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Replacement of fish meal by soybean (*Glycine max*) in the formulation of fish feed ingredients essential for Immunostimulation and growth performance of carps

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ABSTRACT

Fishmeal is the preferred protein ingredient in aquaculture feed and contributes significantly to the variable production cost in the aquaculture industry. However, decreasing fishmeal supply relative to demand and increasing costs threaten the sustainability and growth of the aquaculture industry. The way to sustain the industry is to remove or reduce the use of fishmeal. Past studies have demonstrated that complete or partial substitution of fishmeal with alternative proteins does not adversely affect fish performance. Soybean is probably the most promising alternative protein source for fish meal. It is widely used as the cost effective alternative for high quality fish meal in feeds for many aquaculture fish species due to its high protein content, excellent amino acid profile, low cost, availability and steady supply as compared to the other plant protein sources. Hence taking into account this consideration the present feed formulation has been undertaken, are introduce while formulating the feed. Hence for the present work soybean (*Glycine max*) has been selected as the raw material for formulation of fish feed along with ingredients essential for growth, disease resistance and the binding agent to hold the feed for Nile tilapia (*Oreochromis niloticus*).

Keywords: Formulated feed, Ingredients, Soybean, Grass carp.

1. Introduction

Fisheries have played a very vital role in improving the food security status of the people; it contributes about 15-16% to the total animal protein consumed by 2.9 billion people in low-income and food-deficient countries. An estimated 520 million people nearly 8% of the world population rely on income from fisheries for sustenance. With the continued increase in the awareness of health benefits, the global demand for aquatic foods, even in developed countries is expected to continue to rise. Aquaculture production is responsible for 50% of the global fishery production. Fish is highly nutritive and rich source of animal proteins. For the improvement of fisheries and to achieve maximum yields from resources of fresh water, it is necessary to provide artificial feed, by which fish grows rapidly and attains maximum weight in shortest possible time. Among commonly used feed ingredients, fish meal is considered to be the best ingredients, due to its compatibility with the protein requirement of fish^[1].

Replacement of fish meal with cheaper ingredients of plant origin in fish feed is necessary because of rising cost and uncertain availability of fish meal^[2]. Inclusion of feedstuffs with relatively high levels of carbohydrate in formulated fish feed is preferred in view of its protein-sparing action that may make the diet more cost effective^[3]. According to Rumsey (1993), increased use of plant protein supplements in fish feed can reduce the cost of fish meal. The research has focused on utilizing less expensive and readily available resources to replace fish meal, without reducing the nutritional quality of feed^[4]. The apparent digestibility of protein, energy and individual amino acids are of prime consideration as the basis for feed formulation in fish, with information gained for different raw materials, such as plant byproducts commonly utilized in the feed manufacturing industry.

Numerous investigations have been applied to variety of fish species for several decades with digestibility data obtained for most nutrients^[5]. For commercial culture of fish, the formulation of low-cost balanced diet using locally available agroindustry byproducts is needed. Recently fish meal has become the most expensive protein ingredient in aquaculture feeds.

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Many studies have shown considerable success in partially replacing fishmeal with soybean meal and other soybean products in diet for various fish species [6, 7, 8].

2. Materials and Methods

Feed formulation and preparation is the processes of combining feed ingredients to form mixture that will meet the specific goals of production. It is often a compromise between an ideal formula and practical considerations. While formulating the feed one must take into account some considerations such as price, availability of ingredients used anti-nutritional factors and palatability of mixtures [9]. Along with soybean meal other ingredients such as milk powder, corn flour, eggs, cod liver oil, vitamin mixture containing vitamin B complex and vitamin E, agar powder, garlic paste, pepper powder and cumin powder were used.

2.1 Preparation of feed

Soybean meal (80 gm) was taken in powder form as principal ingredients. Other ingredients like milk powder (60 gm), corn flour (20 gm), and eggs (70 gm) were added and mixed well. Agar powder (4 gm) was added as binding agent; turmeric (0.5 gm) and garlic (1 gm) as antibiotics. The said mixture was boiled, cooled at room temperature. After cooling cod liver oil (3.5 ml), vitamin mixture of vitamin B complex (gm) and vitamin E (1 gm) were added. It was kept under refrigeration for 12 hours. After 12 hours it was squeezed over polythene sheet and dried at room temperature for 48 hours. The dried nodules are crushed into small pellets then pellets were sun dried to avoid fungal infection, weighted and stored in the bottle. Following the above procedure all the feeds were formulated in the percentage composition as:

1. Diet 1 (25%): (soybean meal 25% + groundnut oil cake 75%)
2. Diet 2 (50%): (soybean meal 50% + groundnut oil cake 50%)
3. Diet 3 (75%): (soybean meal 75% + groundnut oil cake 25%)
4. Diet 4 (100%): formulated (totally of soybean meal)
5. Diet 5 (100%): conventional (totally of groundnut oil cake)

2.2 The nutritional importance of ingredients used:

Soybean meal

Soybean meal has been the most frequently studied dietary ingredient as a fish meal replacer in diets for many fish species because of its high protein content, relatively well balanced amino acid profiles, reasonable price and steady supply [10]. Together by weight 60% of dry soybean contains 40% protein and 20% oil and the remainder consists of 35% carbohydrate and about 5% ash [11].

2.3 Nutritional value of soybean/ 100 gms

Carbohydrate: 30.16 gm, Sugars: 7.33 gm, Dietary Fiber: 9.3 gm, Fat: 19.94 gm, Protein: 36.49 gm., Energy: 450 Kcal, [11].

2.4 Milk powder

In present feed formulation Nestle milk powder was used. It contains 20 standard amino acids. It is rich in soluble vitamins A, D and minerals. According to USAID the typical average nutrient in the unreconstituted milk are 36% proteins, 52%

carbohydrates, particularly lactose and calcium 1.3%.

2.5 Nutritional value of milk powder/ 100 gms

Protein: 20.5 gm, Carbohydrates: 52.7 gm, Fats: 19 gm Saturated Fatty Acids: 10.9 gms, Cholesterol: 0.05 gm, Mono Unsaturated Fatty Acid: 4.21 gm, Poly Unsaturated Fatty Acid: 0.41. Partly skimmed milk along with sucrose is the ingredient present in used milk powder [11].

2.6 Egg

Egg albumin was taken. It contains 15% of proteins dissolved in water. It contains about 40 different types of proteins. It has high nutritive value. The proteins in egg white are, ovalbumin: 64%, ovotransferrin: 12%, ovomucoid: 11%, ovomucin: 1.5%, globulin: 8%, lysosomes: 3.5% [11].

2.7 Corn flour

Corn flour was used as filler and binder in present formulation. It contains proteins: 3 gms, carbohydrates: 23 gms and fat: 1 gm. The major ingredients were maize and starch. Starch acts as additive in food processing. It has 110 calories per gm [11].

2.8 Cod liver oil

Cod liver oil is derived from the liver of cod fish *Gadus callarias*. The main ingredients were maize and starch. Starch act as additive in food processing. It has a mild fishy taste and smell. It contains small amount of fish protein [12]. It is a good source of the vitamins A and D, as both the vitamins is nutritionally important to body. Cod liver oil along with vitamin E prevents the body from increasing oxidant stress. It also reduces the tendency of blood forming clot. It helps to reduce generation of free radicals in body. It has been used in form of a gelatin coated capsule named as Sea cod. Each single soft gelatin capsule contains 300 mg of cod liver oil.

2.9 Agar powder

Agar powder were used as binding agent. It helps to form a unique mass of the feed. It is a polymer made up of subunits of sugar galactose. It is also act as an emulsifying and suspending agent in many food products.

2.10 Vitamin mixture

Vitamin B and E were used as vitamin mixture in equal proportions.

2.11 Vitamin B complex

Each capsule of vitamin B complex composed of Thiamine mononitrite, vitamin B2, B6, B12, Nicotinamide, folic acid, biotin and Titanium dioxide. It promotes activity of enzymes. It plays an important role in cell growth and metabolism [13]. Vitamin B is an essential nutrient for the growth, development and some other bodily functions [14].

2.12 Vitamin E

Each soft gelatin capsule contains Tocopherol acetate of about 400 mg. It acts as an antioxidant. It protects the body against the free radicals, which are potentially damaging byproducts of energy metabolism. DL-X Tocopherol acetate is the form used while formulation.

2.13 Turmeric powder (*Curcuma longa*)

It was added as, antibacterial, anti-inflammatory and

hepatoprotective. It functions as an antioxidant & adjunctant in feed preparation. Turmeric used to protect the food from sunlight [15]. It adds warm, mild aroma and distinctive yellow color to the feed. It is also the hypolipidemic [16].

2.14 Nutritional value of turmeric /10 gms.

Protein: 8.6%, Carbohydrates: 63.0%, Fat: 8.9%, Fiber: 6.9%, Total ash: 6.9%, Moisture: 5.8%, Calcium: 6.2% and, Iron: 0.05%. The caloric value is 390 calories per 100 gm. It also contains 5% essential oil, and 3% Curcumin which is a polyphenol [17].

2.15 Garlic paste

It is used as antibiotic and antibacterial properties [18]. It inhibits the growth of bacteria, hence having antibacterial property.

2.16 Nutritional value of garlic/100 g

Moisture: 62.8%, Protein: 6.3%, Carbohydrates: 29%, Fiber: 0.8%, Total fat: 0.1%, Total ash: 1.0%, Calcium: 0.03%, Phosphorus: 0.31% Iron: 0.001%, Vitamin C: 13 mg and Nicotinic acid: 0.4 mg. The caloric value is 142 per 100 gm.

2.17 Pepper powder

It was used as a stimulant for appetite. In India it is used in number of health problems [19]. Pepper has two main components volatile oil and pungent component commonly known as piperine. Black pipers contains 0.6 to 2.6% essential oil nutritionally pepper contains Vitamins A, B and C.

2.18 Cumin powder (*Cuminum cyminum*)

Research in animals indicates that it stimulate the secretion of pancreatic enzymes, important factors in nutrient digestion and assimilation.

2.19 Nutritional value of cumin/ 100 gms

Carbohydrates 44.24 gm, proteins 17.819 gm, fats 1.535, dietary fibers 10.5 gm, iron 66.36 mg, sodium 168 mg, zinc 4.8 mg, calcium 931 mg ,vitamins such as A 64mg, thiamine Bi 0.628 mg, riboflavin B₂ 0.327 mg, niacin B₃ 4.579 mg, foliate B₉ 10 mg, vitamin C 7.7 mg and E 3.38 mg [11].

3. Results and Discussion

Fish meal is one of the most expensive ingredients in prepared fish diets. Fish nutritionists have tried to use less expensive plant protein sources to partially or totally replace fish meal. Of all the plant protein feedstuff, soybean meal is considered to be the most nutritious and is used as the major protein source in many fish diets [20]. Determination of palatability of a feed ingredient is an important criterion in the evaluation of that ingredient for fish.

The growth of fish depends upon the ingredients and its percentage in the formulated feed [21]. The digestibility of a particular feed ingredient reflects in growth of fish. Digestibility depends upon various factors like nature, dietary component, and type of nutrient and level of inclusion. Continuous efforts are being made by the nutritionist to reduce the feed cost as a strategy to sustainable aquaculture. The choice of dietary protein to be used in practical rations is an economic decision, which depends on the protein source as well as on the expected returns from fish growth and value. The particular characteristics of protein sources used in fish

diet are another factor that has to be considered when selecting an economical protein level [22]. Over the years it has been documented that carefully formulated and well-presented fish diet plays a very significant role in fish culture [23].

The details of formulation and processing of fish diets have been studied by several scientists [24, 25, 20]. Bulletin of Food and Agriculture Organization (FAO) and United Nation of Development Programme (UNDP) had also mentioned about feed formulation. Finding alternative protein sources to replace fishmeal in fish feed is important if the growth of the aquaculture industry is to be sustained [26]. Soybean meal is one such potential alternative [24]. Soybean meal are widely used as the most cost effective alternative for high quality fish meal in feeds for many aquaculture fish species due to its high protein content (Approx. 48%) and excellent amino acid profile [4], low cost, availability and steady supply as compared to the other plant protein sources [11]. Essential or indispensable amino acids (EAAs) cannot be synthesized by fish and often remain inadequate but are needed for growth and tissue development [17].

While formulating the feed vitamin mixture is added as it was clearly demonstrated that there are some interactions between vitamins. This is an important factor to be considered while formulating the feed with vitamin mixture [18]. Use of garlic in feed formulation as antioxidant increase antioxidant level and consequently stability of fish flesh (Frigg *et al.* 1990). Antibacterial properties of the ingredients resist the growth of microorganisms thus preventing different disease due to bacteria, fungi and protozoa [27].

The feed is formulated by taking into consideration all the previous investigations. The value of feed ingredients ranges in between Rs. 200/ Kg by taking a trial it was observed that it was well accepted and easily consumed by the fishes. The weights recorded were:

- The total weight of ingredients using Soybean meal - 232.5 g
- The total weight of formulated feed before drying and after pelleting-165 g
- The total weight of formulated feed after drying and pelleting-150 g

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