Nucleotídeos purificados em rações para reprodutores e proles regulam a atividade de enzimas digestivas e a morfologia intestinal em tilápia-do-Nilo

Purified nucleotides in diets for broodstock and offspring regulate digestive enzyme activity and intestinal morphology in Nile tilápia

Los nucleótidos purificados en los piensos para reproductoras y crías regulan la actividad de las enzimas digestivas y la morfología intestinal en la tilapia del Nilo

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ABSTRACT
In this study, the effects of adding nucleotides to diets for broodstock and offspring of Nile tilapia (*Oreochromis niloticus*) were evaluated. Nile tilapia broodstock were fed diets containing 0.0, 2.5, and 5.0 g of Ascogen®/kg for five months. After this period, the eggs were collected and incubated. The offspring, at five days of age, were fed diets containing 0.0, 2.5, 5.0, and 7.5 g of Ascogen®/kg for 100 days. Analyses of digestive enzyme activity and the morphology of hepatic and intestinal tissues of juveniles were conducted. An interactive effect (p<0.05) between the diets of broodstock and offspring on trypsin activity was observed. The highest activity of this enzyme was recorded when diets containing 2.5 g/kg were used for broodstock and 7.5 g/kg for offspring. Juveniles from broodstock fed with 0.0 g/kg and offspring fed with 7.5 g/kg showed a significantly greater (p<0.05) height of enterocytes. Hepatic tissue was not influenced by the diets regarding its morphology. It is concluded that 7.5 g/kg of Ascogen® in offspring’s diet promotes alterations in intestinal morphology in terms of enterocyte height. Under this condition, when associated with 2.5 g/kg of Ascogen® for broodstock, it enhances trypsin activity in the offspring.

Keywords: nucleotides, intestinal villi, hepatocytes, enzymatic activity.
RESUMO
Neste estudo foram avaliados os efeitos da adição de nucleotídeos em dietas para reprodutores e descendentes de tilápia do Nilo (Oreochromis niloticus). Os reprodutores de tilápia do Nilo foram alimentados com dietas contendo 0,0, 2,5 e 5,0 g de Ascogen®/kg durante cinco meses. Após esse período, os ovos foram coletados e incubados. Os filhotes, aos cinco dias de idade, foram alimentados com dietas contendo 0,0, 2,5, 5,0 e 7,5 g de Ascogen®/kg durante 100 dias. Foram realizadas análises da atividade das enzimas digestivas e da morfologia dos tecidos hepáticos e intestinais dos juvenis. Foi observado efeito interativo (p<0,05) entre as dietas dos reprodutores e dos filhotes sobre a atividade da tripsina. A maior atividade desta enzima foi registrada quando foram utilizadas dietas contendo 2,5 g/kg para reprodutores e 7,5 g/kg para descendentes. Juvenis de reprodutores alimentados com 0,0 g/kg e filhotes alimentados com 7,5 g/kg apresentaram altura de enterócitos significativamente maior (p<0,05). O tecido hepático não foi influenciado pelas dietas quanto à sua morfologia. Conclui-se que 7,5 g/kg de Ascogen® na dieta da prole promove alterações na morfologia intestinal em termos de altura dos enterócitos. Nessa condição, quando associado a 2,5 g/kg de Ascogen® para reprodutores, aumenta a atividade da tripsina na prole.

Palavras-chave: nucleotídeos, vilosidades intestinais, hepatócitos, atividade enzimática.

RESUMEN
En este estudio, se evaluaron los efectos de agregar nucleótidos a las dietas para reproductores y crías de tilapia del Nilo (Oreochromis niloticus). Los reproductores de tilapia del Nilo fueron alimentados con dietas que contenían 0,0, 2,5 y 5,0 g de Ascogen®/kg durante cinco meses. Después de este período, los huevos fueron recolectados e incubados. Las crías, a los cinco días de edad, fueron alimentadas con dietas que contenían 0,0, 2,5, 5,0 y 7,5 g de Ascogen®/kg durante 100 días. Se realizaron análisis de la actividad de las enzimas digestivas y de la morfología de los tejidos hepáticos e intestinales de juveniles. Se observó un efecto interactivo (p<0,05) entre las dietas de los reproductores y las crías sobre la actividad de la tripsina. La mayor actividad de esta enzima se registró cuando se utilizaron dietas que contenían 2,5 g/kg para los reproductores y 7,5 g/kg para las crías. Los juveniles de reproductores alimentados con 0,0 g/kg y las crías alimentadas con 7,5 g/kg mostraron una altura de enterocitos significativamente mayor (p<0,05). El tejido hepático no fue influenciado por las dietas en cuanto a su morfología. Se concluye que 7,5 g/kg de Ascogen® en la dieta de las crías promueve alteraciones en la morfología intestinal en cuanto a la altura de los enterocitos. En esta condición, cuando se asocia con 2,5 g/kg de Ascogen® para reproductores, mejora la actividad de la tripsina en la descendencia.

Palabras clave: nucleótilos, vellosidades intestinales, hepatocitos, actividad enzimática.
1 INTRODUCTION

Tilapia aquaculture is growing globally, being the second most produced group of fish worldwide. Nile tilapia (Oreochromis niloticus) is the most important species within this group, raised in over 80 countries, with a production of 9 million tons in 2020 (FAO, 2022). The cost of feed is one of the primary operational expenses in tilapia production. Thus, it is essential to assess nutritional requirements, feed management strategies, and the use of additives or supplements to ensure sustainable and economically viable production (Gule and Geremew, 2022). Tilapia production systems have intensified to enhance productivity and meet market demands (Barroso et al., 2018). However, the availability of high-quality offspring in the market has become a limiting factor for aquaculture (Schulter and Vieira Filho, 2017; Barroso et al., 2018; FAO, 2020; Bombardelli et al., 2021).

In this context, among various strategies, attention to parental nutrition is indispensable due to its direct impact on gamete quality (Izquierdo et al., 2001) and its subsequent influence on reproductive potential (Bombardelli et al., 2017) and offspring quality (Abdelhamid et al., 2009). Proper parental nutrition affects the quality of the yolk, which serves as a source of essential nutrients for the initial stages (Mazorra et al., 2003) and, consequently, the vigor of offspring (Bombardelli et al., 2009; Bombardelli et al., 2021).

In tilapia feed formulation, some additives such as chemotherapeutics and antibiotics are added to promote growth and prevent infections. However, the use of these substances has generated negative perceptions in various countries. In this context, it becomes essential to study additives that promote animal growth without causing productivity and health losses, while maintaining the beneficial effects of antibiotics and chemotherapeutics. Alternatives such as enzyme addition, probiotics, prebiotics, plant extracts, and purified nucleotides are being used as replacements (Stein; Kill, 2006; Bombardelli et al., 2023). Additives based on purified nucleotides have received considerable attention from the production sector as they act on various metabolic pathways, promoting better health, greater resistance to pathogens, and consequently, improved animal growth (Abdel-Tawwab et al., 2008).
The supplementation of nucleotides in fish is imperative to provide insights into the complex interactions within the domains of nutrition and physiological responses (Li and Gatlin, 2006). These nucleotides are structural components of some coenzymes, such as flavin adenine dinucleotide (FAD) and coenzyme A (Champe et al., 2009). They are involved in the coding/decoding of genetic information, as well as in processes of cell growth and repair, and the regulation of various metabolic pathways (Cosgrove, 1998; Hess and Greenberg, 2012). They are also monomers of nucleic acids, acting in the storage and transcription of genetic information in the form of DNA and RNA (Hess and Greenberg, 2012). Furthermore, improving the activity of digestive enzymes through dietary nucleotide supplementation can assist in breaking down macronutrient molecules into smaller pieces, such as carbohydrates, fats, and other proteins, facilitating the nutrient absorption process (Zou et al., 2016).

The addition of these nucleotides to the diet has beneficial effects on lipid metabolism, growth, liver functions, maturation, activation, and proliferation of lymphocytes, macrophage phagocytosis, and cytokine gene expression (Gil, 2002). Moreover, nucleotides are responsible for modulating liver metabolism and improving nutrient absorption by enterocytes in the intestine, thereby improving the utilization of nutrients from the feed for animal development (López-Navarro et al., 1995). Research on the addition of nucleotides to fish diets has yielded positive results, promoting greater animal growth (Li and Gatlin, 2006; de Lima et al., 2020), improvements in stress and immune systems (Barros et al., 2015), gastrointestinal health (Li and Gatlin, 2006), increased digestive enzyme activity (Zou et al., 2016), and benefits for the health of breeders (de Lima et al., 2020; Bombardelli et al., 2023).

Furthermore, it is suggested that nucleotide supplementation may be important for nutritional interaction between broodstock and offspring. It has been observed to improve the immune system of human neonates (Yang et al., 2021). In sows, it leads to piglets with better productive performance and relieves serum oxidative stress (Tan et al., 2021). In rats, during weaning, it regulates energy balance due to low feed intake (Borda et al., 2003). In poultry, it increases egg production, accelerates the formation of intestinal villi in chicks, and improves immunity against bacterial agents (Kruger and Werf, 2018). In
fish, maternal nutrition affects the health and vigor of offspring after three months of age, improves antioxidant status, liver integrity, and resistance to bacterial infection (Nascimento et al., 2013), as well as the initial development of the digestive tract (Gonzalez-Vecino et al., 2015).

Despite these prospects, few studies have been conducted using nucleotides in the diets of fish parents to evaluate their effects on offspring. Therefore, the objective of this study was to assess the interactive effects of parental and offspring nutrition with diets containing purified nucleotides on the activity of digestive enzymes and the morphology of hepatic and intestinal tissues in juvenile Nile tilapia.

2 MATERIAL AND METHODS

2.1 BROODSTOCK FEEDING AND OFFSPRING OBTENTION

All experiments were conducted with GIFT lineage Nile tilapia (*Oreochromis niloticus*). The experiment was approved by the Animal Ethics Committee of the Western Paraná State University (Certificate no. 39/20). The offspring were obtained from 84 males (536 g ± 13.4 g) kept in 12 hapas of 2 m × 1 m, and 252 females (286 g ± 7.15 g) kept in 12 hapas of 2 m × 3 m.

The breeders were fed with extruded feed containing 280 g of digestible protein (DP) and 11.72 MJ of digestible energy (DE) per kg of feed (Table 1) using three added levels of commercial purified nucleotides (0.0, 2.5, and 5.0 g NT kg\(^{-1}\); Ascogen\(^{®}\); 15% nucleotides). Each treatment was carried out with four replications. The animals were fed twice a day at 1% of their body weight and subjected to biometric evaluation every 17 days to correct the feeding rate.
Table 1. Composition of feed and nutrient contents (g kg\(^{-1}\)) of experimental diets at different supplementation levels of purified nucleotides, used to feed males and females of Nile tilapia (\textit{Oreochromis niloticus}).

<table>
<thead>
<tr>
<th>Ingredients (g kg(^{-1}))</th>
<th>Nucleotide (g kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 g</td>
</tr>
<tr>
<td>Soybean meal(^{a})</td>
<td>533.2</td>
</tr>
<tr>
<td>Corn(^{a})</td>
<td>286.8</td>
</tr>
<tr>
<td>Fish flour(^{b})</td>
<td>73.0</td>
</tr>
<tr>
<td>Inert/Bentonite(^{c})</td>
<td>51.6</td>
</tr>
<tr>
<td>Bi-calcium phosphate</td>
<td>25.4</td>
</tr>
<tr>
<td>Limestone</td>
<td>9.2</td>
</tr>
<tr>
<td>Common salt</td>
<td>5.0</td>
</tr>
<tr>
<td>Mineral and Vitamin Suppl.(^{d})</td>
<td>15.0</td>
</tr>
<tr>
<td>Antioxidant(^{e})</td>
<td>0.5</td>
</tr>
<tr>
<td>Nucleotide(^{f})</td>
<td>0.0</td>
</tr>
<tr>
<td>Nutrients (g kg(^{-1}))</td>
<td></td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>9.5</td>
</tr>
<tr>
<td>Starch</td>
<td>210.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>14.5</td>
</tr>
<tr>
<td>Ashes</td>
<td>124.3</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>10.0</td>
</tr>
<tr>
<td>Fat</td>
<td>224.7</td>
</tr>
<tr>
<td>Total lysine</td>
<td>18.8</td>
</tr>
<tr>
<td>Dry matter</td>
<td>895.0</td>
</tr>
<tr>
<td>Total met.+cystine</td>
<td>1.9</td>
</tr>
<tr>
<td>Crude energy (MJ kg(^{-1}))</td>
<td>15.7</td>
</tr>
<tr>
<td>Digestible energy (MJ kg(^{-1}))</td>
<td>11.7</td>
</tr>
<tr>
<td>Crude protein</td>
<td>312.9</td>
</tr>
<tr>
<td>Digestible protein</td>
<td>280.0</td>
</tr>
</tbody>
</table>

\(^{a}\) Digestibility rates of nutrients according to Boscolo et al. (2002). \(^{b}\) Digestibility rates of nutrients according to Meurer et al. (2003). \(^{c}\) Bentonite was used as an inert compound to permit the replacement of purified nucleotide and maintain the same nutrients and energy levels of feeds besides to be used as an adsorbent compound. \(^{d}\) Mineral and vitamin supplement - Basic composition: folic acid: 200 mg; pantothenic acid: 4000 mg; Biotin: 40 mg; Copper: 2000 mg; Iron: 12,500 mg; Iodine: 200 mg; Manganese: 7500 mg; Niacin: 5000 mg; Selenium: 70 mg; Vitamin A: 1,000,000 UI; Vitamin B1: 1900 mg; Vitamin B12: 3500 mg; Vitamin B2: 2000 mg; Vitamin B6: 2400 mg; Vitamin C: 50,000 mg; Vitamin D3: 500,000 UI; Vitamin E: 20,000 UI; Vitamin K3: 500 mg; Zinc: 25,000 mg. \(^{e}\) BHT. \(^{f}\) Ascogen\(^{\circ}\). Source: Research data, prepared by the authors, 2024.
Males and females (1:3) underwent reproductive management in the hapas, remaining 12 days apart with five days of mating (de Lima et al., 2020). At the end of the mating period, the eggs were collected and incubated as described by Bombardelli et al. (2009, 2017). This procedure was repeated over a period of five months, when the offspring were obtained and reared for the experiments.

2.2 45-DAY-OLD TILAPIA REARING

First, 1,200 five-day-old tilapias from each broodstock nucleotide treatment (100 tilapia per hapa) were used to determine the initial body weight. So, the five-day-old tilapias were anesthetized in 75 mg benzocaine L\(^{-1}\) of water, previously diluted in 95% alcohol (CFMV, 2012), and weighed on a digital scale (± 0.0001 g). Then, the five-day-old tilapias were photographed to measure the standard length using IMAGEJ\textsuperscript® 1.60.

Then, for the growth tests, other 8,640 five-day-old tilapias from the broodstock nucleotide treatment were placed in 48 aquariums with a useful volume of 60 L, at a density of 3 five-day-old tilapias L\(^{-1}\). So, the experiment was conducted in a two-factorial experimental design (3 × 4), with twelve treatments and four replications. The treatments consisted of offspring from Nile tilapia broodstock fed with three extruded feed with nucleotides (0.0, 2.5, and 5.0 g NT kg\(^{-1}\)), which also were fed on four powder feed containing 0.0, 2.5, 5.0, and 7.5 g NT kg\(^{-1}\). A 60 L aquarium containing 180 five-day-old tilapias was considered an experimental unit (48 experimental unit; 180 tilapia per aquarium; 720 tilapia per treatment). Were used only three treatments from broodstock feeding owing the facility limitation to carried out the experiment with more than 48 aquariums. The treatment used to feeding offspring with 7.5 g NT kg\(^{-1}\) was used to support the hypothesis that these tilapias could have a higher nucleotide requirement due to their high metabolic rate, imposed during their earlier development stage.

The aquariums were installed in a water recirculation system with constant aeration, mechanical and biological filters, and electric water heating (27.0 ± 1.0 °C). The feed supplied to the offspring was processed as powder and the ingredients were ground in a hammer mill with a 0.3 mm sieve. The feed contained 386 g DP kg\(^{-1}\), 15.91 MJ DE kg\(^{-1}\) (Table 2), and 60 mg of 17-\(\alpha\)-methyltestosterone kg\(^{-1}\).
Table 1. Food composition and nutrient content of experimental feed containing different levels of purified nucleotide used to feed 45 and 105-days-old Nile tilapia (*Oreochromis niloticus*) obtained from broodstock also fed with different levels of purified nucleotide.

<table>
<thead>
<tr>
<th>Ingredients (g kg⁻¹)</th>
<th>0</th>
<th>2.5</th>
<th>5</th>
<th>7.5</th>
<th>0</th>
<th>2.5</th>
<th>5</th>
<th>7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleotide (g kg⁻¹)</strong></td>
<td>264.6</td>
<td>264.6</td>
<td>264.6</td>
<td>264.6</td>
<td>290.0</td>
<td>290.0</td>
<td>290.0</td>
<td>290.0</td>
</tr>
<tr>
<td><strong>Corn</strong></td>
<td>46.9</td>
<td>46.9</td>
<td>46.9</td>
<td>46.9</td>
<td>463.9</td>
<td>463.9</td>
<td>463.9</td>
<td>463.9</td>
</tr>
<tr>
<td><strong>Soybean meal</strong></td>
<td>618.8</td>
<td>618.8</td>
<td>618.8</td>
<td>618.8</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td><strong>Fish flour</strong></td>
<td>10.0</td>
<td>7.5</td>
<td>5.0</td>
<td>2.5</td>
<td>10.0</td>
<td>7.5</td>
<td>5.0</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Soxbean oil</strong></td>
<td>44.4</td>
<td>44.4</td>
<td>44.4</td>
<td>44.4</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
</tr>
<tr>
<td><strong>Common salt</strong></td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Mineral and Vitamin Suppl.</strong></td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Antioxidant</strong></td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Nucleotide</strong></td>
<td>0.0</td>
<td>2.5</td>
<td>5.0</td>
<td>7.5</td>
<td>0.0</td>
<td>2.5</td>
<td>5.0</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>Starch</strong></td>
<td>171.1</td>
<td>171.1</td>
<td>171.1</td>
<td>171.1</td>
<td>208.0</td>
<td>208.0</td>
<td>208.0</td>
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</tr>
<tr>
<td><strong>Linoleic acid</strong></td>
<td>29.4</td>
<td>29.4</td>
<td>29.4</td>
<td>29.4</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
</tr>
<tr>
<td><strong>Calcium</strong></td>
<td>22.4</td>
<td>22.4</td>
<td>22.4</td>
<td>22.4</td>
<td>12.9</td>
<td>12.9</td>
<td>12.9</td>
<td>12.9</td>
</tr>
<tr>
<td><strong>Ashes</strong></td>
<td>116.1</td>
<td>116.1</td>
<td>116.1</td>
<td>116.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total phosphorus</strong></td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td>116.2</td>
<td>116.2</td>
<td>116.2</td>
<td>116.2</td>
<td>38.8</td>
<td>38.8</td>
<td>38.8</td>
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</tr>
<tr>
<td><strong>Total lysine</strong></td>
<td>20.3</td>
<td>20.3</td>
<td>20.3</td>
<td>20.3</td>
<td>22.2</td>
<td>22.2</td>
<td>22.2</td>
<td>22.2</td>
</tr>
<tr>
<td><strong>Dry matter</strong></td>
<td>913.1</td>
<td>913.1</td>
<td>913.1</td>
<td>913.1</td>
<td>891.4</td>
<td>891.4</td>
<td>891.4</td>
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</tr>
<tr>
<td><strong>Total metionine</strong></td>
<td>9.7</td>
<td>9.7</td>
<td>9.7</td>
<td>9.7</td>
<td>5.1</td>
<td>5.1</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Crude energy (MJ kg⁻¹)</strong></td>
<td>19.5</td>
<td>19.5</td>
<td>19.5</td>
<td>19.5</td>
<td>17.4</td>
<td>17.4</td>
<td>17.4</td>
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<tr>
<td><strong>Digestible energy (MJ kg⁻¹)</strong></td>
<td>15.9</td>
<td>15.9</td>
<td>15.9</td>
<td>15.9</td>
<td>13.4</td>
<td>13.4</td>
<td>13.4</td>
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<tr>
<td><strong>Crude protein</strong></td>
<td>451.0</td>
<td>451.0</td>
<td>451.0</td>
<td>451.0</td>
<td>352.0</td>
<td>352.0</td>
<td>352.0</td>
<td>352.0</td>
</tr>
<tr>
<td><strong>Digestible protein</strong></td>
<td>386.0</td>
<td>386.0</td>
<td>386.0</td>
<td>386.0</td>
<td>320.0</td>
<td>320.0</td>
<td>320.0</td>
<td>320.0</td>
</tr>
</tbody>
</table>

- *Digestibility rates of nutrients according to Boscolo et al. (2002).* 
- *Digestibility rates of nutrients according to Meurer et al. (2003).* 
- *Bentonite was used as an inert compound to permit the replacement of purified nucleotide and maintain the same nutrients and energy levels of feeds besides to be used as an adsorbent compound.* 
- *Mineral and vitamin supplement - Basic composition: folic acid: 200 mg; pantothenic acid: 4000 mg; Biotin: 40 mg; Copper: 2000 mg; Iron: 12,500 mg; Iodine: 200 mg; Manganese: 7500 mg; Niacin: 5000 mg; Selenium: 70 mg; Vitamin A: 1,000,000 UI; Vitamin B1: 1900 mg; Vitamin B12: 3500 mg; Vitamin B2: 2000 mg; Vitamin B6: 2400 mg; Vitamin C: 50,000 mg; Vitamin D3: 500,000 UI; Vitamin E: 20,000 UI; Vitamin K3: 500 mg; Zinc: 25,000 mg.* 
- *BHT.* 
- *Ascogen®. Source: Research data, prepared by the authors, 2024.*
The fish were fed seven times a day. The residues were removed daily and 30% of water volume was exchanged. Biometry was performed every 10 d to correct the feeding rate (Nascimento et al., 2023). In the first 10 d of rearing, the offspring were fed with 30% of their biomass (Nascimento et al., 2023). Between d 11 and 20 they were fed with 20% of their biomass, and subsequently with 10% of their biomass until d 40 (Nascimento et al., 2023).

Water temperature (27.01 ± 1.3 °C) was measured daily using a digital minimum/maximum thermometer. Dissolved oxygen levels (4.93 ± 1.75 mg/L) were also measured daily using a YSI Professional Plus multiparameter meter, in addition to conductivity (85.07 ± 3.9 µS/cm), total dissolved solid content (68.8 ± 3.65 mg/L), pH (7.66 ± 0.25), ammonia (0.026 ± 0.01 mg/L), nitrite (0.11 ± 0.1 mg/L), and nitrate (0.13 ± 0.1 mg/L) levels.

At the end of 40 d, the 45-days-old tilapia were anesthetized by immersion in a solution containing 75 mg benzocaine L⁻¹, as previously described, and then were transferred to another structure (hapas; 2 × 4 mm mesh; 95 × 95 × 56 cm) for the subsequent rearing tests.

2.3 105-DAY-OLD TILAPIA REARING

Using an identical experimental design as described previously, 960 forty-five-day-old tilapia from the previous trial were placed in 48 hapas (2 × 4 mm mesh) measuring 95 × 95 × 56 cm and with a useful volume of 500 L (Nascimento et al., 2023). The hapas were installed in a 10 × 20 m earth pond, covered with greenhouse plastic and supplied with water only to compensate infiltration and evaporation losses. These fish were fed for 60 d with diets containing 320 g DP and 13.4 MJ DE kg⁻¹ and supplemented with 0.0, 2.5, 5.0, and 7.5 g NT kg⁻¹ (Table 2). During the first 30 d of rearing, the tilapias were fed four times a day with 10% of their biomass. Then, they were fed with 4% of their biomass until the end of the experiment (Nascimento et al., 2023). Feed rates were corrected biweekly using biometry. Minimum and maximum water temperatures (23.4 ± 2.1 °C and 27 ± 2.3 °C), dissolved oxygen levels (5.94 ± 1.57 mg/L), electrical conductivity (73.2 ± 1.7 µS/cm), total dissolved solids (69.9 ± 19 mg/L), pH (7.07 ± 09),
total ammonia (0.11 ± 0.01 mg/L), and water nitrite (0.29 ± 0.02 mg/L) were evaluated as in the previous experiment.

2.4 GUT AND LIVER SAMPLES COLLECTION

At the end of the experimental period, 105-day-old tilapias were fed and 60 minutes after feeding the fish were anesthetized as described above. This procedure was used to guarantee the full gut on collection time. Then, five fish from each experimental unit were euthanized through spinal cord section (CFMV, 2012) and dissected for gut and liver samples collection.

After euthanasia, the fish were dissected to obtain samples corresponding to five centimeters from the anterior part of the intestine, measured from the end of the stomach. These intestinal samples were immediately preserved in liquid nitrogen and, after 48 hours, stored in an ultrafreezer at -85°C for subsequent determination of digestive enzyme activity. From the same fish, other samples of 3 cm from the anterior intestine and the livers were fixed in 10% formaldehyde for subsequent processing and histological evaluation of intestinal and hepatic tissues.

2.5 ANALYSIS OF DIGESTIVE ENZYME ACTIVITY

The intestinal samples designated for the evaluation of digestive enzyme activity were crushed and homogenized in 0.8% saline solution using a tissue homogenizer (IKA® T10 basic, IKA, Campinas-SP, Brazil). Subsequently, the homogenate was centrifuged (Sigma®, 3-16 KL, Osterode am Harz, Germany) at 4°C for 10 minutes at 12,000 x g. The supernatant obtained after centrifugation was collected and used for the determination of the activities of maltase, sucrase, amylase, lipase, chymotrypsin, and trypsin. The protein content of each sample was determined using the Bradford method (1976), with albumin used to generate the standard curve, and readings were taken at the absorbance of 595 nm.

For the measurement of disaccharidases (maltase and sucrase), 50 µL of the sample was incubated with 20 µL of maleate buffer (pH 6) for 5 minutes. Then, 30 µL of the respective substrate (maltose and sucrose) was added to initiate the reaction for 5
minutes. Glucose was subsequently measured using a 12oeficien kit from Gold Analisa®, following the manufacturer’s recommendations. The samples were incubated in a water bath with controlled temperature at 25°C and read at tem absorbance of 505 nm. The results were expressed in U/mg of protein, where U = nmol of glucose/min/mg of protein (Pereira et al., 2011 and Dahlqvist, 1984).

The activity of amylase was determined using a 12oeficien kit, adapted for a microplate with a wavelength set to 660 nm at 30°C. The results were expressed in kU/L/mg of protein, where U = the amount of enzyme that hydrolyzes starch/min/mg of protein. The activity of lipase was determined using a 12oeficien kit, adapted for a microplate with a wavelength set to 412 nm at 37°C. The results were expressed in U/L/mg of protein, where U = the amount of enzyme that catalyzes the release of 1 μmol of fatty acid/min/mg of protein.

The activity of chymotrypsin was determined according to the method of Hummel (1959), adapted for a microplate. For the determination of this enzyme’s activity, the substrate benzoyl tyrosine ethyl ester (BTEE) was used. The extracts were incubated in a reaction 12oefic containing BTEE and 12oefici in Tris/CaCl2 buffer at pH 7.8 for two minutes in a microplate, with readings taken at tem-second intervals, at a wavelength of 256 nm, and a temperature of 30°C. The result was expressed in U/mg of protein, with one unit of chymotrypsin being the amount of enzyme required to form 1 μmol of 12oefic/minute. The molar extinction 12oeficiente of 12oefic used for the enzyme calculation was 964 M.

The activity of trypsin was determined according to the method of Hummel (1959), adapted for a microplate. The substrate used for the determination of this enzyme’s activity was α-p-toluenesulphonyl-L-arginine methyl ester hydrochloride (TAME). The extracts were incubated in a reaction 12oefic containing TAME in Tris/CaCl2 buffer at pH 8.1 for two minutes in a microplate. Readings were taken at tem-second intervals, at a wavelength of 247 nm, and a temperature of 30°C. The result was expressed as U/mg of protein, with one unit of trypsin being the amount of enzyme required to form 1 μmol of Na-p-Tosyl-L-Arginine/minute. The molar extinction 12oeficiente of Na-p-Tosyl-L-Arginine used for the enzyme calculation was 540 M.
Digestive enzyme analyses were performed in triplicates for each collected fish, and readings were taken in 96-well flat-bottom microplates using a Microplate Reader (Multiskan® FC, Thermo Scientific, Rastatie, Finland).

2.6 HISTOLOGY OF GUT AND LIVER

The gut and liver of the same five tilapias from each hapa (20 per treatment) were obtained for morphological evaluation. The organs were placed in a Karnovsky solution. The material was then dehydrated by passing it through an increasing alcohol series and was placed in histological paraffin. Next, 5 μm thick transversal cuts were removed from the guts and stained with hematoxylin and eosin. The laminas were mounted on Permont®, evaluated, and photographed under an optical microscope (Olympus CX31) with a digital camera (Olympus SC30). Fifty images were obtained from each gut or liver sample. In the gut were measure at least 10 villus from each image. Were measured the villus height, enterocytes height and thickness of the tunic (Bombardelli et al., 2022).

In the liver, the evaluation was based on the observation of general aspects of the hepatic tissue and verification of alterations in the tissue pattern. The pattern considered normal, was the one in which the tissue had well-defined characteristics and structures, such as well-defined blood and biliary vessels, well-marked blood cells, and evident cell nuclei. Liver tissue assessments were performed by capturing images with a 40X objective, in random fields in areas adjacent to the central lobular veins. The areas and high of 200 hepatocytes of each liver were measured (Tessaro et al., 2014).

Morphometric evaluations of the gut and hepatic tissue were undertaken by images of random fields at 400x magnification. All morphometric analyses were performed using the Image Pro-plus 4.0® software, according to Ha et al. (2017).

2.7 STATISTICAL ANALYSIS

All the data were subjected to the Shapiro-Wilk test for data and residual normality and to the Bartlett’s test for homogeneity of variance. If the assumptions were not met, the data were subjected to the arcsine square root transformation. The data are presented as mean ± standard error. The results of the two-factorial experiments were
subjected to two-factor analysis of variance (two-way ANOVA). In the bacterial challenge, the data were subjected to one-factor analysis of variance (one-way ANOVA). The difference between means was analyzed using the Tukey’s multiple comparison test. Statistica 10.0® (StatSoft, 2011) was used for all analyses and significance was set at 5%.

3 RESULTS

The feeding of broodstock, and offspring with diets containing purified nucleotides interactively influenced (p<0.05) trypsin activity (Table 3). Juveniles fed with 7.5 g/kg, descendants of broodstock fed with 2.5 g/kg, exhibited the highest (p<0.05) trypsin activity (80.25 ± 10.81 µm/min/mg of protein) in the anterior region of the intestine (Table 3; Figure 1). The activity of other digestive enzymes was not altered (p>0.05) by the diets provided to the broodstock or the offspring (Table 3).

Intestinal tissue morphology was influenced (p<0.05) only by the feeding of the offspring with diets containing purified nucleotides (Table 4). Juveniles fed with 7.5 g/kg showed the highest enterocyte height (50.07 ± 0.24 µm; Table 4; Figure 2).

Other parameters related to intestinal tissue morphology (Table 4) and hepatocyte morphology (Table 4) were not altered (p>0.05) by the feeding of the broodstock or offspring with diets containing purified nucleotides.
Figure 1 - Trypsin enzyme activity in the anterior intestine of Nile tilapia (*Oreochromis niloticus*) juveniles, which were descendants of broodstock fed diets containing purified nucleotides and were subjected to a 100-day feeding regime with diets supplemented with purified nucleotides. Different letters indicate differences between the means, according to the Tukey test (p<0.05).

Source: Research data, prepared by the authors, 2024.

Figure 2 - Enterocyte height in the anterior intestine of Nile tilapia (*Oreochromis niloticus*) juveniles, which were descendants of broodstock fed diets containing purified nucleotides and were fed for 100 days with diets supplemented with purified nucleotides. Different letters indicate differences between the means, according to the Tukey test (p<0.05).

Source: Research data, prepared by the authors, 2024.
Table 3 - Digestive enzyme activity in the anterior intestine of Nile tilapia (*Oreochromis niloticus*) juveniles, which were descendants of breeders fed diets containing purified nucleotides and were subjected to a 100-day feeding regime with diets supplemented with purified nucleotides.

<table>
<thead>
<tr>
<th>Broodstock (g) AS Kg⁻¹</th>
<th>Offspring (g) AS Kg⁻¹</th>
<th>MS¹</th>
<th>SS²</th>
<th>AM³</th>
<th>LP⁴</th>
<th>TS⁵</th>
<th>QS⁶</th>
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<tr>
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<td>2.5</td>
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<table>
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<th>Two-way</th>
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</table>

AS: Ascogen®; ¹ MS: maltase (U/mg of protein; U=nmol of glucose/min/mg of protein); ² SS: sucrase (U/mg of protein; U=nmol of glucose/min/mg of protein); ³ AM: amylase (kU/L); ⁴ LP: lipase (U/L); ⁵ TS: trypsin (µm/min/mg of protein); ⁶ QS: chymotrypsin (µm/min/mg of protein). Different letters indicate differences between the means, according to the Tukey test (p<0.05) Source: Research data, prepared by the authors, 2024.
Table 4 - Intestinal and hepatic morphology of Nile tilapia (Oreochromis niloticus) juveniles, which were descendants of broodstock fed diets containing purified nucleotides and were subjected to a 100-day feeding regime with diets supplemented with purified nucleotides.

<table>
<thead>
<tr>
<th>Broodstock (g) AS Kg⁻¹</th>
<th>Offspring (g) AS Kg⁻¹</th>
<th>HV¹</th>
<th>HE²</th>
<th>TB³</th>
<th>AH⁴</th>
<th>HMİH⁵</th>
<th>HMİH⁶</th>
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<tr>
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Two-way ANOVA

<table>
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P: Ascorgen®; ¹ VH: villus height (µm); ² EH: enterocyte height (µm); ³ BB: basement membrane (µm); ⁴ AH: hepatocyte area (µm²); ⁵ MİM: minimum hepatocyte height (µm); ⁶ MİM: maximum hepatocyte height (µm). Different letters indicate differences between the means, according to the Tukey test (p<0.05) Source: Research data, prepared by the authors, 2024.
4 DISCUSSION

The supplementation of purified nucleotides in the diet of Nile tilapia showed an interactive effect between the feeding of the parents and their offspring. The main positive aspects of this study were the enhancement of trypsin enzyme activity and the height of enterocytes in the offspring. This can improve the digestion and absorption of nutrients in the early stages of the fish, consequently accelerating their development and providing better productivity. Adequate nutrition for broodstock enhances organ vascularization during embryonic development, which is essential for nutrient transport to the embryo through the bloodstream and organ formation (Waterland, Garza, 1999). The morphological characteristics of the fish's digestive system, from the formation of the primitive intestine, undergo variations related to the ontogenetic evolution period (Seixas Filho et al., 2000). In other words, the development of the digestive tract and other organs in fish is closely linked to the early phase of their development, which is directly influenced by the good nutrition inherited from their parents.

The relationship between nutrition and offspring quality occurs when the reserves of the parent fish are mobilized and transported to the gametes (Bhujel, 2000; El-Sayed, 2006). Maternal immunity is also transferred to the offspring through the yolk (Zhang et al., 2013). Reserves are essential for the survival of the offspring until the onset of exogenous feeding (Mazorra et al., 2003). The relationship between parental nutrition and the production of high-quality offspring is crucial for the success of aquaculture production (Bombardelli et al., 2010; Migaud et al., 2013).

In fish, offspring vigor is directly affected by the quality of the parent's diet, especially in species where embryogenesis occurs outside the maternal body, as in Oreochromis niloticus (Nascimento et al., 2023). In such cases, the eggs function as a closed system, with no external nutrient supply, only yolk reserves (Rosa et al., 2007). It is well known that appropriate artificial diets for fish breeders have a significant impact on the quality of their offspring (Navarro et al., 2010). The nutritional requirements of larvae are influenced by the nutritional composition of the yolk and the biochemical composition of their bodies, which depend on yolk composition and are altered by the nutritional status of the parents (Rotta, 2003).
The benefits of dietary supplementation of nucleotides on fish reproduction and positive effects have been reported for female (Gonzalez Vecino, 2005; de Lima et al., 2020) and male fish (Bombardelli et al., 2023). In this case, dietary supplementation ensures the supply of nucleotides to breeders under conditions of high metabolic demand. These demands are unlikely to be met by natural sources present in conventional feeds (Li and Gatlin, 2006; Hess and Greenberg, 2012; Hossain et al., 2019). The need for the addition of nucleotides may vary depending on the species, source, and nucleotide composition (Li and Gatlin, 2006; Li et al., 2007).

The trypsin enzyme showed an increase in its activity compared to the others. Trypsin is likely the key enzyme in the regulation and digestive process in young fish (Moura et al., 2009). This enzyme's function is to digest ingested proteins, which is crucial during the initial phase when protein demands are higher. Its reaction is characterized by the participation of a serine residue in the active center and catalyzes the hydrolysis of peptide bonds (Moraes et al., 2009). The increase in trypsin can be explained by the improvement of enzymatic activities in the intestinal mucosa, which activates trypsinogen. Ran et al. (2016), when testing yeast in the diet of Nile tilapia, observed that 0.1% of inactive yeast significantly increased trypsin enzyme activity compared to the control, while the activities of lipase and amylase did not show significant differences. This was also observed by Zhao et al. (2017), where a yeast source showed an increase in trypsin activity. The nucleotide source used in the formulation contains inactive yeast, which is also a source of nucleotides. Horii (1997) describes that inactive yeast contains about 38 to 50% protein, and trypsin is an enzyme responsible for breaking down proteins into smaller molecules.

Dietary supplementation of purified nucleotides improved the intestinal morphology of juveniles, showing an effect on the height of the enterocytes in offspring that received diets with 7.5 g/kg⁻¹ and un-supplemented parents. This effect may be related to nucleotides being absorbed by the enterocytes after being acted upon by digestive enzymes (Uauy, 1998), serving as an energy substrate for these cells, contributing to the energy supply required for metabolic processes, thus improving nutrient absorption and digestion (Wu et al., 1996), and promoting the development of these cells. McCauley et
al. (1998) state that nucleotide supplementation is important for enterocytes during development, maturation, and intestinal repair. According to Sato et al. (1999), nucleotides increase the proliferation and maturation of enterocytes.

Dietary nucleotide supplementation in fish acts on the function of the gastrointestinal tract (Xu et al., 2015), increasing the surface area of the intestine (Burrells et al., 2001), the height of enterocytes, and microvilli (Peng et al., 2013). Besides nucleotides serving as an energy substrate for enterocytes, it can also be hypothesized that in the early development phase, tissues with high cell proliferation, such as the intestine, depend on a greater demand for nucleotides (Cheng et al., 2011). Therefore, with supplementation, nucleotides are available to the cells that depend on this demand, resulting in their absorption in the enterocytes.

Nucleotides are also important in tissues that require rapid renewal, such as the intestinal mucosa (Guo et al., 2016). They are commonly associated with intestinal recovery after any type of injury or physiological modification (Domeneghini et al., 2004; Wu et al., 2018). Dietary nucleotides and their metabolite/intermediary products also have a significant positive effect on the development and growth of the intestines in Nile tilapia (Xu et al., 2015; Tie et al., 2021), as well as intestinal microvilli and enterocytes of Pagrus major (Hossain et al., 2016). The benefits of dietary nucleotides in intestinal development are important because they increase the activity of digestive enzymes, enhance nutrient utilization, and ensure better intestinal digestion (Hunt et al., 2014).

Dietary nucleotide supplementation promotes intestinal morphological changes in young fish during growth (Burrells et al., 2001; Borda et al., 2003; Cheng et al., 2011). However, these changes are not observed in breeders (De Lima et al., 2020) because they are not in a phase of intense development of the intestinal mucosa (Graciano, 2012).

The diets provided to Nile tilapia juveniles did not interfere with the hepatic morphology of the animals in any of the treatments. The liver plays an essential role in controlling many vital functions in the body. In fish, it is an indicator of the animal's nutritional status, and changes in hepatic tissue can be considered influenced by the diet (Caballero et al., 1999). If the diet is inadequate, histological changes in the liver are easily visible (Tacon, 1992). Nucleotide synthesis occurs in the cytoplasm of hepatic cells.
since the enzymes for purine and pyrimidine synthesis are available in this organ. Nucleotides enter via the hepatic portal vein and are transported to the hepatocytes (Rossi et al., 2007). Carver and Walker (1995) report that when nucleotides are restricted in the body, it can result in the accumulation of lipids in the hepatocyte. The lack of morphological changes in hepatic tissue observed in this study demonstrates that the animals were likely in favorable rearing conditions.

Dietary nucleotide supplementation is also essential for improving fish growth (Shiau et al., 2015; Hossain et al., 2016; Guo et al., 2017; Tie et al., 2021). In intensive tilapia farming, exogenous supplementation is essential to meet nutritional requirements and assist during the reproductive period (Mewes et al., 2016). In breeders, the supply of nucleotides improves the physiological status of high-production tilapia under conditions of high metabolic demand and challenge (El-Noorashy et al., 2021; Bombardelli et al., 2023). These demands are unlikely to be met by natural sources present in conventional feeds (Li and Gatlin, 2006; Hess and Greenberg, 2012; Hossain et al., 2020). Furthermore, feeding tilapia breeders with diets containing purified nucleotides does not have deleterious effects on fish health, maintaining adequate hematological parameters (De Lima et al., 2020; Bombardelli et al., 2023).

The results obtained in this study indicate that feeding both the parents and offspring of Nile tilapia with diets supplemented with purified nucleotides allows the transfer of nutritional factors via the yolk, which improves the quality of the offspring by stimulating the digestive enzyme activity of juveniles. Furthermore, the improvement in gastric activity may also stimulate greater intestinal tissue development, reflecting better nutrient absorption, and overall health and vigor in juveniles.

Despite these results, the mechanisms that regulate the transfer of nutritional factors from females to their offspring via the yolk are not yet fully understood. This has stimulated the development of new research focused on fish breeder nutrition and its influence on offspring quality and vigor. Results such as those presented in this article also contribute to a new perspective on experimentation, as maternal nutrition effects not only the offspring's development in the first days of life (Bombardelli et al., 2009; Sousa
et al., 2013) but also result in physiological changes after extended breeding periods, extending up to 105 days of life.

5 CONCLUSION

The inclusion of nucleotides in the diet of both parents and offspring resulted in benefits for juveniles by increasing trypsin activity in the intestines of 105-day-old juveniles. Thus, feeding parents with diets containing 2.5 g/kg and offspring with 7.5 g/kg is recommended as it positively affects gastric activity and nutrient absorption.
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