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EFFECT OF SOYABEAN AND SPIRULINA SUPPLEMENTATION ON PROXIMATE COMPOSITION OF COMMON CARP AS *Catla catla* AND *Labeo rohita*

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ABSTRACT

In the present study to investigate the proximate analysis of Indian Major Carp Catla catla and Labeo Rohita, fingerlings for period of 30, 90, and 180 days. In the present study to examine the effects of Spirulina and soya bean on biochemical parameters such as carbohydrates, protein and lipids in common carp (Catla catla and Labeo Rohita). Spirulina plantensis, soya bean and combined feed were incorporated into diets at concentrations of 1%. The control diet contained no supplement. Combined diet at 1% concentration produced the best and statistically significant ($p<0.05$) on carbohydrates, protein and lipids content in Catla catla and Labeo Rohita as compared to soya bean, spirulina and control diet. Soya bean diet at 1% produced the best and statistically significant ($p<0.05$) protein content in Catla catla and Labeo Rohita as compared to Spirulina, combined diet and control diet. The present investigation shows that incorporation of spirulina in diets for common carp results in increased carbohydrate and lipid content while protein content was increased in soya bean diets.

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INTRODUCTION

India is the third largest producer of fish in the world next only to China and Peru and it ranks second in the production of Inland fishes. Fish is very important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged in developed or developing countries. According to nutritionists, fish is an excellent substitute for red meat and an excellent source of protein. Fish flesh contains all the essential amino acids and minerals viz., iodine, phosphorus, potassium, iron, copper and vitamin A and D in desirable concentrations. It also serves as valuable ingredient to a healthy diet because of its low carbohydrate and unsaturated fat contents. So the inclusion of fish in our diet can make a valuable contribution to any diet that contain mainly of cereals, starchy roots and sugar for the healthy growth.

Carp farming is the backbone of Indian aquaculture contributing more than 85% of the total production. In recent years, technological advancement and modification of supplementation of feed, culture practice from traditional to semi-intensive and intensive system has led to various environmental related stressors in the culture species (Eddy and Williams, 1987). The Indian carps, *catla*, rohu, *mrigal* and kalbasu are fast growing and highly preferred food fishes in India. These carps have also gained popularity in other Southeast Asian countries. The Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* are the most important commercial fishes in India with a maximum market demand and acceptability as food by the consumers due to their taste and

flesh. They contribute about 67% of total freshwater fish production (ICLARM, 2001). In India, the aquaculture practices mainly revolve around a few species of finfish and shellfish, among which the Indian Major Carps viz. *Catla catla* contributes substantially to the inland production. Although carp culture is widely practiced, the non- availability of appropriate compounded feed to meet the demands of the species still remains as a major constraint.

Food is a major requirement for all living organisms including fish for reproduction, growth and maintenance. Feeding management plays a critical role in the success of fish culture. The current trend in fish culture is towards increased intensification whereby, provision of feeds becomes necessary and success depends significantly on the availability of well balanced nutritionally complete and cost effective compounded feeds. In fish culture systems, the importance of artificial feed supplementation cannot be over emphasized. Since feed is the main variable cost in culture fish production, precise information on the nutritional requirement is necessary in order to formulate and produce economical and nutritionally balanced and complete diet tailored towards the needs of the fish (Al Ogaily *et al.*, 1996).

Fish require adequate nutrition in order to grow and survive. Survival and growth of the fish was depending on the feed and fertilizer in cultural practices. Nature offers a great diversity of food to fish including plants and animals. Artificial feed plays an important role in semi intensive fish culture where it is required to maintain a high density of fish than the natural fertility of the water can support. The role of artificial feed in intensive fish farming cannot be ignored as nutritional requirements of fish depend upon the feed supplied. The quantity and quality of feed consumed have a pronounced effect on growth rate, efficiency of feed conversion and biochemical composition of fish. Development of aquaculture will be greatly enhanced by finding alternative and less expensive ingredients. Therefore the present study was focused to evaluate the effects of *Spirulina* and soyabean on proximate composition (protein, total fats and carbohydrates) of common carp as *C. catla* and *L. rohita* on 30, 90 and 180 days.

MATERIALS AND METHODS

Collection and acclimation of experimental fishes

Catla catla and *Labeo rohita* (4.85 ± 1.12 g) were procured from Fish farm, Orathanadu, Thanjavur District, Tamil Nadu, India, using cast net and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water with continuous aeration for two weeks prior to experimentation. During this period, fishes were fed with a known amount of fish food.

Preparation of Diet

Four diets were prepared: an unenriched control diet plus three diets containing *Spirulina plantensis*), soya bean and combination of soya bean and *Spirulina plantensis* each at a concentration of 1% (Table 1). The *Spirulina* was obtained from PARRY Nutraceuticals (Division of EID Parry (India) Ltd. at Pannangudi, Pudukkottai Dist. Tamilnadu, India. The soya bean purchased from General stores, Kelavasal, Thanjavur, Tamilnadu, India. Soya bean further made as formulate of fine powder and used for the experimental. The fingerlings were fed with (3% of their body weight) twice a day for 180 days. Every third day, tanks were partially cleaned and water was partially changed. The temperature was 28±1.5°C, dissolved oxygen 7.4±0.6 mg/l, and total ammonia were 0.5±0.2 mg/l.

Table 1 Ingredients and proximate composition of formulated diets.

Ingredients (%)	All diets
Fishmeal	35.0
Soybean meal	17.0
Rice bran	11.0
Groundnut oil cake	10.0
Tapioca flour	10.0
Mineral premix	1.5
Vitamin premix	0.5

Ingredients	*Control*	*Spirulina*	*Soyabean*	*Combined diet*
		1%	1%	1%
Wheat flour	15	14.0	14.0	13.0
Spirulina platensis	-	1.0	-	0.5
Soyabean		-	1.0	0.5
Proximate composition (%)				
Crude protein	36.2	40.6	40.6	40.6
Crude lipid	7.6	8.1	8.1	8.1
Crude carbohydrate	21.2	18.7	18.7	18.7
Ash	8.4	8.8	8.8	8.8

Sampling of the fish

Fishe were sampled once a month for six months using drags net. Length and weight of each species was measured separately to assess the health of condition of fish and their growth. The length (mm) and weight (g) of individual fish was recorded separately on treatment wise with the help of measuring scale and portable sensitive balance. Fishes were captured randomly from each experimental treatment and these fishes were used for hematological and serological analysis.

Experimental design

The fishes were fed (3% of their body weight) twice a day for 180 days. Every third day, tanks were partially cleaned and water was partially changed. The temperature was 28±1.5°C, dissolved oxygen 7.4±0.6 mg/l, and total ammonia were maintained 0.5±0.2 mg/l. Fishes were weighed at 30-day intervals to determine weight gain, specific growth rate (SGR) and feed conservation ratio (FCR), survival. At the end of the experiments, animals were sacrificed and the body tissues were dissected out, washed with ice-cold physiological saline. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris Hcl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

Proximate analysis

Protein was estimated by the method of Lowry *et al.*, (1951). Total lipids in tissues were estimated by the method of Folch *et al.*, (1957). The amount of carbohydrate present in the given sample by using Anthrone method.

Statistical Analysis:

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 was used and $p< 0.05$ and $p<0.01$ were considered to be significant.

RESULTS

Food is a major requirement for all living organisms including fish for reproduction, growth and maintenance. The aim of the present study is to evaluate the effects of *Spirulina*, soyabean and combined feed (*Spirulina* and soyabean) supplementation on proximate composition of common carp *C. catla* and *L. rohita* for a period of 30, 90 and 180 days. The observations made on different feed supplementations of experimental and control fish were compared.

Proximate composition:

In the observation on 30 days, *Catla* fish has showed (Table 2) significant increase in protein, (3.92± 0.11), carbohydrate (1.40 ± 0.07) and lipid (0.44 ± 0.01) in 1% combined diet, while the 1% *Spirulina* supplemented *Catla* showed increase in protein (3.79 ± 0.07), carbohydrate, (1.34 ± 0.06) and lipid (0.51 ± 0.02). 1% soyabean supplemented *Catla* showed increase protein (4.16 ± 0.08), carbohydrate, (1.3 ± 0.05) and lipid (0.41 ± 0.01while in control *Catla*, the protein (3.31± 0.14), carbohydrate, (1.29±0.05) and lipid (0.32±0.01were observed. The proximate composition of freshwater fish *Catla* was higher in combined diet followed by *Spirulina* and soyabean supplementation respectively.

In the observation on 90 days, *Catla* showed (Table 3) a significant increase in protein, (4.64 ± 0.08), carbohydrate (3.12 ± 0.17) and lipid (1.82 ± 0.08 in 1% combined diet while 1% *Spirulina* supplemented *Catla catla* showed increase protein (4.49 ± 0.13), carbohydrate, (2.88 ± 0.11) and lipid (1.02 ± 0.06. 1% soyabean supplemented *Catla* showed increase protein (5.21 ± 0.18), carbohydrate, (2.85 ± 0.09) and lipid (0.95 ± 0.04while the control *Catla* showed protein (4.32 ± 0.04), carbohydrate (1.76 ± 0.07) and lipid (0.53 ± 0.02. The proximate composition of freshwater fish *Catla* was highest in combined diet followed by *Spirulina* and soyabean supplementation.

Table 2 The proximate composition of freshwater fish *C. catla* and *L. rohita* with different feeding regimes (30 days)

Types of Feed	Protein (mg/gm)	Carbohydrate (mg/gm)	Lipids (mg/gm)
C. catla			
Control	3.31± 0.14	1.29±0.05	0.32±0.01
Soyabean (1%)	4.16 ± 0.08**	1.3 ± 0.05*	0.41 ± 0.01*
***Spirulina* (1%)**	3.79 ± 0.07*	1.34 ± 0.06*	0.51 ± 0.02*
Combined diet (Soyabean and *Spirulina*) (1%)	3.92 ± 0.11*	1.40 ± 0.07**	0.44 ± 0.01**
L. rohita			
Control	3.43± 0.14	1.32±0.05	0.38±0.01
Soyabean (1%)	4.71 ± 0.04**	1.76 ± 0.07*	0.53 ± 0.02*
***Spirulina* (1%)**	4.41 ± 0.08*	1.85 ± 0.09*	0.90± 0.04*
Combined diet (Soyabean and *Spirulina*) (1%)	4.32 ± 0.04*	1.87 ± 0.07**	0.95± 0.05*

Values were expressed as Mean ± standard deviation (number of trials, 3)
*Significantly different from control (*p<0.05; **p<0.01)

Table 3 The proximate composition of freshwater fish *C. catla* and *L. rohita* with different feeding regimes (90 days)

Types of Feed	Protein (mg/gm)	Carbohydrate (mg/gm)	Lipids (mg/gm)
C. catla			
Control	4.32 ± 0.04	1.76 ± 0.07	0.53 ± 0.02
Soyabean (1%)	5.21±0.18**	2.85 ± 0.09*	0.95 ± 0.04*
***Spirulina* (1%)**	4.49±0.13*	2.88 ± 0.11*	1.02 ± 0.06*
Combined diet (Soyabean and *Spirulina*) (1%)	4.64 ± 0.08*	3.12 ± 0.17**	1.82± 0.08**
L. rohita			
Control	4.53 ± 0.13	1.85 ± 0.11	0.97 ± 0.05
Soyabean (1%)	4.97±0.14**	2.87 ± 0.07*	0.85 ± 0.03*
***Spirulina* (1%)**	4.90 ± 0.09*	2.88 ± 0.09*	0.99 ± 0.04*
Combined diet (Soyabean and *Spirulina*) (1%)	4.88 ± 0.04*	3.25 ± 0.11**	1.37 ± 0.06**

Values were expressed as Mean ± standard deviation (number of trials, 3)
*Significantly different from control (*p<0.05; **p<0.01)

In the observation on 180 days, *Catla* showed (Table 4) significant increase in protein, (6.85 ± 0.04), carbohydrate (4.56 ± 0.06) and lipid (2.35 ± 0.08), in 1% combined diet while the 1% *Spirulina* supplemented *Catla* showed increased protein (6.78 ± 0.04), carbohydrate, (4.27 ± 0.09) and lipid (2.26 ± 0.06). 1% soyabean supplemented Catla showed increase protein (6.94 ± 0.09), carbohydrate, (4.23 ± 0.08) and lipid (2.16 ± 0.04) were observed while the control *Catla* showed protein (5.74 ± 0.04), carbohydrate (3.18 ± 0.06) and lipid (1.28 ± 0.06). The proximate composition of freshwater fish *Catla* was highest in combined diet followed by *Spirulina* and soyabean supplementation.

DISCUSSION

Global fishery production has been reported to be 142 million tonnes in 2008 and the contribution of aquaculture was more than 60%. The total fisheries production continued to grow rising from 34.50% in 2006 to 36.90% in 2008. It has been estimated that the total fish production will be 53.64 million metric tonnes in 2030, based on the annual growth rate. In contrast to world capture fisheries, which have almost stopped growing since the mid-1980's, the aquaculture sector maintains an average annual growth rate of 8.30% worldwide. In aquaculture, the contribution of inland fishery production is 4.66 metric tonnes of which almost 90% is contributed from freshwater aquaculture. India now ranks second and third in world fishery production and freshwater aquaculture respectively (Umaa Rani *et al*., 2014).

Table 4 The proximate composition of freshwater fish *C. catla* and *L. rohita* with different feeding regimes (180 days)

Types of Feed	Protein (mg/gm)	Carbohydrate (mg/gm)	Lipids (mg/gm)
C. catla			
Control	5.74 ± 0.04	3.18 ± 0.06	1.28 ± 0.06
Soyabean (1%)	6.94 ± 0.09**	4.23 ± 0.08*	2.16 ± 0.04*
***Spirulina* (1%)**	6.78 ± 0.04*	4.27 ± 0.09*	2.35 ± 0.08*
Combined diet (Soyabean and *Spirulina*) (1%)	6.85 ± 0.04*	4.56 ± 0.06**	2.26 ± 0.06**
L. rohita			
Control	5.95 ± 0.14	3.27 ± 0.09	1.21 ± 0.04
Soyabean (1%)	6.33 ± 0.12**	3.29 ± 0.06*	2.33 ± 0.06*
***Spirulina* (1%)**	6.17 ± 0.10*	3.31 ± 0.09*	2.43 ± 0.08*
Combined diet (Soyabean and *Spirulina*) (1%)	6.22 ± 0.15*	3.36 ± 0.11**	2.85 ± 0.03**

Values were expressed as Mean ± standard deviation (number of trials, 3)
*Significantly different from control (*p<0.05; **p<0.01)

Proximate body composition is the analysis of carbohydrates, proteins and lipids contents of fish. The biochemical composition of a particular living system in the level of organic compounds like proteins, lipids, carbohydrates, amino acids and importantly nucleic acids act as a source of energy for various physiological functions. The different tissues and organs in an animal are structurally and functionally designed to carry out different physiological processes. It is possible that they have different organic compositions. The biochemical analysis of *C. catla* and *L. rohita* indicated that 1% of combined diets results in significant increase in the level of Protein, carbohydrate and lipids in 180 days when compared to control. The changes in carbohydrate, protein and lipidis contents of fish body could be related to the changes in their synthesis and deposition rate in fish muscles (Abdel-Tawwab *et al.*, 2006). The biochemical analyses often provide vital information for health-assessment and management of cultured fish (Ghosh *et al.*, 2003; Cnaani *et al.*, 2004).

The results on biochemical composition, such as protein, carbohydrate and lipids contents of soyabean and *Spirulina* supplementation fed were recorded. After the feeding in the trial experiment of every 30 days for six months, the total protein, carbohydrate and lipids contents were found to be maximum in 1% of combined diet followed by *Spirulina* and soyabean than control. The statistical analysis made on the biochemical constituents between control and experimental diets revealed the significant variation between them.

Biochemical studies are very important from the nutritional point of view. Protein is the essential substenance of life and accordingly exists in largest quantity of all the nutrients as a component of the human body (Sudhakar *et al.*, 2011). In various fish species, proteins are important as structural compounds, biocatalysts and hormones for control of growth and differentiations (Amal and Naheb, 2012). Protein in fish is a main constituent of tissue and organs. They are precursors of other nitrogen compounds (enzymes, hormones, slurry, neurotransmitters, cofactors etc.,) and constitute an important energy source. The effect of dietary protein levels on fish growth performance varies considerably within species, size, age, diet and composition, range of proteins level tested and rearing conditions (Arredondo, 2012). Inadequate protein levels in the diets result in a reduction of growth and loss of weight. However, when an excess of protein is supplied in the diet, only part of it is used for protein synthesis (growth) and the remaining is

transformed into energy (Arredondo *et al*., 2012). Each body cell is composed mainly of protein. Protein makes up the membrane surrounding the cell and also occurs within the cell. Protein plays a vital role in the formation of enzymes, antibodies and hormones and other substances that regulate the body process. The study revealed that high protein contents were observed in soyabean supplemented fish than other diet. The high content of protein is due to rich source of protein content in soyabean. Results of the present study are in agreement with earlier reports (Storebakken, 2000).

Lipids and fatty acids play a significant role in membrane and have a direct impact on membrane mediated processes such as osmoregulation, nutrient assimilation and transport. On the other hand, the nature and quantity of these lipids in fish vary according to species and habit. Previous studies, Kumaran *et al*., 2012 correlate with our present investigation pertaining to lipid observations.

The effects of *Spirulina* on whole-body lipid contents are correlated with their synthesis and accumulation rate in muscle, as well as the growth rate of the organisms (Abdel-Tawwab *et al*., 2006; Abdel-Tawwab and Ahmad, 2009). Nandeesha *et al*., (2001) stated that the effect of dietary *Spirulina* on whole-body lipid content is dependent on species of the *Spirulina* used. Also, its effect on whole-body lipid content differs among species (Nandeesha *et al*., 1998). In some studies, a raw spirulina source was used. It has been shown that rearing conditions can affect crude protein and lipid contents of *spirulina*, resulting in different effects on body composition of fish compared with those of commercial *Spirulina* powder (Takeuchi *et al*., 2002). Junming Deng *et al*., (2007) investigated the effects of dietary soybean oligosaccharides (SBOS) on the levels of lipids in plasma and liver, and the fatty acid composition in muscle and liver of juvenile Japanese flounder (*Paralichthys olivaceus*). These results indicate that dietary SBOS supplementation (stachyose, 2.61%; raffinose, 0.61%) does not negatively affect the lipid metabolism of the fish fed fish meal (FM)-based diets, but decrease the incidences of fatty liver of the fish fed soy protein isolate (SPI)-based diets. The biochemical analysis of *C. catla* and *L. rohita* fingerlings indicated that 1% combined diet increase in the level of lipids in 180 days when compared to control, 30 and 90 days supplementation of *Spirulina* and soyabean

Components like carbohydrate play a vital role as energy precursors for fish under stress conditions (Umminger, 1970). Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Lucas, 1996). Changes in carbohydrate metabolism measured as plasma glucose (energy substrate whose production is thought to metabolically assist the animal to cope with an increased energy demand caused by stress) used as general stress indicators in fish (Teles *et al*., 2007). Glucose (or glucose 6-phosphate) is released through the degradation of glycogen by glycogen phosphorylase (GP) (Roach *et al*., 1998), and energy is mainly supplied by the oxidation of glucose and lactate as a result of carbohydrate metabolism (Morgan *et al*., 1997). Silbergeld, (1974) stated that an assay of this important parameter can serve as an indicator for environmental stress. In the present study increased carbohydrate contents were observed in combined diet of fish than other diets. The increased content of carbohydrate is due to the source of carbohydrate content in soyabean and *Spirulina*. Results of the present study were in agreement with earlier reports of Gumus and Ikiz, (2009).

The effect of carbohydrate and lipid levels on body composition (lipid, protein, ash and water) of fish has been investigated by a number of researchers (Kaushik and Oliva-Teles, 1985). The lipid composition of the fish body was influenced by the dietary carbohydrate and lipid levels. The lipid content of muscle was higher in fish fed with the high lipid level diet. These increments of body lipid by increasing dietary lipid level have been reported in previous studies (Kaushik and Oliva-Teles, 1985; Medale *et al*., 1991). A similar relationship between dietary carbohydrate level and whole body lipid content exists in 180 days as compared with 30 and 90 days of supplementation. The increase in dietary carbohydrates in *C. catla* and *L. rohita* results in an increase in body lipid content (Hilton and Atkinson, 1982). This effect can be due to the excess carbohydrate of fish converted into lipids, resulting in increase weight achieved with these diets. Dietary lipid level plays an important role in influencing growth rate and muscle composition of these species.

The proximate analysis of *C.catla* and *L.rohita* indicated that 1% combined diets results in significant increase in the level of carbohydrate and lipids in 180 days when compared to control in 30 and 90 days supplementation of *Spirulina* and soyabean while protein content was increased in soyabean supplemented fish.

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