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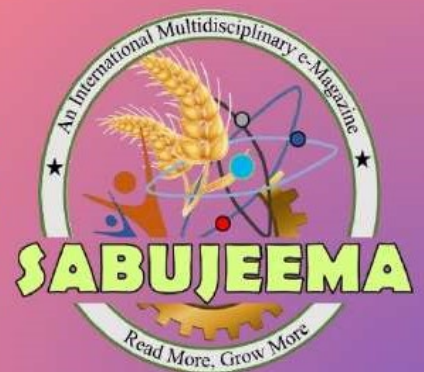
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# OPTIMIZATION OF GOSSYPOL LEVELS IN COTTON SEED CAKE BY SOLID-STATE FERMENTATION AND FURTHER ITS APPLICATION IN AQUAFEEDS.

[Article ID: SIMM0069]

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The worldwide aquaculture industry depends on the availability of low cost, high quality feeds. Due to increasing demand, decreasing supply and the high cost of fishmeal, fish nutritionists have concentrated their efforts to find alternative sources of proteins to substitute fishmeal in fish diet. However, interrelationships between nutrition, immunity and disease resistance in fish are poorly understood. Several studies have directed to an efficient and cost-effective supplementary fish feed because of the importance of fish as a protein source for human and animal's diet. Aquafeed is the primary determinant that influences the successful growth and intensification of aquaculture production.

Fish feeding constitutes over 50% of operating cost in intensive aquaculture, where protein is the most expensive dietary source. Fish meal is one of the primary ingredients in making fish feed, considering its high importance as a source of protein. However, it is an expensive feed ingredient, and the supplies often vary unpredictably due to overfishing or large-scale transient oceanic changes.

## ALTERNATIVE PROTEIN SOURCES:

Researchers are looking for cheaper and alternative viable protein source for non-ruminants feed. The dietary replacement of fishmeal by plant origin by-products, such as soybean meal (SBM), cottonseed meal (CSM) and rapeseed meal (RSM), has been increasing in the aquaculture industry because of their low price, high market availabilities and sufficient protein contents. Recommended plant proteins can be used as a total replacement for animal protein without detrimental effects. The protein of plant origin is preferred compared to animal origin protein in carps' culture with fermented and beneficial microorganisms in recent times.

## SOLID-STATE FERMENTATION:

Solid-state fermentation is defined as the fermentation involving solids in the absence (or near absence) of free water; however, the substrate must possess enough moisture to support the growth and metabolism of the microorganism. In recent years, there has been a resurgence of interest in solid-state fermentation and its applicability to the production of enzymes, metabolites and organic compounds.

**AGRO-INDUSTRIAL SOLID****SUBSTRATES:**

Agro-industrial residues are generally considered the best substrates for the SSF processes to produce industrially important enzymes. Some of the substrates available locally with low cost are wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, sawdust, corncobs, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, cornflour, steamed rice. Raw material, wheat bran, has been reported as a good carbon source for protease production from *Aspergillus* spp.

**COTTON SEED CAKE:**

Cottonseed, *Gossypium hirsute* Linnaeus, is the third leading plant protein by weight (after soybean and rapeseed) used worldwide. Cottonseed meal (CSM) has 41-45% protein and is widely cultivated. Owing to its high protein value for human consumption and animals and low market price compared with other legumes and fishmeal, cottonseed meal consequently has immense potential for incorporation in high-protein aquafeeds. Nutritionally, CSM contains high levels of proteins and is very palatable to fish. However, these studies most frequently used replacing one plant protein source with the other; whereas, more challenging and profitable would replace fishmeal or other animal protein sources. CSM that is abundant throughout the world and global production amounted to 44.84 million metric tons and is available at a relatively lower cost than animal proteins. The level of cottonseed meal inclusion in fish diets varies widely among fish species. The amount of CSM included in feeds depends

on the animal species, developmental stages, levels of free gossypol, dietary protein and available lysine. Studies on the Utilization of SBM and CSM as a partial or complete replacement for fishmeal in catfish diets have been conducted and successfully reported. Among plant proteins, CSM, a byproduct of the cottonseed processing industry, has been tested in several fish species, including tilapia, *Sarotherodon mossambicus*, Channel catfish *Ictalurus punctatus*, Chinook salmon, *Oncorhynchus tshawytscha* and Coho salmon, *Oncorhynchus kisutch* and Rainbow trout, *Oncorhynchus mykiss*.

However, cottonseed meal contains gossypol, a polyphenolic compound that, at high levels, can be toxic to aquatic animals. Also, CSM is low in some essential amino acids, mainly lysine and sulphur-containing amino acids methionine and cystine. The amount of CSM that can be used in aquatic animal feeds depends on the tolerance of animals to gossypol, amino acid composition, and the relative cost of CSM compared with other protein sources. As the lysine binds with the gossypol in CSM, its presence will be unavailable. The presence of the bound gossypol decreases the digestibility of amino acids during enzymatic digestion and thus reduces the nutritional value of cottonseed protein. Microbial fermentation is a viable method for detoxification of gossypol and improving CSM's nutritional properties in solid-state fermentation. Processing plant materials through a simple and inexpensive process like fermentation might considerably decrease the antinutritional factors and crude fibre content, increasing their nutritional value.

Gossypol is toxic to a wide range of animals. Its gossypol content limits the use

of CSM in fish feeds. However, its effect on fishes is species dependent. In few findings, 0.03% free gossypol was toxic to rainbow trout. But in few other cases, channel catfish can tolerate up to 0.09% free gossypol in their diets without any suppressive effect on their growth. Furthermore, investigations up to 0.2% free gossypol can be safely added to the diets of *T. aurea*, and the reduced growth of the fish was due to cyclopropionic acids contained in the gland and glandless cotton seeds, not to the free gossypol content.

#### **REDUCTION OF GOSSYPOL IN CSM:**

Much research has been conducted to reduce or eliminate gossypol in CSM to improve its utilization as an ingredient in the feeds of animals and fish. Although several approaches have been undertaken, two methods have been most effective. The first approach was to prepare CSM from naturally mutant “glandless” seed that lacked the lysigenous glands and most of the gossypol. Cotton plants that produced glandless seeds were first noted in the late 1950s when farmers visually noticed the existence of mutant plants without the dark-coloured glands rendering the seeds largely gossypol free. The second approach, which has been used sporadically, was to extract the gossypol from a meal with an acidified polar solvent. Although requiring additional processing, this approach has been found to eliminate 90–95% of the gossypol present in regular high-gossypol CSM.

In recent approaches, the detoxification of gossypol in CSM through microbial fermentation is a promising method since, unlike chemical methods, the biodegradation of gossypol occurs during the fermentation process. The fermented CSM usually contains some kinds of exoenzyme (secreted by microorganisms) such as a

cellulolytic enzyme, amylase, protease and lipolytic enzyme, vitamins and other active substances apart from the detoxification of free gossypol. Solid-state fermentation (SSF) was used to produce industrial products, including enzymes and amino acids, and is an attractive process due to its low capital investment and operating expenses.

#### **CONCLUSION:**

The use of CSM in non-ruminants feed is limited due to the presence of gossypol and high fibre content. As the SSF process was optimized, the fermented CSM had a significantly lower level of free gossypol, bound gossypol and crude fibre contents and higher crude protein compared to untreated. Few past works reveal that chemical disinfection of CSM with 0.5 % lactic acid would be a suitable alternative for heat sterilization of CSM, thus saves energy and cost of SSF process for gossypol detoxification and nutritive quality improvement. The free gossypol and bound gossypol levels in fermented CSM meets the international standard requirements, which show its potential to be used as safer non-ruminants feed in the aquafeeds. Further, the possibility of this is a simple, economic and eco-friendly process for its industrial adoption may be explored.

