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Effect of Formulated Feeds on Growth Performance and Pigmentation in Ornamental Fishes - A Cohort Study

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ABSTRACT

This study assesses the effect of formulated feed and pigmentation in ornamental fishes. Ornamental fishes are the most popular and fastest growing categories of pets. The culture of ornamental fishes is called as aquariculture. Ornamental fish culture (aquariculture) is the culture of attractive, colourful fishes of various characteristics, which are reared in a confined aquatic system. Coloration is one essential sales factor deciding the market value of ornamental fish. Ornamental fishes are also known as living jewels. There are more than 30,000 fish species reported around the world; of this about 800 belong to ornamental fishes. Most of the ornamental fishes survive in fresh water. Pigmentation is one of the major quality attributes of the aquarium fish for market acceptability. Carotenoids are responsible for pigmentation of skin color in ornamental fish. Like other animals, de novo synthesis of carotenoids does not occur in fish, therefore natural and supplementary feed sources are need to be incorporated as pigment sources for fish. Properly formulated feed is the major backbone of successful culture of ornamental fish in confined environment.

KEYWORDS

Growth Performance, Immune Response, Nutritional Level, Skin Pigmentation, Supplemented Diet

INTRODUCTION

Ornamental fish are those small sized, live and colourful fish kept in home or public aquaria. The trade value of ornamental fish is determined by the beauty and intensity of colouration patterns [1]. The colour of ornamental fish is mainly due to scattering or accumulation of chromatophores consisting of four chromatin groups of melanin, purine, pteridine and carotenoids present in the skin. Of these, carotenoids are the most important group representing more than 600 natural fat-soluble highly associated polyprenoids pigments [2]. The higher plants and microorganisms synthesize carotenoids *de novo* and it is not feasible with aquatic animals. Therefore, the carotenoid sources for fish must be transmitted to through feed [3]. Natural and synthetic carotenoids have been included in fish diets to strengthen skin and fillet colour [4]. They are diverse family of organic compounds that act as immune stimulant and antioxidant. Carotenoids also supply the basis for an all-inclusive of animal pigmentation.

Nutrition is another most essential element influencing the ability of cultured fish to reveal its **genetic potential** for growth and reproduction. Live Food is the best to feed to fish as it is natural and healthy [5]. Increasing costs and short supply of Fish feed has created the need to search for an alternative. So, the feed needs to be secured with some additives. Feed additives are edible substances that are added to animal feeds in small quantity to improve the feed quality so that it enhances **growth performance** and reduces mortality in fish [6]. Feed additives generally used in aquaculture are growth stimulators, immune enhancers, antimicrobialagents and antioxidants [7]. They are added in the fish feeds to increase the physical or chemicalproperties of the feed in terms of increasing fish performance or the quality of resulting product.

To enhance the immune system of fish, Immune stimulants are added to the fish feed. Immune stimulants are naturally occurring compounds that regulate the immune system by increasing the host's resistance in opposition to diseases caused by pathogens [8]. Dosages of immune stimulants that have been given to fish play an important role in the stimulation of immune response [9]. Including of probiotics to the feed stimulates the feed conversion ratio and decreases the mortality rate [10]. Probiotics are also proved to have increase the immune response and improve immune system in fish.

SKIN PIGMENTATION

Pigments are responsible for the wide spectrum of colours in fishes which is an importantcondition for the quality as they get higher price in the trade market. Fish colouration can be determined by Genetics, Nervous system and glandular factors, Dietary factor [11].

Varieties of carotenoids pigments are used in fish diet for colour enhancement, most preside of them with their colors are tunaxanthin (yellow), lutein (greenish-yellow), beta-carotene (orange), alpha, betadoradexanthins (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange-red), asthaxanthin (red), eichinenone (red) and taraxanthin (yellow). Among these, dominant carotenoid is astaxanthin, which is common in red fishes [12].

A. Carotenoids

The pigmentation of skin colour in ornamental fish is occurred by carotenoids. Carotenoids are also vital nutrients for healthy growth, metabolism, and reproduction. As fish is not capable of producing carotenoids *de novo* there is a need to integrate carotenoids in the diet of cultured species [13]. Carotenoids are tetraterpenoid organic pigments that are naturally present in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some bacteria, and some types of fungus [14].

When carotenoids bind to proteins or lipoproteins they also form complexes of carotenoproteins and carotenolipoproteins [15]. Carotenoids and their complexes produce biological pigment that can be used to display the visible spectral colours from red and orange to yellow, green, blue and violet [16]. Leutin, zeaxanthin and astaxanthin are among the most powerful of the carotenoids tested for colouring in fishes, which are also produce synthetically and obtainable commercially [17]. Dietary supply of carotenoids can enhance the skin colour as well as trade value of ornamental fishes.

The commercial natural astaxanthin production make use in byproducts of crustacean such as the Antarctic krill, crayfish meal, shrimp meal, crab meal, etc. These are enrich sources of carotenoid astaxanthin and are used in fishfeed formulation as additive [18]. Plant based carotenoids are mainly obtained from the micro algal pigment.

B. Types of Carotenoids

1. **β-Carotene:** The β-carotene is a thermo labile orange pigment, light, and oxygen sensitive, and is associated with protection from heart disease and cancer due to its potential protection mechanisms already mentioned. However, LDL-cholesterol oxidation is animportant factor in the development of atherosclerosis and β-carotene works by inhibiting the lipoprotein oxidation.
2. **Lycopene:** The pigment lycopene belongs to the subset of non-oxygenated carotenoids, characterized by a symmetric structure consisting 11 conjugated double bonds. Due to its chemical composition, lycopene stands as one of the best biological suppressors of free radicals.
3. **Lutein and Zeaxanthin:** Lutein and zeaxanthin are carotenoids stored in our body in the retina and lens of eyes. Some studies have shown that high lutein and zeaxanthin intake, especially from xanthophylls rich foods in such as spinach, broccoli, and eggs, are related to the significant reduction of cataract and age related macular degeneration.
4. **Astaxanthin:** Astaxanthin is a pigment found in aquatic animals, such as lobster, crab and shrimp. There is growing interest in the use of astaxanthin for poultry and fish cultureis developed, and when this pigment is not integratedwith animals,it should be added to foods to make it attractive to consumers.

C. Methods for Separating Carotenoids

High-performance Liquid Chromatography (HPLC) analysis is the preferred method of separating, identifying, and quantifying carotenoids. The first methods for separation of carotenoids and chlorophylls using open column chromatography weredeveloped in 1906. The comparison of different carotenoid concentration is tested using Duncan test (95% confidence interval) with One Way Analysis of Variance (ANOVA). Analysis of variance is used to analyze the differences between statistical models and their associated assessment procedures in a model.

D. Procedures

Whenever possible, all operations are carried out in soft and controlled light and in an atmosphere of nitrogen. Solutions placed overnight are stored under nitrogen at +4°C or -18°C in the dark. All chemicals should be of analytical quality.

Preparation of Samples

The skin from an individual frozen fish is removed and immediately frozen with liquid nitrogen, ground, and dried with Na_2SO_4. The mixture is washed several times with acetone until the solvent remained colorless. Organic phases are combined and reduced in isolation. The oily residue is dissolved in acetone-hexane (14:86) and separated by thin-layer chromatography on MgO with acetone-hexane (14:86). Two bands are observed: a yellow band near the solvent front, and a red one with a low Rfvalue. In some experiments, an additional weak yellow band in the middle of the other two bands is observed. The bands are cut out, the yellow ones dissolved in acetone-hexane (14:86), and the red one in acetone. The solutions are filtered and the solvent removed in a gentle nitrogen jet.

HPLC

Each sample is dissolved in 100μl acetone-hexane (14:86) and 30 μl is injected into the HPLC apparatus (at least two injections per sample). The mobile phase is acetone-hexane (14:86) at a flow rate of 2 ml/min and the substances were detected at 470 nm. The separation time is 10 min.

Because the saponification is practically omitted, the esters of lutein and tunaxanthin are not separated and only the total amount of these esters is determined. The combined lutein and tunaxanthin, and the astaxanthin esters are measured from calibration curves based on standard solutions of lutein ester and astaxanthin ester, respectively. Due to small sample amounts, only comparative measurement is possible. However, it is considered sufficient to discriminate between different groups of colour categories. In cases where no band is known in the thin-layer chromatography (for astaxanthin: 13 of 40 fish samples, and for tunaxanthin/ lutein: 2 of 40 fish samples), a value of zero is assigned for statistical analyses. This may sometimes be inaccurate, but it does not at least create a systematic error in which our results depend; rather, it is restricted to increasing the variability within groups.

GROWTH AND IMMUNE RESPONSE

Under natural conditions, fish may restrict and maintain their food intake, thus reducing their nutritional requirements, reducing the possibility of suffering malnutrition [19]. Development of functional feed additives enhancing growth, immune response; induce the physiological functions and health performance of the fishes over the normal feed additives [20].

FEED ADDITIVES

The nutritional status of fish diet is important in determining the fish's ability to resist various diseases. Therefore, there is a clear need for a proper diet to promote health and to prevent the outbreaks of disease [19]. Phytobiotic or phytogenic compounds are plant derivatives that are incorporated into the feed to improve the fish growth and health performance [21]. In plant extracts, turmeric (*Curcuma longa*) rhizome has potential as a food additive to reduce stress responses and improving fish growth and health due to its antimicrobial, antioxidant, anti-inflammatory, immunostimulatory properties and its stimulating effect on digestive enzymes secretion [22]. Turmeric also has potential for skin pigmentation in ornamental fish due to its rich yellow-orange pigments [23]. Microalgae are used as a live feed for fish and shellfish larvae. Protein and vitamin content is animportant factor determining the nutritional value of microalgae [11]. Yeast, enzymes, organic acids, mycotoxin binders, probiotics, prebiotics, seaweeds and mushroom are also used as effective fish feed for ornamental fishes.

CONCLUSION

The above study concluded that carotenoids are an essential part of the commercial ornamental fish industry. Due to the unfavorable effects of synthetic carotenoids on the aquatic environment, natural plant sources can be made productive and incorporated in formulated feeds for colour enhancement in a captive environment. As the aquafeed industry seeks a natural, environment friendly source of pigment to improve coloration and to enhance commercial acceptability, there is a great potential for use of natural plant based carotenoids for pigmentation in aquaculture. It will create avenues for promotion of the ornamental fish industry as well as a colour enhancer feed industry and employment generation. And the above study also concluded that Functional feed additives are used for, higher productivity and enhanced resistance to infectious disease, which would ultimately lead to sustainable aquaculture.

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