



Advances in biology through agronomy, aquaculture, coastal and environmental sciences

— Volume II —

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**Advances in biology through
agronomy, aquaculture, coastal and
environmental sciences**
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Advances in Biology through Agronomy, Aquaculture, Coastal and Environmental Sciences, Volume II, is an e-book that continues the work of the first version (<https://editorapantanal.com.br/ebooks/2023/advances-in-biology-through-agronomy-aquaculture-coastal-and-environmental-sciences/ebook.pdf>), published in 2023 by Leandris Argente-Martínez and Ofelda Peñuelas-Rubio.

Our e-book compiles 25 manuscripts that present scientific results and reviews based on biological aspects. All of them contribute to explaining the responses of living organisms to agricultural, aquaculture, and coastal ecosystems, as well as those facing fragility or threat of climate change. This e-book was edited by Leandris Argente-Martínez, Ofelda Peñuelas-Rubio, and Joe Luis Arias-Moscato, members of the Consolidated Academic Group ITVAYA-CA-3.

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Partial substitution of pechita (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*) flour with commercial flour in diets for tilapia (*Oreochromis niloticus*) farming

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ABSTRACT

Feed represents between 50% and 80% of operating costs in aquaculture production, with protein ingredients constituting more than two-thirds of diet costs (FAO, 2024). Therefore, the search for alternative, economical, and locally available plant-based ingredients has become a priority for the sustainability of tilapia (*Oreochromis niloticus*) farming. In this study, the effects of partial substitution of commercial fish meal with mesquite (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*) meal in the diets of juvenile tilapia were evaluated. Three treatments were formulated: a diet including mesquite meal, a diet including alfalfa meal, and a control diet (commercial feed without substitution). A total of 135 juveniles with an initial weight of 5 ± 2 g were used, distributed in nine tanks with 15 organisms each, and fed for 41 days at 4% of their total biomass three times daily. Water quality parameters (dissolved oxygen, temperature, and pH) were monitored daily, and biometrics were performed every 15 days to evaluate growth. The results revealed that although plants-maintained survival above 77% in the plants-based diet treatment group, their growth was significantly lower than that in the control treatment group. The control diet reached a final weight of 20.0 ± 0.9 g, with a feed conversion ratio (FCR) of 4.1, while the weights of the alfalfa and mesquite diets reached 12.0 ± 0.9 g (FCR=10) and 8.0 ± 0.9 g (FCR=9.5), respectively. Water quality parameters remained mostly within tolerable ranges for the species, although extreme values of dissolved oxygen (1.5 mg/L) and temperature (33°C) were recorded, which may have

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affected fish metabolism. Mesquite and alfalfa meals can be used as supplements in tilapia feed, but formulation optimization is needed to improve the digestibility and essential amino acid profile.

Keywords: *Oreochromis niloticus*, feeding, plant ingredients, mesquite, alfalfa, aquaculture.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is the predominant farmed species worldwide, accounting for more than half of global tilapia production since 1992 (FAO, 2024). This species is particularly suitable for aquaculture: it is omnivorous with the ability to filter phytoplankton, tolerates a wide range of environmental conditions, and grows rapidly at optimal temperatures between 31°C and 36°C (FAO, 2024). However, the success of intensive farming critically depends on the availability of low-cost formulated feeds.

In aquaculture, feed can represent between 50% and 80% of operating costs, and protein ingredients constitute more than two-thirds of diet costs (Padhan, 2022). Traditionally, fish meal has been the main protein source in fish feeds because of its excellent amino acid profile and high digestibility (Watanabe, 2002). However, global fish meal production has declined, while its price has increased, making it necessary to identify and produce sustainable alternatives to replace this feed source (FAO, 2024).

In this context, plant-based ingredients have emerged as promising alternatives (FAO, 2024). Alfalfa (*Medicago sativa*) is a legume with high protein (15–22%), vitamin, and mineral contents and is widely available and low in cost. Mesquite (*Prosopis glandulosa*), known as sweet mesquite, is a tree native to the southwestern United States and northern Mexico whose pods have been traditionally used for human and animal feed. Mesquite pods contain sugars, proteins, and fibers, representing an underutilized local resource with potential for use in aquaculture feed (Botanic Gardens Conservation International, 2020; Becker; Grosjean, 1980).

When new ingredients in tilapia diets are being evaluated, it is essential to maintain constant protein and energy levels (isoproteic and isoenergetic diets) so that any observed differences can be attributed to the ingredient being evaluated and not to nutritional imbalance (García Meilán, 2016). In addition to protein levels, the balance of essential amino acids such as lysine, methionine, and threonine is decisive, since when one of them falls below the ideal level, protein synthesis is reduced, even if the total protein content appears adequate (Furuya et al., 2023).

Previous research has shown that the inclusion of plant ingredients in tilapia diets can be successful, when necessary, adjustments are made. Studies with soybean meal, sunflower meal, and pea concentrate have shown that it is possible to partially substitute fish meal without significantly affecting growth, as long as a balanced amino acid profile is maintained (Refaey et al., 2025). Similarly, the use of chaya leaves (*Cnidoscolus chayamansa*) as a partial substitute for formulated feed has been evaluated, and

substitution levels of 25–50% can maintain weight gains similar to those of the control (Poot-López et al., 2012).

The present study aimed to evaluate the effects of partial substitution of commercial fish meal with mesquite (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*) meals on the growth, survival, and feed conversion of juvenile tilapia (*Oreochromis niloticus*) and to monitor water quality parameters during bioassays.

MATERIALS AND METHODS

Study area

This work was carried out in the aquaculture laboratories of the Technological Institute of the Yaqui Valley, located in the municipality of Bâcum, Block 611, using its facilities and tools for the bioassay and the production of the evaluated feeds.

Feed preparation

For the preparation of experimental feeds, nutritious, low-cost, and easily available ingredients, namely, mesquite (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*), which provide vitamins, protein, and other nutrients (Becker & Grosjean, 1980), were selected. The fish meal (commercial feed) used as a base and control was obtained from the aquaculture laboratory.

To obtain mesquite and alfalfa meals, the ingredients were placed in industrial blenders for grinding, yielding 1 kg of meal from each raw material. The same grinding process was applied to commercial fish meal to homogenize all the ingredients, following standard plant ingredient processing methods (Hernández, 2019).

Feed formulation

Using alfalfa, mesquite, and fish meal (commercial feed), the feeds to be evaluated were prepared. Each meal was placed in clean buckets, enriching each formulation as shown in Table 1 so that each feed matched the profile of the commercial feed used as a control. Afterward, 1.5 L of hot water was added to bind and mix all the ingredients until a uniform dough was obtained. The dough was introduced into a pelletizer, and each formulation was processed separately. The obtained pellets were left to dry and cut to a size that allowed the juveniles to ingest them easily. The finished feed was kept refrigerated to avoid contamination. Throughout the preparation process, good personal hygiene and clean instruments were maintained.

Table 1. Composition of experimental feeds (per kg of diet).

Ingredients	Mesquite Diet	Alfalfa Diet
Mesquite meal	700 g	-
Alfalfa meal	-	700 g
Corn meal	140 g	140 g
Fish meal	50 g	50 g
Vitamins (Vitamix)	2 g	2 g
Vegetable oil	52 g	52 g
Gelatin	6 g	6 g
Starch	20 g	20 g
Lard	30 g	30 g

Source: Own elaboration.

Experimental organisms

Juvenile black tilapia (*Oreochromis niloticus*), a species characterized by its ease of handling and high tolerance to adverse environmental conditions (FAO, 2024), were used. The organisms came from the fishing cooperative “La Cochi de Marcela,” located on the Esperanza to Hornos highway, from which they were transferred to the aquaculture laboratory of the Technological Institute of the Yaqui Valley.

Juvenile fish with body weights between 4 and 8 g were selected, and those with poor physical appearance, bruises, or obvious signs of disease were discarded, following the standard selection criteria for bioassays (Martínez, 2003).

To avoid high mortality rates and stress during transport, the organisms were placed in plastic bags with oxygen at a density of 15 organisms per bag, yielding a total of 135 transported organisms, following recommended protocols for aquatic organism handling (Martínez, 2003).

Once in the laboratory, the bags with the organisms were placed in the experimental tanks to equalize the temperature and avoid thermal stress. The fish were subsequently fed according to the stipulated feed during a 7-day adaptation period.

Bioassay

Distribution of organisms

After the acclimatization period, the organisms were placed in nine experimental tanks with a density of 15 organisms per tank. Through random assignment, the type of feed each tank received was

determined, with three replicates per treatment (control, alfalfa, and mesquite), following a completely randomized design (Poot-López et al., 2012).

The fish were fed the three treatments (control diet, alfalfa meal diet, and mesquite meal diet). The daily ratio was calculated on the basis of the total biomass of the organisms in each tank, which accounted for 4% of the total body weight and was divided into three daily rations administered every four hours, in accordance with the recommendations for juvenile tilapia (El-Sayed, 2004).

To maintain water quality and inhibit contaminants, temperature and dissolved oxygen were monitored daily with the help of an oximeter. The pH was measured periodically. Dissolved oxygen was maintained using a constant aeration system. Water exchanges of 50–60% were performed every three days and 90% every week, supplemented with siphoning to remove feces and feed residues, following recommended practices for static systems (Fagbenro; Jauncey, 1995).

Biometrics of all the organisms were performed every 15 days from the start of the bioassay, and individual weight and size were recorded. These data allowed the evaluation of growth and adjustment of the feed ratio on the basis of increased biomass (Olvera-Novoa, 1994).

Statistical analysis

The growth, survival, and physicochemical parameter data were analyzed using descriptive statistics, and the results are presented as the means and standard deviations and were identified at the 5% level of significance using Tukey's one-way ANOVA.

RESULTS AND DISCULTS

Water quality parameters

Water quality parameters during the bioassay varied across treatments and over time. Dissolved oxygen (DO) fluctuated because of the increase in organic matter (feces and uneaten feed) and water exchange. The highest value was recorded in the 15.73 mg/L alfalfa treatment on day 34, whereas the lowest value was observed in the 1.5 mg/L mesquite treatment on day 22.

These extreme values deserve special attention. The concentration of dissolved oxygen (1.5 mg/L) recorded in the mesquite treatment was below the recommended range for tilapia, which should be maintained above 3 mg/L to avoid severe stress and hypoxia, with optimal concentrations above 4–5 mg/L to maximize feed conversion (Matter et al., 2025). Such low values can cause reduced feed intake and directly affect growth, which could partially explain the poor performance of the fish in this treatment. Studies in recirculating systems have shown that dissolved oxygen is among the most critical parameters for tilapia welfare and growth (Refaey et al., 2025).

The temperature progressively increased from the beginning of the bioassay and stabilized after day 15. The highest value was recorded in the alfalfa treatment on day 41 at 33°C, whereas the lowest value was observed in the male treatment on day 1 at 27.8°C. Tilapia is a tropical species whose optimal

temperature range is between 26°C and 30°C, although it can tolerate temperatures up to 36°C (Nivelle et al., 2019). The recorded temperatures (27.8–33°C) are within the tolerable range, although values close to 33°C increase oxygen consumption by fish (Leonard; Skov, 2022), which, combined with low DO concentrations, could generate metabolic stress. Research with tilapia has shown that temperature directly affects the feeding rate and conversion efficiency (Verdegem et al., 2016; Leonard; Skov, 2022).

The pH in the three treatments remained similar throughout the experiment, ranging between 5.0 and 5.5, indicating acidic conditions. The optimal pH range for tilapia is between 6.5 and 8.5, although they can tolerate values from 5 to 11 (Matter et al., 2025). pH values less than 6.5 can reduce growth and cause damage to the gills and skin (Boyd, 1998). The acidic pH recorded (5.0–5.5) could have negatively affected the metabolism and feed efficiency of the organisms in all the treatments. Studies in recirculating systems have reported that the pH tends to decrease because of nitrification and respiration, which require constant monitoring (Refaey et al., 2025).

Overall, water quality parameters, although mostly within tolerable ranges, presented suboptimal conditions (low DO and acidic pH) that likely contributed to the low growth observed in all the treatments, including the control. Previous studies have shown that when ammonia begins to accumulate or when water quality conditions deteriorate, fish respond by reducing their feeding activity, which directly affects growth and feed conversion (Zhang et al., 2016).

Growth, survival, and feed conversion ratio

The results of the productive parameters are presented in Table 2. Survival was greater than 70% in all the treatments, with 93.33% in the alfalfa treatment, 77.77% in the mesquite treatment and 71.11% in the control. These values are acceptable considering the experimental conditions and management and are similar to those reported in other studies with plant ingredients (González-González, 2023; Lan et al., 2023).

Table 2. Productive parameters of juvenile tilapia.

Treatment	Initial weight (g)	Survival (%)	Final weight (g)	FCR
Mesquite	5 ± 0.28	77.77	8.0 ± 0.76	9.5
Alfalfa	5 ± 0.34	93.33	12.0 ± 0.85	10.0
Control	5 ± 0.31	71.11	20.0 ± 0.91	4.1

The final weight differed notably between the treatments. In the control treatment, the yield reached 20.0 ± 0.91 g, which was significantly greater than that in the alfalfa (12.0 ± 0.85 g) and mesquite (8.0 ± 0.76 g) treatments. This difference suggests that partial substitution of fish meal with plant meals,

in terms of proportions used, did not maintain the same growth level as the commercial diet did. Similar results were reported by Barragán et al. (2017), who, when evaluating different inclusion levels of plant meals in tilapia diets, reported that a diet with higher fish meal content (54.9%) resulted in better growth than a diet with a higher proportion of plant ingredients, although the FCRs obtained in their study (2.53–2.60) were notably better than those of the present work (Mohammadi et al., 2020).

The feed conversion ratio (FCR), which indicates the amount of feed needed to produce a unit of live weight, was notably more efficient in the control treatment (4.1) than in the alfalfa (10.0) and mesquite (9.5) treatments were. These values indicate that fish fed plant-based diets required more than twice the amount of feed to gain the same weight as those on the control diet did. These FCRs are higher than those reported in the literature for tilapia under optimal conditions, which usually range between 1.5 and 2.5 (El-Sayed, 1998), suggesting that the general experimental conditions (water quality, diet formulation) were not adequate.

The results obtained can be explained by multiple factors. First, the nutritional quality of the plant meals used likely differed significantly from that of the fish meal in terms of the amino acid profile and digestibility. Studies with protein hydrolysates in tilapia have shown that the peptide and free amino acid profile of the ingredient influences acceptance and digestibility (Afuang et al., 2003). Alfalfa and mesquite meals, although they provide protein, may be deficient in essential amino acids such as lysine, methionine, and threonine, which are frequently limiting plant ingredients (Yousif et al., 1994).

Second, the presence of antinutritional factors in plant meals may reduce protein digestibility and nutrient availability. Like other legumes, alfalfa may contain saponins, tannins, and protease inhibitors that affect digestion (Francis et al., 2001). Studies in rainbow trout have shown that phytic acid, which is present in many vegetables, can reduce feed intake and growth when it exceeds certain threshold levels (Kaiser et al., 2021). When new ingredients are evaluated in the literature, ensuring that digestible protein, essential amino acids, and energy remain balanced is essential (García Meilán, 2016).

Similar results have been reported in studies with moringa meal included in tilapia diets, where González-González (2023) reported that the inclusion levels of 25–100% moringa meal did not significantly differ between treatments, although survival was 100% for the lowest inclusion levels. In contrast, Rivas-Vega et al. (2010) evaluated the inclusion of moringa leaf meal in tilapia cultured in seawater and reported that up to 20% substitution of sardine meal protein did not affect growth, with a protein digestibility of 89.1%.

Studies with other plant ingredients, such as chaya, have shown promising results. Poot-López et al. (2012) evaluated the partial substitution of formulated feed with raw chaya leaves in tilapia and reported that compared with the control treatment, treatments with 50–75% formulated feed resulted in weight gains similar to those of the control, and the feed conversion ratio of formulated feed decreased between 5.38 and 40.23% depending on the climatic season.

The high survival in the alfalfa treatment (93.33%) suggests that, although growth was limited, plant-based diets were not toxic and allowed the maintenance of organisms. This finding is consistent with reports indicating that moderate inclusion of alternative ingredients can maintain survival even if growth is compromised (Fasakin et al., 2005). The lower survival in the control treatment (71.11%) is atypical and could be related to uncontrolled factors, such as water quality in those specific experimental units.

Notably, the FCR values obtained in this study (4.1–10.0) are high compared with those reported in the literature. Fontainhas-Fernandes et al. (1999) reported FCRs between 1.5 and 2.5 for tilapia fed plant-protein-based diets. Köprücü and Özdemir (2005) reported apparent digestibility coefficients for various plant ingredients in tilapia ranging from 70–90%, suggesting that good efficiency can be achieved with adequately processed plant ingredients.

The processing of plant ingredients is crucial for improving their digestibility. Studies on snook have shown that extrusion can significantly improve the digestibility coefficients of plant meals, which can reach protein digestibility values between 73.4 and 96.9% (Hernández, 2019). Microbial fermentation has also been consolidated as an effective strategy to reduce antinutritional factors and improve nutrient bioavailability in plant ingredients for use in aquaculture (Ahmed et al., 2025).

CONCLUSIONS

Compared with the control diet, partial substitution of commercial fish meal with mesquite (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*) meals in the diet for juvenile tilapia (*Oreochromis niloticus*) maintained acceptable survival (above 77%) but resulted in significantly lower growth and higher feed conversion ratios.

In terms of final weight (12.0 g vs 8.0 g) and survival (93.33% vs 77.77%), the alfalfa meal treatment resulted in better performance than the mesquite treatment did, suggesting that alfalfa could be a more promising alternative for future formulations.

Water quality parameters (temperature, dissolved oxygen, and pH) remained mostly within tolerable ranges for the species, although suboptimal conditions (mainly low oxygen and acidic pH) were recorded that may have affected the metabolism and feed efficiency of the organisms.

Mesquite and alfalfa meals represent local resources with potential for use in aquaculture, but further research is needed to optimize their use and maximize their productive efficiency, considering aspects such as processing, amino acid supplementation, and optimal inclusion levels.

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