Fuel Cells Bulletin
ISSN: 1464-2859

Evaluation of Fermented Apple Bagasse (Manzarine) In Tilapia Feed (*Oreochromis Niloticus,* Rocky Mountain Variety) In a Semi-Controlled System

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SUMMARY

The objective of this work was to evaluate the use of a fermented apple (manzarine) compound rich in functional compounds and yeasts in the behavior of Tilapia (*Oreochromis niloticus*) Rocky Mountain variety in a semi-controlled aquaculture system. The variables evaluated were weight gain, length, width and height of the fish, as well as dissolved oxygen, temperature and pH. They were separated into two diets, including 10% Manzarina (MAN Treatment), comparing it against a control food. During the study, 210 three-week-old fingerlings were used, which for evaluation 21 fish tanks in the growth stage and two 1,800-liter Rotoplás ® containers for the development stage were used. For the statistical analysis of the data, an ANOVA analysis of variance was used to evaluate the productive variables. In all variables, the interaction of treatment with the covariates "day, oxygen, pH, and temperature" was tested. There was no statistical difference between treatments based on fish growth ($P > 0.05$), however, there was a statistical difference in the influence of the environment on growth ($P \leq 0.05$), where dissolved oxygen had a significant influence ($P \leq 0.05$) with daily gain in weight, length and height, and temperature and pH significantly influenced ($P < 0.05$) the width of the fish.

Keywords: Oregano essential oil, zeolite, yeast, molasses.

INTRODUCTION

Aquaculture plays a key role in the growth and stability of the food system, contributing to the conservation of aquatic species, increasing nutrition levels, reducing environmental impacts, producing raw materials for industry and pharmacology, as well as promoting self-employment. Aquaculture is the development of aquatic organisms under controlled or semi-controlled conditions, this being one of the most economical options for obtaining protein of animal origin. In the production of fish by aquaculture, 50% or more of the expenditures are destined for feed, so it is necessary to develop feed alternatives with high digestibility with added nutritional benefits, economic and high protein quality, as well as nutraceutical supplements that optimize the health and growth of the fish, thus achieving a greater productive benefit. Thiessen *et al.*, 2003; Kaiser *et al.,* 2022.

The main challenge in aquaculture lies in the fact that this sector is experiencing very rapid growth, currently being the one with the highest rate of expansion within food production. It is estimated that, by 2050, the world's population will reach 9 billion people, which will generate greater pressure on the food supply chain Sadigov, (2022). However, fishing, as a source of protein for both human consumption and animal production, is not a long-term sustainable solution, as there are no fish stocks capable of supporting COMEPESCA, (2022).

Therefore, there are protein sources of vegetable origin available on the market such as soybean paste, which has an excellent amino acid profile which represents an excellent opportunity as a source of food for fish but unfortunately due to its high demand it is expensive and scarce, Dong *et al.*, 2013; FAO, 2020.

Manza fruit waste has a high nutritional value; however, it is still little explored, so it is a source of current study by researchers to quantify its nutritional potential and how nutritionists use it as a strategy in aquaculture diets. Fermented apple bagasse (manzarine) represents an option in the diet of fish produced through aquaculture, because being a product of microbial fermentation of by-products of the apple industry, it represents an economical alternative to add protein of microbial origin with high digestibility to the diet, in addition to offering added value due to the presence of antioxidant compounds (phenolic compounds) of the apple as well as fermentation yeasts that add probiotic capacity to the diet, Díaz-Plascencia, (2011); Hodar *et al.,* 2020; Guavara-Valdez *et al.,* 2024.

Therefore, the objective of this work was to evaluate the use of a fermented apple compound (manzarine) rich in functional compounds and yeasts in the behavior of Tilapia (*Oreochromis niloticus*) Rocky Mountain variety in a semi-controlled aquaculture system.

MATERIALS AND METHODS

The experiment was carried out in the fish farming module of the Faculty of Zootechnics and Ecology of the Autonomous University of Chihuahua, in the Francisco R. Almada Peripheral, km 1. Located in the municipality of Chihuahua, in the state of Chihuahua, Mexico, at coordinates 28°5´44.88 North latitude, 106°37´19.2 West longitude, at 1440 meters above sea level (INEGI, 2018).

This experiment was divided into two stages, the growth stage carried out in a controlled system of fish tanks and then the development stage, carried out in an experimental greenhouse.

In the growth stage, a controlled system composed of 21 fish tanks with a recirculating hydraulic system and a physicochemical filtration system was used to sustain life and obtain optimal development of aquatic organisms.

For water recirculation, a 0.5 hp pump, Pedrollo® PKm 60, was used, at an expense of 40 l/min for the entire system, obtaining adequate oxygenation. The filtration system is composed of a 6 mm thick glass tank that contains physicochemical filtration compounds, such as activated carbon, pellet and zeolite, which also help the establishment of colonies of denitrifying bacteria, which favors an adequate level of nitrogenous compounds from waste in the water, as well as an ultraviolet light system. which through two 15 W tubes, irradiate the water eliminating possible parasites, maintaining aquaculture health and water quality.

The water was monitored daily at 8:00 am, reviewing its quality through parameters of oxygen concentration, temperature and pH, so that they were always in the optimal ranges for this species.

The diets used were formulated using the Nutrion 5 formulation software based on the N.R.C. tables (N R C, 1998) for Tilapia (*Oreochromis niloticus*), to which it was modified to add the Manzarine without altering the nutritional aspects of the feed. The diets were formulated based on (Table 1) using the inclusion level of 10% for Manzarine (MAN Treatment), contrasted with a control diet (CONTROL Treatment). These diets were prepared in the animal nutrition laboratory of the Faculty of Zootechnics and Ecology of the UACH, all the ingredients were pulverized with a hammer mill and mixed with a humidification of 24% in a 19 L can until it was completely homogeneous. The mixed feed was passed through a Tor-Rey® meat mill obtaining pellets of 0.5 cm in diameter, which facilitated the handling of the feed. The pellets were left to dry on permeable trays for 48 h, until a humidity of 8% was reached, obtaining a yellow food in the CONTROL diet and brown in the MAN diet, both with a pleasant smell and taste for the fish. Once the diets were formulated, a proximal analysis was carried out using conventional methods of the A.O.A.C (Association of Official Analytical Chemistry) to evaluate their nutritional contents and determine if there were differences between them.

The fish used were 2 weeks old tilapia mojarra (*Oreochromis niloticus*) Rocky Mountain variety, obtained from the aquaculture center "La Boquilla" of SAGARPA, located in San Francisco de Conchos, Chihuahua, Mexico.

Glass fish tanks with 10 fish each were used as an experimental unit, with 5 repetitions of each giving a total of 21 fish tanks. From each fish tank, a sample of 3 fish was obtained at random each week until the eighth week to obtain the measurements, having three replicates for each measurement.

Starting in the ninth week, it was carried out in a greenhouse where two 1,800-liter Rotoplás ® containers were used. For the water recirculation system, a 0.5 hp Pedrollo ® PKm 60 pump was used for both containers, because due to the size of the fish they needed a larger space, to start their development stage.

The fish were caught with nets and transported in a cooler with water and ice in order to have proper handling without causing damage to the fish, since when the water temperature drops, the fish reduces the speed of metabolism, entering a state of relaxation, facilitating its handling.

Water quality and daily feeding were monitored using the same diets. A sample of 10 fish was randomly obtained from each container weekly to obtain their morphological measurements, having ten replicates for each measurement.

Ethical aspects

The study was carried out in accordance with the internal code of bioethics and taking into account both the animal welfare regulations of the FZyE of the UACH and the regulations of the Official Mexican Standard on animal care published by the Ministry of Agriculture and Rural Development (SAGARPA 1999).

Two treatments were established: the 10% Manzarine diet (MAN) and the CONTROL diet.

Table 1. Percentage of inclusion of each food used in the CONTROL and MAN diets

Ingredients (kg)	Diet	
	CONTROL %	MAN[1] %
Soy paste	62.9	53.7
Vegetable oil	6.2	6.4
Milk powder	11	10
Corn powder	12.5	12.5
Meat meal	5	5
Vitamin and mineral premix	0.3	0.3
Calcium carbonate	1.1	1.1
Will	1	1
Manzarina	0	10
Calculated Nutrient Analysis		
E. M. Mcal kg^{-1}	3.510	3.447
E. D. Mcal kg^{-1}	3.841	3.789
E. N. Mcal kg^{-1}	2.485	2.470
TDN %	83.844	83.180
Total Protein %	36.123	36.166
Digestible Protein %	31.666	27.745
Crude Fiber %	3.062	2.938
Grasa Cruda %	8.165	9.242
Ash %	8.520	11.698
Calcium %	1.210	2.179

[1] MAN indicates treatment with 10% Manzarina

Response Variables

Weight gain. Throughout the experiment, the fish were weighed and measured every seven days, as described by Crivelenti (2011), weighing them on an electronic scale in triplicate until the eighth week, taken randomly from each of the 10 fish tanks and individually from each container from the ninth week.

Longitude. The length was measured with a digital millimeter vernier. To facilitate their handling, they were passed through cold water once removed from the fish tank or container, where they remained for a few seconds until they managed to reduce their activity and be able to handle them correctly, without causing damage to the fish.

Height The height was measured with a digital millimeter vernier at the same time as the length for ease of handling. In the same way, they were passed through cold water once removed from the fish tank or container, where they remained for a few seconds until they managed to reduce their activity and be able to have a correct handling, without causing damage to the fish.

Width. The width was measured with a digital millimeter vernier at the same time as the length and height for ease of handling. In the same way, they were passed through cold water once removed from the fish tank or container, where they remained for a few seconds until they managed to reduce their activity and be able to have a correct handling, without causing damage to the fish.

Temperature. During the 17 weeks of the experiment, its daily measurement was carried out, for which a Hanna waterproof digital submersible potentiometer, HI-98130, was placed on the surface of the fish tank or container, obtaining temperature data.

pH. Its daily measurement was carried out, for which a Hanna waterproof HI-98130 digital submersible potentiometer was used, placed on the surface of the fish tank or container, obtaining pH data.

Dissolved oxygen. Its measurement was carried out daily using a digital oximeter Hach 850045, placed at the bottom of the fish tank to take its measurement.

Experimental design. For the statistical analysis of the data, an ANOVA analysis of variance was used to evaluate the productive variables. In all variables, the interaction of the treatment with the covariates "Day, Oxygen, pH, and Temperature" was tested, leaving in the final model the statistically significant (P<0.05) as shown in the following equations:

$$PV = \mu + T_i + \beta_D + \beta_O + \beta_{pH} + \beta_T + T_i\beta_O$$
$$L = \mu + T_i + \beta_D + \beta_O + \beta_{pH} + \beta_T + T_i\beta_O$$
$$A_L = \mu + T_i + \beta_D + \beta_O + \beta_{pH} + \beta_T + T_i\beta_O$$
$$A_N = \mu + T_i + \beta_D + \beta_O + \beta_{pH} + \beta_T + T_i\beta_{pH} + T_i\beta_T$$

Where:
PV = It is the response variable of Live weight.
L = It is the answer variable of Length.
A_L = This is the response variable of Height.
A_N = This is the response variable of Width.
μ = This is the overall mean between treatments.
T_i = It is the effect of the -th treatment.i
β_D = It is the Covariate of Day.
β_O = It is the covariate of Oxygen.
β_{pH} = It is the pH covariate.
β_T = It is the covariate of Temperature.
$T_i\beta_O$ = It is the interaction between the effect - th treatment with the Oxygen covariate.i
$T_i\beta_{pH}$ = It is the interaction between the effect - th treatment with the pH covariate.i
$T_i\beta_T$ = It is the interaction between the effect - th treatment with the covariate of Temperature.i
The analysis was performed in the SAS computational package, version 9.0 (SAS, 2004)

RESULTS AND DISCUSSION

Productive Behavior

Daily weight gain. In the present study, it was observed that there was no statistical difference ($P > 0.05$) in live weight (Figure 1) between treatments in relation to daily weight gain (GDP). The treatments had a GDP of 0.38 g for the CONTROL and -0.19 g for MAN during the 17 weeks of the duration of the experiment. However, the results were not as expected according to the growth table of Cantor, (2007) where he mentions that the GDP should be 3.29 g. Having a decrease between weeks 14 and 17 of experiment, where the fish begin to decrease their weight, so there was an influence on the part of dissolved oxygen (Graph 2) in both treatments MAN ($P < 0.05$) with a dissolved oxygen of 11.55 ppm and CONTROL ($P = 0.05$) with 10.15 ppm. Dissolved oxygen for proper tilapia growth should be 3 to 10 ppm (Cantor, 2007; Perea-Ganchou *et al.,* 2017). Cantor (2007) mentions that tilapia in the adult stage has a preference for the consumption of plankton and filamentous algae. Filamentous algae feed on nitrogen and phosphorus and with the help of oxygen grow rapidly, these algae generate toxins that adversely affect aquatic life. (Peña, 2005); Rueda Liberato (2023). Generating a decrease in fish growth.

Longitude. Similarly, it was observed that in the growth of fish length (Graph 3) there was no statistical difference between treatments ($P > 0.05$), obtaining a length in week 12 of the experiment (week 14 of life) of 9.47 cm for the CONTROL and 9.89 cm for MAN. The growth was similar to what was said by Arango *et al.,* (2000); Carpio *et al.,* (2023) who reported that the length of the fish at week 14 of life was 9.62 cm. As in the live weight, there was a statistical influence of oxygen in the MAN ($P < 0.05$) and CONTROL ($P < 0.05$) treatments.

Height. In the height growth of the fish (Graph 4) it was also observed that there was no statistical difference between treatments ($P > 0.05$), obtaining a height in week 12 of the experiment (week 14 of life) of 2.89 cm for the CONTROL and 3.07 cm for MAN. The growth was similar to what Ruiz (2009) said; Guevara Valdez *et al.,* (2024), who reported a height of the fish at week 14 of life was 2.9 cm. As in live weight and length, there was a statistical influence of oxygen in the MAN ($P < 0.05$) and CONTROL ($P < 0.05$) treatments.

Width. In the growth of fish width (Graph 5) there was no statistical difference between treatments ($P > 0.05$), obtaining a width of the fish in week 12 of the experiment (week 14 of life) of 1.46 cm for the CONTROL and 1.57 cm for MAN. The growth was lower than what was reported by Arango *et al.,* (2000), who reported that the width of the fish at week 14 of life was 3.38cm. Unlike the other variables, this one had a statistical influence on the part of the temperature (Graph 6) in week 17 of the experiment (week 19 of life), the MAN treatment ($P < 0.05$) which had a temperature of 18.7 °C and CONTROL ($P < 0.05$) a temperature of 18.4 °C. Pullin (1982); Guevara Valdez *et al.,* (2024) mentioned that tilapia reduces its activity and feeding when the temperature is below 20 °C.

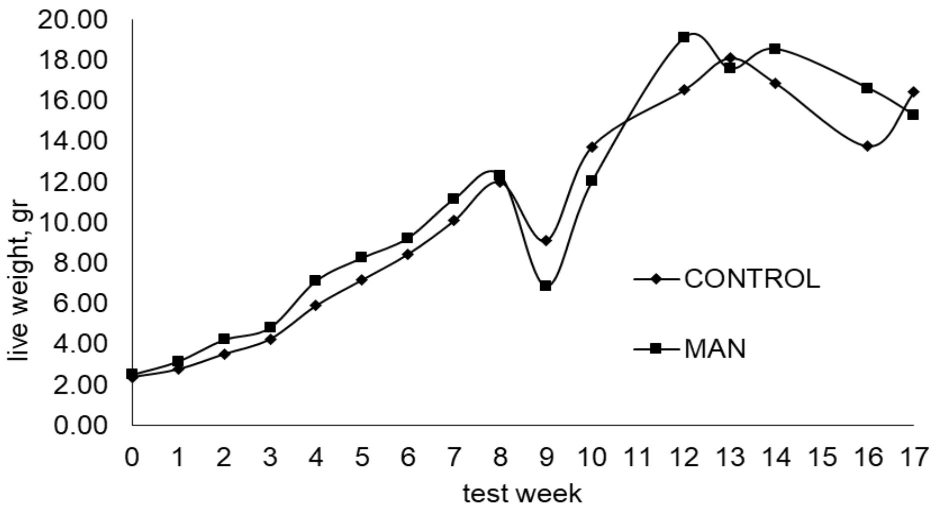

Figure 1. Average live weight growth line of the CONTROL and MAN treatments during the experiment.

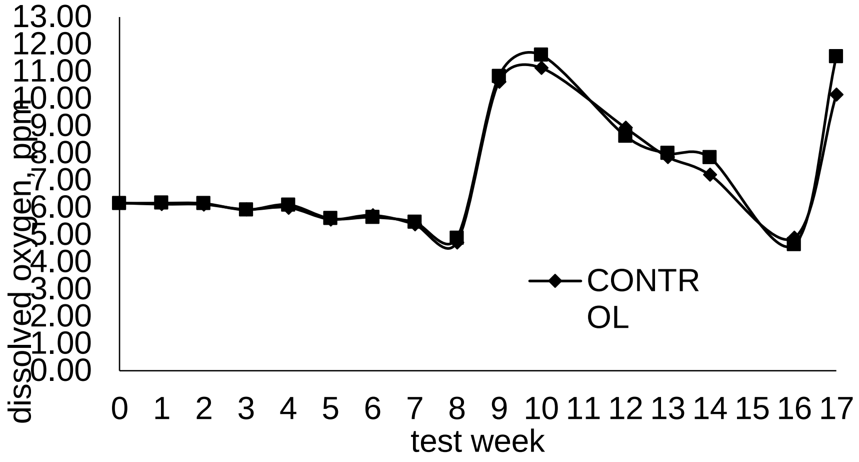

Figure 2. Amount of oxygen dissolved in the average water during the experiment.

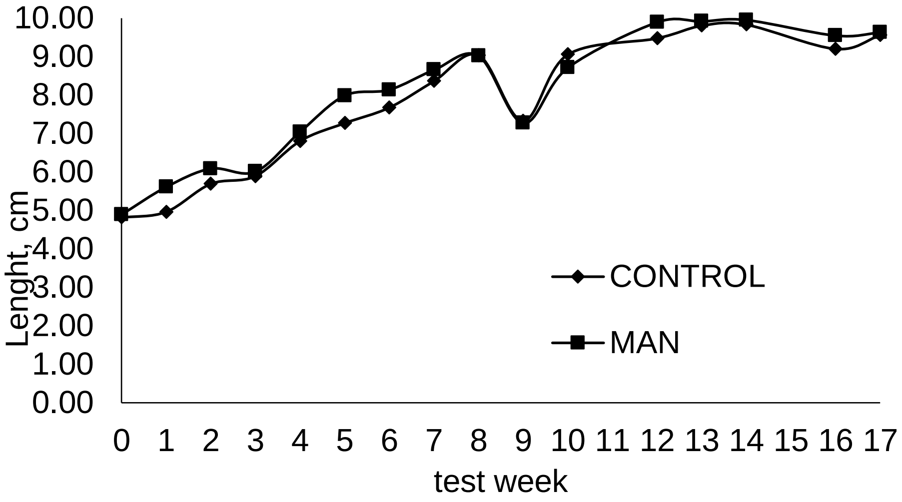

Figure 3. Growth line of the average length of the CONTROL and MAN treatments during the experiment.

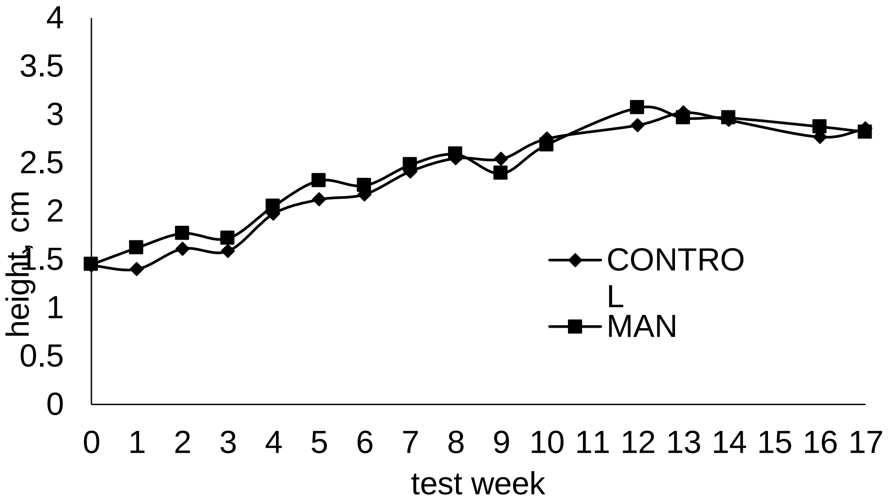

Figure 4. Growth line of the average height of the CONTROL and MAN treatments during the experiment.

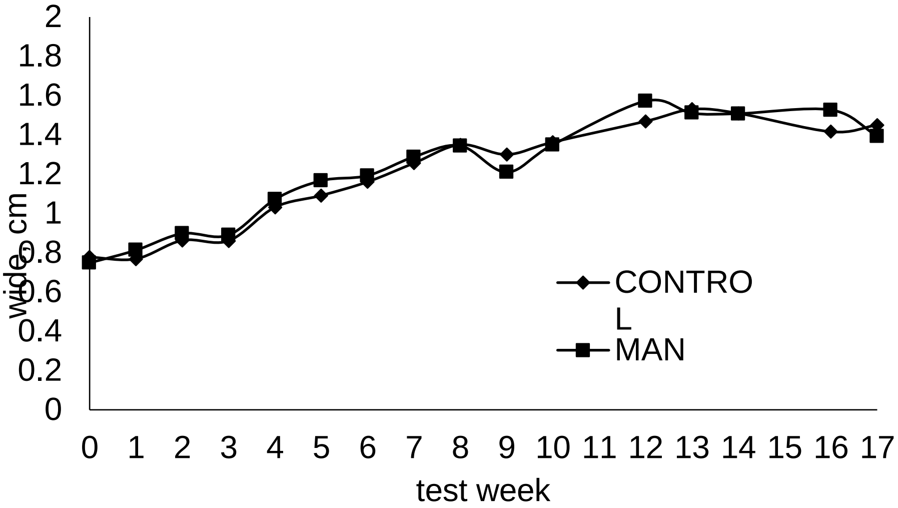

Figure 5. Growth line of the average width of the CONTROL and MAN treatments during the experiment.

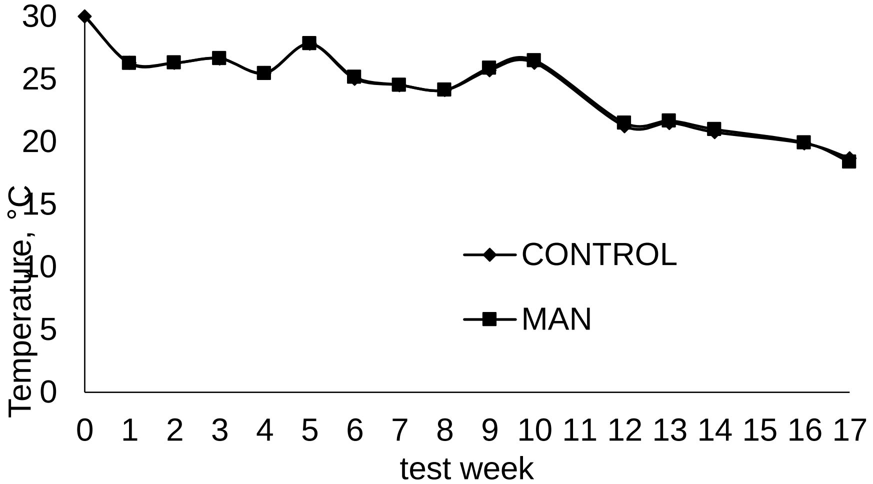

Figure 6. Graph of the average temperature of the CONTROL and MAN treatments during the experiment.

CONCLUSIONS AND RECOMMENDATIONS

From the present work it is concluded that there was no statistical difference between treatments based on the growth of the fish, however there was a statistical difference in the influence of growth (weight, height and length), due to the high levels of oxygen there was an accelerated growth of algae, so the fish had a higher consumption of these. which release toxins that inhibit the growth of the fish, the temperature has an influence on the width of the fish, because when the temperature decreases the fish reduces its activity and feeding so more research is needed to show that the manzarine works or not as a protein source for fish, because during the experiment there were external factors that directly affected the consumption and growth of the fish.

LITERATURE CITED

1. Arango, J. F. 2000. Mass selection by weight and size of two generations of red tilapia (*Oreochromis spp.)*. PhD thesis. National University of Colombia. Valle de la Cauca. Colombia.
2. Cantor, A. 2007. Tilapia production manual. Secretariat of Rural Development. State of Puebla. Mexico.
3. Carpio, B. A. B., Cañarte, C. A. N., Mestanza, A. L. B., Herrera, W. A. V., & Tobar, J. M. R. (2023). Characterization of the frequency of feeding in different rations in juveniles of red tilapia (Oreochromis mossambicus), based on growth indicators (length and weight). Revista Científica Referada Multidisciplinaria PENTACIENCIAS, 5(2), 449-462.

4. COMEPESCA. 2022. Mexican Council for the Promotion of Fisheries and Aquaculture Products. https://comepesca.com/soya-una-solucion-para-la-acuicultura/ Accessed January 2025.
5. Crivelenti, L. Z. and A. V Mundim. 2011. Serum biochemical values of Nile tilapia (*Oreochromis niloticus*) in intensive farming. Rev. Investig. Vet. Peru 22:318–323.
6. Díaz-Plascencia, D. 2011. Development of a yeast-based inoculum and its effect on in vitro fermentation kinetics in rations for high-yielding Holstein cows. Doctoral Thesis. Faculty of Zootechnics and Ecology. Autonomous University of Chihuahua. Chihuahua, Chih. Mexico.
7. Dong, G. F., Y. O. Yang, X. M. Song, L. Yu, T. T. Zhao, G. L. Huang, Z. J. Hu y J. L. Zhang. 2013. Comparative effects of dietary supplementation with maggot meal and soybean meal in gibel carp (*Carassius auratus gibelio*) and darkbarbel catfish (*Pelteobagrus vachelli*): growth performance and antioxidant responses. Aquac. Nutr. 19:543-554. doi: 10.1111/anu.12006.
8. FAO. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Food and Agriculture Organization of the United Nations. 2020.
9. Guevara Valdez José Luis, Daniel Díaz Plascencia, Pablo Fidel Mancillas Flores, José Roberto Espinoza Prieto, Diana González López. Rosario Martínez Yáñez. 2024. Inclusion of fermented apple bagasse and a probiotic (*kluyveromyces lactis*) in the tilapia diet (*oreochromis niloticus*) during their youth stage. African Journal of Biological Sciences, 6. (8). 2461-2476. doi: 10.48047/AFJBS.6.8.2024.2461-2476
10. Hodar AR, Vasava RJ, Mahavadiya DR, Joshi NH. Replacement of fishmeal and fish oil for aquaculture feed formulation using alternative sources: a review. *J Exp Zool India* . 2020 ; **23** (1): 13 – 21.
11. INEGI. National Institute of Statistics and Geography. 2018. https://www.inegi.org.mx/app/mapa/espacioydatos/default.aspx?ag=08019. Retrieved June 2022.
12. Kaiser, F., Harbach, H., & Schulz, C. (2022). Rapeseed proteins as fishmeal alternatives: A review. *Reviews in Aquaculture*, *14*(4), 1887-1911.
13. NRC. 1998. Nutrient Requirements of Fish. National Research Council. USA.
14. Peña-Salamanca, E.J., M. L. Palacios-Peñaranda and N. Ospina-Álvarez. 2005. Algae as indicators of pollution. Universidad del Valle. Cali. Colombia.
15. Perea-Ganchou, F., Perdomo-Carrillo, D. A., Corredor-Zambrano, Z., Moreno-Torre, R., Pereira-Morales, M., & González-Estopiñán, M. (2017). Factors affecting the reproductive performance of tilapia of the genus Oreochromis in the lower zone of the state of Trujillo, Venezuela. Scientific Journal, 27(2), 78-86.
16. Pullin R. S. V. y R. H Lowe. 1982. The biology and culture of tilapia. ICLARM Conference Proceedings 7, 432 p. International Center for Living Aquatic Resources Management. Manila: Philippines.
17. Rueda Liberato, A. C. (2023). Evaluation of three culture densities of Orechromis niloticus (nilotic tilapia) in aquaponics under laboratory conditions.
18. Ruiz-Peña, M. A. 2009. Masal selection by weight and coloration in red tilapia. National University of Colombia. Valle de la Cauca. Colombia.
19. Sadigov, R. (2022). Rapid growth of the world population and its socioeconomic results. The Scientific World Journal, 2022(1), 8110229.
20. SAGARPA (1999) Official Mexican Standard NOM-062-ZOO-1999 Technical specifications for the production, care and use of laboratory animals. Official Gazette of the Federation, August 22, 2001. Mexico City, Mexico. https://www.gob.mx/cms/uploads/attachment/file/203498/NOM-062-ZOO-1999'_220801. pdf. Accessed on: 10 November 2023.
21. Thiessen, D. L., G. L. Campbell y P. D. Adelizi. 2003. Digestibility and growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with pea and canola products. Aquac. Nutr. 9:67–7.