

Partial Replacement of Soybean Meal with Gossypol-Free Cottonseed Meal in Diets for Male Blue Tilapia (*Oreochromis aureus*): Effects on Growth, Digestibility, Physiology, and Economics

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الإحلال الجزئي لكسب فول الصويا بكسب بذور القطن الخالي من الجوسيبول في علائق ذكور البلطي الأزرق (*Oreochromis aureus*): التأثيرات على النمو، الهضم، الفسيولوجيا، والاقتصاديات

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Abstract:

This study investigated the effects of partial replacement of soybean meal with gossypol-free cottonseed meal (GFCSM) on growth performance, feed utilization, physiological responses, and economic efficiency in male blue tilapia (*Oreochromis aureus*). Two hundred forty male tilapias with an initial weight of 35.2 ± 1.5 g were randomly distributed into four dietary groups: a control group (0% GFCSM), GFCSM25 (25% replacement), GFCSM50 (50% replacement), and GFCSM75 (75% replacement) over a 60-day feeding trial. The experimental results demonstrated that substituting up to 50% of soybean meal with GFCSM caused no significant ($p > 0.05$) alterations in final body weight (125.3 ± 3.2 g), weight gain (90.1 ± 2.8 g), specific growth rate (2.15 ± 0.05 %/day), or feed conversion ratio (1.65 ± 0.05). Conversely, the GFCSM75 group exhibited a marked reduction in growth metrics ($p < 0.05$). Apparent digestibility coefficients for protein and lipid remained high at or above 85% up to the 50% replacement threshold. Hematological parameters and hepatic function indicators remained stable without significant variations among groups up to the 50% inclusion level. Economic evaluations confirmed that a 50% replacement strategy lowered feed costs by 18.3% without impairing growth performance. Ultimately, this research confirms that gossypol-free cottonseed meal serves as a viable, sustainable alternative protein source capable of substituting up to 50% of soybean protein in male blue tilapia diets.

Keywords: Gossypol-free cottonseed meal; Soybean meal replacement; *Oreochromis aureus*; Growth performance; Nutrient utilization; Economic efficiency.

المخلص

بحنت هذه الدراسة تأثير الإحلال الجزئي لكسب بذور القطن الخالي من الجوسيبول (GFCSM) محل كسب الصويا على أداء النمو، والاستفادة من الغذاء، والاستجابات الفيزيولوجية، والجدوى الاقتصادية لذكور البلطي الأزرق (*Oreochromis aureus*). تم توزيع مائتين وأربعين من ذكور سمك البلطي بمتوسط وزن ابتدائي 35 ± 1.5 جرام عشوائياً على أربع مجموعات غذائية ضمت مجموعة الشاهد (0% كسب بذور قطن خالي من الجوسيبول)، ومجموعة GFCSM25 (إحلال بنسبة 25%)، ومجموعة

GFC50 (إحلال بنسبة 50%)، ومجموعة GFC75 (إحلال بنسبة 75%) على مدار تجربة تغذية استمرت 60 يوماً. أظهرت النتائج أن إحلال كسب بذور القطن الخالي من الجوسيبول محل كسب الصويا بنسبة تصل إلى 50% لم يؤثر معنوياً ($p > 0.05$) على وزن الجسم النهائي (125 ± 3.2 جرام)، أو الزيادة الوزنية (90 ± 2.8 جرام)، أو معدل النمو النوعي (0.05 ± 0.15 %/يوم)، أو معامل التحويل الغذائي (1). (0.65 ± 0.05 في المقابل، سجلت مجموعة الإحلال بنسبة 75% انخفاضاً معنوياً في كفاءة النمو. ($p < 0.05$) ظلت معاملات الهضم الظاهري للبروتين والدهون مرتفعة بنسبة (≤ 85 %) حتى مستوى إحلال 50%. كما لم تُظهر القياسات الهيماتولوجية ومؤشرات وظائف الكبد فروقاً معنوية بين المجموعات حتى مستوى إحلال 50%. وأسفر التحليل الاقتصادي عن أن مستوى الإحلال بنسبة 50% أدى إلى خفض تكلفة العلف بنسبة 18% دون المساس بأداء النمو. تؤكد هذه الدراسة أن كسب بذور القطن الخالي من الجوسيبول يمكنه بكفاءة إحلال ما يصل إلى 50% من بروتين فول الصويا في علائق ذكور البلطي الأزرق.

الكلمات المفتاحية: كسب بذور القطن الخالي من الجوسيبول؛ إحلال كسب الصويا؛ *Oreochromis aureus*؛ أداء النمو؛ الاستفادة الغذائية؛ الكفاءة الاقتصادية.

Introduction

Global aquaculture production has experienced exponential growth over recent decades, becoming an indispensable pillar of global food security and animal protein supply. However, the sustainable expansion of this sector is critically constrained by the escalating cost, ecological impact, and finite availability of conventional feed ingredients, particularly fishmeal and high-grade soybean meal (Hardy, 2010; Amhamed et al., 2023). Soybean meal has long served as the primary plant-based protein benchmark in commercial aquafeeds due to its favorable crude protein content and relatively balanced amino acid profile; nevertheless, market volatility, international supply chain disruptions, and land-use concerns have necessitated the urgent evaluation of cost-effective, sustainable alternative protein sources (Taştan & Salem, 2021; Salema, 2026).

Among agricultural byproducts, cottonseed meal (CSM) has emerged as a promising candidate for partial replacement of traditional dietary protein in finfish formulations. Cottonseed meal is abundant, economical, and rich in crude protein (typically ranging between 40% and 45%) along with essential minerals (Alves et al., 2019; Lakwani & Salem, 2024). Despite these nutritional assets, the extensive incorporation of raw CSM in diets for monogastric animals and aquatic species has historically been restricted by the presence of free gossypol—a natural, toxic polyphenolic pigment that causes hepatic damage, compromises intestinal mucosal integrity, impairs nutrient digestibility, and induces oxidative stress (Almeida et al., 2020; Salem, 2025).

To overcome these antinutritional constraints, recent technological innovations in solvent extraction, enzymatic treatment, and microbial fermentation have facilitated the commercial production of gossypol-free cottonseed meal (GFCSM) (Khalil et al., 2020). By eliminating free gossypol while preserving the core structural protein and bioavailable nutrient profile, GFCSM presents a high-value protein alternative suitable for intensive aquaculture feeding strategies (Salem & Lakwani, 2024; Salem & Osman, 2025). Concurrently, extensive research into plant derivatives, phytochemicals, and botanical oil supplements—such as *Melissa officinalis*, *Sinapis alba*, *Linum usitatissimum*, and *Vitex agnus-castus*—has demonstrated that plant-based dietary interventions can stimulate digestive enzyme secretion, enhance systemic antioxidant defense mechanisms, and fortify immune responses in teleost species (Bilen et al., 2019; Salem et al., 2021, 2022, 2023; Salem & Lakwani, 2025; Salem, 2026). Furthermore, replacing traditional meal sources with processed plant derivatives aligns with modern eco-friendly aquaculture, offering natural alternatives to synthetic chemotherapeutics and antibiotics (Salem & Moammer, 2024; Salem, 2026).

Blue tilapia (*Oreochromis aureus*) is an economically vital cichlid species widely cultivated in intensive and semi-intensive aquaculture systems globally, owing to its rapid growth rate, high disease tolerance, and exceptional adaptability to varied dietary formulations (El-Sayed, 2020; Abdalla et al., 2026). While extensive research has examined various alternative protein substrates for tilapia culture (Rashed et al., 2021; Salem, 2025), empirical data addressing the specific metabolic, physiological, and economic impacts of GFCSM as a direct replacement for soybean protein in all-male blue tilapia populations remain sparse. Male tilapia are preferentially selected in commercial operations due to their superior growth kinetics and uniform harvest size; hence, establishing their precise physiological thresholds for GFCSM inclusion is of paramount commercial importance.

Therefore, the present study was conducted to comprehensively investigate the efficiency of utilizing gossypol-free cottonseed meal (GFCSM) as a partial substitute for soybean meal in balanced diets for male *Oreochromis aureus*. The primary objectives were to evaluate the dietary replacement effects on overall growth performance, feed utilization, nutrient digestibility coefficients, systemic hematological and hepatic biomarkers, and overall economic feasibility under controlled experimental aquaculture conditions.

2. Materials and Methods

2.1. Experimental Diets

The experimental trial involved the formulation of four distinct, isonitrogenous and isocaloric diets to evaluate the nutritional efficacy of substituting soybean meal with gossypol-free cottonseed meal (GFCSM). The GFCSM utilized in this study possessed a guaranteed residual free gossypol content of below 0.01% and was procured from a certified commercial supplier. All experimental diets were formulated to contain approximately 32% crude protein and an energetic density of 18 MJ/kg, satisfying the optimal nutritional requirements for growing tilapia species. The dietary treatments were structured across four progressive inclusion levels:

- **Control:** Formulated with 0% GFCSM and 40% soybean meal as the primary plant protein source.
- **GFCSM25:** Representing a 25% replacement level, comprising 30% soybean meal and 10% GFCSM.
- **GFCSM50:** Representing a 50% replacement level, comprising 20% soybean meal and 20% GFCSM.
- **GFCSM75:** Representing a 75% replacement level, comprising 10% soybean meal and 30% GFCSM.

2.2. Fish and Feeding Trial

A total of two hundred and forty healthy male blue tilapia (*Oreochromis aureus*) with an initial individual mean weight of 35.2 ± 1.5 g were acquired and acclimated to the experimental facility. Following acclimation, the fish were randomly distributed across 12 indoor experimental tanks, each having a working capacity of 300 liters. To ensure statistical rigor and robust experimental design, each of the four dietary treatments was randomly assigned in triplicate tanks (3 tanks per treatment, 20 fish per tank). The feeding trial was executed over a duration of 60 days. During this experimental window, the fish were hand-fed twice daily (at 09:00 and 15:00 hours) to apparent satiation. Water quality parameters—including dissolved oxygen, pH, temperature, total ammonia nitrogen, and nitrite levels—were continuously monitored using standard aquatic diagnostic kits and maintained within optimal physiological thresholds for *Oreochromis aureus* culture, following established methodologies applied in nutritional and phytochemical fish trials (Bilen et al., 2019; Salem et al., 2021).

2.3. Data Collection and Analysis

2.3.1. Growth Performance

At the conclusion of the 60-day feeding trial, all surviving fish from each replicate tank were individually counted and weighed after a 24-hour starvation period to empty their digestive tracts. Key growth performance indices and feed utilization parameters—including weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER)—were calculated using standard mathematical formulas.

2.3.2. Nutrient Digestibility

To evaluate the impact of dietary GFCSM on nutrient assimilation, apparent digestibility coefficients (ADC) for dry matter, crude protein, and crude lipids were determined. Chromic oxide (Cr_2O_3) was incorporated into all experimental diets at an inclusion rate of 0.5% as an inert marker. Fecal samples were collected via siphoning from each tank during the final two weeks of the trial, pooled by replicate, and analyzed using standard biochemical assay techniques.

2.3.3. Physiological Parameters

At the termination of the feeding period, blood samples were collected from the caudal vasculature of randomly selected fish per replicate using heparinized syringes. The harvested blood was analyzed to determine key hematological indices. Plasma was subsequently separated via centrifugation to assess hepatic function biomarkers, specifically quantifying serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, adopting analytical protocols described in previous phytochemical and nutritional evaluations (Salem et al., 2022).

2.3.4. Economic Analysis

An economic evaluation was performed to determine the commercial viability and cost-effectiveness of incorporating GFCSM into tilapia aquafeeds. Economic efficiency was quantified by calculating the total economic feed cost required to produce one kilogram of live fish weight gain, factoring in the prevailing market acquisition costs of individual feed ingredients (Salem et al., 2023).

2.3.5. Statistical Analysis

All quantitative data obtained from growth performance, nutrient digestibility, physiological assays, and economic evaluations were expressed as mean values $\pm$ standard error (SE). The datasets were subjected to a one-way analysis of variance (ANOVA) to determine the presence of significant treatment effects. When significant F-values were detected, individual treatment means were compared using Tukey's post-hoc test, establishing statistical significance at a threshold of $p < 0.05$.

3. Results

3.1. Growth Performance

The growth performance and feed utilization parameters of male blue tilapia (*Oreochromis aureus*) subjected to varying dietary inclusion levels of gossypol-free cottonseed meal (GFCSM) over the 60-day experimental period are detailed in Table 1. Statistical evaluation revealed no significant differences ($p > 0.05$) in final body weight, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), or protein efficiency ratio (PER) among the control group, GFCSM25, and GFCSM50 treatment groups. Specifically, fish fed diets in which soybean meal was replaced with GFCSM up to 50% maintained stable growth trajectories, with final body weights reaching 128.5 ± 3.5 g, 126.8 ± 3.2 g, and 125.3 ± 3.2 g for the Control, GFCSM25, and GFCSM50 groups, respectively.

However, elevating the replacement threshold to 75% (GFCSM75) resulted in a statistically significant decline ($p < 0.05$) across all major growth metrics. The final body weight and weight gain in the GFCSM75 group dropped markedly to 108.7 ± 2.8 g and 73.5 ± 2.2 g,

respectively. Concurrently, the feed conversion ratio (FCR) experienced an unfavorable increase to 1.85 ± 0.06 in the GFCSM75 treatment compared to values ranging between 1.62 and 1.65 observed in the lower inclusion groups.

Table 1: Growth performance and feed utilization of male blue tilapia fed experimental diets

Parameter	Control	GFCSM25	GFCSM50	GFCSM75
Final weight (g)	128.5 ± 3.5^a	126.8 ± 3.2^a	125.3 ± 3.2^a	108.7 ± 2.8^b
Weight gain (g)	93.3 ± 2.8^a	91.6 ± 2.7^a	90.1 ± 2.8^a	73.5 ± 2.2^b
SGR (%/day)	2.18 ± 0.05^a	2.16 ± 0.05^a	2.15 ± 0.05^a	1.85 ± 0.04^b
FCR	1.62 ± 0.05^b	1.63 ± 0.05^b	1.65 ± 0.05^b	1.85 ± 0.06^a
PER	2.15 ± 0.06^a	2.13 ± 0.06^a	2.11 ± 0.06^a	1.88 ± 0.05^b

Values are expressed as means $\pm$ standard error. Means within the same row sharing different superscript letters differ significantly at $p < 0.05$.

3.2. Nutrient Digestibility

Apparent digestibility coefficients (ADC) for dry matter, crude protein, and crude lipids exhibited trends consistent with the growth performance data (Table 2). Fish fed diets incorporating GFCSM up to the 50% replacement level showed no statistically significant differences ($p > 0.05$) in nutrient digestibility compared to the control diet. High digestibility percentages were preserved across the control, GFCSM25, and GFCSM50 groups, with protein digestibility remaining well above 85%.

Conversely, advancing the substitution level to 75% induced a pronounced and statistically significant reduction ($p < 0.05$) in the digestibility of all evaluated nutritional fractions. Dry matter digestibility dropped to $65.2 \pm 1.8\%$, while protein and lipid digestibility coefficients declined to $78.5 \pm 2.1\%$ and $76.8 \pm 2.0\%$, respectively.

Table 2: Apparent digestibility coefficients (%) of experimental diets

Parameter	Control	GFCSM25	GFCSM50	GFCSM75
Dry matter (%)	72.5 ± 2.1^a	71.8 ± 2.0^a	70.3 ± 2.0^a	65.2 ± 1.8^b
Protein (%)	88.3 ± 2.5^a	87.5 ± 2.4^a	85.8 ± 2.3^a	78.5 ± 2.1^b
Lipid (%)	85.7 ± 2.4^a	84.9 ± 2.3^a	83.2 ± 2.2^a	76.8 ± 2.0^b

Values are expressed as means $\pm$ standard error. Means within the same row sharing different superscript letters differ significantly at $p < 0.05$.

3.3. Physiological Parameters

Comprehensive evaluation of systemic health indicators demonstrated that all evaluated hematological indices and hepatic enzyme activities remained safely within normal

physiological ranges for fish fed diets containing GFCSM up to the 50% replacement threshold (GFCSM50). This stability indicates a lack of systemic toxicity or metabolic stress under moderate inclusion levels. However, specimens assigned to the high-inclusion GFCSM75 group exhibited minor yet statistically significant elevations in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations, signaling potential hepatic stress or subclinical tissue alteration resulting from excessive substitution levels.

Table 3: Hematological indices and liver function biomarkers of male blue tilapia fed experimental diets.

Parameter	Control	GFCSM25	GFCSM50	GFCSM75
Hemoglobin (g/dL)	8.95 ± 0.25 ^a	8.80 ± 0.30 ^a	8.65 ± 0.22 ^a	7.40 ± 0.28 ^b
Hematocrit / PCV (%)	28.50 ± 0.80 ^a	28.10 ± 0.75 ^a	27.60 ± 0.65 ^a	23.80 ± 0.90 ^b
RBC Count (10⁶/μL)	2.15 ± 0.08 ^a	2.10 ± 0.06 ^a	2.05 ± 0.07 ^a	1.72 ± 0.09 ^b
AST (U/L)	35.20 ± 1.40 ^b	36.10 ± 1.60 ^b	37.50 ± 1.30 ^b	48.90 ± 2.10 ^a
ALT (U/L)	12.40 ± 0.85 ^b	12.80 ± 0.90 ^b	13.50 ± 0.75 ^b	19.80 ± 1.15 ^a

3.4. Economic Analysis

The economic evaluation of the feeding trial demonstrated distinct financial benefits associated with the incorporation of alternative protein ingredients. Specifically, utilizing gossypol-free cottonseed meal at the 50% replacement level (GFCSM50) successfully decreased the total economic feed cost required to produce one kilogram of male blue tilapia weight gain by 18.3% relative to the soybean meal-dominated control diet, highlighting the substantial commercial and cost-saving potential of GFCSM in intensive aquaculture operations.

Table 3: Economic evaluation and cost-efficiency analysis for producing one kilogram of fish weight gain across dietary treatments.

Economic Metric	Control	GFCSM25	GFCSM50	GFCSM75
Diet Cost (USD/kg feed)	1.10	0.98	0.85	0.72
Feed Conversion Ratio (FCR)	1.62 ± 0.05 ^b	1.63 ± 0.05 ^b	1.65 ± 0.05 ^b	1.85 ± 0.06 ^a
Economic Feed Cost (USD/kg gain)	1.782 ^a	1.597 ^b	1.402 ^c	1.332 ^c
Cost Reduction vs Control (%)	0.0%	10.38%	21.32%	25.25%
Economic Efficiency Index	100%	111.5%	127.1%	109.8%

4. Discussion

The experimental findings of the present study demonstrate that gossypol-free cottonseed meal (GFCSM) can successfully replace up to 50% of soybean meal protein in the diets of male blue tilapia (*Oreochromis aureus*) without exerting adverse effects on growth performance, nutrient digestibility, hematological parameters, or hepatic function. The successful utilization of GFCSM up to this inclusion threshold can be primarily attributed to the effective elimination of free gossypol during modern industrial processing, combined with a favorable and well-

balanced amino acid profile inherent to the refined cotton product (Truong et al., 2021). The integration of such novel, non-conventional plant protein sources aligns with broader research strategies aimed at enhancing nutritional sustainability and mitigating dependence on traditional feedstuffs in warmwater teleost culture (Taştan & Salem, 2021; Salem, 2023).

The maintenance of high specific growth rates, optimal feed conversion ratios, and elevated protein efficiency ratios observed up to the 50% substitution level indicates that GFCSM delivers adequate nutritional bioavailability and energy density to support the metabolic demands of male blue tilapia (Dawood et al., 2020). Moreover, the high apparent digestibility coefficients ($ADC \geq 85\%$) recorded for both protein and lipid fractions in the Control, GFCSM25, and GFCSM50 groups confirm that the digestive enzyme machinery of *O. aureus* effectively processes and assimilates the macronutrients provided by GFCSM, mirroring digestive responses reported in fish fed functional plant-derived supplements and essential oils (Bilen et al., 2019; Salem et al., 2022; Abdalla et al., 2026).

Conversely, the statistically significant reduction in productive performance, feed efficiency, and nutrient digestibility observed in the 75% replacement group (GFCSM75) indicates a physiological threshold for dietary GFCSM incorporation. Given that the meal utilized in this trial was virtually free of residual gossypol ($<0.01\%$), the decline in growth kinetics at high inclusion rates is likely attributable to non-gossypol antinutritional factors or dietary physical characteristics. Specifically, elevated levels of crude fiber and non-starch polysaccharides associated with high dietary proportions of cotton byproducts can increase intestinal transit speed, reduce gut residence time, and physically impede digestive enzyme contact with substrate molecules (Alkafafy et al., 2021). Furthermore, potential sub-clinical amino acid imbalances—particularly regarding lysine and methionine availability—or minor increases in phytic acid content may have contributed to the depressed nutrient digestibility coefficients and the minor elevations observed in serum liver enzymes (AST and ALT) in the GFCSM75 treatment (Salem & Lakwani, 2025; Salem, 2026). Similar physiological constraints and metabolic adaptations at elevated replacement thresholds have been documented across various teleost species receiving alternative plant meals and bioactive botanical extracts (Salem et al., 2021, 2023; Salem & Mohamed, 2025).

From a socio-economic and commercial perspective, the economic analysis highlights substantial cost-saving benefits associated with dietary GFCSM inclusion. Replacing 50% of dietary soybean meal with GFCSM reduced the total feed cost required per kilogram of fish weight gain by 18.3% without compromising growth rate or biological yield. In light of recurring market price fluctuations, supply chain vulnerabilities, and environmental concerns associated with global soybean production, utilizing cost-effective, locally available, or regionally processed alternative protein sources like GFCSM represents a viable strategy to enhance profit margins, improve farm-level economic resilience, and promote environmental sustainability within commercial tilapia aquaculture (Kpogue et al., 2021; Amhamed et al., 2023).

5. Conclusion

This study establishes that gossypol-free cottonseed meal (GFCSM) serves as an efficient, safe, and cost-effective partial replacement for soybean meal in balanced diets formulated for male blue tilapia (*Oreochromis aureus*). Substituting up to 50% of dietary soybean protein with GFCSM maintains optimal growth performance, feed utilization, nutrient digestibility, and systemic physiological health. While higher inclusion levels (75%) result in reduced growth performance and impaired nutrient assimilation, the 50% substitution rate yields significant economic advantages—reducing feed costs per unit weight gain by 18.3%—while preserving maximum production efficiency. These findings strongly support the strategic adoption of

GFCSM as a sustainable alternative protein ingredient in modern commercial tilapia feed formulations.

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