

**EVALUATION OF THE NUTRITIONAL POTENTIAL OF THE  
BY-PRODUCT OF OIL EXTRACTION IN FEED FOR NILE TILAPIA<sup>1</sup>*****AVALIAÇÃO DO POTENCIAL NUTRICIONAL DO SUBPRODUTO DA EXTRAÇÃO  
OLEÍCOLA EM RAÇÕES PARA TILÁPIA-DO-NILO***

**Carolina Rapachi Fortes<sup>2</sup>, Aline Rossato<sup>3</sup>, Larissa da Silva Silveira<sup>3</sup>,  
Matheus Dellaméa Baldissera<sup>3</sup>, Roger Wagner<sup>4</sup>, Carla Cristina Zeppenfeld<sup>5</sup>,  
Bernardo Baldisserotto<sup>4</sup>, Isabela Maraschin Vieira<sup>6</sup>, Marcelo Leite da Veiga<sup>5</sup>,  
Luiz Fernando Rodrigues Jr<sup>3</sup>, Michele Rorato Sagrillo<sup>3</sup> e Liana da Silva Fernandes<sup>3</sup>**

**ABSTRACT**

The growing production of olive oil in Brazil has resulted in a significant increase in agro-industrial waste, such as olive pomace, whose improper disposal can cause numerous environmental impacts. This study evaluated the feasibility of using olive pomace as a functional ingredient in feed for Nile tilapia (*Oreochromis niloticus*), aiming to utilize this waste, its nutritional potential, and promote sustainable practices. Feeds containing different percentages of olive pomace were formulated, namely 0% (control), 50%, and 100%. The tilapia used in this experiment had an initial weight of 1 to 5 g and was fed for 45 days. Growth, muscle fatty acid profile, and intestinal histological changes were evaluated. Feeds containing olive pomace had a bromatological composition similar to the control feed. The feeds containing olive pomace had a bromatological composition similar to the control feed. The groups treated with 50% and 100% pomace showed significant weight and length gain, with no intestinal histopathological changes. In addition, an increase in polyunsaturated fatty acids (PUFAs) and a reduction in saturated fatty acids were observed in the fish in the 100% group, indicating an improvement in the nutritional value of the meat. The results demonstrate that olive pomace can be a viable alternative in fish feed, contributing to zootechnical performance, final product quality, and environmental sustainability through waste recovery.

**Keywords:** animal nutrition; agroindustrial waste; fatty acids; olive pomace; sustainability.

---

1 Part of the doctoral thesis presented to the Graduate Program in Nanosciences, Universidade Franciscana - UFN, Santa Maria, RS, Brazil.

2 Universidade Franciscana - UFN, Santa Maria, RS, Brazil. Email: carolinarapachi@ufn.edu.br. ORCID: <https://orcid.org/0009-0001-2356-025X>.

3 Graduate Program in Nanosciences, Universidade Franciscana - UFN, Santa Maria, RS, Brazil. Emails: aline.rossato@ufn.edu.br. ORCID: <https://orcid.org/0000-0001-6464-6255>; larissa.silveira@ufn.edu.br. ORCID: <https://orcid.org/0000-0003-4460-9857>; matheus.dellamea@ufn.edu.br. ORCID: <https://orcid.org/0000-0002-3280-8528>; luiz.fernando@ufn.edu.br. ORCID: <https://orcid.org/0000-0002-5753-5503>; sagrillomr@ufn.edu.br. ORCID: <https://orcid.org/0000-0001-5659-159X>; liana@ufn.edu.br. ORCID: <https://orcid.org/0000-0003-4745-7617>.

4 Department of Food Technology and Science - Universidade Federal de Santa Maria, RS, Brazil. Email: rogerwag@gmail.com. ORCID: <https://orcid.org/0000-0002-6176-7913>.

5 Fish Physiology Laboratory - LAFIPE, Universidade Federal de Santa Maria, RS, Brazil. Emails: carlazeppenfeld@hotmail.com. ORCID: <https://orcid.org/0000-0002-6399-1935>; bbaldisserotto@gmail.com. ORCID: <https://orcid.org/0000-0002-8770-0100>.

6 Experimental morphophysiology laboratory - LABITEX, Universidade Federal de Santa Maria, RS, Brazil. Emails: belamaraschinvieira@gmail.com. ORCID: <https://orcid.org/0000-0001-9098-2658>; marceloveiga@gmail.com. ORCID: <https://orcid.org/0000-0002-9303-3324>.

## RESUMO

*A crescente produção de azeite de oliva no Brasil tem resultado em um aumento significativo de resíduos agroindustriais, como o bagaço de oliva, cuja destinação inadequada pode causar inúmeros impactos ambientais. Este estudo avaliou a viabilidade do uso do bagaço de oliva como ingrediente funcional em rações para Tilápia-do-Nilo (*Oreochromis niloticus*), visando o aproveitamento deste resíduo, seu potencial nutricional, além de ser uma prática sustentável. Foram formuladas rações contendo diferentes percentuais do bagaço de oliveira, sendo 0% (controle), 50% e 100%. Tilápias utilizadas neste experimento, tinham peso inicial de 1 a 5 g, as quais foram alimentadas durante 45 dias. Avaliou-se o crescimento, o perfil de ácidos graxos no músculo e alterações histológicas intestinais. As rações contendo bagaço de oliva apresentaram composição bromatológica semelhante à ração controle. Os grupos tratados com 50% e 100% de bagaço apresentaram ganho de peso e comprimento significativo, sem alterações histopatológicas intestinais. Além disso, foi observado aumento de ácidos graxos poli-insaturados (PUFAs) e redução de saturados nos peixes do grupo 100%, indicando uma melhora no valor nutricional da carne. Os resultados demonstram que o bagaço de oliva pode ser uma alternativa viável na alimentação de peixes, contribuindo para o desempenho zootécnico, a qualidade do produto final e a sustentabilidade ambiental por meio da valorização de resíduos.*

**Palavras-chave:** *nutrição animal; resíduos agroindustriais; ácidos graxos; bagaço de azeitona; sustentabilidade.*

## 1 INTRODUCTION

Aquaculture plays a fundamental role in global food security, representing one of the fastest-growing sectors in animal protein production. Among farmed species, tilapia (*Oreochromis niloticus*) stands out due to its rapid growth, adaptability to different production systems, and high consumer acceptance, particularly in developing countries. In 2023, global tilapia production was estimated at 6.7 million tons (Global Seafood Alliance, 2024). In Brazil, fish farming reached 887.000 tons in 2023, of which 579.000 tons corresponded to tilapia, increasing to 662.200 tons in 2024 and representing approximately 68% of national aquaculture production (Epagri, 2024). In the same period, Brazil exported 13.792 tons of tilapia, with 94% destined for the United States, consolidating its position as the world's second largest supplier of fresh fillets of this species (Embrapa, 2025).

Despite the significant growth of tilapia farming, production costs remain a major limiting factor for the expansion and profitability of the sector. In this context, feed represents the highest operational cost, accounting for 65% to 84% of total production expenses, depending on the region (Epagri, 2024; CNA, 2024). Consequently, the increase in feed prices has stimulated the search for alternative ingredients that are economically viable and environmentally sustainable. Agro-industrial by-products have emerged as promising alternatives, as they can partially replace conventional ingredients while reducing production costs and minimizing environmental impacts.

In recent years, several studies have evaluated the inclusion of plant-based by-products in aquaculture feeds, demonstrating their potential to maintain or even improve growth performance, feed efficiency, and nutrient utilization in fish (Gatlin *et al.*, 2007; Hardy, 2010; Glencross *et al.*, 2020). More recently, a systematic review highlighted the increasing use of non-conventional ingredients in

tilapia diets, emphasizing their relevance in sustainable aquaculture systems (Tacon; Metian, 2015; Furuya *et al.*, 2023). In addition to zootechnical performance, such ingredients may also influence product quality, including fillet composition and oxidative stability.

Within this context, olive pomace (*Olea europaea* L.) has gained attention as a potential feed ingredient. Olive cultivation in Brazil has expanded considerably in recent years, particularly in the southern and southeastern regions. Rio Grande do Sul, the country's largest producer of olive oil and table olives, recorded a production of 448.500 liters of extra virgin olive oil in the 2022/2023 harvest, representing a 122% increase compared to the previous year (IBRAOLIVA, 2023). However, the expansion of olive oil production is directly associated with the generation of large volumes of agro-industrial waste. Among these, olive pomace stands out due to its high production and potential environmental impact. The most widely used extraction method in Brazil is the continuous two-phase system, which, although considered more environmentally friendly than traditional methods, still generates significant quantities of residue. Approximately 80 kg of olive pomace are produced for every 100 kg of processed olives (Lozano-Sánchez *et al.*, 2017).

This residue is characterized by high moisture content and is composed of olive pulp, fragments of pits, and vegetation water. Due to its high organic load and biochemical oxygen demand, its improper disposal can lead to environmental problems such as soil degradation, water contamination, and phytotoxicity, mainly associated with its high content of phenolic compounds, lipids, and organic acids (Dermeche *et al.*, 2013). Therefore, strategies aimed at the reuse of this by-product are essential to mitigate its environmental impact.

Despite being considered a waste product, olive pomace contains nutritionally valuable components, including residual oil rich in unsaturated fatty acids, dietary fiber (cellulose, hemicellulose, and pectins), proteins, minerals, and phenolic compounds with antioxidant properties (Monteiro; Speroni; Emanuelli, 2021). These characteristics make it a promising candidate for use in animal nutrition, particularly in aquaculture.

Recent studies have demonstrated that tilapia can consume diets containing olive pomace, indicating its feasibility as a feed ingredient, although its effects may vary depending on inclusion levels and processing methods (Krasue *et al.*, 2020). In addition, the incorporation of olive by-products in fish diets has been associated with improvements in oxidative status and modulation of inflammatory responses, suggesting potential functional benefits (Petsini *et al.*, 2022). Furthermore, studies in other animal species indicate that olive pomace may influence productive performance and physiological responses due to its fiber and phenolic content (Braga *et al.*, 2023).

Therefore, although olive pomace presents significant potential as a sustainable ingredient in aquafeeds, further studies are needed to better understand its effects on nutrient digestibility, growth performance, and product quality in tilapia. In this context, the aim of this study was to formulate a diet containing olive pomace and to evaluate its nutritional potential for use in fish feed.

## 2 MATERIALS AND METHODS

### 2.1 OBTAINING AND PREPARING OLIVE POMACE

The by-product resulting from olive oil extraction was collected at a company located in Caçapava do Sul, Rio Grande do Sul. This material comes from a mixture of different olive cultivars. Initially, it was pressed to remove residual oil and then dried in an oven at 40 °C to be used in feed production (Figure 1).

**Figure 1** - Dry by-product of olive oil extraction.



Source: Author's construction.

### 2.2 EXPERIMENTAL DIET

The diets were prepared in the Fish Physiology Laboratory (LAFIPE, Universidade Federal de Santa Maria), according to the protocol described by Zeppenfeld *et al.* (2017). Initially, the majority ingredients (protein sources, wheat bran, and corn) were mixed, and after complete homogenization, the ingredients at less proportions (vitamins, minerals, and amino acids) were added. A premix of vegetable oil with olive pomace was incorporated into the main ingredients to create the experimental diets, which was moistened (water at room temperature), pelletized, and placed in a forced-air oven for 24 hours at 40 °C, as shown in Figure 2. The rations were prepared according to nutritional requirements and calculated to meet the isoenergetic and isonitrogenous nutritional needs of the species studied (Table 1). Soybean meal was replaced by olive oil extraction by-product at a proportion of 50% (treatment S 50) and 100% (treatment S 100).

**Table 1** - Formulation of basal diet.

| Ingredient                              | g/kg |
|---|------|
| Meat and bone meal                      | 350  |
| Soybean meal                            | 300  |
| Corn                                    | 150  |
| Rice bran                               | 120  |
| Canola oil                              | 30   |
| Vitamin and mineral premix <sup>1</sup> | 30   |
| Common salt                             | 10   |
| Dicalcium phosphate                     | 10   |

<sup>1</sup> Vitamin and mineral mixture (per kilogram of product): 200 mg folic acid, 5,000 mg pantothenic acid, 0.60 g antioxidant, 125 mg biotin, 25 mg cobalt, 2,000 mg copper, 820 mg iron, 100 mg iodine, 3,750 mg manganese, 5,000 mg niacin, 75 mg selenium, 1,000,000 UI vitamin A, 1,250 mg vitamin B1, 2,500 mg vitamin B2, 2,485 mg vitamin B6, 3,750 mg vitamin B12, 28,000 mg vitamin C, 500,000 UI vitamin D3, 20,000 UI vitamin E, 500 mg vitamin K and 17,500 mg zinc.

Analyses of the nutritional composition of these rations were performed. The moisture content was determined in an oven at 105 °C for 24 hours. The total nitrogen content was determined using the method approved by American Association of Cereal Chemists (AACC) No. 46-13 (1995), and the crude protein content was obtained by using a factor of 6.25 to convert nitrogen to protein. The ash content was determined according to AACC (1995) method No. 08-01, using a temperature of 550 °C until constant weight. The lipid content was determined according to Association of Official Analytical Chemists (AOAC) (1995) in a Soxhlet extractor, using petroleum ether as a solvent. The dietary fiber content was determined according to enzymatic-gravimetric methods No. 985.29 and No. 991.42 (McCleary *et al.*, 2023).

**Figure 2** - Pelleted feed containing olive oil extraction by-products.

Source: Author's construction.

## 2.3 *IN VIVO* MODEL EXPERIMENT: FISH

### 2.3.1 Animals

Fingerlings of Nile tilapia (1 to 5 g) were purchased from local suppliers and kept for acclimatization for one week before the start of the experiments in a closed recirculation system containing biological filters at the LAFIPE). Water quality parameters, such as temperature ( $27.5 \pm 0.2$  °C), dissolved oxygen ( $6.7 \pm 0.3$  mg/L), pH ( $6.5 \pm 0.3$ ), total ammonia ( $0.80 \pm 0.06$  mg/L) and non-ionized ammonia ( $0.0041 \pm 0.00022$  mg/L) were evaluated daily, once a day, remaining appropriate for Nile tilapia physiology.

### 2.3.2 Ethical statement

The experimental protocols involving animals were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Santa Maria, UFSM, Santa Maria, RS, Brazil (Protocol 9790120623) and complied with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA).

### 2.3.3 Experiment

After acclimatization, fifty-four Nile tilapia (1-5 g) were used to evaluate feed containing olive oil extraction by-products added to the feed. The experiment lasted 45 days. At the beginning of the experiment (day 0), the weight and length of the tilapia were measured, and they were divided into three groups, in triplicate each group containing 18 fish per group, six fish per box, as listed below:

- **Control Group (C):** received feed without the addition of olive oil extraction by-product;
- **Group S 50:** received 50% olive oil extraction by-product in the feed;
- **Group S 100:** received 100% olive oil extraction by-product in the feed.

The animals were placed in a recirculation system in 60 L plastic boxes, with 6 fish per box, in triplicate each box. All groups received experimental feed once a day (10% total biomass) at 2 p.m. for 45 consecutive days. To remove excess feces and feed residues, the tanks were cleaned by siphoning 2 h after feeding. At the end of the experiment (day 45), final weight was measured to calculate weight gain (WG) -  $WG \text{ (g/day)} = (\text{final mean fish weight (g)} - \text{initial mean fish weight (g)}) / \text{duration of experiment (days)}$ . After, fish were anesthetized (eugenol; 150 mg/L) and muscle tissue was collected for fatty acid analysis. Two animals per box (totalizing 6 fish per group) were used to quantify fatty acids in fish muscles.

### 2.3.4 Quantification of fatty acids in fish muscle

Lipid extraction was performed according to the method of Bligh & Dyer (1959) with modifications. 0.5 g of fish muscle samples were placed in a mixture of methanol:chloroform. Subsequently, fatty acid methyl esters (FAME) were obtained by acid catalysis, as described by Hartman and Lago (1973). The samples were injected (1  $\mu\text{L}$ ) in split mode (20:1) into a Varian 3400CX gas chromatograph (USA). The chromatograph was equipped with a flame ionization detector (GC-FID) and the GC column was an HP-88 (Agilent Technologies, USA) (100 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ). The column temperature was maintained at 50  $^{\circ}\text{C}$  for 0.5 min, after which the temperature was increased to 185  $^{\circ}\text{C}$  at a rate of 10  $^{\circ}\text{C min}^{-1}$ , followed by an increase of 0.5  $^{\circ}\text{C min}^{-1}$  to 190  $^{\circ}\text{C}$  and increased by 15  $^{\circ}\text{C min}^{-1}$  to 230  $^{\circ}\text{C}$ , where it was maintained for 5 min. The injector and detector temperature was 250  $^{\circ}\text{C}$ . The FAMEs were identified by comparison with the standard certificate FAME Mix 37 (Supelco, USA) (Hartman and Lago, 1973). The results were expressed as a percentage of the total area of chromatograms, considering the correction factors of the flame ionization detector and ester-to-acid conversion.

### 2.3.5 Histopathological analysis

Intestinal samples were collected and fixed in 10 % buffered formalin solution. After fixation, the samples underwent routine histological processing. Initially, dehydration was performed in ascending series of alcohol (70%, 80%, 90%, 95%, and 100%), followed by clearing in xylene, impregnation, and embedding in histological paraffin. Paraffin blocks were sectioned on a rotary microtome, obtaining sections approximately 5 to 7  $\mu\text{m}$  thick. For morphological analysis, slides were stained with hematoxylin/eosin (HE). After staining, the slides were rehydrated in ascending series of alcohol, cleared in xylene, and mounted with permanent mounting medium and coverslip Dunn *et al.* (2024). All slides were examined by two histopathologists in a double-blind manner to detect microarchitecture and changes using an optical microscope, as well as intestinal villi height.

## 2.4 STATISTICAL ANALYSIS

The data met the assumption of parametric testing according to the Kolmogorov-Smirnov (normality) and Levene (homoscedasticity) tests, except for some data linked to fish performance computations. These data were log transformed to obtain normally distributed data. Statistical analysis was performed using bilateral one-way analysis of variance (ANOVA) followed by the Tukey post hoc analysis. Significance was set at  $p < 0.05$ , and data were expressed as mean  $\pm$  standard deviation.

### 3 RESULTS AND DISCUSSION

#### 3.1 BROMATOLOGICAL COMPOSITION OF FEED

Bromatological analyses were performed on feed formulated with 50% (S50) and 100% (S100) concentrations of olive oil extraction by-products before administration to fish (Table 1).

The nutritional profiles of the formulated diets (Table 2) show that the inclusion of olive pomace resulted in slight variations compared to the control diet.

**Table 2** - Bromatological analysis of feed containing olive oil extraction by-products.

| GROUPS  | DM (%) | % in DM |       |      |       |      |
|---------|--------|---------|-------|------|-------|------|
|         |        | MM      | CP    | EE   | NDF   | ADF  |
| Control | 93.96  | 25.53   | 30.32 | 7.49 | 25.12 | 1.78 |
| S 50    | 92.70  | 20.01   | 31.28 | 7.51 | 26.01 | 1.08 |
| S 100   | 93.43  | 19.98   | 30.05 | 6.93 | 25.13 | 1.18 |

Caption: DM: dry matter; MM: mineral matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber.

Source: Author's construction.

A study conducted by Amaral *et al.* (2022) evaluated the composition of olive pomace and reported a composition of 6.75% protein, 14.95% total lipids, 3.49% ash, 7.30% moisture, and 63.39% dietary fiber.

Meanwhile, the study conducted by Uribe *et al.* (2015) reported ash values ranging from 5.92% to 6.73%, lipid content between 4.59% and 9.11%, protein from 6.24% to 8.62%, and crude fiber between 29.1% and 36.37%. Similar results were reported by other authors, indicating that olive pomace typically presents high fiber content, moderate lipid levels, and relatively low protein values (Nefzaoui, 1991; Molina-Alcaide; Yáñez-Ruiz, 2008).

More recently, studies have demonstrated that fermentation processes can improve the nutritional value of olive by-products by increasing lipid availability, enhancing fatty acid profiles such as oleic acid, and improving amino acid composition without significant protein losses (Molina-Alcaide; Yáñez-Ruiz, 2008; Reboredo-Rodríguez *et al.*, 2015).

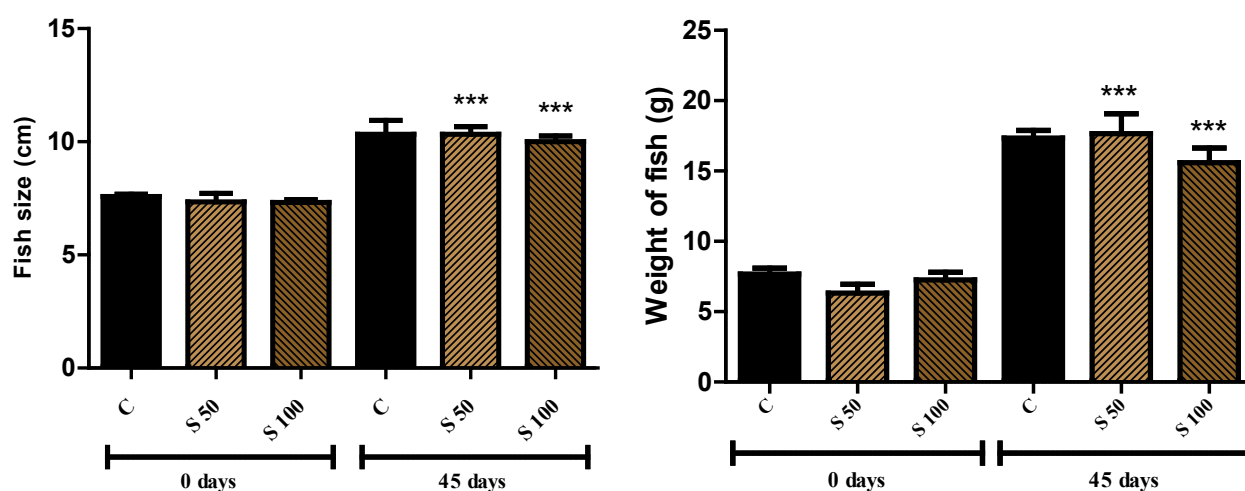
In turn, Hazreen-Nita (2022) points out that bagasse, although low in protein, is rich in bioactive compounds such as polyphenols and lipids, and can replace up to 20-25% of traditional ingredients without adverse effects on growth or feed efficiency in tilapia.

### 3.2 ANIMAL SIZE AND WEIGHT

As shown in Figure 3, no significant differences were observed in initial weight and length (day 0) between the experimental groups. After 45 days, the treatments with olive pomace inclusion (S 50 and S 100) showed statistically significant differences in body length compared to the control. However, these differences were of small magnitude.

For weight, the S 50 group showed higher values than the control, while the S 100 group did not show the same pattern, presenting similar or lower values. These results suggest that the inclusion of olive pomace can influence zootechnical performance, but in a way that depends on the inclusion level, indicating that moderate concentrations may be more efficient than higher levels.

**Figure 3** - Analysis of fish size and weight on the first day and after 45 days of treatment.



Data were expressed as mean  $\pm$  standard deviation (SD). Analyses were performed using one-way ANOVA followed by Tukey's post hoc test. Values with  $p < 0.05$  were considered statistically significant. Where \*\*\*  $p < 0.001$ .

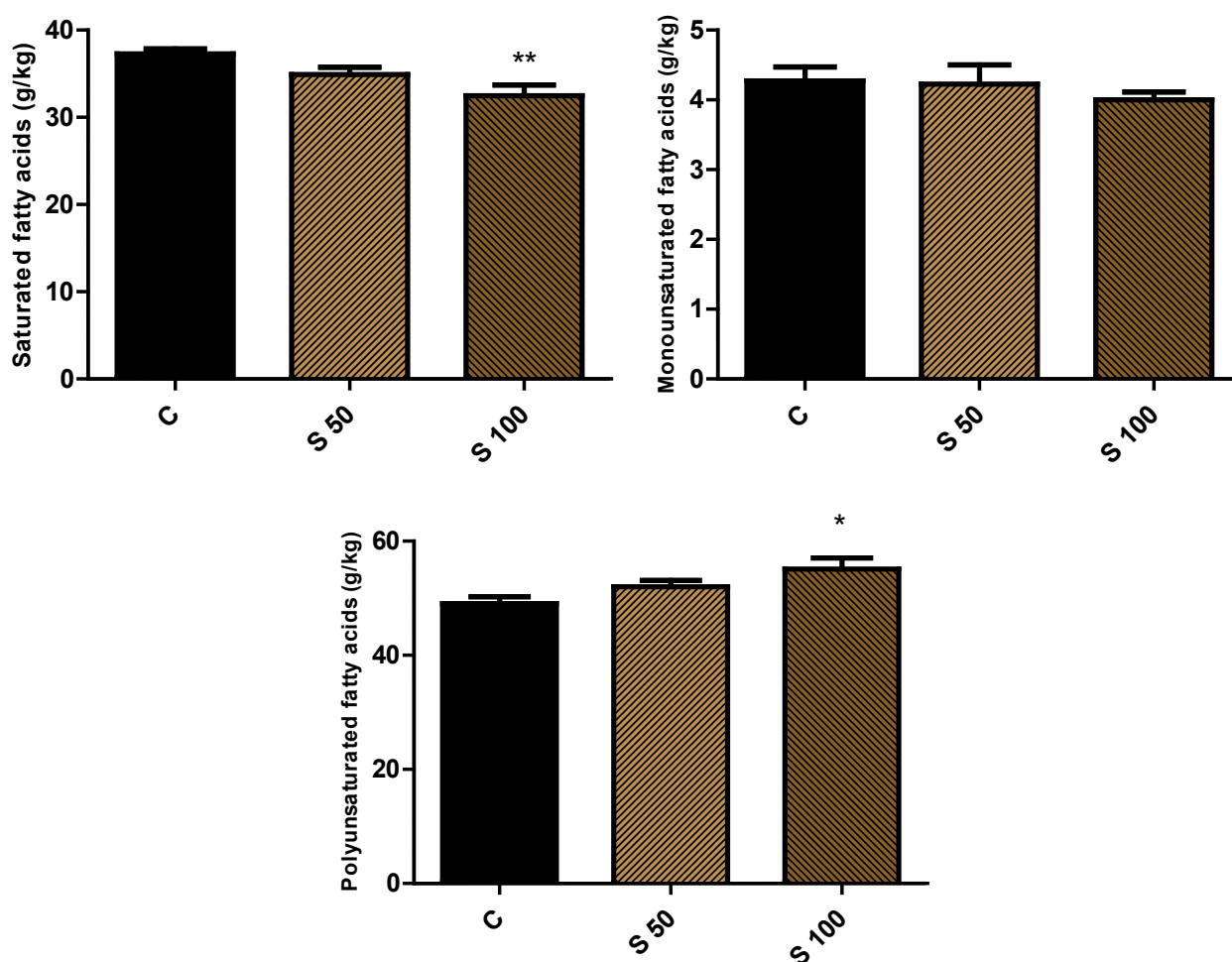
This result is consistent with recent studies that have demonstrated the potential of agro-industrial by-products in feeding tilapia and other fish. Krasue *et al.* (2020), for example, observed the acceptability of olive pomace in diets of *O. mossambicus*, suggesting the feasibility of its use without compromising consumption. Similarly, Hazreen-Nita (2022) highlighted that olive by-products can improve not only growth but also intestinal health, immune response, and antioxidant capacity.

More comprehensive reviews (Zuluaga-Hernández *et al.*, 2023; Lourenço *et al.*, 2025) reinforce those unconventional ingredients, such as fruit, vegetable, and olive oil industry waste, can be incorporated into aquaculture diets with positive effects on weight gain, feed conversion, and production sustainability. These studies highlight that, in addition to reducing feed costs, the main component of tilapia farming operating costs, such ingredients contribute to the sustainable use of agro-industrial waste.

### 3.3 QUANTIFICATION OF FATTY ACIDS IN MUSCLE

The results (Figure 4) show that fish fed the S 100 diet had a significantly lower concentration of saturated fatty acids and a significantly higher concentration of polyunsaturated fatty acids compared to the control group. Pentadecanoic acid (C15:0), eicosatrienoic acid (C20:3n3) and tetracosanoic acid (C24:0) were significantly higher in fish feed with a diet S 50 compared to control group, while alpha-linolenic acid (C18:3n3) was significantly lower. On the other hand, heneicosanoic acid (C21:0) was significantly lower in fish feed with a diet of S 100 compared to control group (Table 3).

**Figure 4** - Quantification of saturated, monounsaturated, and polyunsaturated fatty acids after 45 days of treatment in fish.



Data were expressed as mean  $\pm$  standard deviation (SD). Analyses were performed using one-way ANOVA followed by Tukey's post hoc test. Values with  $p < 0.05$  were considered statistically significant. Where \*  $p < 0,05$

The fatty acid profile in fish fillets is mainly determined by their diet. Fish oil, often used in commercial feed, is expensive and can be partially or totally replaced by vegetable oils, which apparently do not negatively affect the growth of aquatic animals (Alhazzaa *et al.*, 2013). This is because, in addition to meeting the basic nutritional needs of fish, fatty acids in the diet, when provided at adequate levels, promote good fish development and efficient feed conversion in aquaculture, resulting in high levels of essential fatty acids in the meat (Al-Souti *et al.*, 2012). For fish health, augmentation on polyunsaturated fatty acids has been associated with improvement in fish performance. In this sense, Santigosa *et al.* (2024) reported that supplementation with algae oil, a plant source of polyunsaturated fatty acids, improved the body composition and lipid profile of fish, including sturgeons. These studies reinforce the importance of including omega-3 fatty acids in fish feed to optimize zootechnical performance and the quality of the final product, as observed in our present study.

Beyond positive effects for fish growth, the fatty acid composition of fish muscle/meat is an important trait for consumers because some fatty acids (particularly monounsaturated fatty acids and polyunsaturated fatty acids) promote health (Mititelu *et al.*, 2025). In the present study, dietary supplementation with olive pomace (S 100) reduced the total content of saturated fatty acids, which can be considered a positive effect, because saturated fatty acids are considered to be unhealthy for the cardiovascular system, secondary to their effects on cholesterol metabolism and higher LDL cholesterol levels, both of which are risk factors for coronary heart disease (CHD) (Mensink, 2016). The augmentation in polyunsaturated fatty acids should be considered desirable to human health, because recent studies have shown that deficiency in this type of fatty acid is associated with increased vulnerability to cardiovascular and neurological diseases, including myocardial infarction and stroke (Peng *et al.*, 2024).

**Table 3** - Quantification of fatty acids found in the muscles of Nile tilapia fed diets containing olive oil extraction by-products at concentrations of 50 and 100%.

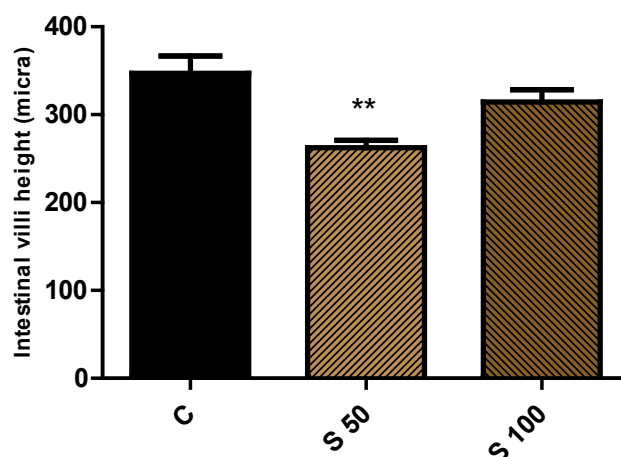
| Fatty Acids                   | Groups      |                 |              |
|-------------------------------|-------------|-----------------|--------------|
|                               | C           | S 50            | S 100        |
| <b>C11:0</b>                  | 0.344±0.111 | 0.496±0.077     | 0.388±0.054  |
| <b>C13:0</b>                  | 0.265±0.093 | 0.455±0.088     | 0.324±0.054  |
| <b>C14:0</b>                  | 0.461±0.144 | 0.338±0.144     | 0.447± 0.321 |
| <b>C15:0</b>                  | 0.421±0.134 | 0.743±0.096 *** | 0.588±0.113  |
| <b>C16:0</b>                  | 21.52±1.205 | 19.82±1.415     | 19.08±1.588  |
| <b>C17:0</b>                  | 0.908±0.077 | 1.22±0.257      | 1.07±0.219   |
| <b>C18:0</b>                  | 11.98±1.453 | 10.51±1.130     | 9.567±1.907  |
| <b>C20:0</b>                  | 0.398±0.044 | 0.326±0.034     | 0.32±0.056   |
| <b>C21:0</b>                  | 0.083±0.016 | 0.053±0.008     | 0.04±0.012 * |
| <b>C22:0</b>                  | 0.415±0.045 | 0.343±0.047     | 0.373±0.053  |
| <b>C23:0</b>                  | 0.386±0.120 | 0.588±0.097     | 0.353±0.088  |
| <b>C14:1</b>                  | 0.54±0.082  | 0.733±0.094     | 0.591±0.169  |
| <b>C16:1</b>                  | 2.783±0.318 | 2.335±0.503     | 2.378±0.284  |
| <b>C17:1</b>                  | 0.39±0.121  | 0.416±0.094     | 0.266±0.053  |
| <b>C22:1</b>                  | 0.225±0.015 | 0.203±0.059     | 0.211±0.046  |
| <b>C24:1</b>                  | 0.505±0.216 | 0.538±0.144     | 0.525±0.137  |
| <b>C18:1n9cis</b>             | 18.85±3.512 | 20.07±4.010     | 23.29±4.191  |
| <b>C18:1n9trans</b>           | 0.62±0.175  | 0.858±0.211     | 0.788±0.270  |
| <b><u>C18:2n6cis</u></b>      | 13.33±2.316 | 10.93±1.087     | 11.54±1.298  |
| <b><u>C18:3n3</u></b>         | 2.105±0.323 | 1.555±0.309 *   | 1.958±0.347  |
| <b><u>C18:3n6</u></b>         | 0.461±0.168 | 0.335±0.058     | 0.383±0.099  |
| <b><u>C20:3n3</u></b>         | 0.075±0.019 | 0.214±0.089 *** | 0.085±0.025  |
| <b><u>C20:3n6</u></b>         | 1.092±0.106 | 1.015±0.123     | 1.015±0.271  |
| <b><u>C20:4</u></b>           | 4.332±1.016 | 8.085±1.952 *** | 6.236±2.137  |
| <b><u>C22:5n3</u></b>         | 1.19±0.334  | 1.29±0.359      | 1.43±0.416   |
| <b><u>C22:6n3</u></b>         | 3.953±1.589 | 5.315±1.434     | 4.637±1.583  |
| <b><u>C24:0 + C20:5n3</u></b> | 0.93±0.188  | 0.68±0.197      | 0.768±0.095  |

The analyses were performed using two-way ANOVA followed by Tukey's post hoc test. Values with  $p < 0.05$  were considered statistically significant. Where \*  $p < 0.05$  and \*\*\*  $p < 0.001$ . Fatty acids only bold marked = saturated fatty acids; Fatty acids bold and italic marked = monounsaturated fatty acids; Fatty acids bold and underlined marked = polyunsaturated fatty acids.

### 3.4 HISTOPATHOLOGICAL FINDINGS

Histopathological analysis revealed a reduction in the height of intestinal microvilli in Nile tilapia fed a diet containing 50% olive pomace compared to the control group. On the other hand, tilapia that received the diet with 100% pomace had microvilli with a height similar to those of the control group, showing no significant morphological changes, as shown in Figure 5.

Figure 5 - Histological findings of intestinal villi height in treated fish.



Data were expressed as mean  $\pm$  standard deviation (SD). Analyses were performed using one-way ANOVA followed by Tukey's post hoc test. Values with  $p < 0.05$  were considered statistically significant. Where \*\*  $p < 0.01$ .

Histopathological analysis revealed a decrease in the height of intestinal microvilli in Nile tilapia fed a diet containing 50% olive pomace (S 50), compared to the control group. This finding may be related to the presence of phenolic compounds, insoluble fibers, or other antinutrients present in the pomace, which, at certain concentrations, can interfere with the integrity of the intestinal mucosa or nutrient absorption, as reported by Ullah *et al.* (2022) and Zhou *et al.* (2018) in studies with other fibrous ingredients.

On the other hand, fish fed the diet containing 100% olive pomace had microvilli with a height similar to that of the control group, suggesting a possible adaptation of the intestinal epithelium to the higher proportion of fiber or, alternatively, a potential protective effect of the bioactive compounds in the residue, such as polyphenols, which have moderate antioxidant and anti-inflammatory action (Reverter *et al.*, 2017). Recent findings indicate that olive-derived bioactive compounds, including oleuropein, hydroxytyrosol, and tyrosol, interact with the gut microbiota and can promote beneficial shifts in microbial composition, increasing short-chain fatty acid (SCFA) production and reinforcing intestinal barrier integrity (Garrido-Romero *et al.*, 2025). Moreover, human and animal studies reviewed by Garrido-Romero *et al.* (2025) also show that polyphenols from olive residues not only inhibit the proliferation of potentially pathogenic bacteria but also support beneficial genera such as *Lactobacillus* and *Bifidobacterium*, contributing to epithelial health and systemic immune balance. Thus, we hypothesize that polyphenols present in the olive pomace can be responsible for adaptation of the intestinal epithelium.

Therefore, the results of the present study suggest nonlinear behavior, in which intermediate concentrations of pomace (S 50) can impair intestinal structure, while higher levels (S 100) favor epithelial adaptation, possibly mediated by the antioxidant and immunomodulatory action of phenolic

compounds. However, biological variation, sample size limitations, and diet formulation effects can influence this result.

#### 4 CONCLUSION

Evaluating the potential for reuse of agroindustrial waste can be an alternative to promote sustainable practices and add value to plant-based by-products. Inclusion of 100% pomace diet resulted in a more favorable lipid profile in the fish muscles, with a reduction in saturated fatty acids and an increase in polyunsaturated fatty acids, suggesting an improvement in the nutritional value of the final product. These results suggest that olive pomace can be used as a functional ingredient in aquaculture feed, may promoting zootechnical and nutritional benefits, while contributing to the valorization of agro-industrial waste and the sustainability of the production chain. However, observed histological changes warrant further investigation to ensure the safety of olive pomace for Nile tilapia.

#### ACKNOWLEDGEMENTS

We would like to thank Don José Azeites de Oliva de Caçapava do Sul/RS for providing the waste used in this study. We would also like to thank Universidade Franciscana, the Laboratory of Bioprospecting and Experimental Biology (LABBIE), and CNPq for funding the author's scholarship.

#### REFERENCES

- ALHAZZAA, R. et al. Coping with sub-optimal water temperature: modifications in fatty acid profile of barramundi as influenced by dietary lipid. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, [s. l.], v. 165, p. 243-253, 2013. DOI: <https://doi.org/10.1016/j.cbpa.2013.03.019>.
- AL-SOUTI, A. et al. Effects of fish oil replacement with vegetable oils on growth performance, feed utilization and fatty acid composition of tissues of sobaity seabream (*Sparidentex hasta*). **Aquaculture Research**, [s. l.], v. 43, n. 7, p. 1004-1016, 2012. DOI: <https://doi.org/10.1111/j.1365-2109.2011.02898.x>.
- AMARAL, V. F. et al. Composição centesimal e potencial bioativo de um resíduo da extração comercial de azeite de oliva. In: **Inovação e Sustentabilidade em Saúde, Nutrição e Alimentação**. São Leopoldo: UNISINOS, 2022. v. 1, p. 65-70.
- AMERICAN ASSOCIATION CEREAL CHEMISTS (AACC). **Official methods of analysis**. 9. ed. Saint Paul: AACC, 1995. v. 2.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC). **Official methods of analysis of the Association of Official Analytical Chemists**. 16. ed. Washington, DC: AOAC, 1995.

BLIGH, E. G.; DYER, W. J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology**, [s. l.], v. 37, p. 911-917, 1959.

BRAGA, C. V. et al. Agro-industrial by-products in animal nutrition: physiological impacts. **Scientific Reports**, [s. l.], v. 13, 2023. DOI: <https://doi.org/10.1038/s41598-023-30252-6>.

CONFEDERAÇÃO DA AGRICULTURA E PECUÁRIA DO BRASIL (CNA). **CNA analisa custos de produção de tilápia, tabaqui e camarão**. Brasília: CNA, 2024. Disponível em: <https://cnabrasil.org.br>. Acesso em: 12 ago. 2025.

DERMECHE, S. et al. Olive mill wastes: biochemical characterizations and valorization strategies. **Process Biochemistry**, [s. l.], v. 48, n. 10, p. 1532-1552, 2013. DOI: <https://doi.org/10.1016/j.procbio.2013.07.010>.

DUNN, C. et al. Quantitative assessment of H&E staining for pathology: development and clinical evaluation of a novel system. **Diagnostic Pathology**, [s. l.], v. 19, p. 42, 2024. DOI: <https://doi.org/10.1186/s13000-024-01461-x>.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA (EMBRAPA). **Primeiro trimestre de 2025 registra aumento de 112% nas exportações da piscicultura brasileira**. Palmas: Embrapa Pesca e Aquicultura, 2025. Disponível em: <https://www.infoteca.cnptia.embrapa.br>. Acesso em: 15 out. 2025.

EMPRESA DE PESQUISA AGROPECUÁRIA E EXTENSÃO RURAL DE SANTA CATARINA (EPAGRI). **Relatórios técnicos de custos de produção aquícola**. Florianópolis: EPAGRI, 2024. Disponível em: <https://infopeixe.com.br>. Acesso em: 10 ago. 2025.

FURUYA, W. M. et al. Use of alternative ingredients in tilapia diets: a review. **Fishes**, [s. l.], v. 8, p. 556, 2023. DOI: <https://doi.org/10.3390/fishes8110556>.

GARRIDO-ROMERO, M.; DÍEZ-MUNICIO, M.; MORENO, F. J. Exploring the impact of olive-derived bioactive components on gut microbiota: implications for digestive health. **Foods**, [s. l.], v. 14, n. 14, p. 2413, 2025. DOI: <https://doi.org/10.3390/foods14142413>.

GATLIN, D. M. et al. Expanding the utilization of sustainable plant products in aquafeeds. **Aquaculture Research**, [s. l.], v. 38, p. 551-579, 2007. DOI: <https://doi.org/10.1111/j.1365-2109.2007.01704.x>.

GLENCROSS, B. D. et al. A feed is only as good as its ingredients. **Aquaculture Nutrition**, [s. l.], v. 26, p. 561-573, 2020. DOI: <https://doi.org/10.1111/anu.13005>.

GLOBAL SEAFOOD ALLIANCE. **Global tilapia production back on track after Covid-related pause**. [S. l.]: GSA, 2024. Disponível em: <https://www.seafoodsource.com>. Acesso em: 14 ago. 2025.

HARDY, R. W. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. **Aquaculture Research**, [s. l.], v. 41, p. 770-776, 2010. DOI: <https://doi.org/10.1111/j.1365-2109.2009.02349.x>.

HARTMAN, L.; LAGO, R. C. Rapid preparation of fatty acid methyl esters from lipids. **Laboratory Practice**, London, v. 22, p. 475-477, 1973.

HAZREEN-NITA, M. K. et al. Olive oil by-products in aquafeeds: opportunities and challenges. **Aquaculture Reports**, [s. l.], v. 22, p. 100998, 2022. DOI: <https://doi.org/10.1016/j.aqrep.2021.100998>.

INSTITUTO BRASILEIRO DE OLIVICULTURA (IBRAOLIVA). **Portal Ibraoliva**. Porto Alegre: IBRAOLIVA, 2023. Disponível em: <https://www.ibraoliva.com.br>. Acesso em: 15 maio 2025.

KRASUE, E. et al. Palatability of olive pomace in Mozambique Tilapia (*Oreochromis mossambicus*). **Journal of Animal Science**, [s. l.], v. 98, p. 13-14, 2020. DOI: <https://doi.org/10.1093/jdsci/98.1.13>.

LOURENÇO, S. et al. Application of fruit and vegetable processing by-products as ingredients in aquafeed. **Discover Food**, [s. l.], v. 5, p. 45, 2025. DOI: <https://doi.org/10.1007/s44187-025-00309-6>.

LOZANO-SÁNCHEZ, J. et al. Macro and micro functional components of a spreadable olive by-product (pâté) generated by new concept of two-phase decanter. **European Journal of Lipid Science and Technology**, [s. l.], v. 119, n. 1, p. 1600096, 2017. DOI: <https://doi.org/10.1002/ejlt.201600096>.

MCCLEARY, B. V. Measurement of dietary fiber: which AOAC official method of analysis to use. **Journal of AOAC International**, [s. l.], v. 106, p. 917-930, 2023. DOI: <https://doi.org/10.1093/jaoacint/qsad047>.

MENSINK, R. P. **Effects of saturated fatty acids on serum lipids and lipoproteins: a systematic review and regression analysis**. Geneva: World Health Organization, 2016. Disponível em: <https://apps.who.int/iris/handle/10665/246104>. Acesso em: 20 ago. 2025.

MITITELU, M. et al. Polyunsaturated fatty acids and human health: a key to modern nutritional balance in association with polyphenolic compounds from food sources. **Foods**, [s. l.], v. 14, n. 1, p. 46, 2025. DOI: <https://doi.org/10.3390/foods14010046>.

MOLINA-ALCAIDE, E.; YÁÑEZ-RUIZ, D. R. Potential use of olive by-products in ruminant feeding: a review. **Animal Feed Science and Technology**, Amsterdam, v. 147, n. 1-3, p. 247-264, 2008. DOI: <https://doi.org/10.1016/j.anifeedsci.2007.09.021>.

MONTEIRO, C. S.; SPERONI, C. S.; EMANUELLI, T. **Aproveitamento de bagaço de oliva através do fracionamento por peneiras**. Santa Maria: UFSM, 2021. (Informe Técnico Centro de Ciências Rurais, n. 92).

NEFZAOU, A. Studies on the utilization of olive tree by-products in ruminant feeding in Tunisia. **Options Méditerranéennes**, Série A, n. 16, p. 101-106, 1991.

PENG, X. et al. The association between circulating fatty acids and stroke in hypertensive patients. **Nutrition, Metabolism and Cardiovascular Diseases**, [s. l.], v. 34, n. 2, p. 370-378, 2024. DOI: <https://doi.org/10.1016/j.numecd.2023.10.021>.

PETSINI, F. et al. Dietary olive pomace improves fish health status. **Foods**, [s. l.], v. 11, n. 3, p. 385, 2022. DOI: <https://doi.org/10.3390/foods11030385>.

REBOREDO-RODRÍGUEZ, P. et al. Characterisation of virgin olive oil obtained from fermented olives. **Food Chemistry**, [s. l.], v. 171, p. 336-344, 2015. DOI: <https://doi.org/10.1016/j.foodchem.2014.09.141>.

REVERTER, M. et al. Biological and ecological roles of natural products from marine microalgae. **Marine Drugs**, [s. l.], v. 15, n. 3, p. 81, 2017. DOI: <https://doi.org/10.3390/md15030081>.

SANTIGOSA, E. et al. Omega-3 EPA and DHA from algal oil improve Pacific fish performance and composition. **Journal of Aquatic Food Product Technology**, [s. l.], v. 32, n. 1, p. 1-10, 2024. DOI: <https://doi.org/10.1080/10498850.2023.2294156>.

TACON, A. G. J.; METIAN, M. Feed matters: satisfying the feed demand of aquaculture. **Reviews in Fisheries Science & Aquaculture**, [s. l.], v. 23, n. 1, p. 1-10, 2015. DOI: <https://doi.org/10.1080/23308249.2014.987209>.

ULLAH, R. et al. Dietary fiber and gut health in aquaculture: a review. **Aquaculture Reports**, [s. l.], v. 22, p. 100940, 2022. DOI: <https://doi.org/10.1016/j.aqrep.2022.100940>.

URIBE, E. et al. Comparison of chemical composition, bioactive compounds and antioxidant activity of three olive-waste cakes. **Chilean Journal of Agricultural Research**, Chillán, v. 74, n. 3, p. 337-343, 2015. DOI: <http://dx.doi.org/10.4067/S0718-58392014000300007>.

ZEPPENFELD, C. C. et al. *Aloysia triphylla* essential oil as food additive for *Rhamdia quelen* – stress and antioxidant parameters. **Aquaculture Nutrition**, [s. l.], v. 23, p. 1362-1367, 2017. DOI: <https://doi.org/10.1111/anu.12511>.

ZHOU, Z. et al. Effect of dietary insoluble fiber on growth performance and gut morphology in hybrid tilapia. **Aquaculture Nutrition**, [s. l.], v. 24, p. 38-47, 2018. DOI: <https://doi.org/10.1111/anu.12518>.

ZULUAGA-HERNÁNDEZ, C. D.; HINCAPIÉ, C. A.; OSORIO, M. Non-conventional ingredients for Tilapia (*Oreochromis* spp.) feed: a systematic review. **Fishes**, [s. l.], v. 8, n. 11, p. 556, 2023. DOI: <https://doi.org/10.3390/fishes8110556>.