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Effect of different levels of spirulina (blue green algae) on the growth performance and body composition of cirrhinus mrigala

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Abstract

Fish is considered a high-quality protein source and a replacement of red meat in developing countries and Asia. *Cirrhinus mrigala* was chosen for this study due to high survival rate and low diseases. An experiment was conducted for 30 days to evaluate the effects of Spirulina used as feed additives on growth performance and body composition of *Cirrhinus mrigala.* There were three aquariums under three treatments. Three types of experimental diets were formed. Experimental group T0 contained a control diet without addition of feed additives, T1 was fed with 5%(50/1000kg) spirulina and T2 fed with7%(70/1000kg) spirulina along with control diet. Data was statistically analyzed by one way ANOVA. All the water quality parameters were highly monitored and maintained.The body composition in terms of crude protein, crude fat, ash and moisture were determined by Kjeldhal method, solvent extraction and dry ash. Higher value of weight gain was found in T1(2.04±0.52g) and lowest value shown in T0 (1.35± 0.364g). The lowest Feed conversion ratio (FCR) was found for T1 while the highest was measured in T0. The values of average daily gain (ADG) and specific growth rate (SGR) were greater in T1 and T2 as compared to T0. Fish fed with the spirulina T1 and T2 had the highest protein and lowest fat contents as compared to T0. The fish that received supplemented diets had slightly higher moisture and ash contents in comparison to the fish fed the control diet. The experimental conditions in T1 and T2 showed increased feed intake and net weight gain when compared to T0. Results from this study indicate that using Spirulina-added control diet as feed attractants led to enhanced growth performance together with reduced FCR and improved SGR and ADG and body composition.

Keywords: Spirulina, *Cirrhinus mrigala*, Proximate composition, Growth rate, Weight

Introduction

Fisheries play a social and economic role in many countries. It is a significant source of animal protein in the human diet (Venkatramalingam and Saravanan, 2020). Fish is considered a high-quality protein source and a replacement for red meat in developing countries and Asia. Proteins are important for the growth and body tissues, synthesis other enzymes and hormones (Takeuchi *et al*., 2002). All essential amino acids, vitamins A and D, minerals like iodine, potassium, copper, phosphorous and iron are present in a desirable concentration in fish flesh. Fish flesh contains low concentrations of unsaturated fat and carbohydrates. Fish is an excellent source of polyunsaturated fatty acids that is considered as appreciated component of a healthy diet (Subramanian and Balasubramanian,2016).

Fish is a highly edible food that accounts for up to 40% of all animal protein consumed in developing countries. Sixty-five percent of tilapia and eighty percent of carps *(Catla catla*, *Labeo rohita* and *Cirrhinus marigala*) are cultured without modern composite feeds around the world (Khan, 2012). The semi-intensive fish culture system, which relies on minimal inputs, is the dominant method for fish production, supplying the majority of fish for both domestic and commercial consumption. This system helps to effectively utilize all the ecological niches in the aquatic environment with the stocking of fish species having different feeding habits (Nhan,2007).

Aquaculture sector is one of the most significant and fastest growing industries in term of fisheries production to meet the increasing sea food. It is recognized as a major food source providing 50% of worldwide fish consumption and achieves significant scale in Asia in recent decade. Different resources apply to progress in

the fish industry through aquaculture practice (Khan, 2012). China has initiated intensive culture of carps and other omnivorous species which are more feed intensive to enhance per unit fish production (Khan, 2012). India is the second-largest aquaculture producer in the World about 80% of India's aquaculture production is dominated by carp production (Venkatramalingam and Saravanan, 2020). Major carps including *Catla catla, Labeo rohita* and *Cirrhinus mrigala* constitute the most commercially relevant fishes of Asian nations because consumers value their taste and meat quality. They contribute about 67% of total freshwater fish production (Kadhar, 2012).

Aquaculture production in Bangladesh reached 914752 tones which made the country rank first in the world while inland capture fisheries produced 732000 tones making Bangladesh third globally. This remarkable achievement is largely attributed to substantial development investments from both the government and donor organizations. Investments in the fisheries sector have been spent on aquaculture development 54% in Bangladesh (Mazid, 2002).Fish farming is a relatively new activity in Pakistan and it has huge development potential due to country abundant aquatic resources. Estimates that fish ponds cover approximately 60,470 hectares of land (Khan, 2012). The Economic Survey of Pakistan states in 2006-2007 (ESP, 2007) fisheries are the principal source of livelihoods for many rural communities inhabiting the long coastline as well as inland along the major rivers and in the vicinity of lakes and dams.

Fish are also an important source of nutrition for many people particularly in rural areas containing 15-20% protein with high levels of vitamin A, phosphorous and many other useful dietary supplements in addition to low cholesterol content (Mazid, 2002). The major carps, *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*, are the most commercially significant fish in Pakistan, highly demanded in the market and widely preferred by consumers for their taste and flesh quality. They contribute about 67% of total freshwater fish production (Kadhar.2012).

Cirrhinus mrigala is very suitable for commercial fish farming. The *mrigala* fish, a species of ray-finned fish in the carp family, is highly valued for its rapid growth, high fecundity, disease resistance, excellent flesh quality, and strong consumer demand. It is one of the most widely farmed fish species worldwide. It is native to streams and rivers in Pakistan. The fish is important in polyculture with other native fish species especially *Rohu* and *Catla.* As the global food demand is increasing, so the popularity of freshwater fish species like *mrigala* is also increasing.

Fish meal is the primary protein source for aquaculture diets because of its high protein content well balanced amino acid and fatty acid composition, high digestibility and palatability (Yildirim *et al*., 2014). Fish meal is the most expensive ingredient however and is highly desired by other livestock industries (Kristofersson and Anderson, 2006). The exploration of cost-effective protein alternatives to partially or completely replace fish meal is a key focus of global research. Protein is the most important component in aquaculture feed therefore the formulation of fish feed attempts to provide a certain level of protein that will supply essential amino acids for the growth and good health of the farmed fish (Naz and Javed, 2013). A strategy to minimize fish meal usage is substituting it with more affordable animal or plant-based protein sources. The most common alternative to traditional fishmeal is plant-based feed. (Ishimi *et al*.,2006).

Various algal species have been utilized in aquaculture, primarily for their nutritional benefits. Microalgae play important roles in farming aquatic animals including mollusks, shrimp and fish. (Kim *et al*.,2011). Aquaculture feeds use Spirulina as a main ingredient because it contains a rich mixture of proteins along with vitamins and essential amino acids and minerals and essential fat components as well as antioxidant carotenoids. Presence of chlorophyII (green) and phycocyanin (blue) colors in its cellular structure spirulina is known as blue green algae (Nakagawa and Montgomery, 2007). Spirulina is easily and cost-effectively cultivated using an inexpensive culture medium. It thrives in water, is simple to harvest and process, and is rich in both macro- and micronutrients.

Commercial spirulina powder contains 60% protein, 20% carbohydrates, 3 to 6 % moisture, 7% minerals and 5% fats. It is use to substitute fishmeal either partially or entirely in the formulation of aqua feeds. It also can be used to establish immune-potentiating functions in carp (Watanuki *et al*. 2006). Multiple studies demonstrate how spirulina enhances fish growth along with improving feed efficiency and body composition and strengthening disease resistance in cultured fish populations. Various compounds found in the algae include phycobiliproteins and carotenoids together with phycocyanin and polysaccharides in addition to unsaturated fatty acids and superoxide dismutase and additional compounds.

The organic value of proteins in spirulina is very high. The essential amino acids make up roughly 47% of total protein weight. (Fulfillment *et al.,* 2014). Isoleucine amino acid present in spirulina is required in the body for proper growth and nitrogen balance. Leucine amino acid present in it boost the muscular energy

.Spirulina helps to increase lean body mass because of its high protein content particularly of the branched-chain amino acids, valine, and isoleucine..(Milasius *et al* 2009).

Materials and methods

Experimental location and designs

The research took place inside the laboratory facilities of the Zoology and Wildlife department at the University of Agriculture Faisalabad. The experiment involved obtaining commercial feed and feed additives namely spirulina. For the feeding trial of *Cirrhinus mrigala* fingerlings total of three glass aquariums were utilized. Each aquarium was stocked with a density of thirty-three fingerlings resulting in a total of one hundred fingerlings across all the aquariums. The three aquariums were divided into three treatment group, consisting of one control diet without any supplements and two control diets with feed additives. The fingerlings were randomly released into the aquariums. Each fingerling had an average weighted of 3-4 grams and measured approximately 5-8cm in length. They underwent a one-week acclimatization period, during which they were fed a maintenance ration equivalent to 8% of their body weight.

Preparation of the experimental diets

The experimental diets included three formulations which were designed as follows:

- The first control diet used commercial feed as the base product.
- The second diet contained commercial feed with 50g/1000g Spirulina added as a dietary supplement to the control diet.
- The third diet contained commercial feed with 7% (70g/1000g) Spirulina supplemented through feed additives.

Feeding trial

Each aquarium received a daily diet equivalent to 8% of the total weight of fish within it. The fingerlings were fed twice a day: once from 08:30-09:30 am and again from 03:00-04:00 pm. The trials lasted for a duration of 30 days. To account for changes in body weight 5% of the fish were randomly sampled and weighed weekly to adjust the feed intake accordingly.

Water quality parameters

The aquariums were regularly monitored to assess the water quality, specifically pH levels and dissolved oxygen content using various procedures. Temperature measurements were taken daily while dissolved oxygen and pH were measured on a weekly basis.

Growth performance

Information regarding the growth of the fish was collected. The fish were weighed precisely using an electronic balance measuring the weight in grams. To determine the fish lengths a measuring scale was utilized measuring in centimeters. Various growth parameters were then calculated based on performance including the mean final fish weight, daily weight gain (in grams per fish per day), survival rate and specific growth rate (in percentage per day) of the fish.

Weight gain

$$\textbf{Weight gain} = \text{Final weight} - \text{Initial weight}$$

Average daily weight gain

$$\textbf{(ADG)} = \text{Final weight} - \text{Initial weight/ Days}$$

Specific growth rate (SGR) %

$$\textbf{(SGR)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{experimental days}} \times 100$$

Feed conversion ratio

$$\text{Daily feed intake} = \frac{\text{Total dry feed intake(g)}}{\text{total weight gain(g)}}$$

Survival rate (%) =Final no. of fish survived/ No. of actual fish stocked ×100

Biochemical Analysis of Fish

The specimens were collected, measured and weighted after 30 days of rearing fish. The samples were then sent to the lab in Nayab Research center Jhang Road Faisalabad for analysis in order to determine the percentages of moisture, protein, fat and ash in the entire body. The Kjeldahl method was used to determine the crude protein content. Fat content was extracted using petroleum ether as a solvent following the Soxhlet extraction method. Moisture content was measured using a moisture analyzer (drying apparatus) at a temperature range of 105°C to 110°C. The drying process took 24 hours.

A pre-weighed and labeled crucible received the weighed sample as part of ash determination. A muffle furnace received the crucible at the temperature needed for the procedure (550-600°C range). The sample was incinerated for a sufficient period to ensure complete combustion of organic matter.

Statically analysis:

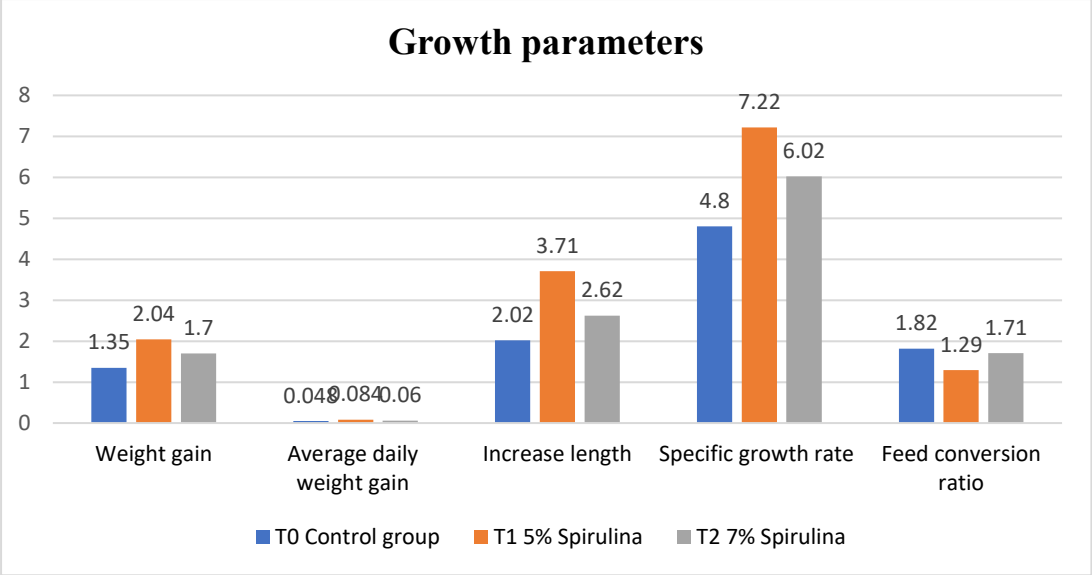
One way ANOVA served to analyze the statistical data. The study considers p values below 0.05($p<0.05$) as statistically significant.

Results:

Table 1.1: Effect of different levels of spirulina on the growth performance of *Cirrhinus mrigala*.

Growth parameters	T0 Control group	T1 5% Spirulina	T2 7% Spirulina
Total body weight (g) Initial final Weight gain(g) Average daily weight gain (g)	 3.17 4.77 1.35±0.364 0.048± 0.013	 3.93 5.97 2.04±0.524 0.084± 0.018	 3.87 5.57 1.70±0.360 0.06± 0.012
Total length(inch) Initial length Final length Increase length	 5.25 7.27 2.02	 5.29 9 3.71	 5.33 8 2.67
Specific growth rate SGR (%)	4.8±1.303	7.22±1.865	6.02±1.297
Survival rate (%)	94	100	100
Feed conversion ratio	1.82±0.121	1.29±1.725	1.71±0.125

Fig 1.1: Effect of different levels of spirulina on the growth performance of *Cirrhinus mrigala*.

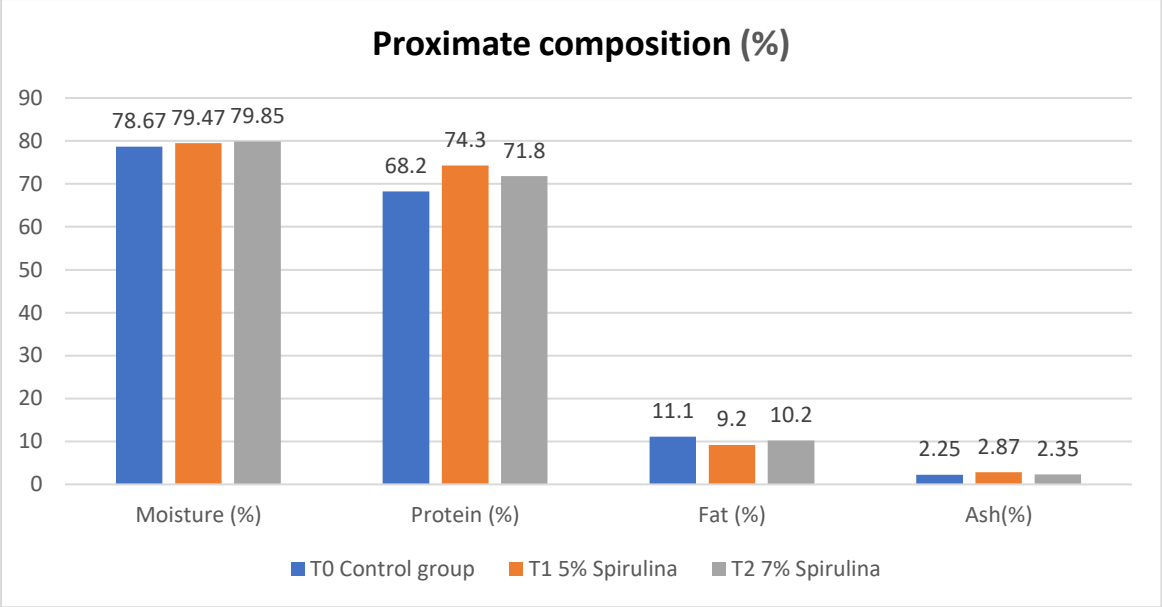


The results of the present study indicated that spirulina significantly enhanced the growth performance of *Cirrhinus mrigala*. The fingerlings of *Cirrhinus mrigala*, with initial weights of 3.17±0.14g, 3.93±0.75g, and 3.87±0.96g, and initial lengths of 6.25±0.47cm, 6.29±0.91cm, and 6.33±0.27cm for the three treatments (T0, T1, and T2, respectively), were fed on three different experimental diets. The experimental group in T1 showed the highest weight increase (2.04±0.52) as compared to the control group in T0 (1.35±0.036). The group in T1 exhibited the lowest feed conversion ratio among all treatments yet showed the highest feed conversion ratio in T0. The treatment groups of T1 and T2 recorded higher average daily gain (ADG) and specific growth rate (SGR) than T0. Both T1 and T2 maintained a full survival rate throughout the study.

Table 1.2: Effect of different levels of spirulina on proximate composition of *Cirrhinus mrigala.*

Proximate composition (%)	**T0 Control group**	**T1 5% Spirulina**	**T2 7% Spirulina**
Moisture **(%)**	78.67±0.315	79.47±0.221	79.85±0.400
Protein **(%)**	68.2±0.094	74.3±0.141	71.8±0.377
Fat (%)	11.1±0.0471	9.2±0.0471	10.2±0.094
Ash**(%)**	2.25±0.142	2.87±0.018	2.35±0.072

Fig1.2: Effect of different levels of spirulina on proximate composition of *Cirrhinus mrigala.*



Fish fed diet with spirulina T1 and T2 maintained the highest protein content while displaying the lowest fat content relative to T0. Fish that received supplementalfeeds showed a slight rise in their moisture and ash levels when compared to the fish in the control group.

Discussion

The research investigated how various spirulina consumption doses influenced *Cirrhinus mrigala* (mori or morakhi) body structure as well as their feed conversion ability and growth developments. All growth performance metrics at their best levels appeared across the three treatment groups. Results of the study showed fish fed with spirulina-containing diets had superior growth rate performance combined with better food utilization ratios that led them to increase their weight significantly beyond the control diet.

The present results of this research confirmed that incorporating spirulina into the diet resulted in superior growth outcomes for *Cirrhinus mrigala*. Test group T1 achieved the greatest weight increase of 2.04±0.524g because it received a 5% spirulina diet but control fish in group T0 showed the lowest weight change of 1.35±0. 364g.The weight gain value in T2 (1.70± 0.360g) with 7% spirulina was highest as compared to T0 and lowest compared to T1. This is due to feed hardness increases as spirulina contents increases making ingestion difficult. It is due to high concentration of protein present in the composition of microalga, which increases the competition between protein and starch for water during the extrusion process, leading to reduced gelatinization and resulting in a harder texture with less expansion. This hardness makes it challenging for fish to consume the feed. This may also affect to reduced intake and subsequently lower growth performance in diets with high spirulina content. The results demonstrated a greater improvement in fish growth in the T1 treatment, where 5% of the fish meal protein was replaced with spirulina. However, higher substitution levels did not lead to further enhancement.

Results of present study regarding length gain showed that highest length gain was recorded in T1(3.17 ± 0.110) which fed with 5% spirulina and lowest was found in T0(2.02±0.012) fed with control diet. The measured growth pattern showed a strong connection between fish length and weight development. Present results correlated the previous study that reported by Tongsiri *et al.* (2010) who found that replacement of 5% fish meal with spirulina resulted in the best growth performance of *P. gigas* but higher replacement levels lowered the fish weight gain. Present results also correlated with Kadhar *et al.* (2012) *Catla catla* fingerlings showed maximum increase in length and weight were observed in 4% spirulina The present findings align with those of Kim et al. (2013), who reported that spirulina had positive effects on the length gain of fish fingerlings. Similarly, the research by Olvera-Novoa et al. (2018) showed spirulina could account for 20% of fish protein in diets for O. mossambicus although surpassing this amount negatively affected growth alongside feed consumption.

The survival rates during the experimental trial were 100% for two groups T1 and T2 that treated with spirulina. Spirulina-based diets administered to fish proved beneficial because they did not result in mortality or disease incidents similar to control group populations. Spirulina includes phycocyanin which represents a robust plant-based protein responsible for this effect. Phycocyanin has antioxidant, pain-relief and anti-inflammatory effects in body. Polyphenolic compounds are another key class of antioxidants found in spirulina. These compounds exert their effects by chelating redox-active metals and accepting electrons from reactive oxygen species. The presence of spirulina in the diet enhances antioxidant protection which blocks the formation of reactive oxygen derivatives thus keeping fish tissues safe. Present results was resembled with the previous study of Hayashi *et al.,* (2000) who reported that feeding Spirulina to fish improved survival and growth rates. Sorrenti, V *et al.,* (2021) reported that high survival rate of Climbing perch by culturing in garden ditch fed with spirulina as compared to control diet.

Results regarding feed conversion ratio (FCR) expressed that increase FCR was observed in T0(1.82± 0.121) which was fed with control diet and lowest was recorded in T1 (1.29±0.172) which was fed with the diet containing spirulina. This may due to the beneficial microbe present in digestive system increasing digestibility and absorption of nutrients due to addition of spirulina which lead to enhance growth and improved FCR. This was also due spirulina functions against oxidative stress leading to better growth and feed conversion ratio. The present study resembled with previous study of Ghosh *et al*., (2003) who reported that feed conversion ratio was significantly improved in spirulina treated groups which established variation between control and treated groups. Present results also correlated with Amit *et al.,* (2014) who reported that feed supplemented with spirulina powder promoted the feed conversion ratio. The FCR results in the present study were in accordance with the findings of Tan *et al.*, (2017) who has reported that the feed conversion ratio was significantly altered when fish meal was supplemented with spirulina at 30% replacement level.

The present results indicated that highest whole-body protein content in fish was found in T1 (74.3%) which was fed with 5% spirulina as compared to T2(71.8%) fed with 7% spirulina and T0 (68.2%) which was fed with control diets. Present results showed that spirulina increased protein contents in whole body. It was due to improvements in protein and fat metabolism by spirulina due to some mechanisms such as spirulina exhibits soft cell walls. This cell wall made up of digestible mucoprotein. Mucoprotein is responsible for its high digestibility. It is also due to the protein contents in fish body increased due to the high protein content in dietary Spirulina and its ability to activate protein synthesis and somatic growth of fishes. Present study established similar correlation with the study of Morais, (2009) regarding Improvement in feed digestibility, increasing protein contents digestive enzymes, and growth performance had been reported as benefits of spirulina supplementation. Nandeesha *et al*. (2001) stated that the positive effect of dietary Spirulina on whole-body lipid and protein contents as compared to control group.

Results regarding fat that expressed fat contents decreased by dietary Spirulina in T1 (9.2%) which feed with 5% spirulina and T2 (10.2%) with 7% spirulina as compared to T0 (11.1%) feed with control diet. The results investigation revealed that spirulina decreased fat content in whole body. The antioxidant and fat-reducing properties of spirulina occur from its polyphenol compounds. Improvement in feed digestibility, lipid peroxidation and growth performance had been previously reported as benefits of spirulina supplementation Morais, (2009). Similarly, results were reported by Velasquez *et al.,* (2016) significant effects of spirulina in decreasing whole-body fat content in Nile tilapia. Present findings also resembled the previous study of Nandeesha *et al.,* (2001) who was stated that the positive effect of dietary Spirulina on whole-body lipid and protein contents as compared to control group.

The fish who received spirulina as part of their diet contained slightly more moisture in comparison to fish who received a standard diet. The moisture content of fish fed spirulina-rich diet reached T1(79±0.046%) moisture level and control-fed fish reached T0(78.66±0.053%) moisture level. The moisture content analysis showed no significant differences regarding treatment methods for the fish samples. The fish muscle's heat penetration together with water loss occurs simultaneously which may explain this observation. The study findings indicated that dietary spirulina did not impact fish moisture content according to Roohani et al., (2019). The findings from the present study matched those reported by Islam M. F. (2017) who studied freshness content among twenty-seven freshwater fish species which displayed levels between 72.18% and 83.65%.

Ash contains various minerals that play a crucial role in the body structure of each organism. Present results indicated that the maximum value of ash content was found in T1(2.87±0.786%) and T2 (2.35±0.072%) fed with spirulina as compared to T0 (2.25±0.0145%) fed with control diet. Spirulina contains significant mineral content including calcium as well as magnesium and phosphorus and iron together with zinc and other minerals. These minerals are associated to organic compounds involved in the muscles contraction process and its value increases the fish growth. Results of present study resembled with the previous study of Valverde

et al., (2000) who was reported that fish treated with dietary spirulina had high ash contents as compared to control group. The gradual increase in ash composition with the increase of dietary spirulina is supported by different studies Abdulrahman (2014).

A study was conducted to evaluate how spirulina used as a feed supplement affects *Cirrhinus mrigala* body composition alongside growth outcomes during the research period. Spirulina platensis functions well as fishmeal replacement in *Cirrhinus mrigala* diets since it provides high protein values while maintaining fish performance standards. The addition of spirulina in the diet produced superior growth performance and feed utilization results than using commercial feed ($p<0.05$). Tests revealed a reduced growth performance ($p<0.05$) among *Cirrhinus mrigala* fed with commercial diet which resulted in decreased weight gain (WG), average daily gain (ADG), specific growth rate (SGR) and increased food conversion ratio (FCR). The dietary protein quality in *Cirrhinus mrigala* showed a significant decrease ($p<0.05$) when commercial feed was used as the main component. Fish that received diets with spirulina showed better protein and, in their bodies, compared to fish provided the standard diet.

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