

## Original Research Articles

# Effects of Adding Purslane (*Portulaca oleracea*) as a Source of Omega-3 Fatty Acids on Muscle Composition, Fatty Acid Profile, and Productive Performance in Tilapia (*Oreochromis niloticus*)

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This study evaluated the effect of diets enriched with purslane flour (*P. oleracea*) on production variables, muscle composition, and fatty acid profile in Nile tilapia. Four isonitrogenous and isolipidic diets were prepared, incorporating 0% (CTRL), 5% (V-5), 10% (V-10), and 15% (V-15) of *P. oleracea* flour. Trials were conducted in triplicate with an initial average weight ( $2.93 \pm 0.17$  g), and the fish were fed the experimental diets for 60 days. The inclusion of 100 g/kg of purslane significantly increased ( $p < 0.05$ ) the lipid content in the fillet. However, at 150 g/kg, protein content decreased ( $p < 0.05$ ), while levels of 20:5 n-3 (EPA) (3.27%) and 22:6 n-3 (DHA) (4.14%) in the tilapia fillet increased ( $p < 0.05$ ), with a 100% survival rate reported in all treatments. The results demonstrate that up to 100 g/kg of purslane flour can be included in diets for Nile tilapia without negatively affecting productive performance, and up to 150 g/kg when the objective is to improve the nutritional quality of the tilapia fillet.

## INTRODUCTION

The contribution of aquaculture to the global supply of aquatic animals was 51% in 2022, surpassing capture fisheries for the first time.<sup>1</sup> Among the different groups of farmed species, freshwater fish contributed the largest volume of production. Within this group, the Nile tilapia (*Oreochromis niloticus*) is the most commercially important species with an annual production of 5.3 million tons in 2022 with a tendency to increase in the coming years.<sup>1</sup>

*O. niloticus* is the second most valued species in the aquaculture sector, after carp (*Ctenopharingodon idellus*)<sup>1,2</sup>; due to its rapid growth, disease resistance, adaptability to various captive conditions and high productivity.<sup>3</sup> Furthermore, tilapia is highly appreciated for its high nutritional value, mainly as a valuable protein source, lipids and other nutrients.<sup>4</sup> Tilapia has the majority of important fatty acids. However, it has a greater content of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) such as palmitic acid (16:0) and stearic acid (18:0), oleic acid (18:1n-9) and linoleic acid (18:2n-6), respectively.<sup>5</sup> Moreover, the levels of omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (20:5n-3/EPA) and do-

cosahexaenoic acid (22:6n-3/DHA), are considered deficient compared to marine fish.<sup>4,6</sup>

The fatty acid composition in farmed fish largely depends on dietary lipids.<sup>7</sup> It has also been shown that tilapia has the metabolic capacity to efficiently convert n-3 and n-6 precursor fatty acids into long-chain PUFAs (n-3 and n-6) through enzymatic desaturation and elongation systems.<sup>8</sup>

Traditionally, fish oil has been the most important lipid source for the preparation of diets for aquatic organisms, as it meets dietary requirements for essential fatty acids such as EPA and DHA, which are beneficial for fish health and fillet quality.<sup>9,10</sup> However, inclusion rates in aquaculture diets are showing a downward trend due to high costs and supply fluctuations.<sup>1</sup> Additionally, it has been proposed to include plant-based ingredients that are more affordable and readily available as alternatives to fish oil in the production of balanced diets for aquatic organisms.<sup>10</sup> Therefore, it is necessary not only to replace fish oil with alternative plant- or animal-based oils but also to improve the lipid composition of the fillet, as this is one of the main challenges in maintaining the sustainability of the aquaculture industry.<sup>4,11</sup>

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As a result, numerous studies in fish have reported the efficacy of incorporating plant-derived fatty acids into fish muscle through diet.<sup>5,7,12</sup> Among the ingredients successfully tested are oils from flaxseed,<sup>7</sup> soybean,<sup>2,13</sup> canola and peanut,<sup>14</sup> palm,<sup>15</sup> various vegetable oils,<sup>16</sup> and chia and flaxseed.<sup>17</sup> Scientific evidence indicates that it is possible to improve the lipid deficit in tilapia by incorporating plant based sources of omega-3 fatty acids into the diet.<sup>13,18</sup>

Therefore, a plant ingredient with great lipid potential that could be used as an alternative source of fatty acids in the nutrition of cultured fish is purslane. *P. oleracea* belongs to the family *Portulacaceae*; it is an herbaceous plant classified among the most common in the world and is widely distributed in tropical and subtropical regions, with a productive season throughout the year.<sup>19,20</sup> Since it is considered an arvense plant, its availability is high and has no economic value.<sup>19</sup>

Purslane has been considered a nutraceutical plant since it is highly nutritious and has bioactive compounds that provide multiple medicinal, pharmacological, and nutritional benefits.<sup>21</sup> In addition, recent studies have shown that *P. oleracea* flour is an important source of PUFA, due to a high concentration of omega-3 fatty acids, mainly linoleic acid (18:2n-6) (12%) and  $\alpha$ -linolenic acid (18:3n-3) (46 %).<sup>19</sup> These fatty acids are generally present in fish oils and fats, but are not normally synthesized in terrestrial plants.<sup>22</sup>  $\alpha$ -linolenic acid and linoleic acid are precursor fatty acids that can be metabolized through enzymatic desaturation and elongation systems, resulting in the production of long-chain PUFAs such as EPA, DHA, and arachidonic acid (ARA), respectively.<sup>5</sup>

The objective of this study was to evaluate the effects of incorporating purslane, a plant-based source of omega-3 fatty acids into the diet, on productive performance, chemical composition, and fatty acid profile in the muscle tissue of Nile tilapia (*Oreochromis niloticus*).

## MATERIALS AND METHODS

*P. oleracea* plants were collected in the municipality of San Blas, Nayarit, Mexico. They were then washed, dehydrated at 45°C for 36 h, ground in an electric mill, sieved to 80  $\mu$ m, and stored at 4°C until use.

### EXPERIMENTAL DIETS

A control diet (CTRL) and three experimental diets based on purslane flour were formulated at inclusion levels of 5% (V-5), 10% (V-10), and 15% (V-15). The ingredients were homogenized and moistened to 30%. The resulting moist mixture was pelleted (2.0 mm diameter) using a Torrey® meat grinder, Monterrey. The prepared diets were dehydrated in an oven at 45°C for 12 h. Subsequently, they were broken into appropriate sizes for the fish's mouth and stored at 4°C.

## ORGANISMS AND EXPERIMENTAL CONDITIONS

The study was conducted at the National School of Fisheries Engineering, Autonomous University of Nayarit, San Blas, Nayarit, Mexico. Masculinized Nile tilapia (*O. niloticus*) with an average weight of  $2.93 \pm 0.17$  g were randomly distributed across 4 treatments in triplicate, with 10 fish per replicate/experimental unit (240 L). Dissolved oxygen was maintained between 5.7 and 6.2 mg/L, and water temperature was kept at  $27 \pm 4^\circ\text{C}$ . Fish were fed three times daily (9:00, 13:00, and 16:00) at a rate of 5% of their body weight. The feeding trial lasted 60 days, with biometric measurements taken every seven days.

The following conventional formulas were used to calculate the productive variables:

1. *Weight Gain (WG)* = (final weight (g) - initial weight (g))
2. *Feed Conversion Ratio (FCR)* = Total feed consumed / Total weight gain
3. *Daily Growth Rate (DGR)* = (Final weight - Initial weight) / days
4. *Survival Percentage (S)* =  $100 \times (\text{initial number of fish} - \text{final number of fish})$

## CHEMICAL ANALYSIS

The chemical composition of the ingredients, diets, and tilapia fillets was analyzed following standard methods.<sup>23</sup> Moisture content was determined by dehydration at 60°C for 24 h, protein (crude protein = Nitrogen  $\times$  6.25) was measured using the microKjeldahl method with a Kjeltex system (model 1009 and 1002, Tecator, Sweden), total lipids were determined by petroleum ether extraction using a Soxtec system (Soxtec System HT6, Tecator, Sweden), fiber was determined by drying and burning the sample after extraction with 0.5 M H<sub>2</sub>SO<sub>4</sub> and 0.5 M NaOH, ash content was measured by ashing the sample in a muffle furnace (Terlab® TE-M12D) at 550°C for 6 h, and nitrogen-free extract was calculated by difference.

## FATTY ACID ANALYSIS

At the end of the feeding trial, fish were fasted for 24 hours, then sacrificed and filleted (muscle tissue). Fillets were stored in polyethylene bags (under N<sub>2</sub> atmosphere) and frozen at -18°C. Fatty acid profiling was performed following the method described by Folch *et al.*<sup>24</sup> The extraction was done with a 1:1 sample (wet tissue or flour): extracting solution ratio (2 methanol: 1 dichloromethane). The mixture was sonicated for 2 min and refrigerated for 12 h. The suspension was centrifuged at 3000 rpm/10 min at 4°C, and extraction was repeated three times with a methanol: dichloromethane mixture (1:1). The supernatant was extracted, 1 mL of distilled water and one drop of HCl (0.6N) were added, followed by centrifugation at 3000 rpm/10 min at 4°C to separate the solution from the water. The extracted solution was stored in 2 mL amber vials after evaporation with nitrogen (N<sub>2</sub>).

## SAPONIFICATION

To each extracted solution, 100  $\mu$ L of a methanolic solution (10 mL KOH 3N/90 mL methanol) was added and transferred to a water bath at 60°C for 30 min. Next, 300  $\mu$ L of distilled water + 200  $\mu$ L of hexane were added, shaken vigorously for 2 min, centrifuged at 3000 rpm/2 min at 4°C, and the lower layer was extracted (discarding the supernatant). To extract the saponifiable lipids, 300  $\mu$ L of distilled water + one drop of HCl 6N + 200  $\mu$ L of hexane were added. The supernatant was extracted and stored in vials.

## FATTY ACID METHYLATION

Each sample was mixed with 500  $\mu$ L of esterification reagent (boron trifluoride in methanol  $\text{BF}_3/\text{CH}_3\text{OH}$  at 14% in methanol) and heated at 60°C for 15 min. Then, 300  $\mu$ L of distilled water + 200  $\mu$ L of hexane were added, shaken vigorously for 2 min, and centrifuged at 2000 rpm/5 min at 4°C. The supernatant was extracted and stored in amber vials, dried with  $\text{N}_2$ .

Fatty acid methyl esters (FAME) were separated and quantified using a gas chromatograph (Agilent GC6880, Agilent Technologies, Santa Clara, CA, EE. UU.) equipped with a flame ionization detector and a capillary column GC (60 m x 0.25  $\mu$ m; Agilent 122-2362 dB-23). The initial temperature was 150°C. Five minutes after sample injection (2  $\mu$ L), it was increased to 260°C and held for 27.06 min. Hydrogen was used as the carrier gas and nitrogen as the makeup gas. Fatty acids were identified by comparing their retention times with known standards (Supelco/Sigma-Aldrich, St. Louis, MO, EE. UU.) Each fatty acid concentration was calculated from the corresponding area on the GC850 integrator chromatogram.

## STATISTICAL ANALYSIS

The data obtained were subjected to normality and homogeneity tests. A one-way analysis of variance (ANOVA,  $p < 0.05$ ) was performed to determine if the obtained data differed. The software STATISTICA® 7 was used, and Tukey's multiple range test was applied to classify the treatments.

## RESULTS

### PROXIMAL COMPOSITION OF DIETS

The results of the proximal chemical composition of the experimental diets are shown in [Table 1](#). The protein and lipid content in the diets were adjusted to the formulated percentages, confirming that they are isoproteic and isolipidic. Fourteen fatty acids were identified in the total fatty acid content of the diets, with the most abundant being palmitic (24.81% - 26.01%),  $\alpha$ -linolenic (20.52% - 25.89%), and oleic (17.36% - 19.95%). The results indicated that the experimental diets containing purslane flour (V-5, V-10, and V-15) had the highest values (20.52%, 21.93%, and 25.89%, respectively) of  $\alpha$ -linolenic acid (18:3n-3) compared to the CTRL diet (0.96%) ([Table 2](#)).

## PRODUCTIVE PERFORMANCE

The productive performance parameters (weight gain, daily growth rate, feed conversion ratio, and survival rate) are summarized in [Table 3](#). After 60 days of feeding, the fish showed a 100% survival rate across all treatments. Weight gain was significantly higher ( $p < 0.05$ ) in fish fed the CTRL diet (24.88 g), showing similar results with the V-10 diet (21.60 g). Conversely, the V-5 and V-15 diets had the lowest values (19.26 g and 18.02 g, respectively). FCR values ranged from 1.61 to 1.84 across the four diets, with no significant differences ( $p < 0.05$ ).

## FILLET COMPOSITION

[Table 4](#) shows changes in the proximal chemical composition in Nile tilapia fillets in response to different treatments. There were no significant differences ( $p < 0.05$ ) in ash and moisture content across all diets. However, the protein percentage in the fillets from the CTRL treatment was higher (86.33%) than in the experimental treatments with purslane flour (84.80%). Additionally, lipid content in tilapia fillets increased significantly with purslane supplementation, as indicated by fillet samples from the V-15 diet, which recorded the highest lipid content (1.96%), with no significant differences ( $p > 0.05$ ) from the CTRL diet fillets (2.04%), while the lowest lipid content was found in the V-5 diet (1.78%).

## FATTY ACID PROFILE OF THE FILLET

The fatty acid profile of tilapia fillets is presented in [Table 5](#). Twenty fatty acids were identified, including seven saturated fatty acids (SFA), four monounsaturated fatty acids (MUFA), five polyunsaturated fatty acids in the n-6 group (PUFA n-6), and four in the n-3 group (PUFA n-3). The most abundant group across all treatments was SFA (47.39%), followed by MUFA (21.76%), while long-chain n-6 and n-3 polyunsaturated fatty acids were found in lower percentages (15.40% and 8.18%, respectively). The most abundant fatty acids in fish fillets from all four treatments were palmitic (22.90%), oleic (16.61%), and linoleic (9.56%). Fish fed the V-10 and V-15 diets with purslane flour showed significantly higher ( $p < 0.05$ ) EPA (2.93% and 3.27%, respectively) and DHA (3.31% and 4.14%, respectively) content compared to fish fed the CTRL diet (1.94% and 2.99%, respectively). The significant differences ( $p < 0.05$ ) found in fish fed different diets indicate that the effects on the fillet's lipid profile are related to the enriching ingredient (purslane) used and the inclusion levels (5%, 10%, and 15%). An upward trend in PUFA levels was observed in fish fed diets with higher inclusion levels of *P. oleracea* flour.

## DISCUSSION

Since the aquaculture industry heavily relies on fishmeal and fish oil, an interesting strategy to optimize this sector's sustainable development is the incorporation of alternative feed ingredients in fish diets.<sup>25,26</sup> Therefore, efforts are being made to find new sustainable alternatives for use in

**Table 1. Ingredients and proximate composition of experimental diets (% dry matter).**

| Ingredients                        | Experimental diets g/kg |       |       |       |
|------------------------------------|-------------------------|-------|-------|-------|
|                                    | CTRL                    | V-5   | V-10  | V-15  |
| Purslane flour                     | 0                       | 50    | 100   | 150   |
| Fish meal                          | 299                     | 295   | 297   | 296   |
| Soybean past                       | 170                     | 108   | 66.4  | 59.4  |
| Wheat flour                        | 356                     | 87    | 80    | 40    |
| Mesquite flour                     | 10                      | 300   | 300   | 300   |
| Fish oil                           | 26                      | 23.5  | 21.8  | 20.8  |
| Soy lecithin                       | 26                      | 23.5  | 21.8  | 20.8  |
| Vitamins*                          | 3                       | 3     | 3     | 3     |
| Minerals**                         | 10                      | 10    | 10    | 10    |
| Grenetina                          | 40                      | 40    | 40    | 40    |
| Starch                             | 60                      | 60    | 60    | 60    |
| <b>Proximal composition (g/kg)</b> |                         |       |       |       |
| Moisture (%)                       | 2.81                    | 2.90  | 3.24  | 3.02  |
| Protein (%)                        | 35.39                   | 35.57 | 35.20 | 34.98 |
| Lipid (%)                          | 10.02                   | 9.87  | 10.51 | 10.93 |
| Ash (%)                            | 8.53                    | 6.93  | 7.76  | 8.42  |
| Fiber (%)                          | 1.54                    | 4.19  | 4.89  | 5.64  |
| NFE                                | 40.10                   | 39.57 | 37.62 | 37.01 |

\*Vitamin premix contains (mg kg dry matter) = Vit. A, 50 million IU; Vit. E, 130 g; thiamine monohydrate (150), riboflavin (100), pyridoxine HCl (50), pantothenic acid (75), niacin (300), biotin (1), inositol (500), folic acid (10), cyanocobalamin (0.1).

\*\*Mineral premix contains (g kg dry matter) = FeSO<sub>4</sub> (25), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.5), ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.09), KCl (0.5), NaCl, (0.6), MnCl<sub>2</sub>·4H<sub>2</sub>O (0.0234), KI (0.05), CoCl<sub>2</sub>·6H<sub>2</sub>O (0.0025). NFE: Nitrogen-free extract, calculated as 100-(Protein + Lipids + Ash + Fiber) g/kg.

**Table 2. Fatty acids composition (g/100 g) of diets for tilapia (*O. niloticus*).**

| Fatty acids | Experimental diets |       |       |       |
|-------------|--------------------|-------|-------|-------|
|             | CTRL               | V-5   | V-10  | V-15  |
| C14:0       | 5.11               | 5.58  | 5.26  | 4.66  |
| C15:0       | 1.28               | 1.26  | 1.52  | 1.31  |
| C16:0       | 30.26              | 25.49 | 24.81 | 26.01 |
| C17:0       | 1.43               | 0.93  | 1.08  | 1.42  |
| C18:0       | 13.46              | 3.11  | 4.55  | 4.12  |
| C23:0       | 4.30               | 5.02  | 6.10  | 4.89  |
| SFA         | 55.84              | 41.39 | 43.32 | 36.41 |
| C16:1       | 4.65               | 4.04  | 4.30  | 2.35  |
| C18:1n-9    | 24.06              | 19.95 | 17.49 | 17.36 |
| C20:1n-9    | 1.43               | 0.99  | 0.80  | 1.33  |
| MUFA        | 30.14              | 24.98 | 19.59 | 21.04 |
| C18:3n-3    | 0.96               | 20.52 | 21.93 | 25.89 |
| C20:3n-3    | 7.16               | 1.25  | 1.27  | 2.30  |
| C20:4n-6    | 2.11               | 2.40  | 2.95  | 1.60  |
| C20:5n-3    | 2.19               | 1.13  | 1.90  | 1.71  |
| C22:6n-3    | 2.0                | 1.30  | 1.56  | 1.64  |
| PUFA        | 13.06              | 26.6  | 29.61 | 33.14 |
| Others      | 1.0                | 5.69  | 2.71  | 5.99  |

SFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids.

**Table 3. Productive performance of Nile tilapia (*O. niloticus*) fed diets with different inclusion levels of purslane (*P. oleracea*).**

| Parameters | Experimental diets      |                         |                          |                         |
|------------|-------------------------|-------------------------|--------------------------|-------------------------|
|            | CTRL                    | V-5                     | V-10                     | V-15                    |
| IW (g)     | 2.90±0.06 <sup>a</sup>  | 2.94±0.05 <sup>a</sup>  | 2.93±0.04 <sup>a</sup>   | 2.96±0.07 <sup>a</sup>  |
| FW (g)     | 27.28±2.59 <sup>a</sup> | 22.21±1.05 <sup>b</sup> | 24.53±0.67 <sup>ab</sup> | 20.98±0.30 <sup>b</sup> |
| WG (g)     | 24.88±2.63 <sup>a</sup> | 19.26±1.03 <sup>b</sup> | 21.60±0.62 <sup>ab</sup> | 18.02±0.28 <sup>b</sup> |
| FCR        | 1.72±0.10 <sup>a</sup>  | 1.76±0.08 <sup>a</sup>  | 1.61±0.06 <sup>a</sup>   | 1.84±0.10 <sup>a</sup>  |
| DGR        | 0.41±0.18 <sup>a</sup>  | 0.32±0.07 <sup>bc</sup> | 0.36±0.02 <sup>ab</sup>  | 0.30±0.04 <sup>c</sup>  |
| S (%)      | 100                     | 100                     | 100                      | 100                     |

IW = Initial Weight, FW = Final Weight, WG = Weight Gain, FCR = Feed Conversion Ratio, DGR = Daily Growth Rate, and S = Survival Percentage. Values are presented as mean ± standard deviation, n=3. Means that do not share a letter are significantly different (p<0.05), based on the Tukey test.

**Table 4. Muscle composition (g/kg dry matter) of Nile tilapia (*O. niloticus*) after 60 days of feeding.**

| Nutrients | Experimental diets      |                          |                         |                         |
|-----------|-------------------------|--------------------------|-------------------------|-------------------------|
|           | CTRL                    | V-5                      | V-10                    | V-15                    |
| Moisture  | 79.92±0.05 <sup>a</sup> | 80.06±0.59 <sup>a</sup>  | 80.21±0.48 <sup>a</sup> | 80.17±0.38 <sup>a</sup> |
| Ash       | 7.20±0.23 <sup>a</sup>  | 7.54±0.22 <sup>a</sup>   | 7.71±1.01 <sup>a</sup>  | 7.85±0.51 <sup>a</sup>  |
| Protein   | 86.33±1.33 <sup>a</sup> | 85.45±2.67 <sup>ab</sup> | 84.87±0.87 <sup>b</sup> | 84.09±1.25 <sup>b</sup> |
| Lipids    | 2.04±0.10 <sup>a</sup>  | 1.78±0.90 <sup>b</sup>   | 1.92±0.64 <sup>ab</sup> | 1.96±0.48 <sup>a</sup>  |

Values are presented as mean ± standard deviation, n=3. Means that do not share a letter are significantly different (p<0.05), based on the Tukey test.

feed.<sup>9</sup> The results of this study demonstrate that incorporating *P. oleracea* flour in the diet formulation for tilapia (*O. niloticus*) is feasible at inclusion levels of up to 100 g/kg without negative effects on productive performance parameters (WG, DGR, and PF) and feed efficiency (FCR).

Previous studies have also reported that using plant-based ingredients in fish diets improves the productive performance of various fish species, including Nile tilapia (*O. niloticus*),<sup>2</sup> rohu (*Labeo rohita*),<sup>27</sup> Atlantic salmon (*Salmo salar*),<sup>28</sup> and hybrid sturgeon (*Acipenser baerii*),<sup>29</sup> among others, as long as low inclusion levels are used, or some form of improvement is applied. A study by Cámara Ruiz *et al.*<sup>30</sup> suggests the inclusion of 20 g/kg of *P. oleracea* in diets for gilthead sea bream (*Sparus aurata*) without affecting productive variables.

On the other hand, fish fed diets containing *P. oleracea* flour at inclusion levels of 150 g/kg had the lowest values in WG and DGR compared to the CTRL diet and the V-10 diet. This aligns with the findings of Abdel-Razek *et al.*<sup>25</sup> who reported a significant reduction in fish growth, WG, and DGR in a feeding trial on Nile tilapia when inclusion levels exceeded 30 g/kg of *P. oleracea*.

These negative effects could be associated with the high fiber content typical of plant sources,<sup>29,31</sup> which influences nutrient absorption and movement through the gastrointestinal tract, as fiber can bind with proteins, lipids, or minerals, reducing their bioavailability.<sup>32</sup> This may also be related to the lower energy digestibility of plant protein, compared to animal protein, possibly due to the high content of non-starch polysaccharides that influence the absorption, metabolism, and utilization of nutrients since they bind to bile salts and obstruct the action of digestive

enzymes and increase energy requirements.<sup>33</sup> Deng *et al.*<sup>2</sup> linked the low productive performance in fish fed plant-protein-based diets to the low digestibility of the ingredients, which reduces nutrient bioavailability. The 100% survival rate across treatments indicates that *P. oleracea* flour is not harmful to Nile tilapia and can be included in feed diets.

The proximal composition of the fillets indicated that moisture and ash content showed no significant differences (p> 0.05) across treatments. This is comparable to results obtained for the same species using chia (*Salvia hispanica*) and flaxseed (*Linum usitatissimum*) flours.<sup>17</sup> Therefore, it can be said that the lipid source affects the chemical composition of fish muscle tissues.

Protein content showed a significant decrease (p< 0.05) when inclusion levels increased to 150 g/kg of *P. oleracea* flour. This same trend has been reported in other studies when the inclusion level is exceeded.<sup>26</sup> The protein decrease is primarily related to the nutrient composition of the protein source,<sup>18</sup> but it may also be attributed to the stimulation of extracellular enzymes by the gut microflora, which enhances diet digestion and absorption.<sup>34</sup> On the other hand, considering that the diets were isoproteic and isolipidic, the protein variation could be associated with the quality and nutritional value of the ingredients used.<sup>14</sup>

Additionally, the inclusion of *P. oleracea* flour in diets led to a significant increase (p<0.05) in lipid content, which could be attributed to the lipid composition of the diet. Similar results were reported in numerous studies: Luo *et al.*<sup>35</sup> studied the inclusion of cottonseed meal for rainbow trout (*Oncorhynchus mykiss*); Szabo *et al.*<sup>13</sup> characterized the incorporation dynamics of fatty acids from soybean and

**Table 5. Fatty acid composition (% fatty acid) of muscle of tilapia (*O. niloticus*) fed diets containing purslane flour (*P. oleracea*).**

| Fatty acids | Experimental diets |             |             |              |
|-------------|--------------------|-------------|-------------|--------------|
|             | CTRL               | V-5         | V-10        | V-15         |
| C14         | 3.37±1.46◇         | 3.27±0.50◇  | 3.87±0.61◇  | 1.58±0.25◇   |
| C14:1       | 0.46±0.32◇         | 0.50±0.22◇  | 0.82±0.09◇  | 1.03±0.51◇   |
| C15         | 1.38±0.10◇         | 0.64±0.42◇  | 0.94±0.08◇  | 0.65±0.16◇   |
| C16         | 23.59±4.01◇        | 22.85±1.57◇ | 22.60±0.63◇ | 22.58±1.12◇  |
| C16:1       | 4.82±1.02◇         | 4.30±0.38◇  | 4.13±0.27◇  | 3.68±0.17◇   |
| C17         | 0.35±0.10◇         | 0.57±0.27◇  | 0.76±0.14◇  | 0.77±0.26◇   |
| C17:1       | 0.91±0.20◇         | 0.58±0.25◇  | 0.56±0.31◇  | 0.73±0.10◇   |
| C18         | 8.81±0.93◇         | 7.13±0.54◇  | 8.07±0.71◇  | 7.35±0.27◇   |
| C18:1n-9    | 17.18±0.23◇        | 15.61±0.55◇ | 16.48±0.68◇ | 17.20±0.26◇  |
| C18:2n-6    | 11.89±0.77◇        | 10.94±0.26◇ | 9.11±0.76◇  | 6.33±0.34◇   |
| C18:3n-6    | 0.72±0.06◇         | 1.02±0.16◇  | 1.10±0.19◇  | 1.71±0.27◇   |
| C18:3n-3    | 1.43±0.68◇         | 1.56±0.23◇  | 1.26±0.20◇  | 1.02±0.10◇   |
| C20         | 1.22±1.52◇         | 0.48±0.12◇  | 0.71±0.06◇  | 0.55±0.14◇   |
| C20:2       | 1.43±0.01◇         | 2.10±0.21◇  | 2.36±0.44◇  | 2.40±0.46◇   |
| C20:3n-6    | 1.40±0.36◇         | 2.05±0.07◇  | 2.49±0.45◇  | 2.23±0.30◇   |
| C20:3n-3    | 0.89±0.08◇         | 2.48±0.44◇  | 1.93±0.47◇◇ | 1.82±0.35◇   |
| C20:4n-6    | 2.54±0.87◇         | 2.35±0.46◇  | 2.53±0.51◇  | 2.22±0.14◇   |
| C20:5n-3    | 1.94±0.68◇         | 1.54±0.19◇  | 2.93±0.19◇  | 3.27±0.12◇   |
| C22:6n-3    | 2.99±0.81◇◇        | 2.86±0.26◇  | 3.31±0.28◇  | 4.14±0.07◇   |
| C23         | 7.67±0.02◇         | 11.47±1.01◇ | 9.80±0.71◇  | 10.86±0.85◇◇ |
| SFA         | 46.39±0.38◇        | 46.41±0.15◇ | 46.75±0.12◇ | 44.34±0.38◇  |
| MUFA        | 24.80±0.12◇        | 20.99±0.42◇ | 21.99±0.85◇ | 22.64±0.26◇  |
| PUFA n-6    | 17.98±0.41◇        | 18.46±0.29◇ | 17.59±0.19◇ | 14.89±0.44◇  |
| PUFA n-3    | 7.25±0.25◇         | 8.44±0.37◇  | 9.47±0.25◇  | 10.25±0.34◇  |
| Others      | 3.58±0.61          | 5.69±3.70   | 2.71±1.05   | 5.99±1.00    |
| DHA/EPA     | 1.54               | 1.86        | 1.13        | 1.27         |

SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, PUFA= Polyunsaturated Fatty Acids, DHA= Docosahexaenoic Acid, EPA= Eicosapentaenoic Acid. Values are presented as mean ± standard deviation, n=3. Means that do not share a letter are significantly different (p<0.05), based on the Tukey test.

flaxseed oils in tilapia muscle tissue; Ayisi *et al.*<sup>15</sup> observed that lipid levels in whole body tilapia increased with high levels of palm oil; Qiu *et al.*<sup>14</sup> evaluated different plant lipid sources in diets for large yellow croaker (*Larimichthys crocea*). These studies have shown that incorporating plant oils into aquaculture diets positively reflects on fish muscle tissue composition.

There is growing scientific evidence demonstrating the potential to modify the fatty acid profile in fish muscle tissue through diet,<sup>7,8,36</sup> and it has been proven that vegetable oils represent a promising replacement alternative.<sup>14,15,37</sup> The results of the present study show that the fatty acid profile of Nile tilapia filets was influenced by the fatty acid composition of the diets, consistent with numerous studies conducted on *O. niloticus*.<sup>2,13,15,38</sup> SFA (47.39%) and MUFA (21.76%) were the most abundant fatty acids in both diets and fillet samples. In a study on tilapia (*O. niloticus*) fed a palm oil-based diet, Ayisi *et al.*<sup>15</sup> reported a similar trend in SFA (47.09%) and MUFA (37.68%) content. These results once again demonstrate the relationship between dietary inputs and tissue fatty acids in fish.<sup>18</sup> This suggests that

nutrient factors should be considered when using enriched diets, as they are key to modulating *in vivo* fatty acid metabolism.<sup>39</sup>

The 22:6 n-3 (4.14%) and 20:5 n-3 (3.27%) levels reported in this study were significantly higher (p< 0.05) in fish fed the V-15 diet than in those fed the CTRL diet (2.99% and 1.94%, respectively). A similar effect was recorded in tilapia fed diets containing flaxseed oil.<sup>7</sup> Our results differ from the findings of Turchini *et al.*<sup>39</sup> who reported limited bioconverted fatty acids in rainbow trout (*Oncorhynchus mykiss*). Furthermore, the increase in 20:5 n-3 and 22:6 n-3 can be attributed to the desaturation and elongation enzymatic activity (particularly in tilapia), which efficiently converts PUFA to long-chain PUFA from precursor fatty acids like 18:2n-6 and C18:3n-3, favoring the n-6/n-3 ratio.<sup>34</sup> Turchini *et al.*<sup>12</sup> demonstrated that diets with higher levels of precursor fatty acids produced filets with higher EPA and DHA levels. Another study indicated that Nile tilapia had a selective preference for  $\alpha$ -linolenic acid as a substrate for long-chain PUFA synthesis.<sup>37</sup> This phenomenon is assumed to result from positive feedback when precursor

sor fatty acids are present, serving as substrates for long-chain fatty acid transformation.<sup>15</sup> However, further studies on regulatory mechanisms are needed. Although the main lipid sources (fish oil and soybean lecithin) were equivalent among dietary treatments (isolipidic diets), the results indicated that the purslane inclusion level influenced the fillet's fatty acid profile, and as expected, the fatty acid composition affected the tissue. Therefore, purslane being a plant with multiple nutritional benefits, becomes an alternative with great nutritional potential as a food additive in fish diets. Furthermore, due to its abundance, wide availability and because it does not have an economic value assigned, it could be valued as a sustainable and profitable ingredient for the aquaculture sector.

The results obtained in this study confirm that *P. oleracea* can be included in tilapia diets up to 100 g/kg without compromising productive performance and up to 150 g/kg when the goal is to increase EPA and DHA levels in tilapia fillets. Survival was 100% in all treatments. Dietary fatty acids were effectively incorporated into tilapia fillets, attributed mainly to desaturation and elongation enzymatic activities for long-chain PUFA biosynthesis from precursor PUFA (C18). These findings make purslane a possible alternative source of precursor fatty acids that could be used in the aquaculture industry to reduce dependence on fish oil. However, it is recommended that in future research, a characterization of the microbial production suspended in the pond water be carried out to predict whether it favors the development of the experimental organisms.

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## ETHICAL CONDUCT APPROVAL – IACUC

This research was authorized by the university Bioethics committee of the Autonomous University of Nayarit.

## INFORMED CONSENT STATEMENT

The authors declare that they have no conflicts of interest for the publication of this manuscript.

## DATA AVAILABILITY STATEMENT

Data is available upon reasonable request.

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