



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-Mosc!so, members of the Consolidated Academic Group ITVAYA-CA-3.

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The Editors

Prologue	4
Agronomy	
Chapter 1	9
The reduction in chemical fertilization combined with PGPM under a controlled deficit irrigation system for maize	
Chapter 2	22
Effects of sowing date and size on the physical and physiological quality of bread wheat seeds in southern Sonora, Mexico	
Chapter 3	35
Morphophysiological response and yield of tomato (<i>Solanum lycopersicum</i> L.) to growth regulators and glutamic acid	
Chapter 4	49
Influence of benzoxazinoid metabolites in maize roots interacting with diverse microbes	
Chapter 5	64
Comparison of RGB spectrum vegetation indices to estimate agronomic variables in wheat and oats	
Chapter 6	74
Alleviation of saline stress in maize and peanut plants induced by <i>Bacillus aryabhattai</i> strain CMAA 1363	
Chapter 7	92
Bacterial consortia as a sustainable strategy for disease management and productivity improvement in maize	
Chapter 8	109
Agronomic management for oat seed production in the Bajío region, Mexico	
Chapter 9	125
Risk factors in loading and unloading operations of agricultural products in land transport units at a company in Veracruz, Mexico	
Chapter 10	145
Physicochemical characterization of four varieties of durum wheat (<i>Triticum durum</i> Desf.) under full irrigation and reduced irrigation conditions	
Chapter 11	161
Antifungal activity of edible chitosan coatings and <i>Coriandrum sativum</i> L. essential oil against <i>Colletotrichum gloeosporioides</i> and their application on mango fruits	
Chapter 12	175
Agricultural gypsum with micronutrients in citrus cultivation	
Chapter 13	186

Initial Agronomic Adaptation of *Stevia rebaudiana* Bertoni in the High Mountain Region of Veracruz and Sustainable Management of *Planococcus citri* Using a Neem-Based Bioinsecticide

Chapter 14 197

Evaluation of yield components of two F1 maize hybrids under controlled deficit irrigation in a moderately saline vertisol soil in Yaqui Valley, Sonora, Mexico

Aquaculture

Chapter 15 207

Effects of partially replacing fish meal with mesquite pod meal (*Prosopis juliflora*) on the growth of Nile tilapia (*Oreochromis niloticus*)

Chapter 16 220

Consumer preferences and value-added opportunities in the commercialization of *Oreochromis niloticus* in Tenosique, Tabasco, México

Chapter 17 228

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

Chapter 18 239

Hydrolysate of the alga *Spirulina* influences the proliferation, growth, and development of *Spathiphyllum uxpanapense* in vitro

Chapter 19 251

Growth of the wild juvenile common snook *Centropomus undecimalis* in captivity

Coastal Sciences

Chapter 20 260

Notes on the recreational fishing of snook (*Centropomus* spp.) in the coastal zone of Centla, Tabasco, Mexico

Chapter 21 268

Linkage among climatic variation and artisanal fisheries behavior of common snook (*Centropomus undecimalis*) in tropical Atlantic coast

Environmental Sciences

Chapter 22 277

Fungal-based arsenic bioremediation: linking enzymatic detoxification mechanisms with bioreactor engineering

Chapter 23 292

Simplified dynamic modeling for scenario analysis of the anaerobic digestion of fruit and vegetable waste

Chapter 24 303

Impact of biofertilizers on wheat (*Triticum aestivum* L.) yield in different environments of the Bajío region of Guanajuato

Chapter 25 326

Genomic Reprogramming and Epigenetic Memory: Transforming Somaclonal Variation into a Tool for Climate Resilience

Chapter 26	351
Research on the use of rhizobacteria for the biodegradation of herbicides and insecticides in agriculture and their effects on environmental protection	
Index	360
About the editors	362

Effects of partially replacing fish meal with mesquite pod meal (*Prosopis juliflora*) on the growth of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Food represents one of the main costs in aquaculture; therefore, the search for alternative protein sources to fish meal is a priority for the sustainable development of the sector. In this study the effects of partially replacing fish meal with mesquite (*Prosopis juliflora*) pod and seed meal on the growth, survival, and proximate composition of juvenile Nile tilapia (*Oreochromis niloticus*) were evaluated. Mature pods were harvested, dried, ground, and sieved to obtain meals, which were used to formulate experimental diets using the Pearson square method. Three treatments were established: a pod meal diet, a seed meal diet, and a control diet. The fish were fed during a 165-day experimental period, with weight and length recorded every 15 days, in addition to monitoring physicochemical water parameters. Proximate analysis revealed that the formulations containing pod meal had adequate protein and carbohydrate contents. At the end of the experiment, the fish fed pod meal achieved the highest growth values, with an average weight of 175.7 g and a length of 248.49 mm, outperforming the treatments with seed meal and commercial feed. Furthermore, no mortality was recorded in the groups fed plant-based meals, whereas the cumulative mortality rate of the control group was 10%. The results indicate that mesquite pod meal represents a viable and sustainable alternative as a partial substitute for fish meal in tilapia diets, contributing to improved growth and reduced dependence on conventional.

Keywords: Nile tilapia, Fish meal, Alternative protein sources.

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INTRODUCTION

In recent decades, aquaculture has increased significantly, becoming a key source of fish for human consumption (FAO, 2024). Its role in global food security is crucial, as it provides protein, essential fatty acids, and micronutrients, especially in developing countries (Bogard et al., 2017). Furthermore, it has driven economic and agricultural development in various regions (Kariuki et al., 2024). According to the FAO, by 2050, fish and aquaculture production will need to increase by more than 60% to meet global demand (Subasinghe, 2014).

Tilapia is among the most widely farmed species because of its rapid growth, disease resistance, and adaptability to various environmental conditions (Méndez-Martínez et al., 2018). Nile tilapia (*Oreochromis niloticus*) stands out for its ease of rearing and market acceptance, making it a profitable alternative for large-scale aquaculture (Rodríguez-Hernández et al., 2025).

One of the greatest challenges in aquaculture is the use of fish feed, as it accounts for between 30% and 70% of production costs (Munguti et al., 2021). Fish meal is the predominant protein source because of its high nutritional value and digestibility. However, its growing demand has led to economic and environmental problems, increasing costs and reducing its availability. This has prompted the search for sustainable alternatives (Annamalai et al., 2021).

Various plant- and animal-based protein sources have been explored to reduce the dependence on fish meal. A promising resource is mesquite (*Prosopis spp.*), a fast-growing perennial shrub found in semiarid regions worldwide (Armijo-Nájera et al., 2019; Chaturvedi et al., 2013). In Mexico, it is estimated that there are more than four million hectares of mesquite, notably *Prosopis leavigata*, *Prosopis glandulosa*, and *Prosopis juliflora* (Andrade-Montemayor et al., 2011). This species is ecologically significant because of its ability to fix nitrogen in the soil, improve fertility, reduce erosion, and provide shelter for wildlife (Villegas-Espinoza et al., 2014).

Mesquite has been evaluated as a forage alternative in arid regions, where feed availability for livestock is limited (Armijo-Nájera et al., 2019). Its pods have been incorporated into the diets of cattle, sheep, camels, buffalo, rabbits, and poultry in South America, Africa, and India. Its protein and soluble sugar contents improve the quality of animal diets and provide a valuable nutritional resource during times of scarcity (Armijo-Nájera et al., 2019).

The use of mesquite pod meal in fish feed is an area of study that is still underdeveloped but has high potential because of its nutritional characteristics. As a legume, mesquite shares properties with other protein sources widely used in aquaculture, such as soybean meal. The latter has been incorporated into various aquaculture diets because of its availability and nutritional profile, although its complete replacement with fish meal depends on the specific needs of each species (Ahmad et al., 2020). However, the presence of antinutritional factors, such as lectins and protease inhibitors, in soybean meal can negatively impact fish growth and health (Veer et al., 2021). In this context, mesquite pod meal emerges

as a viable alternative, as its protein composition positions it as a potential substitute for soybean meal in the formulation of sustainable aquaculture diets.

Given the high cost of fish meal and the decline in the number of fish populations used in its production, finding sustainable solutions is crucial. The development balanced diets that combine more accessible protein sources without compromising nutritional quality is a viable strategy (McGoogan et al., 1997). Studies have shown that the use of plant proteins and animal byproducts can reduce costs without affecting fish growth or health. However, completely replacing fish meal remains a challenge because of the need to maintain an adequate balance of essential amino acids and minimize the effects of antinutritional factors (Ahmad et al., 2020). Aquaculture faces significant challenges in terms of the sustainability of inputs, particularly in the area of fish feed. Exploring alternative protein sources, such as mesquite pod meal, presents an opportunity to reduce the dependence on fishmeal and mitigate environmental impacts. To achieve an effective transition, it is essential to continue investigating its impact on fish performance, feed conversion, and the quality of the final product (Kariuki et al., 2024). The objective of this study was to evaluate the effects of partially replacing fish meal with mesquite pod meal (*Prosopis juliflora*) on the growth, health, and body composition of juvenile Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Selection and Preparation of Plant Material

The experiments were conducted at the National Technological Institute of Mexico/Yaqui Valley Technological Institute which is located in Bacum, Sonora, Mexico. Mature pods were collected from the mesquite tree (*Prosopis juliflora*) and manually selected in a wild area of southern Sonora, Mexico. The selection process involved careful visual inspection to remove pods showing visible signs of physical damage, insect infestation, or fungal growth, with the aim of ensuring the microbiological quality and safety of the final product. This stage is critical, as the presence of contaminants can affect both the nutritional profile and the safety of the feed intended for animal consumption.

*Production of Mesquite Pod Flour (*Prosopis juliflora*)*

Once selected, the pods underwent a natural drying process for 15 days. The average temperature in the shade reached 37 °C; measurements were taken daily, which promoted a rapid reduction in surface moisture. This combined strategy of solar and shade drying prevents overheating of the plant material, which could lead to the degradation of nutritional and phytochemical compounds, especially polyphenols and other natural antioxidants present in mesquite (Moreno-Limón et al., 2011; Ochoa-Meza et al., 2023).

Grinding and Sifting

After drying, the completely dehydrated pods were subjected to mechanical grinding using a food-grade hammer mill. The objective was to reduce the material to a homogeneous powder. The resulting flour was sieved using a stainless-steel sieve with 80 mesh, thus ensuring the production of fine, uniform particles suitable for incorporation into feed mixtures and to facilitate digestibility in juvenile organisms. This degree of fine grinding is also important for maximizing the bioavailability of the nutrients contained in the plant product (Geankoplis, 1998).

Proximate Chemical Analysis

Quantitative analyses were performed using standardized procedures of the Association of Official Analytical Chemists AOAC (2000).

Moisture Content

The total moisture content of the food was determined using the AOAC method (950.46). To do this, the sample was dried in a forced-air oven at 105 °C for 4 to 5 hours to completely remove the moisture present. The total moisture content was subsequently calculated using the following equation:

$$\text{Moisture Content (\%)} = \frac{\text{Weight loss}}{\text{Starting weight}} \times 100$$

Total ash

Three grams of powdered sample was placed in each crucible and calcined at 550 °C for 6 hours. Once the process was complete, the crucibles were allowed to cool in a desiccator and were then weighed to determine the ash content.

$$\text{Total ashes (\%)} = \frac{W - Z}{N} \times 100$$

where W is the weight of the crucible and the ash; Z is the weight of the empty crucible; and N is the weight of the sample.

Determination of Lipids

The determination of crude fat in the powdered sample was performed using the Soxhlet method, in accordance with AOAC method 920.39 (2000), employing petroleum ether as the solvent. Three zero-point grams of sample were placed in prelabeled thimbles, which were subjected to an extraction process

for 24 hours in a Soxhlet apparatus. Upon completion of the extraction, the ether was evaporated, the flask was cooled, and it was weighed to calculate the amount of fat extracted.

$$\text{Lipids (\%)} = \frac{\text{Weight of lipids}}{\text{Sample weight}} \times 100$$

Crude protein analysis

The crude protein content was determined using the micro-Kjeldahl method, in accordance with the methods of the AOAC (990.04). To do this, 0.15 g of flour was weighed and placed in a 250-mL Kjeldahl flask, along with 2.5 mL of 98% sulfuric acid and a catalyst. The mixture was heated until complete clarification was achieved. It was then allowed to cool, and 7 mL of distilled water was added.

The digested solution was transferred to a micro-Kjeldahl distillation apparatus, where 30% (w/v) sodium hydroxide was added to initiate distillation. The released ammonia was captured in a 2% boric acid solution, with methyl red as an indicator. The color change from red to green indicated complete capture of the ammonia. Finally, the solution was titrated with 0.1 N hydrochloric acid until a color change indicating the endpoint was observed. A blank sample was included in the analysis, and the nitrogen percentage was calculated using the following equation.

$$\text{Protein (\%)} = \frac{(\text{N of HCl})(\text{Volume of HCl used})(1.4 \text{ g/mol})(6.5)}{\text{Sample grams}}$$

Nutritional Design of the Experimental Diet

Crude protein analysis

The diet was designed using Pearson's square method, a tool widely used in animal nutrition to balance ingredients on the basis of their nutrient content and the needs of the target organism. For this study, a four-variable model was used, allowing for precise adjustment of the proportion of each feed ingredient, and ensuring that protein, energy, lipid, and fiber requirements were met, in addition to essential vitamins and minerals.

Sequential mixing

The ingredients were mixed in a horizontal-shaft mixer, starting with those present in the highest proportions, such as vegetable and protein flours. Subsequently, the ingredients with lower volumes, such as vitamin-mineral premixes, were incorporated, and finally, the oils were added. This sequential procedure ensures a homogeneous mixture and an even distribution of nutrients throughout the feed matrix.

Addition of Water and Homogenization

Following dry mixing, potable water was added; it was incorporated slowly while continuous mixing was maintained. Fifty milliliters of water was added to every 100 grams of dry mixture, allowing certain compounds to be activated and facilitating the formation of a cohesive mass suitable for the pelletizing process. Hydrating the mixture also improved the texture of the final pellet and reduced dust production during handling and storage.

Manual pelleting

Once a moist, homogeneous mass was obtained, it was left to rest and aerate at room temperature for approximately two hours. This resting period allows the ingredients to properly absorb moisture, improving the structural integrity of the pellet. The mixture was subsequently subjected to a pelletizing process using a manual meat grinder, fitted with a nozzle to form compact feed cylinders. This artisanal method is efficient for pilot or small-scale studies, where detailed control over the texture and size of the pellets is needed.

Final drying of the pellets

The obtained pellets were placed on clean trays and subjected to a drying process for two days. This extended drying reduces the final moisture content of the product to levels safe for storage, minimizing the risk of microbial growth. Once dry, the pellets were ground into particles suitable for feeding to juvenile organisms.

Measurement of feeding and parameter

During the bioassay, the fish were fed three times a day, and biometric assessments were conducted every 15 days over a 90-day period to adjust the feed ratio of the basis of biomass. The assessments involved measuring the weight and length of each individual. To calculate the biomass of each experimental unit, ten organisms per tank were weighed. The length of each fish was determined using an ichthyometer, measuring from the head to the tip of the caudal fin (Afram et al., 2021; Cadena-Cadena et al., 2023).

To maintain optimal water conditions, parameters such as temperature, pH (using an Ohaus ST10 meter), and dissolved oxygen (measured with a YSI oximeter) were recorded twice daily, at 8:30 a.m. and 1:00 p.m. (Cadena-Cadena et al., 2023). Water was siphoned twice a week to remove settleable particles, and 25% of the total water volume was replaced daily. Additionally, 50% of the water was replaced every ten days by washing the aeration stones (Cadena-Cadena et al., 2023; Claudett et al., 2022). Mortality was determined by counting all the specimens that died during the three replicates of the experiment and was expressed as the ratio of the number of dead fish to the total number of organisms (Afram et al., 2021).

$$Mortality = \left[\frac{\text{Number of dead animals}}{\text{Total number of animals}} \right] \times 100$$

Statistical Analysis

Means and their respective standard deviations were calculated for the data obtained. Prior to the statistical analysis, the assumptions of normality and homogeneity of variance were verified. To evaluate possible differences between treatments, one-way analysis of variance (ANOVA) was performed. Differences for which the p-value was less than 0.05 were considered indicate statistical significance. All analyses were performed using SPSS statistical software, version 23.

RESULTS AND DISCUSSION

Response of Growth Performance and Proximate Composition to Pod and Seed Meal Diets

In this study the growth of tilapia fed mesquite pod and seed meal was compared to that of control group that received commercial feed. The results revealed differences in growth patterns among the different treatments, highlighting the potential of mesquite pod meal (*Prosopis juliflora*) as an effective nutritional source for aquaculture species.

During the 165-day experimental period, both the proximate composition of the feed formulations and the growth in weight and length of the organisms fed pod meal, seed meal, and a control treatment based on commercial feed were evaluated. Overall, all the groups showed a progressive increase in growth parameters, although differences in growth efficiency and rate were observed among the treatments.

From the outset, weight (6.20 g) and height (66.6 mm) of the organisms fed seed meals were greater than those of those fed pod meal (5.5 g; 63.7 mm) and the control group (3.2 g; 47.25 mm). Concurrently, proximate composition analysis indicated that the 50/50 formulation—an equal mixture of pod meal and seed meal—contained 17.69% protein, 6.39% fat, 63.17% carbohydrates, 3.46% ash, and 9.30% moisture, with values intermediate to those of its individual components.

At 60 days, the control group weighed more (38.9 g) than the pod (35.1 g) and seed (30.8 g) treatments did. However, in the later stages, the group fed pod flour exhibited more rapid growth. At 90 days, this treatment resulted in a weight of 62.3 g and a length of 166.76 mm, while the seed group maintained the same weight (62.3 g) but with a shorter length (139.2 mm), and the control reached 72.8 g with a length of 130.51 mm. In terms of nutritional content, the pod/commercial feed and seed/commercial feed formulations resulted in the highest protein levels (29.94% and 26.40%, respectively), whereas the control group had 23.80% protein, along with the highest levels of fat (8.97%) and ash (8.97%).

On day 165, highest values of weight and length were recorded for the fish fed pod meal in the study: 175.7 g and 248.49 mm, respectively. In comparison, the group fed seed meal reached 121.2 g and 196.7 mm, and the control group reached 138.7 g and 162.44 mm. These results, supplemented by proximate analysis, suggest that vegetable meals, particularly pod meal, not only offer a balanced nutritional profile but also promote superior growth in the medium and long term under experimental conditions.

Table 1. Proximate analysis of the 2 treatments based on flour and seeds of the mesquite pod (*Prosopis juliflora*) and the control treatment.

formulation 50/50	%Protein	%Lipids	%Carbohydrates	%Ash	%Moisture
Pod Flour	16.91	7.21	62.9	3.76	9.22
Seed Meal	18.47	5.57	63.43	3.16	9.37
Commercial Pod/Food	29.94	6.59	45.94	8.32	9.21
Seed/Commercial Feed	26.4	4.94	50.55	8.77	9.34
Control	23.8	8.97	48.01	8.97	10.25

Table 2. Mean weight (g) and length (mm) of fish fed pod meal, seed meal, and control diet during the 165-day experimental period.

Time (days)	Sheath		Seed		Control	
	weight (g)	size (mm)	weight (g)	size (mm)	weight (g)	size (mm)
0	5.5	63.70	6.20	66.6	3.20	47.25
15	10.2	88.1	9.70	79.2	12.10	80.37
30	17.3	102.8	13.90	95.8	23.40	86.22
45	22.6	115.2	22.50	101.7	19.80	99.63
60	35.1	130.5	30.80	116.9	38.90	109.89
75	55.9	145.9	54.80	133.3	50.40	122.22
90	62.3	166.76	62.30	139.2	72.80	130.51
105	85.7	199.54	73.80	159.5	66.70	137.65
120	106.7	207.46	80.90	165.8	91.30	143.18
135	135.8	218.79	99.80	173.7	105.00	146.80
150	145.6	243.43	104.80	182.2	112.50	160.01
165	175.7	248.49	121.20	196.7	138.70	162.44

In the initial phase of the study, during the first 30 days, superior growth was observed in the organisms fed mesquite pod meal (*Prosopis juliflora*) compared with those fed mesquite seed meal (*Prosopis juliflora*) and commercial feed. This initial difference could be explained by the high energy density and high bioavailability of nutrients present in the formulated feed, even more so than the commercial feed that was designed specifically for tilapia (Cabrera-B. et al., 2001; Guimarães et al., 2012). Starting on day 75 of the experiment, the fish fed the diet containing mesquite pod flour began to show a more rapid growth pattern compared than those in others treatments did, both in terms of weight gain and increase in length. This behavior suggests a possible progressive physiological adaptation to the feed formulated

with plant-based components, which could be associated with improved feed conversion efficiency or greater bioavailability of the nutrients present in the pod meal during later stages of growth (Armijo-Nájera et al., 2019). On the other hand, regarding survival, none of the fish fed plant-based feeds (pods and seeds) died throughout the experimental period, indicating good tolerance to these alternative diets. However, the control group fed commercial feed exhibited a cumulative mortality rate of 10%, which could be related to factors such as the immune response to specific ingredients in the commercial feed or differences in water quality resulting from the type of diet (Abdel-Tawwab et al., 2007; Li et al., 2018).

These findings suggest that mesquite pod flour not only has an adequate nutritional profile but also may induce positive physiological adaptations in the medium and long term. The progressive increase in size observed in this group could be linked to the presence of slowly digestible carbohydrates, soluble fibers, and secondary compounds that, far from acting as antinutrients, could improve digestive efficiency and energy metabolism (de Melo Cavalcante et al., 2022).

In contrast, the performance of the group fed seed meal was more variable. Although the animals initially grew rapidly, the weight gain rate subsequently stabilized, resulting in a final yield lower than that of the pod meal group. This effect could be related to the presence of antinutritional compounds typical of raw seeds, such as trypsin inhibitors, saponins, or tannins, which interfere with protein digestibility and the utilization of essential amino acids (de Melo Cavalcante et al., 2022). Additionally, the slightly lower lipid and protein contents in the seed meal, than in the pod meal, may have limited tissue synthesis in advanced stages of development (de Souza et al., 2019).

Notably, although the commercial feed showed a continuous and stable growth pattern, the height results were lower than those observed in the groups fed plant-based diets. This could suggest that plant-based meals induce greater somatic elongation, possibly because of a different macronutrient ratio or a fatty acid and fiber profile that stimulates body expansion without necessarily increasing total mass (Figueiredo-Silva et al., 2013).

Several studies have reported that the use of ingredients from nonconventional sources in aquaculture can be a viable strategy provided that the availability of limiting amino acids is ensured and the presence of antinutritional factors is controlled (Cadena-Cadena et al., 2023; de Souza et al., 2019; Tibbetts, 2018). The results of the present study are consistent with those of previous research highlighting the use of nonconventional legumes such as *Canavalia ensiformis* and *Vigna unguiculata*, whose starches present nutritional properties comparable to those of corn in tilapia diets (Olivera-Novoa et al., 1997).

Furthermore, it is important to consider the influence of other environmental factors such as salinity, temperature, and stocking density, which can significantly alter the performance of the culture. Studies conducted on tilapia hybrids have shown that they can grow efficiently in environments with salinities of up to 37‰, and that the stocking density and protein formulation of the diet directly impact

the feed conversion and growth rate (Watanabe, 1991; Watanabe et al., 1997). Although salinity was not controlled as an experimental variable in this study, the sustained increase in the use of vegetable flours suggests good physiological adaptation of the organisms to the farming system. Another study conducted in Brazil using mesquite pods indicated that the nutritional profile is comparable to that of corn in terms of dry matter digestibility, gross energy, and protein, as well as essential amino acids such as lysine and methionine. These findings suggest the high potential for digestive utilization by *Oreochromis niloticus*, which supports its use as an alternative ingredient in extruded diets. However, although high levels of starch and sucrose were observed in the diets, a 20% substitution resulted in lower protein utilization, as evidenced by reduced plasma amino acids and increased activity of transaminase and deaminase enzymes, suggesting increased catabolism of endogenous proteins without signs of physiological stress or adverse effects on overall health or survival of the fishs (de Souza et al., 2019). Furthermore, in the São Francisco Valley in Brazil and Benue, Nigeria, it was demonstrated that mesquite pod meal can be incorporated into extruded diets for Nile tilapia fingerlings at levels up to 38.67% without compromising production performance or fish health. Although significant differences were observed in parameters such as the feed conversion ratio and blood glucose levels, digestive enzyme activity and transaminases, as well as hematological variables, no relevant alterations were detected. These results suggest a viable alternative for reducing the cost of tilapia feed, which contributes to the sustainability of aquaculture without negatively affecting the growth or physiological condition of the fish (Aroyehun et al., 2019; Nascimento et al., 2019). The use of plant-based flours in aquaculture diets represents a sustainable and economically viable alternative, especially in regions where commercial feed costs are high. The use of mesquite pods as a feed ingredient could significantly reduce the dependence on animal-based or imported ingredients, contributing to the development of sustainable production systems.

CONCLUSIONS

Mesquite pod flour represents a promising plant-based alternative for the formulation of extruded diets for Nile tilapia (*Oreochromis niloticus*), and shows performance comparable to or better than that of commercial feeds at certain growth stages. Its inclusion at levels of up to 50% did not negatively affect growth parameters, supporting its potential as a partial substitute that can improve economic and environmental sustainability in aquaculture. However, further studies are needed to optimize inclusion levels and evaluate the environmental factors that influence mesquite growth, as well as to promote management and conservation strategies that enhance its ecological, cultural, and economic value.

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A

Alfalfa, 256, 258, 261
 anaerobic digestion, 324, 325, 326, 330, 331
 Anthracnose, 184, 189
 Aquaculture, 280, 281
Arachis hypogea, 76
 Arsenic, 307, 309, 311, 316

B

Benzoxazinoids, 58
 Biomass yield, 9, 16, 17
 Bioreactor, 310, 316
 bioremediation., 394
 Borlaug, 25, 27, 28, 29, 30

C

calcium prohexadione, 36, 38, 41, 42, 43, 44
 Centla, 289, 290, 291, 292, 293, 294
Centropomus undecimalis, 297, 298, 299, 303, 304

Ch

Chitosan, 184, 185, 191
 Chlorophyll content, 15, 16

C

consortia, 94, 95, 97, 99, 100, 101, 103, 104
Coriandrum sativum L., 184, 185, 186, 190, 191, 194
 CPUE, 289, 291, 292, 294
 cytokinins, 36, 38, 40, 41, 42, 43, 44, 46

D

Dates, 116, 122, 133, 139, 146
 Degradation, 392
 Detoxification, 310, 311

E

ENSO, 297, 298, 300

F

Fertilization, 339
 fish, 232, 233, 234, 237, 239, 240, 242
 fruit and vegetable waste, 324, 325, 331

G

Gluten, 172, 175
 Grain quality, 174
 Growth, 260

H

hybrids, 222, 223, 224, 225, 226, 227, 228
 Hydrolyzed extract, 273
 Hypopótamo, 13, 18

I

In vitro culture, 269
 irrigation, 222, 223, 225, 226, 227, 228

L

Locations, 344

M

maize, 50, 51, 53, 54, 55, 56, 57, 222, 223, 225, 227, 228
 Maize, 94, 95, 97, 98, 99
 Mango, 188
 mesquite, 232, 233, 234, 238, 240, 241, 242
 Mesquite, 256, 258, 261, 262
 Microbial, 99, 101, 104
 Morphometric analysis, 289, 293
 morphophysiological, 36, 37, 38, 45, 46

N

NDVI, 9, 10, 12, 14, 15, 18
 nile tilapia, 232
 NOM-017-PESC-1994, 289, 290, 291, 293, 294

O

Oat, 67

Oreochromis niloticus, 232, 233, 234, 241, 242, 246, 247

P

paclobutrazol, 36, 38, 40, 41, 42, 43, 44, 45, 46, 47

Pantanos de Centla, 290

Particle size, 324

physiological quality, 22, 23, 24, 29, 31

Planting, 115, 116, 119, 122, 123, 131, 133, 135, 139, 140

Prosopis juliflora, 232, 233, 234, 238, 240

Protein content, 171

R

Recreational fishing, 289

Reduced irrigation, 167, 178, 179

RGB, 65, 66, 68, 69, 70

Rheological properties, 175

S

S/I ratios, 324, 328, 329, 330, 331

saline, 222, 223

SDS sedimentation, 171, 176

size, 22, 23, 24, 25, 26, 27, 28, 29, 31

Spirulina sp, 267, 269, 270, 276

substrate-to-inoculum, 324, 325, 329, 331

T

Tabasco, 289, 290, 291, 292, 294, 297, 298, 299, 300, 301, 302, 303, 304

Tilapia, 260

tomato, 36, 37, 38, 40, 42, 43, 44, 45

Treatments, 339, 341, 343, 344, 349, 351, 352

V

Varieties, 119, 122, 123, 136, 139, 141

Vegetation indices, 65, 69

VFAs, 325, 326, 328, 331

volatile fatty acids, 325, 326

W

Water stress, 173

Weight, 116, 118, 119, 122, 123, 133, 135, 136, 139, 140, 141

wheat, 22, 23, 24, 25, 26, 27, 28, 29, 31

Wheat, 67

Wild juveniles, 283

Y

yield, 22, 24, 25, 27, 28, 29, 31

Yield, 116, 118, 119, 120, 122, 123, 125, 133, 135, 136, 137, 139, 140, 141, 143, 343, 345, 351, 352

Z

Zea mays, 75, 76, 77, 85, 222

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