of yellow corresponds to a lower pH, or vice versa.

Conclusions

This study demonstrated that crowding stress at a high density (300 kg m^{-3}) prior to slaughter compromises the technological quality of Nile tilapia fillets during refrigerated storage, particularly when the stress is prolonged for 24 hr. Fish subjected to LS had elevated cortisol, and their fillets had higher ultimate pH, paler coloration, and reduced tenderness, which may negatively affect consumer perception and processing yield. These findings reinforce the need to adopt pre-slaughter handling practices that minimize crowding stress, especially in intensive farming systems. Future research should investigate the threshold of stocking density and duration that can be tolerated without compromising quality, the physiological mechanisms linking stress and post-mortem changes, and potential mitigation strategies, such as resting protocols or natural anesthetics, to improve fish welfare and product quality.

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Conflicts of Interest

The authors declare no conflict of interest.

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