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Fermented cottonseed meal as an alternative for groundnut oil cake in aquafeed

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Abstract
Better nutrition in aquaculture is essential for higher growth and improved yields. Enhancing production through the intensification of cultural practices ensures profitability and a sustained supply of fish protein. However, such intensification of aquaculture practices shifts the emphasis to feed-based culture. Feed cost represents the significant operational cost in animal production. In aquaculture, it represents about 50-60% of the operational costs and plays a vital role in determining the profitability of aquaculture. As fish meal resources are declining, the ingredients for these feeds will have to be sourced mainly from plants. Plant-based ingredients are widely available, renewable, and are already being used in aquafeed. These agro-industrial residues like cottonseed meal can be suitable, especially after fermentation, due to their low production cost and high nutritional value. Improved nutritional quality of agro-waste as feed is considered very important for its practical application in fish feed. The improvement in nutrient quality is best obtained by solid-state fermentation, resulting in decreased antinutritional factors of agro-waste products.

Keywords: Aquafeed, agro-waste, fermentation, cottonseed meal

1. Introduction
Marine fish production is experiencing near-stagnation, and the only hope lies with aquaculture to meet the ever-increasing demand. Inland fish farming is the most common aquaculture practice that affects the economy of developing countries. Fish is a better source of vitamin A, B_1, B_6, D, C, and minerals than other animal protein sources [1]. The demand for a protein-based diet is rising due to the increasing population. The global per capita fish consumption increased from an average of 9.0 kg in the 1960′s to above 20.5 kg in 2018[2]. Carp production is the major aquaculture species, contributing over 71.9% of the freshwater output of the world [3]. Feed-based carp culture is yet to gain momentum due to high feed costs compared to other aquaculture species. A recent report indicates that only 1.3% of farmers use commercial feed, whereas the rest use mash feed only [4]. The mash feed comprises groundnut cake, mustard oil cake, or cottonseed meal. Mash feeding is an age-old practise where farmers use locally available feed ingredients as farm-made feed.
Plant protein is preferred over animal protein in carp culture [5]. Dietary replacement of fishmeal by plant by-products, such as soybean, cottonseed, and rapeseed, is on the rise due to their low price market availability and desirable protein level [6]. Among plant protein sources, oilseed cakes/meals are the dominant choices as an alternative to fishmeal. These are by-products left out after oil extraction from respective oil seeds. Many studies focused on finding cheap, readily available protein sources to satisfy the nutritional need of fish [7, 8]. The major problem with plant-based diet sources is high fiber content and antinutritional factors. These antinutritional factors negate the organism's growth and other physiological activities at higher inclusion levels.

2. Cotton Seed
Cotton generally refers to four species in the genus *Gossypium*, i.e., *G. hirsutum* L., *G. barbadense* L., *G. arboretum* L. *and G. herbaceum* L. *G. hirsutum* and *G. barbadense* are the two most commercially cultivated cotton species [9]. Cottonseed is the second-largest protein source used in animal feed [10]. It is a valuable animal feed component because of its relatively high protein content and easy availability.

Cotton is one of India's most important crops among commercial crops. Cotton is the backbone of the textile industry, which supports 70% of the country's total fiber production. Cottonseed, a by-product of the textile industry, provides a significant quantity of edible oil and protein-rich meal for livestock [11]. The cotton plant produces more food for humans and feeds animals than fiber [12]. After soybean and rapeseed, cottonseed is the leading plant protein source used worldwide by weight [13].

3. Cottonseed Composition

Cottonseed contains chemically reactive cyclopropenoid fatty acids, malvalic acid, and sterculic acid. However, they are deficient in lysine [14] and have antinutritional factors, such as gossypol [15]. The chemical composition of CSM as 51.20% crude protein, 7.02% crude fibre, 1.6% ether extract, 9.3% ash and 2.71 ME (kcal/kg) [16].

4. Antinutritional factors associated with CSM

Cottonseed meal (CSM) contains gossypol, a yellow terpenoid produced and stored in the pigment glands found in the cotton plant [17] and is toxic to fish [18], leading to a restriction of its use as a feed ingredient. Gossypol is available in either bound or free form, the bound form being non-toxic and of little significance since it is unavailable and passes through the gastrointestinal tract unabsorbed [19]. The level of CSM inclusion in fish diets varies widely among fish species, developmental stages, free gossypol (FG) level, dietary protein, and available lysine [20]. Usage of CSM in fish feeds is limited by the free gossypol level and available lysine content [21]. FG binds with protein (amino group of lysine) and hinders its availability in CSM [22]. During oil extraction from cottonseeds, gossypol is extruded as free form from the seeds due to high compression force [23]. Gossypol is not expected to cause toxicity if cottonseed meal from "glandless" varieties of cotton plants is utilized in feeds. FG of CSM has anti-nutritional properties [24]; it affects growth [25] and causes infertility in fish [26]. Therefore, lowering and eliminating gossypol from CSM is necessary to improve CSM availability as a feed protein source for the animal. Unprocessed plant proteins are not suitable as aquafeed ingredients since they contain antinutrients, undesirable for fish [27]. Further, palatability issues and limitations of certain essential amino acids exist [13].

5. Solid-State Fermentation

Solid-state fermentation (SSF) deals with the utilization of water-insoluble material for microbial growth and metabolism [28] in the absence or near absence of free liquid or free water [29]. The technology has the advantage of direct utilization of solid substrates under aerobic conditions to produce microbial biomass products. Solid substrate supplies the required nutrients to the growing microbes and serves as an anchorage for the cells. An ideal substrate should provide all the needed nutrients to the microorganisms growing in it. However, some nutrients may be available in sub-optimal concentrations or even absent in the substrates. Loss of nutrients during fermentation is negligible, and there may be an increase in the nutrient level through microbial synthesis [30]. The advantages of using microorganisms for SSF are that they can grow fast and produce protein in large amounts by utilizing the soluble sugars and organic acids present in the substrates [31].

6. Enrichment of Cottonseed Meal (CSM)

Solid-state fermentation (SSF) is a valuable tool for converting large volumes of agro-industrial biomass to value-added enzymes and chemicals [32]. Microbes draw the nutrients from biomass and, in turn, generate beneficial metabolites and enzymes. SSF based detoxification of agro-industrial wastes has been established for phorbol esters in Jatropha seed cake, ricin in castor bean cake, caffeine in the coffee husk, and gossypol in cottonseed meal and glucosinolates in rapeseed meal. SSF is an efficient and economically viable technique to reduce or remove antinutrients and toxins in agro-wastes [33]. Microbial fermentation reduces antinutritional factors in soybean (SM) and cottonseed meals. The fermentation process of cottonseed meal significantly decreased dietary and liver gossypol concentrations [34]. These findings suggest that soybean or cottonseed meal microbial fermentation with *A. oryzae* can enhance antioxidant activities in diets. The presence of gossypol, low level of lysine, and high fiber levels in CSM limit cottonseed meal incorporation in aquafeeds [21]. It is possible to apply conventional methods and microbial biotechnology to deactivate endogenous antinutritional factors. Amino acids extracted from plants, bacteria, and yeasts are promising ingredients in feed [35]. Microbial fermentation could make plant protein sources more valuable and functional in fish feed. Findings of CSM fermentation with the mixed fungal culture of *Candida tropicalis* and *Saccharomyces cerevisiae* increased the protein content from 20% to 33.5% after 48 h of fermentation [36]. Similar findings were seen in raw cottonseed meal after SSF using yeast wherein the crude protein has improved from 8.74 to 12.67% [37].

7. Cotton Seed Meal in Aquafeed

CSM has been tested in several fish species like *Oreochromis niloticus* where 33.76% CSM could be used in the diets[38], Rainbow trout, *Oncorhynchus mykiss* feed at 15% inclusion level of CSM has not shown much difference in growth[39]; similarly in juvenile hybrid tilapia, (*O. niloticus* × *O. aureus*) [21]; parrotfish, *Oplegnathus fasciatus* [34]; Crucian carp, *Carassius auratus gibelio* [40]; juvenile Southern Flounder, *Paralichthys lethostigma* [41]; Snout bream juvenile, *Megalobrama amblycephala* [42]; silver sillago, *Sillago sihama Forsskal* [26], rohu fry, *Labeo rohita* [37]. But in these fish species, the unprocessed and raw CSM contain antinutrients that are highly undesirable for fish species. Incorporation 30% incorporation rate of raw CSM has shown a moderate growth rate in rohu fry at the nursery phase [37].

8. Solid State Fermented CSM in Aquafeed

Fermented CSM at 50% incorporation exhibited a significant growth rate in Nile tilapia than those fish fed with the control and other experimental diets [34]. Juvenile black sea bream fed with fermented CSM, where no significant difference in the digestive and metabolic enzyme activities between the treatment and control fed diets was observed, indicating less stress, easy digestibility and better acceptance of the fermented feeds at higher incorporation levels [43]. Similar finds at 50% incorporation of fermented CSM by replacing sunflower seed meal to rohu fry at nursery phase showed better growth than the other experimental diets [37].

9. Conclusion

Among the plant-based feeds, cottonseed meal is one potential source of protein for fish. It has often been assessed to replace soybean meal in fish diets due to its high palatable nature. The presence of gossypol, fiber content and low lysine, methionine and cystine in CSM has limited its use in fish farming in the raw form. The application of solid-state fermentation for improving feed quality has opened the doors for higher incorporation levels in aquafeed. This higher incorporation is achieved due to a decrease in antinutritional factors like gossypol, phytic acid and the increase in the essential amino acids during the solid-state fermentation process.

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