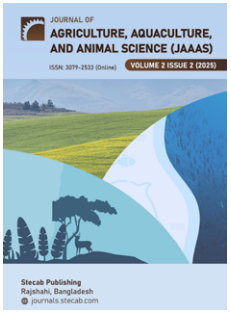

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Effect of Commercial and Artisanal Hormone-Treated Feed on the Sex Reversal of Tilapia (*Oreochromis niloticus*) in the Soconusco Region, Chiapas

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ABSTRACT

This study evaluated the effect of commercial and artisanal feed treated with the hormone 17-alpha-methyltestosterone on the sex reversal of tilapia (*Oreochromis niloticus*) in the Soconusco region of Chiapas, Mexico. A completely randomized experimental design was implemented with three treatments: hormone-treated artisanal feed (T1), hormone-treated commercial feed (T2), and a control group of mixed organisms without hormone treatment (T3), each with three replicates. The fry was fed for 28 days under controlled conditions, and the percentage of males was subsequently determined by direct gonadal analysis. The results showed masculinization rates of 93% in T1, 78% in T2, and 67% in T3. Statistical analysis using the chi-square test revealed significant differences between treatments ($\chi^2 = 12.47$; $p < 0.05$), confirming the greater efficacy of the artisanal feed, possibly associated with better hormone fixation and stability during natural evaporation and refrigerated storage. The results demonstrate that artisanal feed containing 17-alpha-methyltestosterone at 60 mg/kg is a technically viable and low-cost alternative for producing monosex tilapia populations, highlighting the need to standardize preparation processes to optimize reproductive efficiency and environmental safety in tropical aquaculture systems. It is adoption can strengthen the productive efficiency and profitability of aquaculture systems, offering an accessible option for producers in the Soconusco region.

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1. INTRODUCTION

Tilapia farming is one of the most profitable aquaculture activities worldwide due to it is rapid growth, disease resistance, tolerance to high densities, and adaptability to different environmental conditions. However, it is early sexual maturity leads to uncontrolled reproduction, causing overpopulation, competition for food, and increased oxygen demand, which decrease growth and survival rates (Castillo, 2015; Torres-Hernández *et al.*, 2010).

To mitigate this problem, various techniques have been implemented, including manual sex determination, thermal shock, genetic methods, and sex reversal through hormone diets (Hurtado, 2005; Hann *et al.*, 2012; Vega-Galarza, 2018).

Sex reversal using androgens, particularly the hormone 17-alpha-methyltestosterone, had proven effective in generating a high percentage of male populations. Recent studies have shown, for example, that doses of 60 mg/kg can achieve masculinization of more than 90% under certain conditions (Logato *et al.*, 2004; López *et al.*, 2007; Montoya, 2013).

Despite this, a knowledge gap exists regarding the efficacy of commercially prepared hormone-containing foods versus artisanally prepared foods. Commercially prepared foods offer advantages in terms of uniformity and ease of use, but they often involve high costs and less local adaptability. Artisanal foods, on the other hand, can reduce costs; however, their efficacy, standardization, and stability of the active ingredient often vary due to the lack of quality controls (Chisoro *et al.*, 2023).

In this context, comparing both types of food will allow us to establish whether artisanal foods can offer equivalent or superior results to commercial ones, providing scientific evidence to support their application in the aquaculture systems of Soconusco, Chiapas.

2.1. Objective

To determine the effect of commercial and artisanal hormone-treated feed on the sex reversal of tilapia (*Oreochromis niloticus*).

2. LITERATURE REVIEW

Mexico is the world's ninth-largest producer of tilapia, accounting for 94.3% of the national catch of this species. The main producing states are Jalisco, Chiapas, Sinaloa, Nayarit, Michoacán, Veracruz, Tabasco, Guerrero, Hidalgo, and the State of Mexico (FIRCO, 2017). Chiapas leads production, particularly the Malpaso, Angostura, Chicoasén, and La Concordia reservoirs (INAPESCA, 2017).

Tilapia (*Oreochromis sp.* and *Tilapia sp.*) were introduced to Mexico in 1964 and are fundamental for animal protein production in tropical and subtropical regions, with widespread acceptance in developing countries (DOF, 2012). Their cultivation is profitable due to characteristics such as rapid growth, disease resistance, tolerance to high densities, euryhaline capacity, and acceptance of varied diets. In Mexico, 91% of production comes from aquaculture, with a presence in 31 states: Chiapas, Tabasco, Guerrero, the State of Mexico, and Veracruz lead production (FIRCO, 2017).

The Soconusco region in Chiapas possesses ideal conditions for inland aquaculture and mariculture thanks to it is river basins (Coatán, Suchiate and Cacahoatán). However, it faces limitations due to a lack of support programs, a shortage of broodstock, and limited technical assistance (Castillo, 2012). One of the main problems is the early reproduction of the species, which leads to overpopulation, competition for resources, and lower growth rates (Torres *et al.*, 2010). The need to control or eliminate reproduction during the grow-out phase to achieve profitable farming had been demonstrated (Castillo, 2012).

Among the solutions, obtaining monosex offspring, preferably males, stands out, as they reach a larger size in less time (Vega-Galarza, 2018). To achieve this, techniques such as manual sex determination, hybridization, genetic manipulation, heat shock, and sex reversal using diets enriched with hormones containing 17α-methyltestosterone have been developed (Hann Von-Hessberg *et al.*, 2012; Hurtado, 2005). Androgen treatments, administered through diet, immersion, or injection, have demonstrated high efficacy in masculinization (Contreras *et al.*, 2017).

Several studies support this technique: Logato and collaborators in 2004 achieved 100% male populations with doses ≥37.5 mg/kg of feed; López and collaborators in 2007 reported 100% males with a hormone-infused diet and 90% with immersion; Castillo in 2012 obtained 100% males on the diet and 94.67% on immersion; Montoya in 2013 achieved up to 100% with doses of 60–80 mg/kg. The effectiveness of the method has also been confirmed in other species, such as the cichlid *Petenia splendida* (Contreras *et al.*, 2017).

However, a gap in knowledge remains regarding the difference in effectiveness between artisanal and commercial hormone-enriched feeds, making it necessary to comparatively evaluate their efficacy under controlled conditions.

3. METHODOLOGY

This is an experimental and exploratory study. Fieldwork was conducted at the CETAC 22 aquaculture farm, located in Ejido Faja de Oro, Cacahoatán, Chiapas. Three treatments were used, each with three replicates: T1: Homemade feed prepared with the hormone 17-alpha-methyltestosterone; T2: Commercial hormone-treated powdered feed from the Silver Cup brand; and T3: Control group: hormone-free feed from the same brand. Established in a randomized block design, the following physicochemical parameters were measured in all treatments: temperature, oxygen, pH, ammonia, nitrite, nitrate, and light, using a YSI multiparameter probe. One hundred fingerlings were selected that met the requirements: newborn fingerlings between 3 and 4 days old, passing through a 0.5 cm sieve, weighing 1 g, and with a total length of 0.8 cm.

The laboratory work was carried out at the facilities of the Center for Research on Coastal and Continental Systems, located in Puerto Madero, municipality of Tapachula. To prepare the artisanal feed with hormones, crumble-type feed (0 mm) was used as a base for incorporating the hormone 17-alpha-methyltestosterone at a dose of 60 mg per kilogram of feed. The procedure consisted of dissolving the hormone in 90% ethyl alcohol, used as a fixing agent, ensuring a homogeneous distribution of the active substance on the base feed. The mixture was prepared manually on a clean surface, gradually adding the alcohol solution with the 17-alpha-methyltestosterone hormone

until it was uniformly impregnated. Subsequently, the treated feed was dried in the shade for 24 hours in a ventilated area, free from direct sunlight and humidity, to allow for the natural evaporation of the solvent and prevent thermal degradation of the hormone.

Once the food was dry, it was stored under refrigeration (4°C) until use, guaranteeing the stability of the active ingredient and the preservation of it is physicochemical properties.

he one hundred previously selected fry were introduced into ponds 2 m in diameter by 1.10 m high (3.45 m 3 of available water) for treatment for 28 days, they were fed six times a day, providing food at 15% of their initial biomass with 55% protein and after 28 days, they were supplied with 12% of their biomass, so biometry was carried out every 15 days.

To obtain results of sex reversal, 50% of the gonads of the organisms in each treatment were analyzed from 3 to 4 months of age using the direct evaluation technique of the gonads and the squash technique, using methylene blue as a sperm indicator.

For statistical analysis, the significant difference between treatments was evaluated using the chi-square test with a significance of 95% confidence, to evaluate the effectiveness of the commercial feed and the hormone-treated artisanal feed.

4. RESULTS AND DISCUSSION

The use of homemade feed with the hormone 17 alpha-methyltestosterone at a dose of 60 mg/kg of feed, achieved a sex reversal rate of 93% of males, while the treatment with commercial feed showed an effectiveness of 78% (Figure 1).

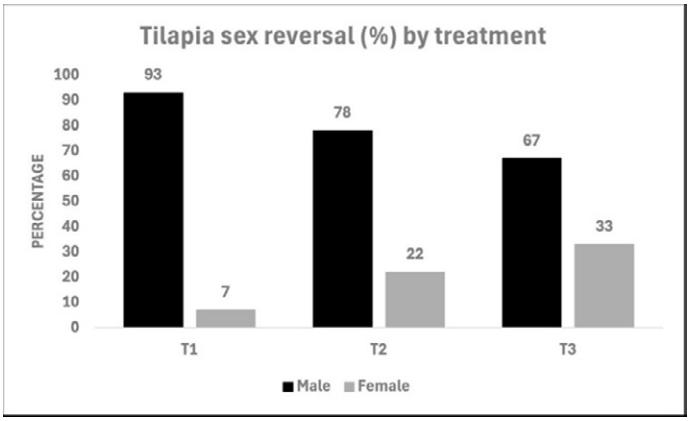

Figure 1. Percentage efficiency of sex reversal among treatments. The letters indicate the treatments: T1: Homemade feed, T2: Commercial feed, T3: Control group with mixed organisms without hormones.

The physicochemical parameters of the water, pH, ammonia, nitrite, nitrate and dissolved oxygen were kept within the optimal ranges for tilapia farming and showed no significant variations between treatments (Table 1), ruling out that water quality influenced the effectiveness of the sex reversal process in the treatments.

Table 1. Physicochemical parameters between treatments, during the 28 days of the hormone treatment process.

Physicochemical parameters					
Treatment	**pH**	**Ammonium (ppm)**	**Nitrite (ppm)**	**Nitrate (ppm)**	**Temperature**
Artisanal fish feed	7	0.5	0.25	5	27
Hormone treated feed	6.9	0.5	0.25	5	27.2
Control group	6.8	0.5	0.25	5	27.1

Statistical analysis showed significant differences ($p < 0.05$) between treatments, demonstrating that the artisanal feed treated with 60 mg/kg of 17-alpha-methyltestosterone had a superior effect on sex reversal compared to the commercial feed and the control group (Table 2). Each subscript identifies subsets of treatments whose proportions do not differ significantly from each other at the 95% confidence level, supporting the consistency of the observed effect.

Although the rate obtained with homemade feed (93%) was slightly lower than the 100% reported by López and collaborators in 2007 and Montoya and collaborators in 2013; this difference can be attributed to factors related to handling, the homogeneity of the hormone mixture, and the stability of the active ingredient during feed preparation. Incomplete solvent evaporation, prolonged exposure to sunlight, or high temperatures during drying could have reduced the hormone's bioavailability.

Table 2. Comparison between treatments with Chi-square statistical analysis.

			Treatment			**Total**
			Artisanal fish feed	**Hormone treated feed**	**Control group**	
Sexed	Male	Count	93^{a}	78^{b}	67^{b}	238
		% sexing	39.1%	32.8%	28.2%	100%
		% treatment	93.0%	78.0%	67.0%	79.3%
		% total	31.0%	26.0%	22.3%	79.3%

Sexed	Female	Count	7[a]	22[b]	33[b]	62
		% sexing	11.3%	35.5%	53.2%	100%
		% treatment	7.0%	22.0%	33.0%	20.7%
		% total	2.3%	7.3%	11.0%	20.7%
	Total	Count	100	100	100	300
		% sexing	33.3%	33.3%	33.3%	100%
		% treatment	100%	100%	100%	100%
		% total	33.3%	33.3%	33.3%	100%

On the other hand, commercial feeds may contain binders or preservatives that alter the adsorption and release of the hormone, limiting it is absorption by the larvae. In contrast, artisanal feed, prepared in a controlled manner and with natural evaporation, promotes greater fixation and stability of the active compound, resulting in more efficient masculinization.

The uniformity of hormone distribution is also crucial. The manual, supervised mixing of the homemade feed allows for a more consistent application of the hormone solution, reducing variability in the effective dose received by each animal. This may explain the differences observed compared to commercial feed, which, although industrially formulated, does not necessarily guarantee the same effectiveness between the hormone and the pellet components.

The results confirm that a dose of 60 mg/kg of 17-alpha-methyltestosterone is effective in inducing masculinization levels exceeding 90%, but they also demonstrate that the quality of the feed manufacturing process is a critical factor in optimizing the physiological response. These findings are consistent with previous studies highlighting that the efficiency of hormonal treatment depends largely on the standardization of the mixing, evaporation, and storage stages, rather than on the dose used.

From a production perspective, the use of hormone-treated artisanal feed is emerging as a technically and economically viable alternative for small- and medium-scale aquaculture systems. It is controlled production allows for recovery rates comparable to those reported by other authors, contributing to the reproductive efficiency and economic sustainability of aquaculture unit is.

5. CONCLUSION

The results obtained in the research demonstrate that the food prepared in a handcrafted way with the hormone 17 alpha-methyltestosterone at a dose of 60 mg/kg had a greater effectiveness in the sexual reversal of tilapia (*Oreochromis niloticus*) compared to the commercial food and the control group, reaching 93% males. This significant difference suggests that the artisanal production process directly influences the stability of the active ingredient, promoting more efficient masculinization.

Hormone-treated artisanal feed represents a viable technical and economic alternative for small and medium-scale aquaculture producers, offering an accessible method with good results. It is controlled implementation could strengthen reproductive efficiency, homogeneous growth, and profitability of aquaculture systems in the Soconusco region of Chiapas.

It is recommended to further study hormone stability, absorption, and residual effects in the environment to standardize the artisanal process and guarantee it is environmental and food safety.

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