ORIGINAL RESEARCH ARTICLES

Effect of environmental and nutritional management during the transport of *O. niloticus* fry: Welfare indicators and productive performance

Efecto del manejo ambiental y nutricional en el transporte de alevines de O. niloticus: indicadores de bienestar y rendimiento productivo

Efeito do manejo ambiental e nutricional no transporte de alevinos de O. niloticus: Indicadores de Bem-Estar e Desempenho Produtivo

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Abstract

Background. The transportation stage of juvenile tilapia (*Oreochromis niloticus*) represents a pivotal phase in aquaculture production, wherein environmental and nutritional management practices can exert a substantial influence on animal welfare and productive performance. **Objectives.** This study aimed to evaluate the combined effect of water-to-oxygen ratios in transport bags and the type of diet provided before and after handling on welfare indicators and growth parameters of tilapia fry. **Methods.** Two experiments (3 × 2 factorial) were conducted, testing three oxygen supplementation levels (10, 15, and 20 L) in transport bags (equivalent to water-to-oxygen ratios of 1:1, 1:1.5, and 1:2), two diets (control and functional) before transport, and two diets (control and functional) after transport. Blood samples were collected at 0, 4, and 28 hours post-transport in order to evaluate glucose, lactate, and hematocrit as indicators of animal welfare. Final weight, specific growth rate and feed efficiency were measured after a 28-day period. **Results.** The 1:2 water-to-oxygen ratio was the most effective, maintaining dissolved oxygen above 3 mg L^{-1} and promoting superior productive performance. All fish exhibited physiological stress responses after 4 hours of transport, as reflected by elevated glucose, lactate, and hematocrit values. However, partial recovery was observed after 28 hours, especially in fish previously fed the functional diet, which showed lower increases in hematological parameters. The best productive performance was recorded in fish feed, a functional diet prior to transport and a control diet afterward, suggesting a beneficial pre-conditioning effect of the functional additives, coupled with an improved post-transport protein supply. **Conclusions.** An integrated management strategy that includes adequate oxygen supplementation (1:2 water-to-oxygen ratio) and the strategic use of functional additives before transport followed by a balanced control diet afterward, enhances both welfare and growth outcomes in tilapia fry. These findings provide valuable insights for optimizing handling practices and supporting sustainable and efficient aquaculture systems.

Keywords: *animal welfare; functional additives; nutritional management; quercetin; transport stress; vitamin C.*

Resumen

Antecedentes. La etapa de transporte de tilapia juvenil (*Oreochromis niloticus*) representa una fase fundamental en la producción acuícola, en la cual las prácticas de manejo ambiental y nutricional pueden ejercer una influencia sustancial sobre el bienestar animal y el desempeño productivo. **Objetivos.** Este estudio tuvo como objetivo evaluar el efecto combinado de las proporciones agua:oxígeno en bolsas de transporte y del tipo de dieta suministrada antes y después del manejo, sobre indicadores de bienestar y parámetros de crecimiento en alevines de tilapia. **Métodos.** Se realizaron dos experimentos con un diseño factorial 3 × 2, evaluando tres niveles de suplementación con oxígeno (10, 15 y 20 L) en bolsas de transporte (equivalentes a relaciones agua:oxígeno de 1:1, 1:1.5 y 1:2), y dos tipos de dieta (control y funcional) antes del transporte, así como dos dietas (control y funcional) después del transporte. Se recolectaron muestras de sangre a las 0, 4 y 28 horas posteriores al transporte con el fin de evaluar glucosa, lactato y hematocrito como indicadores de bienestar animal. El peso final, la tasa específica de crecimiento y la eficiencia alimenticia se midieron después de un período de 28 días. **Resultados.** La relación agua:oxígeno 1:2 fue la más eficaz, ya que mantuvo el oxígeno disuelto por encima de 3 mg L^{-1} y promovió un mejor desempeño productivo. Todos los peces presentaron respuestas fisiológicas de estrés después de 4 horas de transporte, reflejadas en valores elevados de glucosa, lactato y hematocrito. Sin embargo, se observó una recuperación parcial tras 28 horas, especialmente en los peces alimentados previamente con la dieta funcional, los cuales mostraron menores incrementos en los parámetros hematológicos. El mejor desempeño productivo se registró en los peces alimentados con la dieta funcional antes del transporte y con la dieta control posteriormente, lo que sugiere un efecto beneficioso del prea condicionamiento con aditivos funcionales, junto con un mejor suministro proteico post-transporte. **Conclusiones.** Una estrategia de manejo integral que incluya una adecuada suplementación de oxígeno (relación agua:oxígeno de 1:2) y el uso estratégico de aditivos funcionales antes del transporte, seguido de una dieta control balanceada después del mismo, mejora tanto el bienestar como los resultados de crecimiento en alevines de tilapia. Estos hallazgos ofrecen aportes valiosos para la optimización del manejo y la promoción de sistemas acuícolas sostenibles y eficientes.

Palabras clave: *aditivos funcionales; bienestar animal; estrés por transporte; manejo nutricional; quercetina; vitamina C.*

Resumo

Contexto. A etapa de transporte de tilápias juvenis (*Oreochromis niloticus*) representa uma fase crucial na produção aquícola, na qual as práticas de manejo ambiental e nutricional podem exercer uma influência substancial sobre o bem-estar animal e o desempenho produtivo. **Objetivos.** Este estudo teve como objetivo avaliar o efeito combinado das proporções água:oxigênio em sacos de transporte e do tipo de dieta fornecida antes e após o manejo sobre indicadores de bem-estar e parâmetros de crescimento de alevinos de tilápia. **Métodos.** Foram conduzidos dois experimentos com delineamento fatorial 3 × 2, testando três níveis de suplementação de oxigênio (10, 15 e 20 L) em sacos de transporte (equivalentes às proporções água:oxigênio de 1:1, 1:1,5 e 1:2), e dois tipos de dieta (controle e funcional) antes do transporte, bem como duas dietas (controle e funcional) após o transporte. Amostras de sangue foram coletadas às 0, 4 e 28 horas após o transporte a fim de avaliar glicose, lactato e hematócrito como indicadores de bem-estar animal. O peso final, a taxa específica de crescimento e a eficiência alimentar foram medidos após um período de 28 dias. **Resultados.** A proporção água:oxigênio de 1:2 foi a mais eficaz, mantendo o oxigênio dissolvido acima de 3 mg L^{-1} e promovendo melhor desempenho produtivo. Todos os peixes apresentaram respostas fisiológicas ao estresse após 4 horas de transporte, refletidas em valores elevados de glicose, lactato e hematócrito. Contudo, foi observada recuperação parcial após 28 horas, especialmente nos peixes previamente alimentados com a dieta funcional, que apresentaram menores elevações nos parâmetros hematológicos. O melhor desempenho produtivo foi registrado em peixes alimentados com dieta funcional antes do transporte e dieta controle posteriormente, sugerindo um efeito benéfico do pré-condicionamento com aditivos funcionais, aliado ao fornecimento proteico adequado no pós-transporte. **Conclusões.** Uma estratégia de manejo integrada que inclua suplementação adequada de oxigênio (proporção água:oxigênio de 1:2) e o uso estratégico de aditivos funcionais antes do transporte, seguida por uma dieta controle balanceada após o transporte, melhora tanto o bem-estar quanto os resultados de crescimento de alevinos de tilápia. Esses achados fornecem informações valiosas para a otimização das práticas de manejo e o fortalecimento de sistemas aquícolas sustentáveis e eficientes.

Palavras-chave: *aditivos funcionais; Bem-estar animal; Estresse de transporte; Manejo nutricional; Quercetina; Vitamina C.*

Introduction

Fishing and aquaculture have been key activities, with the fundamental goal of providing high-quality protein. In 2022, global fisheries and aquaculture production reached a record 223.2 million tonnes, comprising 185.4 million tonnes of aquatic animals, of which 51 % (94.4 million tonnes) came from aquaculture—never before had aquaculture surpassed capture fisheries in aquatic animal production (FAO, 2024). Of the total aquatic animal production in 2022, 62 percent (115 million tonnes) was harvested in marine areas—comprising 69 percent from capture fisheries and 31 percent from aquaculture—while 38 percent (70 million tonnes) originated from inland waters, with 84 percent from aquaculture and 16 percent from capture fisheries. A total of 89 percent (164.6 million tonnes) of aquatic animal production was directed toward human consumption. Apparent consumption of aquatic foods has grown steadily, with per capita annual supply increasing from 9.1 kg in 1961 to 20.6 kg in 2021, nearly doubling the global population growth rate (FAO, 2024). *Oreochromis niloticus* (tilapia) has been successfully cultured under a wide range of environmental conditions and is one of the most produced fish species worldwide, particularly in tropical and subtropical countries (El-Sayed, 2006; Dagne *et al*, 2013), with the majority of production coming from aquaculture (Kumar & Engle, 2016). It is considered a preferred species for production due to its biological attributes, which offer significant competitive advantages over other fish species. These attributes include rapid body growth, resistance to temperature fluctuations, tolerance to changes in dissolved oxygen, salinity, and pH levels in the water, as well as its robust health, easy reproduction, and adaptability to management and feeding in captivity (Isiordia-Pérez *et al*, 2021; Pérez *et al*, 2015).

In aquaculture, one of the most common practices is the transport of fry, where various factors can significantly affect animal welfare. A key factor is the water-to-oxygen ratio in the transport bags. Dissolved oxygen (DO) is a crucial water parameter, as low concentrations of DO can expose fish to higher mortality risks. DO levels are also affected by water movement: stagnant water leads to a gradual decrease in DO levels, while movement increases oxygenation (Somerville *et al*, 2014). Consequently, the environment to which the fry are exposed during transport can be considered a potential stressor due to its variability. As Schreck and Tort (2016) demonstrate, oxygen concentrations have the capacity to impact metabolic capacity, thereby influencing the magnitude and duration of the allostatic load (the cumulative physiological cost of adapting to and coping with stress over time). Furthermore, a decline in dissolved oxygen (DO) levels to below 5 mg L^{-1} has been demonstrated to induce a hypoxic state in aquatic organisms (Dong *et al*. 2011). This has been shown to result in a number of adverse effects, including a reduction in appetite, an increased susceptibility to disease, a decline in growth rate, and potentially even death (Abdel-Tawwab *et al.* 2019). Hyperoxia, defined as the presence of oxygen concentrations that exceed the organism's tolerance threshold, can also occur, resulting in the production of reactive oxygen species (McArley *et al*. 2022; McArley *et al*. 2020; Hermes-Lima & Zenteno-Savin, 2002; Berschick *et al*. 1987). These reactive oxygen species have the potential to alter oxygen partial pressure and ventilatory frequency.

Another factor influencing physiological responses is nutritional management, particularly the use of additives. It has been shown that vitamin C improves stress resistance and physiological responses during transport by modulating stress-related biochemical parameters and maintaining water quality, which is critical under stressful transport conditions. Vitamin C supplementation has been found to reduce stress indicators such as serum cortisol and glucose levels, while increasing erythrocyte counts and packed cell volume (Adah *et al*, 2023; Peng *et al*, 2013). Similarly, quercetin supplementation has been shown to significantly improve immune responses in fish (Armobin *et al*, 2023; Wang *et al*, 2020), reducing oxidative stress during transport. This study aimed to evaluate the combined effect of the water-to-oxygen ratio (L^{-1}) in transport bags and the pre- and post-transport diet on the welfare and productive performance of tilapia fry, optimizing management conditions to improve their welfare and growth outcomes.

Materials and Methods

Ethical Statement

All animals were handled in accordance with the best practices for tilapia farming (SAGARPA, 2016). The research protocol for the present study was reviewed and approved by the Research Ethics Committee of the University of Guanajuato (CEPIUG-P15-2024).

Pre-Transport Management

Location and Aquaculture System

Pre-experimental activities were conducted at “El Geranio” fish farm, located in the community of Chupícuaro, in Acámbaro, Guanajuato, Mexico. This facility specializes in the production of tilapia fry and supplies producers throughout the central region of the country. Six semicircular tanks were used within the larval production area, as part of an open-flow water system with an average water exchange rate of 25 L/min and a holding capacity of 250 liters per tank.

Experimental Fish and Diets

A total of 12,000 tilapia fry, all hatched at the same farm, were randomly distributed among six tanks (n = 2,000 per tank). For the formulation of the experimental diet, a veterinary-use functional additive was employed, containing per ounce: 3125 g of vitamin C, 190 mg of quercetin, 500 mg of omega-3, 167 mg of omega-6, and 167 mg of omega-9. This additive was incorporated at a 5% inclusion rate into a commercial tilapia fry feed composed of 95.06% dry matter, 49.81% crude protein, and a particle size < 0.35 mm. Three tanks were randomly assigned to receive this supplemented diet, while the remaining tanks were fed only the commercial diet, serving as the control group. Diets were administered for 14 days prior to the fish transport procedure. Following the farm's standard feeding protocol, each tank received approximately 10 g of feed every 2 hours, five times daily. This feeding rate was established based on the stocking density and average biomass of the fry, corresponding to the farm's routine practice to ensure ad libitum access to feed while minimizing waste and maintaining water quality.

Study Variables

Live body weight (g) and total length (cm) were recorded. Blood samples were also collected: 40 fry per tank were randomly selected and anesthetized to establish a physiological baseline. A rapid cooling method was applied (Ferreira *et al*, 2022; Wilson *et al*, 2009) to ensure humane euthanasia and prevent alterations in blood analytes, particularly important given the small size of the fry. A caudal peduncle incision was performed, and with the aid of a micropipette and 5 µL of heparin, blood samples (~10 µL per fry) were collected to create pooled samples (one pool per 20 fish). The assessment of feeding motivation (frenzy) was also utilized as a key welfare indicator, reflecting the fish's ability to engage in natural behaviors and fulfill nutritional needs (Millot *et al*. 2008). This approach aimed to ensure that the fish were transported in optimal condition. As internal welfare indicators (Martínez-Yáñez *et al*. 2025), blood glucose (mg/dL, Accu-Chek Active, Roche), lactate (mg/dL, Accutrend Plus), and microhematocrit (%) were measured. Blood samples were collected into 10 µL heparinized capillary tubes and centrifuged at

1200 rpm for 5 minutes. Following centrifugation, erythrocyte percentages were determined using a hematocrit reader in conjunction with a magnifying lens.

Experiment 1

Assessment of Welfare Indicators During the Transport Process of O. niloticus Fry According to Oxygen Levels and Supplemented Diet

Fish and Experimental Management

From the previously described population, 9,000 tilapia fry were selected for packaging and transport procedures and were randomly assigned to three treatments. The study comprised two dietary groups: fish fed the diet supplemented with the functional additive, and a control group. Standard farm practices for packaging and transport were adhered to. Twelve hours prior to transport, 10 L of water was added to 30 L^{-1} capacity plastic bags, a routine handling procedure at the production unit to ensure there are no leaks. In order to the effect of oxygen concentration relative to biomass during transport on welfare indicators, three treatments were established, corresponding to oxygen supplementation ratios of 1:1, 1:1.5, and 1:2 (water:oxygen, measured in L^{-1}). An oxygen flow meter with a control valve (L min^{-1} O_2) was used to regulate supplementation. For packaging, each bag received a biomass of 430 g, approximately 500 fry with an average weight of 0.86 ± 0.12 g. Transport was conducted by road, and the total process, including packaging, lasted four hours. To ensure consistency in handling, the packaging time of each bag was recorded, enabling sampling at standardized time points.

Study Variables

Before packaging and upon arrival (when bags were opened), water samples were analyzed to determine ammonia (mg L^{-1} NH_3-N, Hanna Checker), dissolved oxygen concentration and temperature (DO, mg L^{-1}, Pro20 YSI oximeter), and pH (EcoSense pH 10). An infrared thermometer (Soonda A50) was also used to assess the temperature of sealed bags at the end of packaging, during loading, and upon arrival. At reception, 40 fry were randomly sampled from each bag for the assessment of internal welfare indicators, as previously described. Additionally, 200 fry were randomly selected from each bag for Experiment 3. The remaining fish were transferred to a system similar to that described in Experiment 3, in which fry from each bag were stocked in a separate tank to evaluate internal welfare indicators 24 hours post-arrival. For these samples, 40 fish per tank (transport bag) were used, and pooled blood samples were obtained as previously described.

Experiment 2

Evaluation of Productive Indicators in O. niloticus Fry According to the Oxygen Level Used During Transport and the Supplemented Diet

Location and Aquaculture Systems

The experiment was conducted at the Aquaculture Laboratory of the "Veterinaria y Zootecnia" department, Universidad de Guanajuato, located at km 9 of the Irapuato–Silao highway in Irapuato, Guanajuato, México. The study was carried out during the month of May 2024. Two recirculating aquaculture systems (RAS) were used, each consisting of 18 tanks with a capacity of 400 L^{-1} and a three-phase filtration system (Figure 1), which included a 1000 L^{-1} sump, a mechanical filter with a capacity of 600 L^{-1}, and a biological filter of 1000 L^{-1}. Each tank was filled with 200 L^{-1} of water. Water was transported from the sump to each tank via PVC pipes ending in a ½ inch outlet, allowing water to fall freely into the tanks. This drop facilitated oxygenation and maintained circulation, with water returning to the sump through a separate set of pipes located at the bottom of the system. A Resun King 6 pump was installed in the sump to move water through the mechanical filter, then the biological filter, and finally to the tanks by gravity flow. Each tank was equipped with an air stone connected to a PVC tubing and hose system, with aeration maintained by an air compressor (160 Lpm, ACQ-009). To ensure proper maturation and oxygenation of the water, circulation and aeration of the systems were initiated 14 days prior to fish transport.

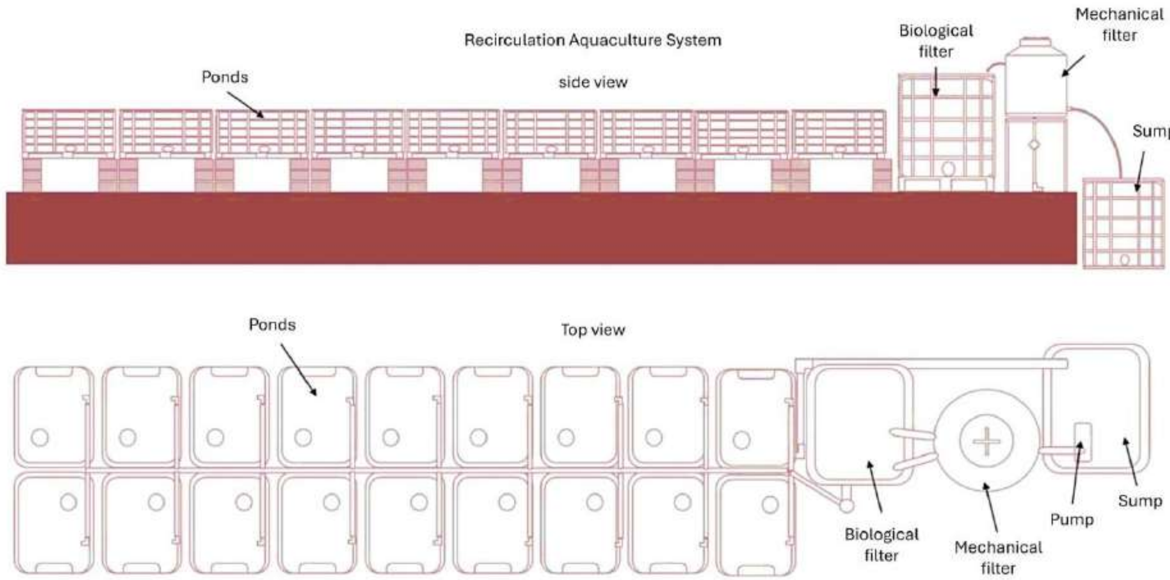

Figure 1. Lateral and top views of the aquaculture recirculating systems used.

Fish and Experimental Management

As mentioned in Experiment 1, fry was randomly selected from each group (fed with the functional additive and control diet) and from each transport bag (oxygen supplementation ratios of 1:1, 1:1.5, and 1:2 water:oxygen). A total of 100 fry (0.88 ± 0.18 g) were stocked into each tank within the recirculating systems. Each RAS system included fry previously fed with the supplemented or control diet, and each of the three water-to-oxygen transport ratios were represented (n = 3). The study lasted 28 days, with one system receiving the diet supplemented with the functional additive, and the other receiving the control diet, at a feeding rate of 8%.

Study Variables

Every third day, water parameters such as pH, ammonia, DO, and temperature were measured in each tank. At the end of the study, individual productive indicators were evaluated in the fish, including wet live weight (g), total length (cm), body condition (Fulton's K), and specific growth rate (SGR, % day^{-1}). At the tank level, survival at 14 and 28 days (%), biomass weight (g), and feed efficiency were determined. A digital scale (AMIR Model US-KA8) and an ichthyometer (Pentair Aquatic Ecosystems) were used. The following formulas were used to calculate body condition, specific growth rate and feed efficiency:

Fulton´s condition factor (Fulton, 1904):

$$K = 100 * \frac{W}{L^3}$$

Where:

K: Fulton´s condition factor

W: Fish weight (g)

L: Fish length (cm)

Specific growth rate (SGR, % day^{-1}) was calculated according to Ricker (1979) and Hepher (1988).

$$SGR = \frac{\ln Wf - \ln Wi}{t} * 100$$

Where:

Wf: Final wet weight of the fish (g)

Wi: Initial wet weight of the fish (g)

ln: Natural logarithm

t: Number of days in the experimental period

Feed efficiency (FE) (Hepher, 1988).

$$FE = \frac{\text{Total weight gain (g)}}{\text{Total feed intake (g)}}$$

Where:

Total weight gain: Difference between final and initial fish weight during the evaluation period

Total feed intake: Total amount of feed supplied over the same period

Finally, to calculate the survival percentage (14 days), daily mortalities of the fry were recorded. Total survival (28 days) was determined as a percentage, based on the number of individuals stocked at the beginning of the experiment.

Statistical analysis

Data on glucose, lactate, and hematocrit levels were analyzed using a two-way factorial ANOVA (3 × 2) with repeated measures over time, as was the case for the temperature of the packaging bags. Repeated-measures ANOVA was chosen given that samples were obtained from the same individuals at different time points, which allows for assessing within-subject effects (Field, 2013). The remaining water quality variables and production parameters were analyzed using a two-way factorial ANOVA (3 × 2), where Factor A represented the oxygen level supplemented in the transport bags (ratios of 1:1, 1:1.5, and 1:2 L^{-1}), and Factor B was the diet fed to the fry prior to transport (control and functional additive). Repeated measures (within-subjects) were defined by the time points 0, 4, and 28 hours, corresponding to the pre-packaging stage (basal), arrival at the laboratory, and the recovery period (24 hours post-transport), respectively. For temperature data, repeated measures were taken at 0, 1, and 4 hours, corresponding to packaging, loading, and the end of transportation. Repeated-measures ANOVA is widely used in aquaculture and physiological research to evaluate physiological responses over time (Zar, 2014; Underwood, 1997). Pearson's correlation coefficient was used to examine the linear relationships among blood variables, as it is appropriate for continuous and normally distributed data (Dancey & Reidy, 2017). Differences between treatment means were analyzed using Tukey's honestly significant difference (HSD) post hoc test (García *et al*, 2000). Data normality was assessed through the inspection of skewness and kurtosis values. These measures provide insight into the distribution of the data and are commonly used as preliminary steps in parametric statistical analyses (Salazar & Del Castillo, 2018; Doane & Seward, 2011). All statistical procedures were conducted using the software PAST version 4.13 (Hammer *et al*, 2001).

Results

Experiment 1

Assessment of water quality and animal welfare indicators during the transport of O. niloticus fry according to oxygen supplementation level and dietary treatment

Water quality

Regarding water quality in the packaging bags at the end of the transport period, a positive correlation was observed between NH_3-N and DO variables (correlation coefficient = 0.6189, P = 0.0062). The values, in relation to the water-to-oxygen supplementation ratios (1:1, 1:1.5, and 1:2; Factor A) and the dietary treatments provided prior to transport (functional additive and control; Factor B), are presented in Figure 2.

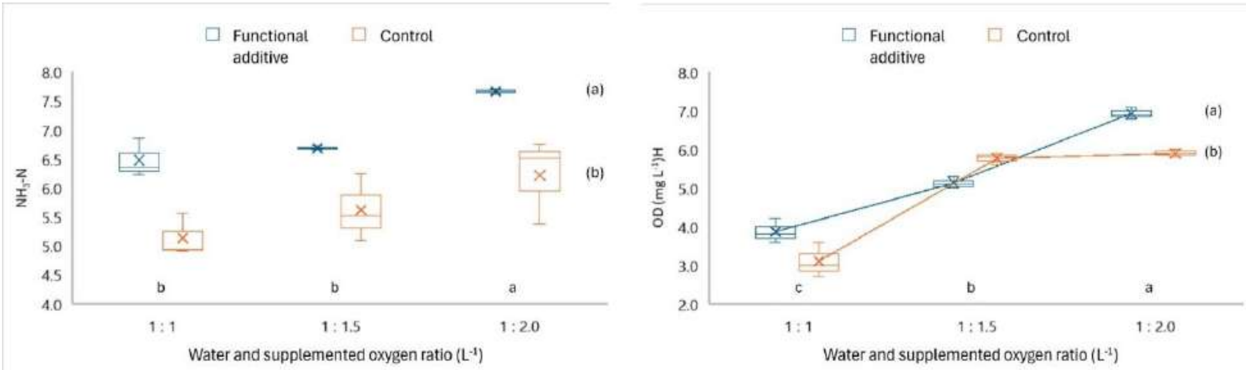

Figure 2. NH_3-N and dissolved oxygen values in the water at the end of the transport process of *O. niloticus* fry according to the water-to-supplemented oxygen ratio (L^{-1}) in the packaging bags (factor A) and the previously provided diet (factor B). 3 × 2 factorial ANOVA, mean differences by Tukey's test (letters), n = 3, mean ± SE.

In the left panel, the NH_3-N concentrations (mg L^{-1}) are shown. The analysis revealed statistically significant differences for both Factor A and Factor B, as determined by Tukey's test. However, no significant interaction between factors was observed. The 1:2 ratio exhibited more pronounced statistically significant differences among the water and oxygen levels used (different letters, $p < 0.05$). Treatments, including the functional additive diet, showed significantly higher NH_3-N concentrations than the control diet across all oxygenation ratios. In the right panel, DO concentrations (mg L^{-1}) are displayed. Values increased proportionally with higher water-to-oxygen supplementation ratios and were significantly higher in treatments with the functional additive compared to the control (different letters, $p < 0.05$). A significant interaction between the two factors was also observed. For Factor A, the 1:2 ratio resulted in the highest DO values, followed by the 1:1.5 ratio with intermediate values, and the 1:1 ratio with the lowest values. For Factor B, the functional additive diet yielded the highest DO levels, whereas the control diet yielded lower values. With respect to the interaction, a notable difference was observed in the 1:1.5 treatment, where DO levels were slightly higher in the control group compared to the additive group, contrary to the pattern observed at the other oxygenation levels, where the additive group generally showed higher DO concentrations. In the 1:2 ratio, treatments with the functional additive reached DO levels close to 6 mg L^{-1}, while the control diet remained steady around 5 mg L^{-1} starting from the 1:1.5 ratio. All treatments and combinations maintained DO concentrations above 3 mg L^{-1}, while water temperature remained stable throughout packaging, loading, and transport (Figure 3).

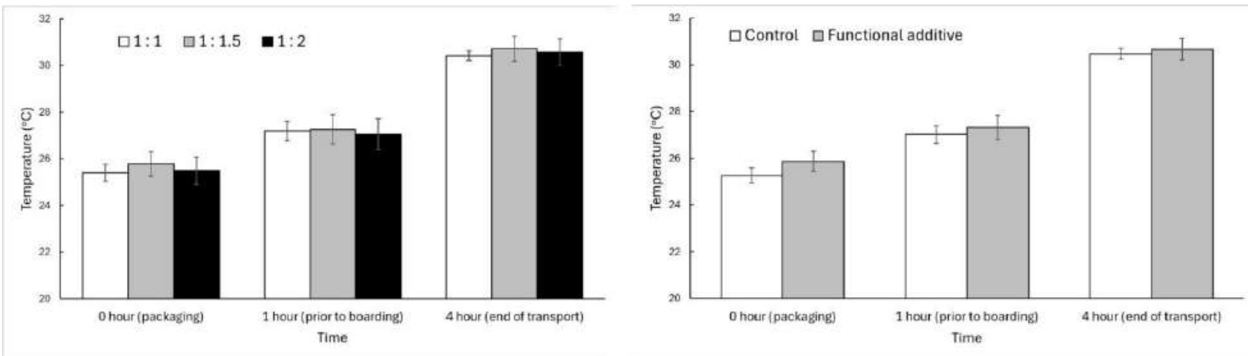

Figure 3. Water temperature values during the transport of *O. niloticus* fry according to the water-to-oxygen ratio (L^{-1}) in the packaging bags (factor A) and the experimental diets (factor B), evaluated at 0, 1, and 4 hours of transport. Data analyzed using a 3 × 2 factorial ANOVA with repeated measures over time; mean differences identified by Tukey's test (letters). n = 3, mean ± SE.

However, highly significant differences were observed among the different time points ($p < 0.01$), as indicated by the distinct letters. In the left panel, water temperature increased progressively in all oxygenation ratio treatments, from approximately 25 °C at the beginning of transport (0 h) to 30 °C at the end (4 h). The right panel shows a similar trend across both diet types, with no significant differences between them. Regarding pH, no statistically significant differences were observed between treatments for either Factor A or B, with an overall mean of 7.16 ± 0.13 SD, demonstrating consistency across treatments. No significant interaction was detected between the factors, suggesting that neither oxygen supplementation level nor dietary treatment had a notable influence on pH by the end of transport.

Internal indicators of welfare

Internal indicators of welfare included blood glucose, lactate, and hematocrit, measured at baseline (0 h), at the end of transport (4 h), and after one day of recovery (28 h). Blood glucose results are shown in Figure 4.

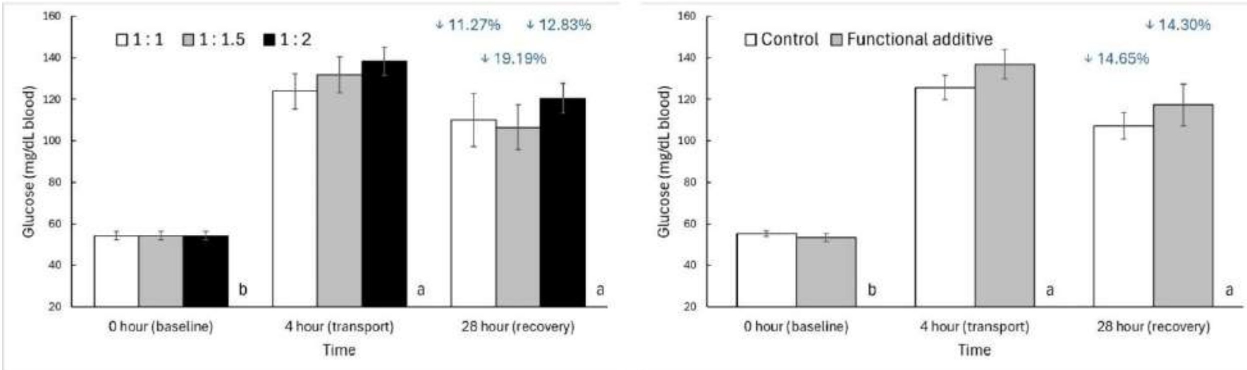

Figure 4. Figure 4. Blood glucose levels in *O. niloticus* fry according to the water-to-supplemented oxygen ratio (L^{-1}) in the packaging bags (factor A) and the experimental diets (factor B), based on 0, 4, and 28 hours of transport. 3 × 2 factorial ANOVA with repeated measures over time, mean differences by Tukey's test (letters), n = 6, mean ± SE.

In both panels (left and right), no statistically significant differences were observed between water-to-oxygen ratios or between dietary treatments. However, significant differences were found among time points ($p < 0.01$), as indicated by the letter. In the left panel, initial glucose levels (~50 mg/dL) were homogeneous among treatments. After 4 hours of transport, glucose concentrations increased significantly in all treatments, reaching values close to 140 mg/dL, suggesting a physiological stress response to transport. After 24 h of recovery, glucose levels decreased, showing reduction percentages of 11.27%, 12.83%, and 19.19% for the 1:1, 1:1.5, and 1:2 ratios, respectively. In the right panel, treatments with the functional additive and control diets followed a similar pattern. Initial glucose levels (~50 mg/dL) significantly increased after 4 h of transport (~140 mg/dL) with no significant differences between the two diets. Following the 28 h recovery period, glucose levels decreased in both treatments, with reduction percentages of 14.30% and 14.65% for the functional additive and control, respectively, indicating a comparable recovery in fry from both dietary groups. Blood lactate results are presented in Figure 5.

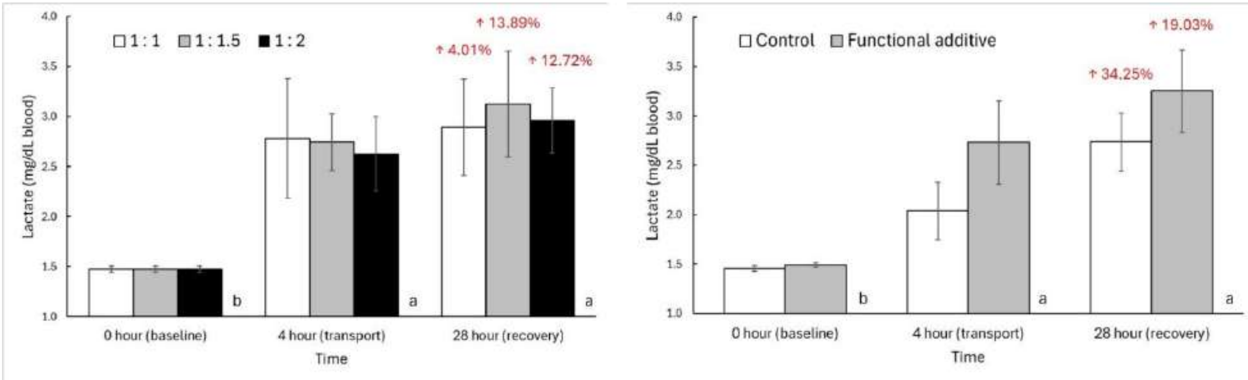

Figure 5. Blood lactate levels in *O. niloticus* fry according to the water-to-supplemented oxygen ratio (L^{-1}) in the packaging bags (Factor A) and the experimental diets (Factor B), measured at 0, 4, and 28 hours of transport. 3 × 2 factorial ANOVA with repeated measures over time; mean differences by Tukey's test (letters), n = 6, mean ± SE.

Baseline blood lactate levels (0 h, ~1.5 mg/dL) showed no significant differences among water-to-oxygen ratios (1:1, 1:1.5, and 1:2) or dietary treatments (functional additive and control). However, after 4 hours of transport, lactate concentrations increased significantly in all treatments, reflecting a metabolic response to stress. At 28 h (recovery), lactate concentrations remained significantly elevated—approximately twice the baseline value—across all treatments. Although no significant differences were detected among water-to-oxygen ratios or diets, the functional additive treatments showed a lower percentage increase in lactate levels compared to the control (19.03% vs. 34.25% at 4 and 28 h), suggesting that fry fed with the control diet experienced greater lactate accumulation during recovery than those receiving the functional additive. Regarding hematocrit values (Figure 6), baseline levels (~30%, at 0 h) did not differ significantly among water-to-oxygen ratios or between diets.

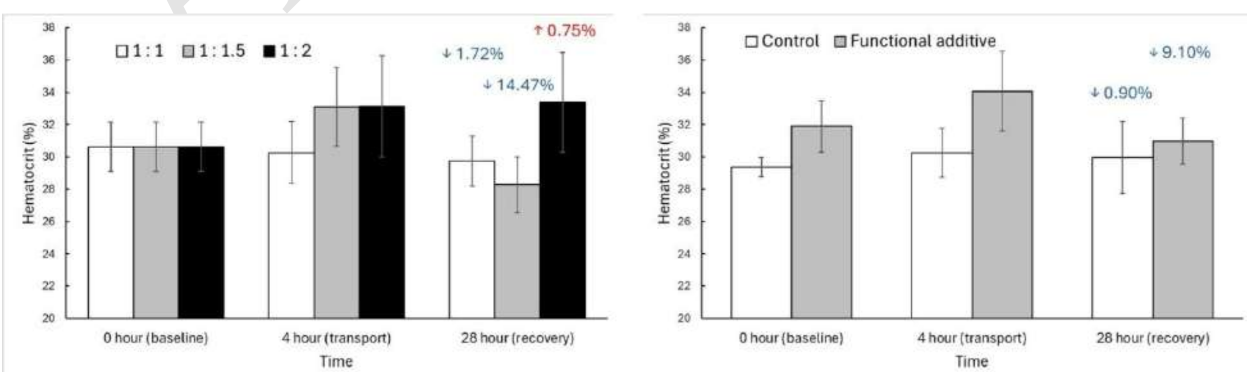

Figure 6. Hematocrit percentage in *O. niloticus* fry according to the water-to-supplemented oxygen ratio (L^{-1}) in the packaging bags (factor A) and the experimental diets (factor B), based on 0, 4, and 28 hours of transport. 3 × 2 factorial ANOVA with repeated measures over time, n = 6, mean ± SE.

After 4 hours of transport, a slight increase in hematocrit was observed across all treatments. At 28 h, values returned to levels similar to baseline across treatments, with no significant differences observed between diets. However, treatments with lower oxygen supplementation showed reductions of 1.72% and 14.47% for the 1:1 and 1:1.5 ratios, respectively, while a slight increase of 0.75% was noted for the 1:2 ratio. The functional additive group exhibited a greater decrease in hematocrit compared to the control (9.10% vs. 0.90%, from 4 to 28 h), indicating a more pronounced reduction during recovery in fry fed with the additive.

Correlations among blood variables are shown in Table 1. A moderate positive correlation was found between glucose and lactate levels (r = 0.5425, p = 0.0001), indicating that higher glucose levels were generally associated with higher lactate levels. The correlation between glucose and hematocrit was weak and not statistically significant (r = 0.1429, p = 0.2925), suggesting no clear relationship between these variables. Lastly, a weak but statistically significant correlation was found between lactate and hematocrit (r = 0.2925, p = 0.0021), indicating a slight trend for lactate levels to increase with hematocrit.

Table 1. Pearson correlations between blood variables.

	Lactate	**Hematocrit**
Glucose	0.5425	0.1429
	0.0000	-----
Lactate		0.2925
		0.0021

Experiment 2

Evaluation of productive indicators of *O. niloticus* fingerlings according to oxygen level used during transport and supplemented diet.

Productive Indicators

Table 2 shows the effects of the water-to-oxygen ratio (factor A) in the packaging bags and the diet (factor B) provided to tilapia fingerlings 14 days prior to transport, on the productive indicators of the fish cultured for 28 days after transport. During this period, fish were fed the diet supplemented with the functional additive. Individual variables such as final weight (g), condition factor (K), and specific growth rate (SGR, % animal day^{-1}) were evaluated, as well as group variables per tank, including survival at 14 and 28 days, final weight per tank, and feed efficiency. Regarding the water-to-oxygen ratio (A), the results revealed statistically significant differences in individual final weight, SGR, and feed efficiency ($p < 0.05$) among the evaluated ratios. Compared to the 1:1 and 1:1.5 ratios, the 1:2 ratio favored higher final weight (5.24 g vs. 3.88 and 3.74 g, respectively), higher SGR (3.45% animal day^{-1} compared to 2.51 and 2.62), and greater feed efficiency (0.78 vs. 0.46 and 0.48). However, survival rates at 14 and 28 days did not show significant differences between the evaluated ratios. As for the effect of the diet provided 14 days before transport (B), no statistically significant differences were found between fish fed the diet supplemented with the functional additive and those fed the control diet in the variables evaluated, although the control group showed a trend towards higher final weights and feed efficiency. Although the interaction between factors A and B was not statistically significant, the combination of the 1:2 ratio with the control diet resulted in the highest values for final weight (6.3 g), final K (1.7), and feed efficiency (0.92), suggesting that it could be the most efficient strategy in terms of productivity.

Table 2. Effect of the water-to-oxygen ratio in packaging bags and diet with functional additives prior to transport on *O. niloticus* performance.

Factors	n	Final weigth, g*	Final K**	SGR % animal day^{-1}	Survival 14 days, %	Survival 28 days, %	Final weigth, g***	Feed efficiency
A. Water and O_2 ratio (L^{-1})		**Variables per fish**			**Variables per pond**			
1:1	6	3.88 ± 0.4^{b}	1.63 ± 0.02	2.51 ± 0.2^{b}	89.0 ± 3.5	82.0 ± 4.3	329.8 ± 54.5	0.46 ± 0.1^{b}
1:1.5	6	3.74 ± 0.4^{b}	1.64 ± 0.02	2.62 ± 0.2^{b}	85.8 ± 3.5	81.8 ± 4.3	315.4 ± 52.1	0.48 ± 0.1^{b}
1:2	6	5.24 ± 0.4^{a}	1.71 ± 0.02	3.45 ± 0.2^{a}	90.1 ± 3.5	85.6 ± 4.3	448.6 ± 53.4	0.78 ± 0.1^{a}
p value		0.0301	---	0.0392	---	---	---	0.0458
B. Diet								
Additive	9	3.76 ± 0.3	1.63 ± 0.01	2.82 ± 0.2	87.1 ± 2.9	83.2 ± 3.5	313.8 ± 43.6	0.51 ± 0.09
Control	9	4.81 ± 0.3	1.69 ± 0.01	2.91 ± 0.2	89.5 ± 2.9	83.1 ± 3.5	415.3 ± 43.6	0.63 ± 0.09
p value		---	---	---	---	---	---	---
A x B								
1:1 x additive	3	3.86 ± 0.6	1.63 ± 0.02	2.33 ± 0.3	91.3 ± 5.0	87.6 ± 6.1	359.2 ± 79.6	0.49 ± 0.1
1:1 x control	3	3.90 ± 0.6	1.63 ± 0.02	2.69 ± 0.3	86.6 ± 5.0	76.3 ± 6.1	300.3 ± 73.5	0.42 ± 0.1
1:1.5 x additive	3	3.26 ± 0.6	1.63 ± 0.02	2.55 ± 0.3	83.0 ± 5.0	79.3 ± 6.1	261.4 ± 75.0	0.40 ± 0.1
1:1.5 x control	3	4.23 ± 0.6	1.66 ± 0.02	2.70 ± 0.3	88.6 ± 5.0	84.3 ± 6.1	369.4 ± 73.8	0.55 ± 0.1
1:2 x additive	3	4.17 ± 0.7	1.64 ± 0.03	3.58 ± 0.3	87.0 ± 5.0	82.6 ± 6.1	320.8 ± 86.7	0.63 ± 0.1
1:2 x control	3	6.30 ± 0.6	1.77 ± 0.02	3.33 ± 0.3	93.3 ± 5.0	88.6 ± 6.1	576.3 ± 76.3	0.92 ± 0.1
p value		---	---	---	---	---	---	---

Factorial ANOVA 3×2 and multiple range test using Tukey's method. Means ± SE. Different letters among rows indicate statistically significant differences ($p < 0.05$). Covariates used: *initial fish weight, **initial Fulton's K per fish, and ***initial pond weight.

Table 3 presents the effects of the water-to-oxygen ratio during transport and the diet provided prior to handling on the productive indicators of tilapia fingerlings fed the control diet. The same variables were evaluated, both individually per fish and per tank. Regarding factor A, no statistically significant differences were observed in the evaluated indicators. However, fish transported with a 1:2 water-to-oxygen ratio showed a trend toward improved productive indicators, with higher final weight (5.6 g) and better feed efficiency (0.85), suggesting that two parts of oxygen per one part of water during transport may enhance fish performance. Regarding the effect of the diet provided prior to transport, fish fed the functional additive had a significantly higher K (1.7; $p < 0.05$) compared to those fed the control diet (1.6). However, the other evaluated variables did not show significant differences between diets. The interaction between factors A and B showed that the combination of the 1:2 ratio with the functional additive diet resulted in the best performance, highlighting a mean final weight of 6.08 g, a K of 1.8, and a feed efficiency of 0.9. Statistically significant differences in SGR ($p < 0.05$) were observed within this interaction, indicating greater growth in fish transported with a 1:2 water-to-oxygen ratio and previously fed with the functional additive. Survival values did not show statistically significant differences between treatments, indicating that both the evaluated water-to-oxygen ratios and diets are sufficient to maintain high post-transport viability, exceeding 80%.

Table 3. Effect of water-to-oxygen ratio in packaging bags, and the control diet supplied prior to transport on *O. niloticus* performance.

Factors	n	Final weigth, g*	Final K**	SGR % animal day^{-1}	Survival 14 days, %	Survival 28 days, %	Final weigth, g***	Feed efficiency
A. Water and O_2 ratio (L^{-1})		**Variables per fish**			**Variables per pond**			
1:1	6	5.02 ± 0.4	1.70 ± 0.03	2.98 ± 0.1	85.5 ± 2.3	79.1 ± 3.1	401.0 ± 40.9	0.66 ± 0.1
1:1.5	6	5.10 ± 0.4	1.69 ± 0.03	3.03 ± 0.1	83.1 ± 2.3	76.6 ± 3.1	398.6 ± 41.3	0.75 ± 0.1
1:2	6	5.66 ± 0.4	1.76 ± 0.03	3.17 ± 0.1	90.0 ± 2.3	86.8 ± 3.1	492.4 ± 41.1	0.85 ± 0.1
p value		---	---	---	---	---	---	---
B. Diet								
Additive	9	5.52 ± 0.3	1.77 ± 0.03[a]	3.12 ± 0.09	88.0 ± 1.8	82.8 ± 2.5	465.3 ± 58.1	0.74 ± 0.1
Control	9	5.00 ± 0.3	1.66 ± 0.03[b]	3.00 ± 0.09	84.4 ± 1.8	78.8 ± 2.5	396.0 ± 33.4	0.64 ± 0.1
p value		---	0.0385	---	---	---	---	---
A x B								
1:1 x additive	3	4.68 ± 0.5	1.75 ± 0.04	2.85 ± 0.15[b]	90.0 ± 3.2	85.3 ± 4.4	409.0 ± 58.1	0.72 ± 0.1
1:1 x control	3	5.35 ± 0.5	1.66 ± 0.05	3.11 ± 0.15[ab]	81.0 ± 3.2	73.0 ± 4.4	392.9 ± 57.8	0.60 ± 0.1
1:1.5 x additive	3	4.78 ± 0.5	1.71 ± 0.04	2.90 ± 0.15[ab]	85.6 ± 3.2	80.3 ± 4.4	387.1 ± 57.9	0.58 ± 0.1
1:1.5 x control	3	5.41 ± 0.5	1.66 ± 0.04	3.15 ± 0.15[ab]	80.6 ± 3.2	73.0 ± 4.4	410.0 ± 58.6	0.56 ± 0.1
1:2 x additive	3	6.08 ± 0.5	1.85 ± 0.05	3.61 ± 0.15[a]	88.3 ± 3.2	83.0 ± 4.4	599.8 ± 58.8	0.93 ± 0.1
1:2 x control	3	4.23 ± 0.5	1.67 ± 0.04	2.73 ± 0.15[b]	91.6 ± 3.2	90.6 ± 4.4	385.0 ± 60.8	0.73 ± 0.1
p value		---	---	0.0049	---	---	---	---

Factorial ANOVA 3×2 and multiple range test using Tukey's method. Means ± SE. Different letters among rows indicate statistically significant differences ($p < 0.05$). Covariates used: *initial fish weight, **initial Fulton's K per fish, and ***initial pond weight.

Discussion

Water quality and animal welfare during transport

The results obtained show that the water-to-oxygen ratios and the pre-transport diet significantly influence water quality at the end of the transport of *O. niloticus* fry. In particular, higher oxygen ratios (1:2) were associated with better dissolved oxygen (DO) conditions, reinforcing the importance of adequate oxygen supply in packaging to minimize stress during transport. These results are consistent with previous studies reporting a direct correlation between the level of supplemented oxygen and the maintenance of appropriate water quality parameters, as it supports fish metabolism and reduces stress (Valdez-Prudencio *et al*, 2021). The presence of hypoxia will trigger a stress response, potentially resulting in problems such as decreased appetite, increased vulnerability to disease, slower growth, or even death (Abdel-Tawwab *et al*, 2019). Moderate hypoxia responses are reflected in blood decreases in pH, pO_2, oxygen saturation, plasma sodium, and chloride levels (Aboagye & Allen, 2018). However, in the present study, the highest water-to-oxygen ratio also showed the highest values of nitrogenous compounds in the transport water, highlighting the relationship between these variables. The relationship between oxygen levels and ammonia excretion in fish is complex and influenced by various factors, including species, diet, and environmental conditions. Higher oxygen levels can exacerbate ammonia toxicity, as observed in zebrafish, where increased dissolved oxygen led to higher plasma ammonia levels and reduced ammonia excretion through the gills due to decreased Na^+/K^+-ATPase activity (Sun *et al*, 2023), a situation contrary to that observed in the tilapia in this study and in other species. In juvenile rainbow trout, higher dissolved oxygen levels (11.84 and 15.80 mg/L) resulted in increased ammonia excretion compared to normoxic conditions (8.82 mg/L) (Tao, 2007). This suggests that while oxygen is crucial for fish metabolism, its interaction with ammonia can be detrimental under certain conditions. The diet supplemented with a functional additive, although it showed advantages in terms of DO concentration, also presented higher NH_3-N levels, probably as a result of more active metabolism associated with the consumption of said diet. For example, black carp fed a high-glucose diet (40%) showed increased oxygen consumption and ammonia excretion, indicating that dietary factors may influence the relationship between oxygen and ammonia excretion (Cai *et al*, 2010). This could imply greater ammonia management during transport when using diets with functional additives, especially when optimizing water-to-oxygen ratios. Nevertheless, all treatments maintained acceptable DO levels (> 3 mg/L), suggesting that the evaluated strategies are viable for the transport of tilapia fry. However, the 10:10 L^{-1} water-to-oxygen ratio (1:1 treatment) could compromise animal welfare if the transport of 430 g of biomass per bag exceeds four hours.

Internal welfare indicators

The physiological responses of fish are generally classified as primary and secondary responses, each associated with alterations in hematological or biochemical parameters that can be used as stress indicators (Zahl *et al*, 2009; Barbosa *et al*, 2007). An increase in blood cortisol concentration is a primary response, while elevated blood glucose and hematological alterations are clear secondary responses (Jiang *et al*, 2017; McDonald & Milligan, 1997). Physiological stress markers such as glucose and lactate showed significant increases after 4 hours of transport, confirming the induction of a metabolic stress response triggered by handling. Transport stress in tilapia is characterized by elevated levels of cortisol and glucose, which are common indicators of stress in fish. For instance, one study found that a 60-minute transport period resulted in higher serum cortisol and plasma glucose levels compared to a 240-minute period, suggesting that shorter transport times may induce more acute stress responses (Goes *et al*, 2018). Similarly, high biomass densities during transport also increased cortisol and glucose levels, indicating greater stress (Odhiambo *et al*, 2020). However, in the fish evaluated in this study, both indicators decreased during the recovery period after 24 hours of rest (hour 28), reflecting the fry's ability to recover after transport. Transport stress generally results in elevated glucose and lactate levels due to increased anaerobic metabolism. Under hypoxic conditions, tilapia utilize glycogen reserves, leading to greater lactate production (Padmavathy & Ramanathan, 2010). It has been shown that the use of salt during transport mitigates stressful effects, resulting in more stable glucose levels post-transport. This suggests that environmental modifications can help accelerate recovery (Alsagheer *et al*, 2024).

Treatments with the functional diet showed a smaller increase in lactate and a greater decrease in hematocrit during recovery, indicating possible mitigation of metabolic stress due to the effects of the functional additive. Specifically, vitamin C and quercetin have been successfully used in fish (Adah *et al*, 2023; Peng *et al*, 2013). The data from the present study may support the inclusion of functional additives as a strategy to improve fish resilience to stress associated with handling during transport. Additionally, the moderate correlation between glucose and lactate highlights the link between glycolytic and metabolic stress responses. During physiological testing conducted by Navarro *et al*. (2016) on tilapia under transport stress, physiological changes were found such as increased blood glucose levels and decreased hematocrit levels, clearly showing compensatory signs of increased energy demand and a secondary stress response. Lactate is associated with stress conditions and reduced ventilatory capacity, resulting in transient hypoxia where glucose is converted into lactate via anaerobic reactions that occur during glycolysis. During transport, fish are often subjected to stressors that can lead to reduced ventilatory capacity and hypoxia. The resulting increase in lactate production is a physiological adaptation to maintain energy homeostasis. Moreover, elevated lactate levels can serve as an indicator of stress and hypoxia (McClerking, 2022), providing insight into the health and welfare of fish during transport. Understanding the role of lactate in fish physiology can inform strategies to mitigate transport stress, such as optimizing oxygen levels and monitoring glucose and lactate in the field as stress biomarkers, particularly with portable devices that can be used on site (Beecham *et al*, 2006). While lactate production is a critical response to hypoxia, it is important to consider the balance between production and clearance. Excessive lactate accumulation can lead to metabolic acidosis and other adverse effects if not properly managed. Meanwhile, the weak correlation between glucose and hematocrit suggests that these parameters respond to independent physiological mechanisms.

Productive indicators after transport

Regarding the group of animals fed the functional additive diet, in terms of performance, the 1:2 water-to-oxygen ratio consistently favored productive indicators. This highlights the importance of providing fish with adequate oxygen levels during transport (Colt & Kroeger, 2013) to ensure optimal performance during subsequent growth phases. Furthermore, although no statistically significant differences in survival were observed, survival rates above 80% across all treatments reflect that both the diets and the oxygenation strategies evaluated are effective in maintaining fry viability during the initial growth phase in culture.

With respect to the diet, fish fed the control diet prior to transport showed better condition factor (K) values and a tendency toward improved growth rates and feed efficiency, especially when combined with the 1:2 water-to-oxygen ratio. These results differ from previous studies that document the positive impact of functional additives on fish growth and health (Alemayehu *et al*, 2018). Although the interaction between diet and oxygenation was not statistically significant across all variables, certain combinations showed interesting effects. For example, the control diet combined with the 1:2 ratio yielded the highest values for final weight, K, and feed efficiency, suggesting that fish may have different needs before and after transport. In the group of fish fed the control diet, a similar performance trend was observed: the 1:2 water-to-oxygen ratio showed the highest productive values, even though no significant differences were found between treatments, underscoring the importance of the oxygen supplementation ratio at the time of fish packaging (Valdez-Prudencio *et al*, 2021). In terms of diet, fish fed the functional additive diet prior to transport showed improved condition factor (K) values and a trend toward better growth rates and feed efficiency, especially when combined with the 1:2 water-to-oxygen ratio, which demonstrates the positive effect of functional additives on fish growth and health (Alemayehu *et al*, 2018). The combination of the 1:2 water-to-oxygen ratio and feeding with the functional additive diet prior to transport showed the best growth values, which could support the previously proposed hypothesis that fry have different nutritional and physiological needs before and after the transport process.

Comparison between both fry groups

In both groups of animals, those fed with the functional additive and those fed the control diet, the best productive indicator values were observed with the 1:2 water-to-oxygen ratio in the packaging bags. This result aligns with previous studies that have reported that adequate levels of dissolved oxygen are essential to minimize stress during transport (Valdez-Prudencio *et al*, 2021) and to ensure better recovery after the process. It is noteworthy that a higher oxygen-to-water ratio helps maintain the fish's metabolic homeostasis (Zhang *et al*, 2010), supporting the findings of this study. Hyperoxia can increase the fish's maximum oxygen uptake capacity, as demonstrated by a 35% higher maximum oxygen consumption rate ($\dot{m}O2max$) under hyperoxia compared to normoxia in rainbow trout (McArley *et al*, 2022). This suggests that Tilapia may also benefit from increased oxygen availability during transport, potentially enhancing their aerobic performance. However, it is also important to consider the potential risk of inducing hyperoxia during handling, which could lead to negative consequences in animals, such as oxidative stress due to the increase in reactive oxygen species (Lushchak VI & Bagnyukova TV, 2006). Thus, studies like the present one provide valuable insights in this regard.

Regarding the use of functional additives prior to transport handling, a very interesting pattern was observed. During the early growth and fattening stage, animals previously fed with the control diet showed the best productive performance values, whereas those fed the functional diet both before and after transport had the lowest productive indicators (Table 2). This suggests a possible difference in the nutritional requirements of fry before and after transport. The functional additive, based on vitamin C and quercetin, seems to metabolically prepare the fry for transport stress, probably by modulating their physiological response and reducing the negative effects of handling. However, once the fish acclimate to their new environment, the benefits of the additive may become limited. This could be explained by the fact that compounds added to the functional diet, such as prebiotics, probiotics, or bioactive compounds, displace a portion of the crude protein in the diet formulation. Therefore, a continuous 5% supplementation of the additive (as used in this study) could reduce the availability of essential proteins for growth, compromising indicators such as final weight and feed efficiency. Conversely, the best productive performance observed in fish fed with the functional additive prior to transport and the control diet afterward (Table 3) suggests that this strategy provides a balanced combination. This outcome is particularly evident when analyzing the interaction between factors, as this combined management strategy (feeding with the functional additive before transport and the control diet during the first fattening phase) resulted in the best growth, body condition, and feed efficiency. Previous studies have demonstrated that providing functional additives before transport can prepare fish to face the physiological stress associated with this handling (Adah *et al*, 2023; Armobin *et al*, 2023; Wang *et al*, 2020; Peng *et al*, 2013), which is reflected in improved metabolic recovery in the following days. The pre-transport functional additive acts as a physiological primer that helps mitigate the impact of transport, while the post-transport control diet ensures an optimal supply of essential nutrients for growth and development, without the possible dilution of key macronutrients caused by the additive.

Consequently, the findings indicate that, in addition to maintaining an optimal water-to-oxygen ratio during transport, careful dietary planning in the pre- and post-transport phases is essential to optimize the productive capacity of fry. These results underscore the importance of considering the metabolic phases of fry and their changing nutritional needs. Prior to transport, supplemented diets may help enhance the stress response, whereas in later stages, a balanced diet without functional additives may support improved productive performance. This integrated approach, together with the proper management of dietary transitions, contributes not only to growth but also to production efficiency and the sustainability of tilapia farming systems.

Practical implications, recommendations, and conclusions

The results of this study provide evidence on the importance of proper oxygen supplementation in packaging bags and the use of functional diets in optimizing the welfare and productive performance of tilapia fry. The present study recommends implementing a 1:2 water-to-supplemental-oxygen ratio during 4-hour transport with a biomass of 430 g, and the inclusion of functional additives in the pre-transport diet to enhance fish resilience and maximise performance in subsequent farming stages. These practices represent a valuable opportunity to enhance sustainability and profitability in the context of tilapia farming.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

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Use of Artificial Intelligence (AI)

During the preparation of this work, ChatGPT (OpenAI) was used for the translation of content from Spanish to English, and the Grammarly program was used for grammar and style revision. After using these tools, the content was reviewed and edited; therefore, the authors assume full responsibility for the content of the publication.

Data availability

The data sets used in the current study are available from the corresponding author on request.

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