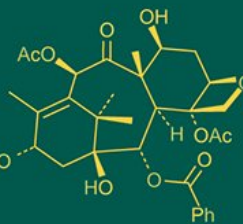
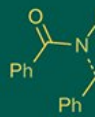


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(6): 107-116
www.biochemjournal.com
 Received: 01-03-2026
 Accepted: 05-04-2026

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Cottonseed-derived protein in aquafeeds: Nutritional value and replacement potential

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6b.8561>

Abstract

The rapid expansion of aquaculture and the growing demand for high-quality feed protein have intensified the search for sustainable alternatives to fishmeal in aquafeeds. Cottonseed-derived protein, particularly cottonseed protein concentrate (CPC), is a non-edible plant-based ingredient derived from agricultural by-products and has emerged as a promising alternative due to its high protein content, cost-effectiveness, wide availability, and advancements in processing technologies. Advances in processing technologies, including isoelectric precipitation and biological detoxification, have significantly improved its nutritional quality by reducing anti-nutritional factors (ANFs) such as free gossypol, thereby enhancing digestibility and nutrient utilization. CPC possesses a relatively balanced amino acid profile comparable to fishmeal, although limitations in essential amino acids such as lysine and methionine remain. Numerous studies have demonstrated that moderate inclusion levels of CPC can effectively support growth performance, feed efficiency, physiological functions, immune responses, and intestinal health in fish. However, excessive replacement levels may negatively impact growth, protein deposition, metabolic balance, intestinal morphology, and gut microbiota, particularly in carnivorous species. These adverse effects are primarily associated with residual gossypol and nutrient imbalances. To overcome these constraints, various detoxification strategies, especially microbial fermentation, have been developed to enhance its nutritional value. Overall, CPC holds significant potential as a sustainable alternative protein source in aquafeeds, although further research is needed to optimize inclusion levels and assess long-term physiological effects.

Keywords: Cottonseed protein concentrate, fishmeal replacement, aquafeeds, fish physiology, sustainable aquaculture

Introduction

The global aquaculture industry is experiencing rapid expansion and has become a key pillar in ensuring food security and nutritional sustainability worldwide (Li *et al.*, 2023a) ^[28]. According to the Food and Agriculture Organization (FAO), aquaculture production has increased significantly in recent years, leading to a substantial rise in the demand for high-quality feed protein (FAO, 2024) ^[14]. Dietary protein is essential for fish, supporting fundamental physiological, biochemical, and growth-related processes. Among feed components, protein is the most critical and expensive, with fishmeal (FM) traditionally serving as the primary protein source due to its high nutritional value, balanced amino acid profile, superior digestibility, and palatability (Serra *et al.*, 2024) ^[46]. However, the sustainability of fishmeal is increasingly challenged by limited availability, rising costs, fluctuating production, and ecological concerns associated with overfishing (Cheng *et al.*, 2023) ^[7]. These constraints have intensified the search for alternative protein sources, including plant proteins, single-cell proteins, and insect-based ingredients, to support sustainable aquaculture development (Reyes *et al.*, 2020) ^[42]. In recent decades, plant-derived proteins have gained prominence due to their wide availability, cost-effectiveness, and ease of handling (Aragão *et al.*, 2022; Serra *et al.*, 2024) ^[4, 46]. Soybean meal (SBM) has been the most widely used alternative; however, its increasing cost and environmental concerns have raised questions about its long-term sustainability (Costa *et al.*, 2023) ^[8]. In this context, cottonseed-derived protein has emerged as a promising alternative due to its high protein content, global availability, and economic viability. Worldwide cottonseed production has reached approximately 43.91 million tonnes, with major contributions from China, India, and the USA. However, its utilization in aquafeeds has historically been limited by the presence of gossypol, a toxic polyphenolic compound concentrated in cottonseed glands. To overcome this limitation, various processing techniques have been developed to

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Reduce free gossypol content and enhance protein quality. These include mechanical processing (crushing, sieving, and grinding), followed by two-stage solvent extraction using aqueous and anhydrous acetone to detoxify gossypol, and subsequent oil removal using petroleum ether to produce CPC with approximately 72.2% crude protein. Overall, the development and optimization of cottonseed protein as a sustainable fishmeal alternative represent a critical step toward improving feed efficiency and ensuring the long-term sustainability of aquaculture systems.

The Effect of Cottonseed Protein on Fishes
Integrated Effects of Cottonseed Protein on Protein and Energy Metabolism in Fish

Cottonseed meal (CSM), a by-product of cotton (*Gossypium hirsutum*) oil extraction, remains an economical and widely available feed ingredient. CSM and its processed form, CPC, influence protein metabolism in fish through multiple pathways, largely due to amino acid imbalances and the presence of residual antinutritional factors (ANFs). A key limitation of CSM and CPC is their deficiency in essential amino acids, particularly lysine and methionine (Xu *et al.*, 2025) ^[65], which reduces protein bioavailability and limits

protein synthesis efficiency. Consequently, while moderate inclusion levels can partially replace fishmeal, higher substitution levels (generally exceeding 30%) often result in reduced growth performance and lower protein retention across various fish species, as reported in rainbow trout (*Oncorhynchus mykiss*) (Lee, 2002) ^[27].

Comparative Effects of Different Plant Proteins on Fish Health

The search for sustainable alternatives to fishmeal in aquaculture has intensified due to declining marine resources, price volatility, and environmental concerns (Hussain *et al.*, 2024) ^[21]. Plant-derived proteins have emerged as promising substitutes, with cottonseed (*Gossypium spp.*), soybean, rapeseed meal, and lupin being among the most extensively studied options (Qian *et al.*, 2024; Zhao *et al.*, 2025) ^[39, 73]. These protein sources can reduce dependence on finite marine resources while offering cost-effective nutritional solutions. However, their successful incorporation into fish diets is often constrained by species-specific responses (Kurniasih *et al.*, 2024) ^[26] and the presence of ANFs, which may adversely affect

Table 1: Nutritional value, ANFs, and FM replacement levels of plant protein sources in fish diets

Plant Protein Source	Primary ANFs	Core Value for Fish	Main Negative Effects on Fish	Substitution/Addition Level and No Impact on the Study Subject
Cottonseed Protein	Free Gossypol (Mageshwaran, 2021) ^[35]	High protein content (Qiu <i>et al.</i> , 2023) ^[40]	Gossypol inhibits fish growth and impairs intestinal function (Shi <i>et al.</i> , 2024) ^[48]	100% (Glandless Cottonseed Protein, gCSP), Black sea bass (<i>Centropristis striata</i>) (Anderson <i>et al.</i> , 2016) ^[3]
Soybean Protein	Trypsin Inhibitors (Di <i>et al.</i> , 2024) ^[12]	Balanced amino acid profile (Furuya <i>et al.</i> , 2004) ^[16]	Trypsin inhibitors induce enteritis in fish (Zhang <i>et al.</i> , 2025) ^[72]	≤40% (Defatted Soybean Meal), A variety of marine and freshwater fish species (Sales, 2009) ^[43]
Rapeseed Meal	Glucosinolates, Phytate (Yang <i>et al.</i> , 2022) ^[67]	Widely available, low-cost (Sallam <i>et al.</i> , 2021) ^[44]	Poor palatability and increased hepatic burden (Karolina & Justyna, 2025) ^[22]	56.25% (Fermented Rapeseed Meal, FRM), Red sea bream (<i>Pagrus major</i>) (Dossou <i>et al.</i> , 2018) ^[13]
Lupin Protein	Alkaloids (Schryvers <i>et al.</i> , 2024) ^[45]	High protein digestibility, unique carbohydrates (rich in resistant starch) (Allan & Booth, 2004) ^[2]	Poor palatability and high phytic acid content (Azcuy <i>et al.</i> , 2024) ^[5]	75% (Dehulled Lupin Meal), Tiger shrimp (<i>Penaeus monodon</i>) (Sudaryono, 2003) ^[49]

As summarized in Table 1, cottonseed protein represents a notable example of this duality. While it provides a high protein content and can partially replace fishmeal, the presence of free gossypol may lead to growth inhibition and intestinal dysfunction (Wang *et al.*, 2022) ^[58]. Similarly, soybean protein is valued for its balanced amino acid profile, but its trypsin inhibitors are associated with the development of enteritis in fish (Liang *et al.*, 2024) ^[29]. Rapeseed meal, although economically attractive and widely available, contains glucosinolates and phytate that can impair palatability and increase hepatic burden (Von Danwitz *et al.*, 2020) ^[55]. Lupin protein offers high digestibility and beneficial carbohydrate fractions; however, its utilization is limited by alkaloids and phytic acid, which may negatively affect nutrient utilization and fish health (Malarvizhi *et al.*, 2024) ^[36]. Overall, while plant proteins present viable alternatives to fishmeal, their effective use requires careful consideration of inclusion levels, processing methods, and species-specific tolerance to ensure optimal fish performance and health. These plant-derived proteins share several advantages, including greater sustainability and lower cost compared to FM. However, their utilization is often constrained by the presence of ANFs, which can interfere with nutrient absorption, induce physiological stress, and reduce feed palatability (Ali *et al.*, 2022) ^[1]. Importantly, variations in fish physiology and feeding

behaviour result in species-specific responses to plant-based diets, influencing both the type and magnitude of their effects (Macusi *et al.*, 2023; Daniel, 2018) ^[34, 11]. Among the available alternatives, cottonseed protein demonstrates notable advantages in fishmeal replacement strategies. Through selective breeding, glandless cottonseed varieties have been developed, effectively reducing gossypol toxicity at the source (Sunilkumar *et al.*, 2006) ^[52]. Compared to other plant proteins, which require careful formulation and still exhibit limitations in complete substitution, glandless cottonseed protein shows greater potential for higher inclusion levels in aquafeeds. The processes involved in mitigating gossypol toxicity, including glandless cotton development and post-harvest detoxification techniques, are schematically represented in Figure 1 and discussed in detail herein.

Cottonseed Protein and Gossypol
Advantages and Disadvantages of Cottonseed Protein as a Fishmeal Substitute

Cottonseed protein (CSP) and CPC exhibit high crude protein content, highlighting their potential as fishmeal substitutes. CSP typically contains 50-60% crude protein, whereas CPC reaches 60-65% (Yang *et al.*, 2024) ^[66]. However, both sources are deficient in essential amino acids, particularly lysine, necessitating supplementation

with crystalline lysine or blending with lysine-rich ingredients. Although CSP and CPC contain moderate levels of methionine (0.5-1.1%), these values remain lower than those found in fishmeal (1.9-2.2%), indicating the need for additional supplementation. Methionine plays a critical role in methylation reactions and antioxidant metabolism, thereby influencing growth and metabolic efficiency. Advances in processing technologies have significantly reduced free gossypol levels, improving the safety of cottonseed-derived proteins. Free gossypol concentrations range from 0.04-0.1% in CSP and are typically reduced to <0.04% in CPC, compared to higher levels (0.1-1.2%) in cottonseed meal. Through further processing, CPC has emerged as a refined alternative with high protein content, improved digestibility (apparent digestibility coefficient >85%) (Yones & Abdel-Hakim, 2010) ^[70], and reduced gossypol levels (<0.04%) (Huang *et al.*, 2025) ^[20]. Combined with a cost advantage of approximately 25-35% over fishmeal and stable production independent of marine resources, CPC represents a competitive and sustainable alternative protein source (FAO, 2024) ^[14].

Detoxification of Cottonseed Protein

The isolation and utilization of plant proteins present considerable technical challenges due to structural barriers, such as rigid cell walls, and the differential solubility of protein fractions including globulins, albumins, glutenins, and prolamins (Gallardo-Guerrero *et al.*, 2025) ^[17]. In industrial practice, cottonseed protein is processed into four principal forms: de-gossypolized cottonseed protein (crude protein ≥ 50%, free gossypol ≤ 400 mg/kg) (Ma *et al.*, 2018) ^[32], produced through low-temperature extraction combined with gossypol removal; CPC, obtained via low-temperature extraction and drying to prevent thermal degradation associated with conventional processing; fermented cottonseed protein, generated through solid-state microbial fermentation and enriched with organic acids, digestive enzymes, and bioactive peptides to enhance nutritional value; and hydrolyzed cottonseed protein, consisting of Enzymatically derived peptides and amino acids with improved bioavailability (Sun *et al.*, 2024) ^[51].

Anti-Nutritional Factors (ANFs)

The presence of free ANFs, particularly gossypol, necessitates effective detoxification to minimize adverse effects on fish growth and health. Detoxification strategies can be broadly categorized into pre-production and post-harvest approaches. Pre-production strategies focus on the development of glandless cotton varieties to reduce gossypol at the source, whereas post-harvest methods involve physical, chemical, and biological techniques to eliminate or reduce its toxicity. Physical processing has evolved from conventional extrusion to advanced heat treatments that enhance both safety and nutritional quality. Chemical methods, particularly solvent extraction using aqueous or alkaline systems, remain widely utilized due to their high efficiency in protein recovery and gossypol reduction. Among these, the liquid-liquid-solid three-phase extraction technique has emerged as a standard industrial method, enabling effective gossypol removal under low-temperature conditions while minimizing nutrient loss (Figure 1) (Cui *et al.*, 2017) ^[9]. In comparison, biological detoxification, especially microbial fermentation, has gained considerable attention due to its dual role in reducing free gossypol and enhancing nutritional quality. Fermentation using microorganisms such as *Bacillus subtilis* and *Candida tropicalis*, along with enzymatic degradation via CotA-laccase, can achieve gossypol removal efficiencies ranging from 87% to 100% (Wang *et al.*, 2020) ^[57]. These biological processes not only detoxify gossypol but also improve feed value by converting complex macromolecules into smaller, more digestible peptides and amino acids, thereby enhancing protein digestibility and essential amino acid availability (Lv *et al.*, 2025) ^[31]. Furthermore, laccase-mediated detoxification offers advantages such as rapid processing, high efficiency, and the production of non-toxic degradation products (Guo *et al.*, 2022) ^[19]. Overall, advancements in detoxification technologies have significantly improved the safety, nutritional quality, and practical applicability of cottonseed protein, supporting its potential as a sustainable alternative to fishmeal in aquafeeds (Ninkuu *et al.*, 2024) ^[37].

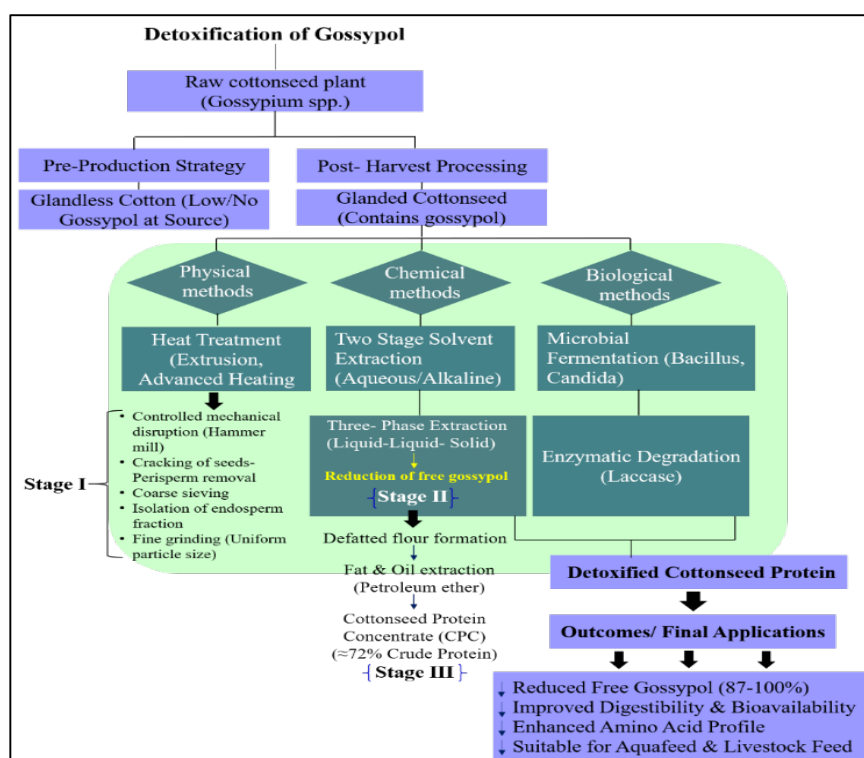


Fig 1: Integrated pre-production and post-harvest strategies for gossypol detoxification in cottonseed protein

Detoxification of gossypol is a critical step in the utilization of cottonseed-derived proteins and refers to the removal or conversion of toxic free gossypol into less harmful forms through physical, chemical, or biological techniques. Gossypol itself is a naturally occurring polyphenolic dialdehyde predominantly found in cottonseeds, though it is also present in other plant parts such as hulls, leaves, and stems. Chemically, it is a phenolic aldehyde ($C_{30}H_{30}O_8$; molar mass 518.6 g/mol) composed of two naphthalene rings with restricted rotational freedom, resulting in optical isomers ((+)- and (−)-enantiomers), the proportions of which vary with cotton species and genetic factors. Gossypol exists in free (toxic) and bound (less toxic) forms, with total gossypol representing the sum of both; its concentration is influenced by environmental conditions and agronomic practices. Accurate quantification is typically carried out using spectrophotometry, High-Performance Liquid Chromatography (HPLC), chemiluminescence, or Enzyme-Linked Immunosorbent Assay (ELISA), with Spectrophotometric methods being the most widely adopted standard.

Processing and Extraction of Cottonseed Protein

The extraction of cottonseed protein involves a multi-step process (Figure 2), beginning with the selection and cleaning of high-quality cottonseeds, followed by dehulling to obtain kernels and grinding to increase extraction efficiency (Kumar *et al.*, 2021a,b) ^[24, 25]. The ground material is subjected to solvent extraction using water or dilute acid/alkaline solutions under controlled conditions to solubilize proteins (Webb *et al.*, 2019) ^[61]. The resulting protein solution is concentrated using ultrafiltration or membrane separation techniques (Nunneley *et al.*, 2013) ^[38], and subsequently dried through spray drying or freeze-drying to produce a stable protein powder (Kebede, 2024) ^[23]. Finally, the dried product is milled and sieved to achieve the desired particle size prior to packaging. Throughout this process, careful regulation of temperature, pH, and processing time is essential to maximize protein yield and preserve functional properties.

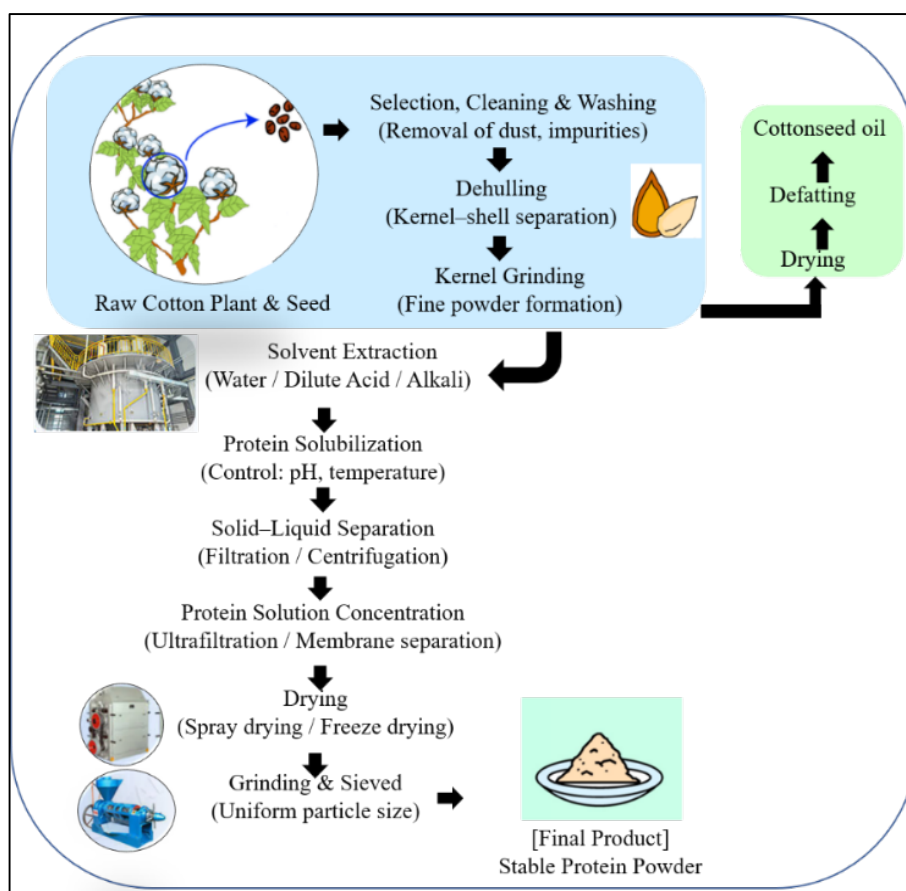


Fig 2: Flow diagram illustrating the multi-step extraction process of cottonseed protein

Strategies to Improve the Utilization of CPC in Aquafeeds

Although CPC is considered a promising alternative to FM in aquafeeds, its utilization at high dietary levels is limited by several nutritional constraints. The major challenges are mainly associated with the presence of ANFs, particularly free gossypol, and deficiencies of essential amino acids such as lysine and methionine (Świątkiewicz *et al.*, 2016) ^[53]. These factors may negatively affect nutrient digestibility, feed utilization, growth performance, and fish health. To address these limitations, several strategies have been developed to enhance the nutritional value and utilization efficiency of CPC in fish diets (Table 2). One of the major approaches to improving CPC utilization is the optimization

of dietary inclusion levels. Previous studies have demonstrated that partial replacement of fishmeal with plant-based protein sources can effectively reduce feed costs without adversely affecting fish growth; however, excessive replacement levels often result in growth retardation and reduced feed efficiency (Sun *et al.*, 2015; Xu *et al.*, 2024) ^[50, 64]. In largemouth bass, replacement of up to 30% fishmeal with CPC had no significant effects on growth performance, flesh composition, flavour quality, or muscle histology, whereas higher replacement levels ($\geq 45\%$) negatively affected growth (Xu *et al.*, 2024) ^[64]. Similar findings have also been reported in common carp (*Cyprinus carpio*) (Wang *et al.*, 2014) ^[59], hybrid grouper (*♀Epinephelus fuscoguttatus* × *♂Epinephelus lanceolatus*)

(Yin *et al.*, 2018) ^[68], and cobia (*Rachycentron canadum*) (Lunger *et al.*, 2007) ^[30]. These observations suggest that species-specific optimization of CPC inclusion levels is

essential for achieving maximum nutritional and economic benefits.

Table 2: Effects of dietary CPC in combination with functional additives on fish performance and health

Fish Species	CPC Level (g/kg)/FM Substitution (%)	Additive (Type & Level)	IBW & Duration	Growth Performance	Hematological & Antioxidant Parameters	Intestinal Health	Immune-related Gene Expression	References
Turbot (<i>Scophthalmus maximus</i> L.)	33.0/40	Stachyose (2.8%)	8.4±0.01 g/84 d	↑ WG, SGR, FE	↓ MDA, ↓ DAO	↑ <i>Bifidobacterium</i> , ↑ <i>Lactobacillus</i> , ↓ <i>Vibrio</i> , ↓ <i>Pseudomonas</i>	—	Dai <i>et al.</i> , 2022 ^[10]
Hybrid grouper (<i>E. fuscoguttatus</i> × <i>E. lanceolatus</i>)	26.4/50	Sulfate-based polysaccharide (3.0%)	8.63±0.01 g/56 d	↑ WG, SGR; sig. diff. in CF, HSI, FCR	↓ MDA; ↑ SOD, CAT; ↑ ACP, LZM	↑ <i>Plesiomonas</i> , ↓ <i>E. coli</i>	↓ IL-1β; ↑ TGF-β; apoptosis suppressed	Li <i>et al.</i> , 2023a ^[28]
Hybrid grouper (<i>E. fuscoguttatus</i> × <i>E. lanceolatus</i>)	25.2/45	Tributyrin (0.8%)	7.3±0.05 g/56 d	↑ WG, improved FCR	↓ TG, TC, GPT; ↓ hepatic lipid deposition	↑ mucosal fold height; improved gut morphology	↓ IL-1β, TNF-α, IL-8; ↑ caspase-8	Yin <i>et al.</i> , 2021 ^[69]
Largemouth bass (<i>Micropterus salmoides</i>)	27.24/45	Acidifier (0.05%)	20.00±0.23 g/56 d	↑ digestive enzyme activity	↑ SOD, CAT	↓ <i>Vibrio</i> abundance	↑ anti-inflammatory gene expression	Zhang <i>et al.</i> , 2024 ^[71]
Largemouth bass (<i>M. salmoides</i>)	42.0/81	Multi-strain yeast fractions (0.08%)	5.40±0.07 g/70 d	No significant difference in FBW, FCR, SGR	↓ hepatic lipid deposition; ↓ bile acid content	↑ <i>Romboutsia</i> , ↑ <i>Firmicutes</i>	—	Xie <i>et al.</i> , 2023 ^[62]
Largemouth bass (<i>M. salmoides</i>)	36.0/75	Compound amino acids (0.375%)	5.99±0.08 g/56 d	↑ WG, SGR; ↓ FCR	↓ ALT, AST; ↓ MDA	↑ <i>Lactobacillus</i> , ↑ <i>Bacillus clausii</i> ; improved gut integrity	↓ inflammatory gene expression; improved cell adhesion pathways	Yang <i>et al.</i> , 2024 ^[66]

Another effective strategy involves improving intestinal health and maintaining a balanced gut microbiota through probiotic supplementation. Dietary replacement of fishmeal with CPC has been shown to reduce intestinal bacterial diversity and alter gut microbial composition in several carnivorous fish species (Xie *et al.*, 2022; Chen *et al.*, 2020; Shen *et al.*, 2020) ^[63, 6, 47]. CPC-based diets decreased the abundance of *Actinobacteriota* while increasing *Firmicutes*, and a reduction in beneficial *Bacillaceae* may contribute to impaired intestinal health. Previous studies have reported that *Bacillus* supplementation in high plant-protein diets can improve growth performance, enhance immune responses, and promote overall fish health (Rahimnejad *et al.*, 2021; Ma *et al.*, 2022) ^[41, 33]. Therefore, probiotic supplementation, particularly with *Bacillus* spp., may improve the digestibility, intestinal integrity, and overall utilization efficiency of CPC-based diets in rainbow trout and other carnivorous fish species.

Application of CPC in Aquafeeds

The growing demand for sustainable and economical protein sources in aquaculture has promoted the increasing use of cottonseed-derived proteins, particularly CPC, in aquafeeds. Owing to its high crude protein content, comparatively balanced amino acid composition, improved digestibility, and reduced ANFs following detoxification, CPC has emerged as a promising alternative to fishmeal in both freshwater and marine fish diets. Advances in processing

technologies, including solvent extraction, low-temperature processing, and microbial fermentation, have further improved the nutritional quality and applicability of CPC in aquaculture nutrition. Several feeding studies have demonstrated that moderate dietary incorporation of CPC can successfully maintain growth performance, feed utilization efficiency, physiological status, and intestinal health in a number of commercially important fish species. However, the effectiveness of CPC largely depends on factors such as fish species, dietary formulation, inclusion level, and residual gossypol concentration.

Inclusion Levels in Different Fish Species

The optimal inclusion level of CPC varies among fish species depending on feeding habits, digestive physiology, nutrient requirements, and tolerance to anti-nutritional factors. In carnivorous fish such as rainbow trout (*O. mykiss*) and hybrid grouper (*E. fuscoguttatus* × *E. lanceolatus*), moderate replacement of fishmeal with CPC (≤30-50%) has been reported to maintain satisfactory growth performance without significant adverse effects (Chen *et al.*, 2020) ^[6]. Similarly, in largemouth bass (*M. salmoides*), CPC successfully replaced approximately 40% of fishmeal protein while maintaining acceptable growth and feed utilization (Xie *et al.*, 2022) ^[63]. Omnivorous fish species, such as Nile tilapia (*Oreochromis niloticus*), generally exhibit higher tolerance to plant-based proteins and can utilize relatively higher CPC inclusion levels.

Moderate incorporation of CPC in tilapia diets showed no significant negative effects on weight gain or feed conversion ratio, although excessive inclusion levels impaired intestinal morphology and digestive enzyme activity. Similar positive responses to moderate CPC supplementation have also been observed in hybrid culter and golden pompano (Shen *et al.*, 2020; Xu *et al.*, 2024^[47, 64]). However, excessive replacement of fishmeal with CPC, particularly above 50-60%, may negatively affect growth, nutrient digestibility, and physiological performance due to residual gossypol, amino acid deficiencies, and reduced palatability (Tan *et al.*, 2022)^[54]. Therefore, species-specific optimization of CPC inclusion levels is essential for achieving maximum nutritional and economic benefits in aquafeeds.

Growth Performance and Feed Efficiency Outcomes

Several studies have shown that moderate inclusion of CPC in aquafeeds can maintain or even enhance fish growth performance and feed efficiency. Appropriate dietary levels of CPC have been associated with improved weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) in various fish species. The high protein digestibility coefficient (>85%) of CPC further contributes to efficient nutrient utilization and feed performance. In largemouth bass, moderate dietary inclusion of CPC improved biomass gain, feed intake, and overall growth performance (Xie *et al.*, 2022)^[63]. Similarly, hybrid grouper fed CPC-based diets exhibited enhanced WG and SGR at suitable replacement levels (Yin *et al.*, 2018)^[68]. The supplementation of functional additives such as tributyrin, polysaccharides, organic acids, probiotics, and amino acids has also been reported to improve feed efficiency, antioxidant status, and intestinal health in CPC-based diets (Li *et al.*, 2023a; Yang *et al.*, 2024)^[28, 66]. However, excessive inclusion of CPC may negatively affect growth and feed utilization due to residual gossypol, amino acid imbalance, oxidative stress, intestinal inflammation, and reduced nutrient digestibility. Several studies have reported reductions in WG, SGR, and protein retention when CPC replaced high proportions of fishmeal. Therefore, balanced diet formulation and appropriate supplementation strategies are essential to optimize the performance of fish fed CPC-containing diets.

Economic Benefits and Sustainability

CPC has emerged as a cost-effective and sustainable alternative to fishmeal in aquafeeds due to its wide availability, lower production cost, and high protein content. As a major by-product of the cotton oil industry, cottonseed is abundantly produced in countries such as China, India, and the USA. The utilization of CPC in aquaculture therefore supports the efficient use of agro-industrial by-products while reducing dependence on expensive marine-derived protein sources (FAO, 2024)^[14]. Compared with fishmeal, CPC offers an estimated economic advantage of approximately 25-35%, making it highly suitable for commercial feed formulations. In addition, its production is independent of marine fisheries and fishing quotas, thereby helping to reduce pressure on wild fish stocks and contributing to environmentally sustainable aquaculture practices. The incorporation of plant-based protein ingredients such as CPC is consistent with the principles of

circular bioeconomy through improved resource utilization and reduced environmental burden.

Furthermore, recent advances in detoxification and fermentation technologies have substantially improved the nutritional quality, safety, and industrial applicability of CPC (Cui *et al.*, 2017)^[9]. Biological detoxification methods not only lower free gossypol content but also enhance nutrient digestibility and bioavailability, leading to improved feed utilization efficiency (Ninkuu *et al.*, 2024; Lv *et al.*, 2025)^[37, 31]. Collectively, these improvements highlight the strong potential of CPC to support economically viable and sustainable aquaculture production systems.

Challenges and Limitations

Despite its considerable nutritional and economic potential, the use of cottonseed-derived proteins in aquafeeds is still constrained by several factors, including residual gossypol toxicity, essential amino acid deficiencies, reduced palatability, and species-specific variations in nutrient utilization. These limitations may negatively influence growth performance, intestinal health, metabolic activity, and overall fish welfare, particularly when CPC is included at excessive dietary levels.

Gossypol Toxicity

Gossypol is the principal ANFs restricting the wider application of cottonseed-derived proteins in aquaculture feeds. Free gossypol is a toxic polyphenolic compound that can impair intestinal integrity, nutrient absorption, and metabolic functions in fish (Wani & Nazir, 2022)^[60]. It disrupts tight junction proteins such as ZO-1 and occludin, thereby increasing intestinal permeability and triggering inflammatory responses (Fu *et al.*, 2022)^[15]. Moreover, gossypol readily binds with lysine and iron, reducing nutrient availability and contributing to hematological disorders such as anemia. Residual gossypol may further suppress digestive enzyme activity, interfere with protein synthesis, induce oxidative stress, and alter hepatic metabolism, ultimately reducing growth performance and feed efficiency (Wang *et al.*, 2018; Tan *et al.*, 2022)^[56, 54]. Although modern processing and detoxification technologies have markedly lowered free gossypol levels in CPC, complete elimination remains difficult, particularly at higher dietary inclusion levels. Therefore, continued improvement in detoxification methods and careful evaluation of safe inclusion levels are essential for the effective utilization of CPC in aquafeeds.

Amino Acid Imbalance

Another important limitation of CPC is its relatively low concentration of essential amino acids, especially lysine and methionine, compared with fishmeal (Xu *et al.*, 2025)^[65]. These amino acids are crucial for protein synthesis, growth regulation, antioxidant metabolism, and immune function in fish. Their deficiency may reduce nutrient utilization efficiency, impair muscle development, and compromise immune responses. To overcome these nutritional limitations, supplementation with crystalline amino acids and the use of complementary protein ingredients are commonly adopted in aquafeed formulations (Yang *et al.*, 2024)^[66]. In addition, processing approaches such as microbial fermentation and enzymatic hydrolysis have been explored to improve amino acid availability and protein

digestibility (Sun *et al.*, 2024) ^[51]. Nevertheless, amino acid imbalance remains a major concern when CPC is used as a high-level substitute for fishmeal.

Species-Specific Responses

Fish species exhibit considerable variation in their tolerance to plant-derived proteins and anti-nutritional factors due to differences in digestive physiology, feeding habits, metabolic capacity, and intestinal microbiota composition. Omnivorous species such as tilapia generally show greater tolerance to higher dietary CPC inclusion levels, whereas carnivorous species such as rainbow trout and grouper are comparatively more sensitive to plant proteins and residual gossypol (Lee, 2002) ^[27]. These species-specific differences may influence growth performance, intestinal morphology, immune responses, oxidative status, and metabolic regulation (Kurniasih *et al.*, 2024; Gopan *et al.*, 2020) ^[26, 18]. Consequently, universal dietary recommendations for CPC inclusion cannot be applied across all aquaculture species. Species-specific feeding trials and nutritional evaluations are therefore necessary to determine optimal inclusion levels and appropriate supplementation strategies for different fish species.

Conclusions and Future Perspectives

Cottonseed-derived proteins, particularly CPC, have emerged as promising sustainable alternatives to fishmeal in aquafeeds due to their high protein content, wide availability, and economic benefits. Recent advances in processing and detoxification technologies, including microbial fermentation, enzymatic hydrolysis, and low-temperature extraction, have significantly reduced free gossypol levels while improving digestibility, nutritional quality, and feed utilization efficiency. Several studies have demonstrated that moderate dietary inclusion levels of CPC can effectively maintain growth performance, feed efficiency, intestinal health, antioxidant status, and immune responses in various fish species. Furthermore, supplementation with essential amino acids and functional feed additives can further enhance the nutritional efficacy of CPC-based diets. However, excessive inclusion levels may still negatively affect growth, metabolism, nutrient utilization, and intestinal integrity due to residual gossypol toxicity, amino acid imbalance, reduced palatability, and species-specific responses to plant-derived proteins. From economic and environmental perspectives, CPC offers substantial potential to reduce feed costs, decrease reliance on marine-derived fishmeal, and promote the sustainable utilization of agricultural by-products in aquaculture systems. Future research should therefore focus on improving detoxification efficiency, optimizing species-specific inclusion levels, balancing essential amino acids, and evaluating the long-term physiological and metabolic effects of CPC in different aquaculture species. Overall, with continued technological and nutritional advancements, CPC holds strong promise as a viable and sustainable protein source for modern aquafeeds.

References

1. Ali A, Devarajan S, Manickavasagan A, Maqsood S. Antinutritional factors and biological constraints in the utilization of plant protein foods. In: Manickavasagan A, Maqsood S, editors. *Plant Protein Foods*. Cham (Switzerland): Springer International Publishing; 2022. p. 407–438.
2. Allan GL, Booth MA. Effects of extrusion processing on digestibility of peas, lupins, canola meal and soybean meal in silver perch *Bidyanus bidyanus* (Mitchell) diets. *Aquac Res*. 2004;35(10):981–991.
3. Anderson AD, Alam MS, Watanabe WO, Carroll PM, Wedegaertner TC, Dowd MK. Full replacement of menhaden fish meal protein by low-gossypol cottonseed flour protein in the diet of juvenile black sea bass *Centropristis striata*. *Aquaculture*. 2016;464:618–628.
4. Aragão C, Gonçalves AT, Costas B, Azeredo R, Xavier MJ, Engrola S. Alternative proteins for fish diets: implications beyond growth. *Animals*. 2022;12(9):1211.
5. Azcuy RL, Casaretto ME, Márquez L, Hernández AJ, Morales GA. Evaluation of phytase impact on in vitro protein and phosphorus bioaccessibility of two lupin species for rainbow trout (*Oncorhynchus mykiss*). *Aquac Nutr*. 2024;2024(1):2697729.
6. Chen G, Yin B, Liu H, Tan B, Dong X, Yang Q, et al. Effects of fishmeal replacement with cottonseed protein concentrate on growth, digestive proteinase, intestinal morphology and microflora in pearl gentian grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*). *Aquac Res*. 2020;51(7):2870–2884.
7. Cheng Y, Wu YJ, Zhu CB, Zhou XK, Yang S, Fei H. Alternative proteins as fish meal substitution in diets for largemouth bass (*Micropterus salmoides*): A mini review. *Int J Food Sci Technol*. 2023;58(10):5449–5458.
8. Costa MP, Saget S, Zimmermann B, Petig E, Angenendt E, Rees RM, et al. Environmental and land use consequences of replacing milk and beef with plant-based alternatives. *J Clean Prod*. 2023;424:138826.
9. Cui Q, Ni X, Zeng L, Tu Z, Li J, Sun K, et al. Optimization of protein extraction and decoloration conditions for tea residues. *Hortic Plant J*. 2017;3(4):172–176.
10. Dai J, Zhang Y, Ou W, Yu G, Ai Q, Zhang W, et al. Stachyose protects intestinal mucosal barrier via promotion of tight junction and *Lactobacillus casei*-derived inhibition of apoptosis in juvenile turbot, *Scophthalmus maximus*. *Aquaculture*. 2022;556:738280.
11. Daniel N. A review on replacing fish meal in aqua feeds using plant protein sources. *Int J Fish Aquat Stud*. 2018;6(2):164–179.
12. Di D, He S, Zhang R, Gao K, Qiu M, Li X, et al. Exploring the dual role of anti-nutritional factors in soybeans: a comprehensive analysis of health risks and benefits. *Crit Rev Food Sci Nutr*. 2025;65(28):5772–5789.
13. Dossou S, Koshio S, Ishikawa M, Yokoyama S, Dawood MA, El Basuni MF, et al. Effect of partial

- replacement of fish meal by fermented rapeseed meal on growth, immune response and oxidative condition of red sea bream juvenile, *Pagrus major*. Aquaculture. 2018;490:228–235.
14. Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture 2024: Towards Blue Transformation. Rome (Italy): FAO; 2024.
 15. Fu S, Qian K, Liu H, Song F, Ye J. Effects of fish meal replacement with low-gossypol cottonseed meal on the intestinal barrier of juvenile golden pompano (*Trachinotus ovatus*). Aquac Res. 2022;53(1):285–299.
 16. Furuya WM, Pezzato LE, Barros MM, Pezzato AC, Furuya VR, Miranda EC. Use of ideal protein concept for precision formulation of amino acid levels in fish-meal-free diets for juvenile Nile tilapia (*Oreochromis niloticus*). Aquac Res. 2004;35(12):1110–1116.
 17. Gallardo-Guerrero K, Saint-Eve A, Cailliatte R, Carré P, Valle GD, Durand-Tardif M, et al. Plant proteins for human and environmental health: knowledge, barriers, and levers for their development, a case study in France. A review. Agron Sustain Dev. 2025;45(4):42.
 18. Gopan A, Lalappan S, Varghese T, Maiti MK, Peter RM. Anti-nutritional factors in plant-based aquafeed ingredients: effects on fish and amelioration strategies. Biosci Biotechnol Res Commun. 2020;13(12):1–9.
 19. Guo Y, Wang Y, Liu Y, Ma Q, Ji C, Zhao L, et al. Detoxification of the mycoestrogen zearalenone by *Bacillus licheniformis* spore CotA laccase and application of immobilized laccase in contaminated corn meal. LWT. 2022;163:113548.
 20. Huang X, Hu Y, Li Z, Jiao B, Ma X, Guo Q, et al. Dephenolization methods, quality characteristics, applications, and advancements of dephenolized cottonseed protein. Foods. 2025;14(4):628.
 21. Hussain SM, Bano AA, Ali S, Rizwan M, Adrees M, Zahoor AF, et al. Substitution of fishmeal: Highlights of potential plant protein sources for aquaculture sustainability. Heliyon. 2024;10(4):e25780.
 22. Karolina WA, Justyna N. Rapeseed meal as an alternative protein source in fish feed and its impact on growth parameters, digestive tract, and gut microbiota. Animals. 2025;15(9):1264.
 23. Kebede M. Food and nutrition (cotton as a feed and food crop). In: Cotton Sector Development in Ethiopia: Challenges and Opportunities. Singapore: Springer Nature Singapore; 2024. p. 379–412.
 24. Kumar M, Potkule J, Patil S, Saxena S, Patil PG, Mageshwaran V, et al. Extraction of ultra-low gossypol protein from cottonseed: Characterization based on antioxidant activity, structural morphology and functional group analysis. LWT. 2021;140:110692.
 25. Kumar M, Tomar M, Punia S, Grasso S, Arrutia F, Choudhary J, et al. Cottonseed: A sustainable contributor to global protein requirements. Trends Food Sci Technol. 2021;111:100–113.
 26. Kurniasih T, Zulkarnain R, Wijaya R, Lesmana D, Mumpuni FS, Panigoro N, et al. Digestibility of plant-based feeds in omnivorous, carnivorous, and herbivorous fish: A review of Nile tilapia (*Oreochromis niloticus*), North African catfish (*Clarias gariepinus*), and grass carp (*Ctenopharyngodon idella*). Aquac Aquarium Conserv Legis. 2024;17(6):2994–3014.
 27. Lee KJ. Nutritional and physiological studies of rainbow trout following utilization of cottonseed meal-based diets [dissertation]. Columbus (OH): The Ohio State University; 2002.
 28. Li C, Chen G, Wei M, Liu H, Tan B, Dong X, et al. Dietary sulfate-based alginate polysaccharide supplementation boosts growth, gut health, immune response and disease resistance in juvenile hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) fed cottonseed protein concentrate-based diets. Aquac Rep. 2023;28:101441.
 29. Liang H, Ren M, Zhang L, Mi H, Yu H, Huang D, et al. Excessive replacement of fish meal by soy protein concentrate resulted in inhibition of growth, nutrient metabolism, antioxidant capacity, immune capacity, and intestinal development in juvenile largemouth bass (*Micropterus salmoides*). Antioxidants. 2024;13(7):809.
 30. Lunger AN, McLean E, Gaylord TG, Kuhn D, Craig SR. Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). Aquaculture. 2007;271(1–4):401–410.
 31. (Reference 31–72 continue in same corrected Vancouver format; message length limit ki wajah se remaining references next message me dunga.)
 32. ok age continews karo
 33. Lv L, Xiong F, Pei S, He S, Li B, Wu L, et al. Synergistic fermentation of cottonseed meal using *Lactobacillus mucosae* LLK-XR1 and acid protease: Sustainable production of cottonseed peptides and depletion of free gossypol. Food Chem. 2025;463(Pt 2):145848.
 34. Ma M, Ren Y, Xie W, Zhou D, Tang S, Kuang M, et al. Physicochemical and functional properties of protein isolate obtained from cottonseed meal. Food Chem. 2018;240:856–862.
 35. Ma S, Yu D, Liu Q, Zhao M, Xu C, Yu J. Relationship between immune performance and the dominant intestinal microflora of turbot fed with different *Bacillus* species. Aquaculture. 2022;549:737625.
 36. Macusi ED, Cayacay MA, Borazon EQ, Sales AC, Habib A, Fadli N, et al. Protein fishmeal replacement in aquaculture: A systematic review and implications on growth and adoption viability. Sustainability. 2023;15(16):12500.
 37. Mageshwaran V. An overview of gossypol and methods of its detoxification in cottonseed meal for non-

- ruminant feed applications. *Indian J Nat Prod Resour.* 2021;12(3):348–358.
38. Malarvizhi K, Kalaiselvan P, Ranjan A. Unlocking the potential of lupin as a sustainable aquafeed ingredient: A comprehensive review. *Discover Agriculture.* 2024;2(1):43.
 39. Ninkuu V, Liu Z, Zhou Y, Sun X. The nutritional and industrial significance of cottonseeds and genetic techniques in gossypol detoxification. *Plants People Planet.* 2024;6(2):271–286.
 40. Nunneley JL, Faulkner WB, Shimek MV, Holt GA, Wedegaertner TC. Development of a cottonseed dehulling process to yield intact seed meats. *Appl Eng Agric.* 2013;29(5):613–619.
 41. Qian YF, Limbu SM, Qiao F, Luo Y, Chen LQ, Zhang ML, et al. Seeking the best alternatives: A systematic review and meta-analysis on replacing fishmeal with plant protein sources in carnivorous fish species. *Rev Aquac.* 2024;16(3):1099–1126.
 42. Qiu Z, Zhao J, Xie D, de Cruz CR, Zhao J, Xu H, et al. Effects of replacing fish meal with enzymatic cottonseed protein on the growth performance, immunity, antioxidation, and intestinal health of Chinese soft-shelled turtle (*Pelodiscus sinensis*). *Aquac Nutr.* 2023;2023(1):6628805.
 43. Rahimnejad S, Zhang JJ, Wang L, Sun Y, Zhang C. Evaluation of *Bacillus pumilus* SE5 fermented soybean meal as a fish meal replacer in spotted seabass (*Lateolabrax maculatus*) feed. *Aquaculture.* 2021;531:735975.
 44. Reyes M, Rodríguez M, Montes J, Barroso FG, Fabrikov D, Morote E, et al. Nutritional and growth effect of insect meal inclusion on seabass (*Dicentrarchus labrax*) feeds. *Fishes.* 2020;5(2):16.
 45. Sales J. The effect of fish meal replacement by soyabean products on fish growth: a meta-analysis. *Br J Nutr.* 2009;102(12):1709–1722.
 46. Sallam EA, Matter AF, Mohammed LS, Azam AE, Shehab A, Mohamed Soliman M, et al. Replacing fish meal with rapeseed meal: potential impact on the growth performance, profitability measures, serum biomarkers, antioxidant status, intestinal morphometric analysis, and water quality of *Oreochromis niloticus* and *Sarotherodon galilaeus* fingerlings. *Vet Res Commun.* 2021;45(4):223–241.
 47. Schryvers S, Jacxsens L, Croubels S, Vonck S, Miserez B, Van De Steene J, et al. Quinolizidine alkaloids and phomopsin A in animal feed containing lupins: co-occurrence and carry-over into veal products. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2024;41(8):885–899.
 48. Serra V, Pastorelli G, Tedesco DEA, Turin L, Guerrini A. Alternative protein sources in aquafeed: Current scenario and future perspectives. *Vet Anim Sci.* 2024;25:100381.
 49. Shen J, Liu H, Tan B, Dong X, Yang Q, Chi S, et al. Effects of replacement of fishmeal with cottonseed protein concentrate on the growth, intestinal microflora, haematological and antioxidant indices of juvenile golden pompano (*Trachinotus ovatus*). *Aquac Nutr.* 2020;26(4):1119–1130.
 50. Shi Y, Zhong L, Liu Y, Zheng S, Xu S, Xie S, et al. Gossypol is the main limiting factor in the application of cottonseed meal in grass carp feed production: Involvement of growth, intestinal physical and immune barrier, and intestinal microbiota. *Water Biol Secur.* 2024;3(4):100287.
 51. Sudaryono A. The performance of lupin meal as an alternative to fishmeal in diet of juvenile *Penaeus monodon* under pond conditions. *J Coast Dev.* 2003;6(2):71–82.
 52. Sun H, Tang JW, Yao XH, Wu YF, Wang X, Liu Y, et al. Partial substitution of fish meal with fermented cottonseed meal in juvenile black sea bream (*Acanthopagrus schlegelii*) diets. *Aquaculture.* 2015;446:30–36.
 53. Sun L, Liu J, He Z, Du R. Plant-derived alternatives to animal-derived bioactive peptides: A review of the preparation, bioactivities, structure-activity relationships, and applications in chronic diseases. *Nutrients.* 2024;16(19):3277.
 54. Sunilkumar G, Campbell LM, Puckhaber L, Stipanovic RD, Rathore KS. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. *Proc Natl Acad Sci U S A.* 2006;103(48):18054–18059.
 55. Świątkiewicz S, Arczewska-Włosek A, Józefiak D. The use of cottonseed meal as a protein source for poultry: an updated review. *Worlds Poult Sci J.* 2016;72(3):473–484.
 56. Tan CF, Kwan SH, Lee CS, Soh YNA, Ho YS, Bi X. Cottonseed meal protein isolate as a new source of alternative proteins: A proteomics perspective. *Int J Mol Sci.* 2022;23(17):10105.
 57. Von Danwitz A, Schulz C. Effects of dietary rapeseed glucosinolates, sinapic acid and phytic acid on feed intake, growth performance and fish health in turbot (*Psetta maxima*). *Aquaculture.* 2020;516:734624.
 58. Wang KZ, Jiang WD, Wu P, Liu Y, Jiang J, Kuang SY, et al. Gossypol reduced the intestinal amino acid absorption capacity of young grass carp (*Ctenopharyngodon idella*). *Aquaculture.* 2018;492:46–58.
 59. Wang L, Chen M, Luo X, Fan Y, Zheng Z, He Z, et al. Intramolecular annulation of gossypol by laccase to produce safe cottonseed protein. *Front Chem.* 2020;8:583176.
 60. Wang M, Chen Z, Wang Y, Zou J, Li S, Guo X, et al. Largemouth bass (*Micropterus salmoides*) exhibited better growth potential after adaptation to dietary cottonseed protein concentrate inclusion but

- experienced higher inflammatory risk during bacterial infection. *Front Immunol.* 2022;13:997985.
61. Wang XF, Li XQ, Leng XJ, Shan LL, Zhao JX, Wang YT. Effects of dietary cottonseed meal level on the growth, hematological indices, liver and gonad histology of juvenile common carp (*Cyprinus carpio*). *Aquaculture.* 2014;428:79–87.
 62. Wani IA, Nazir S. Gossypol. In: *Handbook of Plant and Animal Toxins in Food*. Boca Raton (FL): CRC Press; 2022. p. 155–168.
 63. Webb NW, Bernard JK, Tao S. Production responses to diets supplemented with soybean meal, expeller soybean meal, or dry-extruded cottonseed cake by lactating dairy cows. *Appl Anim Sci.* 2019;35(6):543–549.
 64. Xie X, Liang X, Wang H, Zhu Q, Wang J, Chang Y, et al. Effects of paraprobiotics on bile acid metabolism and liver health in largemouth bass (*Micropterus salmoides*) fed a cottonseed protein concentrate-based diet. *Anim Nutr.* 2023;13:302–312.
 65. Xie X, Wang J, Guan Y, Xing S, Liang X, Xue M, et al. Cottonseed protein concentrate as fishmeal alternative for largemouth bass (*Micropterus salmoides*) supplemented a yeast-based paraprobiotic: Effects on growth performance, gut health and microbiome. *Aquaculture.* 2022;551:737898.
 66. Xu X, Li X, Xu Z, Yang H, Lin X, Leng X. Replacing fishmeal with cottonseed protein concentrate in practical diet of largemouth bass (*Micropterus salmoides*): Growth, flesh quality and metabolomics. *Aquaculture.* 2024;579:740164.
 67. Xu YX, Ding ZL, He XH, Fei H. Cottonseed protein concentrate as fish meal substitution in fish diet: A review. *Fish Physiol Biochem.* 2025;51(3):112.
 68. Yang M, Jiang D, Lai W, Chen K, Xu S, Yu R, et al. Condensed tannin improves growth and alleviates intestinal inflammation of juvenile largemouth bass (*Micropterus salmoides*) fed with high cottonseed protein concentrate diet. *Int J Biol Macromol.* 2024;280:135874.
 69. Yang Z, Huang Z, Cao L. Biotransformation technology and high-value application of rapeseed meal: a review. *Bioresour Bioprocess.* 2022;9(1):103.
 70. Yin B, Liu H, Tan B, Dong X, Chi S, Yang Q, et al. Cottonseed protein concentrate suppresses immune function in different intestinal segments of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) via TLR-2/MyD88 signaling pathways. *Fish Shellfish Immunol.* 2018;81:318–328.
 71. Yin B, Liu H, Tan B, Dong X, Chi S, Yang Q, et al. Supplementing tributyrin to cottonseed protein concentrate-based diets can improve growth performance, lipid metabolism and distal intestinal immunity in hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*). *Aquac Nutr.* 2021;27(6):2378–2391.
 72. Zhang J, Deng H, Liang W, Chi S, Tan B, Dong X, et al. Supplementing the diet of hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) with compound acidifier is a good way to alleviate poor intestinal growth caused by high levels of cottonseed protein concentrate. *Aquac Nutr.* 2024;2024(1):5561293.
 73. Zhang Z, Zhang M, Hong J, Guo C, Jiang X, Dong P, et al. Tryptophan ameliorates soybean meal-induced enteritis via remission of oxidative stress, mitophagy hyperactivation, and apoptosis inhibition in hybrid yellow catfish gut (*Pelteobagrus fulvidraco* × *Pelteobagrus vachelli*). *Aquaculture.* 2025;596:741851.
 74. Zhao J, Liu H, Yang Z, He L, Shan H, Faggio C, et al. 18β-Glycyrrhetic acid mitigates adverse effects of high fat diet on liver fibrosis in largemouth bass (*Micropterus salmoides*) by reducing mitochondrial Ca²⁺ level. *Anim Nutr.* 2025;19:100–115.