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Growth Response, Survival and Phenotypic Sex Ratio of *Oreochromis niloticus* Fry Fed Diets Containing Different Inclusion Levels of *Eriosema psoraloides* Leaf Meal

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ABSTRACT
This study evaluated the potential of *Eriosema psoraloides* leaf meal (EPLM) as a natural sex-modulating feed additive in Nile tilapia (*Oreochromis niloticus*) fry to address aquaculture challenges associated with mixed-sex populations, such as uncontrolled reproduction and stunted growth. A four-week feeding trial was conducted at the National Institute for Freshwater Fisheries Research, Nigeria, using 540 fry randomly assigned to six dietary treatments (D1–D6) with graded EPLM inclusion levels (0–10%) in a completely randomized design. Phenotypic sex was determined using modified acetocarmine squash method, while growth performance was assessed via mean weight gain (MWG), specific growth rate (SGR), and survival rate (SR). Although a visible trend toward male-biased sex ratios was observed peaking at 66.7% male in group D6,Chi-Square analysis revealed no statistically significant association between diet and sex differentiation, χ^2 (10) = 9.516, p = .484. Growth performance was highest at 4% and 10% EPLM inclusion levels, with mean final weights of approximately 0.32±0.01g and 0.33±0.02g, respectively. Survival rate declined progressively with increasing EPLM inclusion, with the lowest rate (87.77±0.56%) recorded in D6, suggesting a potential toxicity threshold. Overall, EPLM demonstrated potential to promote male-biased sex differentiation and enhancing growth under the experimental conditions, presenting a natural alternative to synthetic sex reversal agents. However, due to the lack of statistical significance in sex ratio shifts, further research is needed to validate its masculinizing potential. Dosage optimization remains critical to ensure fish welfare and sustainable production outcomes.

Keywords:
Oreochromis niloticus, *Eriosema psoraloides*, Biotechnology, Gonad squash, Prolific

INTRODUCTION
Aquaculture plays a crucial role in global food security, and Nile tilapia (*Oreochromis niloticus*) is among the most extensively farmed freshwater species owing to its fast growth, adaptability, and high economic importance(FAO, 2022). In Nigeria and across sub-Saharan Africa, Nile tilapia farming plays a critical role in rural livelihoods and protein supply (Wasso *et al.*, 2025). Yet, a major challenge in tilapia production remains the management of mixed-sex populations, which often results in uncontrolled breeding, overcrowding, stunted growth, and ultimately reduced yield and profitability (Opiyo *et al.*, 2020).
To address this, the production of mono-sex male populations is widely practiced, given that male tilapia exhibits superior growth rates compared to females (Bardhan *et al.*, 2021). Traditional sex reversal methods rely on synthetic androgens such as 17α-methyltestosterone, which, despite their effectiveness, raise concerns over environmental safety, regulatory compliance, and consumer health (Megbowon & Mojekwu, 2014). This has prompted a growing interest in natural alternatives, particularly plant-based feed additives with endocrine-modulating properties (Matter *et al.*, 2024).
Eriosema psoraloides, a leguminous plant native to tropical Africa, is known for its rich phytochemical profile, including flavonoids, alkaloids, and phenolic compounds (Awouafack *et al.*, 2015). According to Ateba *et al.* (2021) these bioactive constituents have shown potential in modulating hormonal pathways and influencing physiological processes in animals, including estrogenic effects.Investigations by Elechi and Abo (2020) demonstrated that *E. psoraloides* leaf

extracts possess antidiabetic, antihyperlipidemic, and organ-protective properties, suggesting systemic physiological impacts that may extend to endocrine modulation. However, empirical data on its efficacy, optimal inclusion levels, and safety in tilapia fry diets remain limited, warranting further investigation into its potential as a natural sex-modulating feed additive

Therefore, this study aims to evaluate the efficacy of dietary inclusion of *Eriosema psoraloides* leaf meal (EPLM) as a natural sex-modulating feed additive on the phenotypic sex ratio and growth performance of *O. niloticus* fry. Specifically, it seeks to determine the influence of varying EPLM inclusion levels on early phenotypic sex differentiation, assess growth performance indicators in fry fed EPLM-treated diets, and identify the optimal inclusion level that promotes male-skewed sex ratios without compromising growth and survival.

MATERIALS AND METHODS

Experimental Site

The research was conducted at the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Borgu Local Government Area. (LGA) of Niger State, Nigeria. The experimental site was located at the Hatchery Complex of the Institute located under latitude 9.8675°N and longitude 4.5301°E.

Identification, Collection, and Preparation of Plant Material

Eriosema psoraloides specimens were located on a farm in Karabonde village, Borgu Local Government Area, Niger State, Nigeria. Botanical identification and authentication were conducted by a specialist from the Aquatic Vegetation Programme, Research Operations Department, National Institute for Freshwater Fisheries Research (NIFFR), New Bussa.

Mature pods containing dried seeds were manually harvested and dehulled. The seeds were subsequently cultivated in a designated bed adjacent to the NIFFR feed-mill complex, where they germinated, matured, and served as the source of plant material for the study.

Leaves from the mature *E. psoraloides* plants were manually plucked, detached from branches, and rinsed with distilled water to eliminate surface contaminants and preserve phytochemical integrity, following the recommendations of Elechi and Abo (2020). The cleaned leaves were shade-dried at ambient temperature (~26°C) for seven days to minimize phytochemical degradation, as supported by Awouafack *et al.* (2015). Once dried, the leaves were pulverized into fine powder using a dry matter blender to produce *Eriosema psoraloides* leaf meal (EPLM). The resulting product was packaged in labeled polythene bags and stored under appropriate conditions until use.

Plate 1: *Eriosema psoraloides* leaves on stem with pods and flowers (**Source:** Field work 2023/2024)

Experimental Fish

Freshly hatched mixed-sex *Oreochromis niloticus* fry with mean weightof 0.0084±0.0003g were procured from the tilapia hatchery of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Nigeria. A total of 540 frywere randomly distributed into 18 glass aquaria tanks, each with internal dimensionsof 60 × 30 × 30 cm^3 and filled to three-quarters of their volume with dechlorinated freshwater. To ensure adequate aeration, air stones connected to flexible hoses were installed in each tank to provide a continuous supply of oxygen from an electric pump.

Experimental Designand Duration

The study was conducted using a Completely Randomized Design (CRD) with six dietary treatments, each replicated three times. Each replicate contained 30 fry, and tanks were maintained under controlled laboratory conditions throughout the experimental period.The experimental duration was four weeks, strategically selected to coincide with the critical period of gonadal differentiation in *Oreochromis niloticus* fry.

Experimental Diet, Feeding and Management

Six experimental diets, each containing approximately45% crude protein (CP), were developed using 0.2 mm Coppens® commercial feed as the base, with incremental substitutions of EPLM incorporated.The control diet (D1) contained 0%EPLM, while other experimental diets incorporated graded levels of EPLM as follows:D2 (2% EPLM), D3 (4% EPLM), D4 (6% EPLM), D5 (8% EPLM) and D6 (10% EPLM).These inclusion levels were selected based on the methodology previously described by Murali *et al.* (2020) in evaluating plant-based feed additives for aquaculture applications.

Fish were fed *ad libitum* daily between 09:00am and 6:00pm. Uneaten feed and fecal matter were siphoned out daily and freshwater added to maintain the volume. Photoperiod was at 12L:12D throughout the experiment. Water quality parameters (Dissolved Oxygen, Ammonia, pH, Temperature and Conductivity) were monitored and recorded weekly to ensure good water conditionsduring the experiment.

Phenotypic Sex Ratio Determination

At the end of the 4-week experimental period, 10 fry per replicate (30 fry per treatment) were randomly selected and euthanized via immersion in ice water. Specimens were fixed in 3% neutral buffered formalin for 24 hours to preserve tissue integrity. Microscopic evaluations of gonad tissue were carried out using standard acetocarmine squash method as described by Guerrero III and Shelton (1974)to determine the sex ratio following each treatment.Male gonads showed dense clusters of spermatogonia, while female gonads featured larger oogonia with distinct nuclei and occasional ovarian cavities. Specimens lacking clear sex structures were labeled “undifferentiated” and not categorized either male or female. The modified squash method enabled rapid, non-histological detection of developing gonads, allowing early sex differentiation and evaluation of treatment effects on sexual development.

Growth Response Assessment

The following parameters were used to calculate the growth performance evaluation as previously described and used by Nwangwu *et al.* (2024);

Mean Initial Weight [MIW(g)] =

$$\frac{\text{Total Initial Fish Weight (g)}}{\text{Total number of Fish per Tank at Initial}} \tag{1}$$

Mean Final Weight [MFW(g)] =

$$\frac{\text{Total Final Fish Weight (g)}}{\text{Number of Fish Per Tank at final}} \tag{2}$$

Mean Weight Gain [MWG(g)] = MFW(g)– MIW(g) (3)

$$\text{SGR (\%)} = \frac{(\ln W2 - \ln W1)}{T} \times 100 \tag{4}$$

Where, W1 = Fish initial weight, W2 = Fish Final Weight, ln = Natural Logarithm and T = Number of days in the experiment.

Survival Rate (%) =

$$\frac{\text{Initial Number of Fish Stocked} - \text{Mortality}}{\text{Number of Fish stocked}} \times 100 \tag{5}$$

Table 1: Water Quality Parameters of Experimental AquariumTanks

Parameters	Mean ±SD	D1	**D**2	**D**3	**D**4	**D**5	**D**6
pH	7.47±0.28	7.40±0.25[a]	7.51±0.29[a]	7.48±0.24[a]	7.43±0.30[a]	7.52±0.31[a]	7.45±0.22[a]
Temperature (°C)	28.06±0.50	28.06±0.65[a]	28.08±0.27[a]	28.06±0.38[a]	28.12±0.41[a]	28.01±0.63[a]	28.05±0.56[a]
DO (mg/L)	7.32±0.06	7.27±0.13[a]	7.44±0.15[a]	7.30±0.14[a]	7.34±0.19[a]	7.29±0.28[a]	7.27±0.23[a]
Conductivity (µS/cm)	306.2±1.79	308±1.06[a]	304±1.09[a]	308±1.05[a]	305±1.03[a]	306±9.02[a]	308±1.06[a]
Ammonia (mg/L)	0.05 ± 0.15	0.05 ± 0.12[a]	0.04 ± 0.22[a]	0.05 ± 0.15[a]	0.05 ± 0.12[a]	0.04 ± 0.14[a]	0.05 ± 0.12[a]

DO = Dissolved Oxygen

Data Analysis

Data was processed by Microsoft excel 365.A Pearson Chi-Square test was conducted to evaluate the association between dietary treatments and sex differentiation while growth response data were subjected to one-way ANOVA followed by Duncan's post hoc test to compare treatment means at $p < 0.05$ using IBM SPSS Statistics Version 27.

RESULTS AND DISCUSSION

Results

Phenotypic sex differentiation of *O. niloticus* fry fed graded inclusion levels of *Eriosema psoraloides* leaf meal for four (4) weeks

The effects of diets containing graded inclusion levels of *E. psoraloides* leaf meal on the phenotypic sex differentiation of *O. niloticus*fry is shown in Tables 2. Sex differentiation was assessed microscopically and categorized as male, female or undifferentiated. Each diet group (D1–D6) contained 30 specimens. The Table presents actual counts and corresponding percentages within each dietary group. As shown in the Table, female representation declined from D1 (46.7%) to D6 (16.7%), while male representation increased from D1 (50.0%) to D6 (66.7%). The proportion of undifferentiated fry rose slightly at higher inclusion levels, peaking at D6 (16.7%). These trends suggest a shift toward male-biased differentiation and increased gonadal ambiguity with higher EPLM inclusion, although statistical analysis showed no significant association (*p*> .05).

A Pearson Chi-Square test was conducted to evaluate the association between diet and sex differentiation. The results indicated no statistically significant association, $\chi^2(10) = 9.516$, $p = .484$. The likelihood ratio test also showed no significance, $\chi^2(10) = 10.132$, $p = .429$. A total of 180 valid cases were analyzed.Notably, six cells (33.3%) had expected frequencies below five, with the lowest at 3.83, indicating reduced statistical power and necessitating careful interpretation of the results.

Table 2: Sex Distribution (Counts and Percentages) of *O. niloticus* Fry Across Dietary Treatments Containing Graded Levels of *Eriosema psoraloides* Leaf Meal

SEX	D1 (n=30)	D2 (n=30)	D3 (n=30)	D4 (n=30)	D5 (n=30)	D6 (n=30)	Total (n=180)
Male	15 (50.00)	15 (50.00)	18 (60.00)	18 (60.00)	19 (63.33)	20 (66.67)	105 (58.33)
Female	14 (46.67)	10 (33.33)	8 (26.67)	8 (26.67)	7 (23.33)	5 (16.67)	52 (28.89)
Undifferentiated	1 (3.33)	5 (16.67)	4 (13.33)	4 (13.33)	4 (13.33)	5 (16.67)	23 (12.78)

n = total number of fryselected per group

Growth response and survival of *O. niloticus* fry fed varying inclusion levels of *E. psoraloides* leaf meal for four weeks

The growth performance of *O. niloticus* fry fed diets containing graded levels of *Eriosemapsoraleoides* leaf meal (EPLM) over a four-week period is presented in Table 3.Optimal growth was observed at 4% (D3) and 10% (D6) EPLM inclusions, with significant improvements in mean final weight (MFW) of 0.32± 0.01gand 0.33± 0.02g respectively and mean weight gain (MWG) of 31±0.01gand 0.32±0.02g respectively. Theseare significantly different ($p < 0.05$) than weight gain of approximately 0.29±0.02g observed in groups fed 6% (D4) and 8% (D5) EPLM respectively.

Survival rates varied significantly ($p < 0.05$) across treatments. D1–D3 recorded the highest survival rate of 91.11%, with no significant difference ($p < 0.05$) among them. In contrast, D6 exhibited the lowest survival rate of 87.77%, which was statistically different ($p < 0.05$) from the control (D1) and lower inclusion groups.

Table 3: Growth response and survival rate of *O. niloticus* fry fed varying inclusion levels of *E. psoraloides* leaf meal for four (4) weeks

Parameter	D1	D2	D3	D4	D5	D6
MIW (g)	0.0087± 0.0002[a]	0.0080± 0.0010[a]	0.0085± 0.0003[a]	0.0083± 0.0003[a]	0.0085± 0.0002[a]	0.0086± 0.0004[a]
MFW (g)	0.3133± 0.0150[ab]	0.3050± 0.0180[ab]	0.3180± 0.0120[bc]	0.2980± 0.0200[a]	0.3020± 0.0160[a]	0.3250± 0.0190[c]
MWG (g)	0.3046± 0.0156[ab]	0.2970± 0.0186[ab]	0.3095± 0.0128[bc]	0.2897± 0.0209[ab]	0.2935± 0.0165[a]	0.3164± 0.0197[c]
SGR(% day^{-1})	12.80± 0.23[a]	13.00± 0.48[a]	12.94± 0.27[a]	12.79± 0.49[a]	12.75± 0.36[a]	12.97± 0.36[a]
SUR. (%)	91.11± 0.56 [a]	91.11± 0.56 [a]	91.11± 0.56 [a]	90.00± 1.11 [ab]	88.88± 0.56[bc]	87.77± 0.56[c]

*Means on same rows with different superscripts are significantly different ($p<0.05$)
MIW = Mean initial weight, MFW = Mean final weight, MWG = Mean weight gain, WG = Weight gain, SGR = Specific growth rate and SUR. = Survival rate.

Discussions

From the results of the study, a visible but statistically non-significant trend toward dose-dependent masculinization was observed. The control group (D1, 0% EPLM) exhibited a sex ratio close to the expected 1:1 Mendelian distribution (51.72% male vs. 48.39% female), serving as a valid baseline (Baroiller *et al.*, 2009; Sissao *et al.*, 2019; Triay *et al.*, 2022). As EPLM inclusion increased, the proportion of male fish rose consistently, culminating in 66.67% male and an 80.00% M:F ratio in the D6 (10% EPLM) group. However, Chi-Square analysis revealed no statistically significant association between diet and sex differentiation ($\chi^2(10) = 9.516$, $p = .484$), suggesting that the observed shifts may be due to chance or require larger sample sizes for confirmation.

Despite the lack of statistical significance, the progressive shift in sex ratios suggests that EPLM may contain bioactive phytochemicals capable of influencing sexual differentiation in tilapia. The mechanism is likely nutrigenomic, where dietary constituents modulate gene expression directly or indirectly (Müller & Kersten, 2003). In tilapia, sex determination is primarily genetic (XX/XY), but it remains highly susceptible to environmental and endocrine modulation, particularly during the critical labile period post-hatching (Baroiller *et al.*, 2009; Tao *et al.*, 2018). Phytoestrogens and other plant-derived compounds have been shown to affect gonadal development and sex ratios in fish species (Shohreh *et al.*, 2024)

The gradual increase in male percentage observed across dietary treatments suggests a potential mechanism distinct from the direct receptor agonism typical of synthetic steroids. Instead, it may reflect a nutrigenomic pathway, where dietary phytochemicals subtly influence gene expression networks governing gonadal differentiation (Liu *et al.*, 2022; Xie *et al.*, 2021). However, it is important to note that the Chi-Square analysis revealed no statistically significant association between diet and sex differentiation ($p = .484$), indicating that while the trend is biologically suggestive, it cannot be conclusively attributed to EPLM inclusion under the current experimental conditions.

The gradual, non-binary nature of the response aligns with the behavior of phyto-active compounds, which often act as modulators of endocrine function rather than potent receptor agonists(Burgos-Aceves *et al.*, 2021).These compounds may exert cumulative or threshold-dependent effects that are difficult to detect in short-term trials or small sample sizes (Farooq *et al.*, 2025)

The phytochemical profile of EPLM, may underpin this activity (Shohreh *et al.*, 2024). Specifically, flavonoids are known to act as phytoestrogens or aromatase inhibitors (Bugel *et al.*, 2016; Sepehrfar *et al.*, 2023). Aromatase (*Cyp19a1a*) is the key enzyme that converts androgens to estrogens, and its inhibition is an established mechanism for masculinization in teleost, as it prevents the synthesis of estrogens necessary for ovarian development (Imiuwa, 2020; Rodriguez *et al.*, 2023).

Concurrently, saponins with steroidal structures may act as precursors or stimulants for the endogenous biosynthesis of androgens such as 11-ketotestosterone, further promoting testicular differentiation (Ampofo-Yeboah, 2013; Syanya *et al.*, 2025). These dual actions– estrogen suppression and androgen stimulation could explain the observed male-biased trend, even if not statistically confirmed.

The consistent presence of "undifferentiated" (U) fry across all treatments, particularly in EPLM-fed groups, is a noteworthy biological observation. Although the Chi-Square analysis revealed no statistically significant association between diet and sex differentiation (p = .484), the persistence of undifferentiated individuals suggests that EPLM may not induce direct phenotypic sex reversal but rather interfere with the timing and synchrony of gonadal differentiation. This phenomenon has been reported with other complex plant mixtures containing multiple bioactive agents (Burgos-Aceves *et al.*, 2021; Rodriguez *et al.*, 2023). Such a state of arrested gonadal development may arise from antagonistic or synergistic interactions among phytochemicals that simultaneously target different regulatory nodes of the hypothalamic-pituitary-gonadal (HPG) axis. These compounds can modulate hormonal signaling pathways, delay steroidogenic activation, or disrupt feedback loops essential for sex-specific differentiation (Delbes *et al.*, 2022). Particularly, early-life exposure to endocrine-modulating agents has been shown to alter gene expression patterns and impair gonadal maturation in teleosts and other vertebrates.

The growth performance data reveals a complex, non-linear relationship between EPLM inclusion and zootechnical parameters. Notably, the strong performance observed at low to moderate inclusion levels, particularly in the D3 group (4% EPLM), which recorded a mean final weight (MFW) of 0.3180g and a specific growth rate (SGR) of 12.94% day^{-1}is promising.

This outcome aligns with findings by Gruber *et al.* (2025), who demonstrated that phytogenic feed additives (PFAs) can significantly enhance growth and disease resistance in Nile tilapia. Similarly, Phan *et al.* (2025)reported that low-dose PFAs exert growth-promoting effects through mechanisms such as appetite stimulation, enhanced digestive enzyme activity, and prebiotic modulation of gut microbiota. These interconnected physiological responses likely underpin the improved zootechnical performance observed at moderate EPLM inclusion levels.

The significant growth suppression observed at intermediate inclusion levelsspecifically in groups D4 (6%) and D5 (8%) can be attributed to the activity of antinutritional factors (ANFs). This pattern is consistent with the established understanding that many medicinal plants contain compounds such as tannins and saponins, which can impair nutrient digestibility and absorption. Ahmed *et al.* (2022) reported that tannins can bind irreversibly to dietary proteins and digestive enzymes, thereby reducing their bioavailability and compromising metabolic efficiency. Similarly, Neves *et al.* (2024) demonstrated that elevated levels of plant-based ANFs in tilapia diets lead to growth retardation and poor feed utilization. The decline in specific growth rate (SGR) observed at these inclusion levels reflects a metabolic cost associated with counteracting these compounds. Thus, the growth depression in D4 and D5 suggests that the antinutritional threshold of EPLM may have been exceeded, negatively impacting feed conversion and nutrient assimilationeven as the masculinization process continued.

Notably, the group D6 with highest inclusion level (10%) yielded the highest MFWof 0.3250g and SGR of 12.97%day^{-1}, which appears paradoxical given the presumed increase in antinutritional load. This rebound effect can be explained by two interrelated factors. First, the D6 population exhibited the highest proportion of males (80% M:F ratio), and male tilapias are inherently faster-growing than females due to physiological and endocrinological advantages. El-Sayed and Fitzsimmons (2023) emphasized that male *Oreochromis niloticus* exhibit superior growth performance under identical culture conditions, while Nduku *et al.* (2022) confirmed that male-biased populations consistently outperform mixed-sex groups in terms of weight gain and feed efficiency.

Therefore, the enhanced growth in D6 is likely not a direct nutritional benefit of the 10% EPLM inclusion, but rather a consequence of the highly male-skewed population. Additionally, the reduced survival rate in this group (87.78%) may have lowered intraspecific competition, allowing the surviving individualsto exhibit improved growth. Silva *et al.* (2022) and Abou *et al.* (2007) demonstrated that reduced stocking density and competitive pressure can enhance growth performance in Tilapia.

The observed dose-dependent decline in survivalfrom approximately 91.11% in groups D1 to D3, down to 87.78% in D6 is a critical finding that suggests a toxicity threshold for EPLM. This reduction in survival is likely attributable to the cumulative effects of antinutritional factors (ANFs) and bioactive compounds present at higher inclusion levels. At low inclusion levels (0–4%), these compounds may be tolerable or even beneficial but at higher levels (≥6%), they may become anti-nutritional, impairing digestion, immunity, or organ function leading to increased mortality.Ahmed *et al.* (2022) reported that tannins and saponins in plant-based aquafeeds can impair nutrient absorption and induce physiological stress, while Neves *et al.* (2024) demonstrated that elevated ANF concentrations negatively affect intestinal health and immune function in Nile tilapia. According to Assan *et al.* (2021)high inclusion of leaf meal may reduce feed palatability, causing fry to eat less yet lower feed intake can result in nutritional deficiencies, weakening the fish and making them more susceptible to stress.

Although high-level EPLM inclusion seemingly enhancedmasculinizationin the study, it simultaneously increases mortality, a consequence that is both economically and ethically unsustainable in commercial aquaculture. As Ciliberti *et al.* (2024) emphasized, the narrow margin between efficacy and toxicity in phytogenic applications necessitates precise dosage optimization to avoid compromising fish welfare and production outcomes.

CONCLUSION

This study observed a dose-dependent trend toward masculinization in *Oreochromis niloticus* fry fed diets containing *Eriosema psoraloides* leaf meal (EPLM), with the highest male-biased sex ratio recorded at 10% inclusion. However, Chi-Square analysis revealed no statistically significant association between diet and sex differentiation ($p = .484$), indicating that while the trend is biologically suggestive, it cannot be conclusively attributed to EPLM under the current experimental conditions.

The findings nonetheless support the hypothesis that bioactive phytochemicals in EPLM particularly flavonoids and saponinsmay modulate endocrine pathways involved in gonadal differentiation. These compounds are known to act as aromatase inhibitors or phytoestrogens, influencing the balance of sex steroids during the labile period of sex determination (Bugel *et al.*, 2016; Sepehrfar *et al.*, 2023). Similar effects have been reported in other plant-based interventions aimed at sex control in tilapia (Wokeh *et al.*, 2021).

Moderate EPLM inclusion levels also enhanced growth performance, while higher levels revealed a trade-off between masculinization and survivallikely due to antinutritional factors or phytochemical toxicity. This underscores the importance of precise dosage optimization to balance efficacy with fish welfare and production sustainability.

Overall, EPLM shows promise as a natural alternative to synthetic sex reversal agents, offering a more sustainable and consumer-conscious approach to mono-sex tilapia production.Future research should focus on molecular sexing, gene expression profiling, and long-term reproductive outcomes to validate EPLM's masculinizing potential and elucidate its mechanism of action. Additionally, exploring its compatibility with other functional feed additives could pave the way for multi-targeted nutritional strategies that enhance growth, immunity, and reproductive control in farmed tilapia.

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