PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 13 (2): 93-98, 2014
ISSN 1680-5194

Replacement of Fishmeal with Microalgae *Spirulina* on Common Carp Weight Gain, Meat and Sensitive Composition and Survival

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Abstract: The use of blue green algae *Spirulina* in aquaculture has several potential advantages over the production of fish. This study was designed to investigate the effect of different replacement levels of fishmeal with *Spirulina* on weight gain and Survival rate of common carp *Cyprinus carpio* L., the trail was conducted for 105 days and for this purpose 200 fingerlings common carp with an average weight 32.7 g were brought from a local market. The fish were acclimated to laboratory conditions and fed with control pellets (32% protein) prior to the feeding trials for 21 days. Five experimental diets were used and *Spirulina* replaced fishmeal protein from the standard diet at 0 (T1), 5 (T2), 10 (T3), 15 (T4) and 20% (T5) levels. Analysis of variance for weight gain at this period showed that fish group fed 10% *Spirulina* (T3) had a significant ($p<0.05$) higher body weight as compared with the control ones and other replacements. From the data observed (T2) and (T4) have the higher survival rate and all treatments had significant ($p<0.05$) difference compare to control (T1). No significant differences were observed in all sensitive composition except color in which T2 was higher significantly than others. The percent of protein and lipids increased significantly in T3 and T5.

Key words: Spirulina, fishmeal, replacement, carp, weight gain, meat composition, sensitive, survival

INTRODUCTION

Fishmeal is one of the most expensive ingredients of aquaculture diets, it is estimated that more than 50% of the variable costs of bass production are feed costs, so profitability of production is significantly influenced by feed. Due to its relatively high cost, cost variability and growing environmental concerns about harvesting wild fish to produce fishmeal, it is desirable to replace fishmeal with less expensive protein sources. In spite of being the most important protein source in commercial feeds, production of fishmeal is restricted to certain part of world only; as a result, it is becoming more expensive and slowly getting out of reach by many aquaculture-practicing countries. Furthermore, abundance of FM appears to be ending since the level of FM production is expected to remain stable over the next 10 years (Mazurkiewicz, 2009).

Spirulina are ubiquitous, spirally coiled or filamentous prokaryotic cyanobacteria and possess significant morphological similarity (Henrikson, 2009). The main characteristics of *Spirulina* are the loosely coiled trichomes of varying width with cross-walls, which are visible in light microscopy (Castenholz, 2001).

The chemical composition of *Spirulina* indicates that it has a high nutritional value due to a wide range of essential nutrients, such as vitamins, minerals and proteins (Dillon and Phan, 1993). Microalgae have an important role in aquaculture as a means of enriching zooplankton for feeding to fish and larvae (Chakraborty, *et al*., 2007).

MATERIALS AND METHODS

This experiment work of this study was conducted in the Fish Laboratory for the Department of Animal Production, Faculty of Agricultural Sciences, University of Sulaimaniya, Iraq.

Experimental diet: Five practical diets were formulated based on the proximate composition of the feed ingredients. Diet 1 (Control diet contained no *Spirulina*), diets 2, 3, 4 and 5 contained 5, 10, 15 and 20% dried *Spirulina* respectively by the replacement of fish meal on an equivalent protein basis. Composition and proximate analysis of algae and different experimental diets were shown in Table 1.

Fish and feeding regime: Common carp (*Cyprinus carpio*) fingerlings with an average weight 32.7 g were brought from a local aquarium fish supplier located in kuit, in mid of Iraq and acclimatized in plastic aquaria for three weeks before to be used in the experiment. Fish were randomly allocated on the aquaria (7/aquarium).

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Each treatment was represented in four aquariums (4 replicates). A feeding regime of 3% body weight per day was employed throughout the trail and readjusted weekly according to change in the body weight. He experimental facility consisted of 20 plastic Aquaria (100 L each). Each aquarium was supplied with aerated and dechlorinated tap water.

RESULTS

In this experiment, the feed consisted of isonitrogenous and isocaloric feed in all treatments. These are shown in Table 1. The weight gain and survival rates shown in Table 3.

The presented data in Table 3 showed that the average body weight at the start of the experiment for all groups [control 0% *Spirulina* (T1), 5% *Spirulina* (T2), 10% *Spirulina* (T3), 15% *Spirulina* (T4), 20% *Spirulina* (T5), as compared to the final weeks (week 12) were found to be 8.375, 12.663, 16.593, 13.000 and 15.033 g, respectively where there were significant ($p>0.05$) differences among these fish groups. Analysis of variance for weight gain at this period showed that fish group fed 10% *Spirulina* (T3) had a significant ($p<0.05$) higher body weight as compared with the control ones and other replacements.

Analysis of variance for body weight showed that the T3 and T5 groups had a significantly ($p<0.05$) higher weight gain as compared to the control ones. This means that the body weight of T3 and T5 increased by 16.593 and 15.033 g, respectively.

The data in Table 4 showed that fish meat composition data of protein were 24.145, 24.005, 20.935, 20.910 and 17.865% for the T5, T3, T2, T1 and T4, respectively, there were T5 had significant ($p<0.05$) differences as compared with the control ones and other treatments no significant ($p<0.05$) difference between T5 and T3.

Data for lipids were 1.623, 1.527, 1.483, 1.047 and 0.737% for the T1, T3, T5, T4 and T2, respectively, observed T1 had significant ($p<0.05$) difference.

The ash in Table 4 presented 1.193, 1.177, 1.110, 1.057 and 0.967% for T2, T5, T3, T1 and T4, respectively, observed no significant ($p<0.05$) difference among treatments.

Moisture data showed 76.400, 74.397, 73.530, 73.503 and 71.663% for T5, T3, T4, T1 and T2, respectively, observed T5 had significant ($p<0.05$) difference among treatments.

The presented data in Table 5 showed that T3 had a significant ($p<0.05$) effect on flesh's color of *C. carpio* all over the experimental period. Flesh's color of all treatments were 2.571, 2.286, 2.143, 2.000 and 1.857 for the T2, T3, T5, T1 and T4, respectively, which mean that the T2 was significantly higher ($p<0.05$) than control and other treatments. However, data 2.857, 2.571, 2.286, 2.143 and 2.143 for the T3, T4, T5, T2 and T1, respectively, observed that the *Spirulina* supplementation had no significant ($p>0.05$) effect on the flesh's freshness in all different treatments.

The data in Juiciness 2.571, 2.571, 2.286, 2.143 and 2.000 for the T3, T4, T2, T5 and T1, respectively, observed all treatments higher than control but there were no significant ($p>0.05$) difference.

With regard to the Flavor 3.143, 3.143, 3.000, 3.000 and 2.857 to the T5, T1, T4, T3 and T2, respectively, there were no significant ($p>0.05$) differences among the treatments compared to the control.

The results were 3.000, 2.714, 2.714, 2.286 and 2.143 to the T5, T4, T3, T1 and T2 respectively, of complete acceptable there were no significant ($p<0.05$) differences among the treatments compared to the control.

The presented data in Table 5 showed that T3 had a significant ($p<0.05$) effect on flesh's color of *C. carpio* all over the experimental period. Flesh's color of all treatments were 2.571, 2.286, 2.143, 2.000 and 1.857 for the T2, T3, T 5, T1 and T4, respectively, which mean that the T2 significantly ($p<0.05$) than control and other treatments. However, data 2.857, 2.571, 2.286, 2.143 and 2.143 for the T3, T4, T5, T2 and T1, respectively, observed that the *Spirulina* supplementation had no significant ($p>0.05$) effect on the flesh's freshness in all different treatments.

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The results were 3.000, 2.714, 2.714, 2.286 and 2.143 to the T5, T4, T3, T1 and T2, respectively, of complete acceptable there were no significant ($p<0.05$) differences among the treatments compared to the control.

DISCUSSION

These results are in agreement with those obtained by many studies (Ibrahim, 2001; Dawah *et al*., 2002; Colla *et al*., 2004; Stander, 2004; Zhou *et al*., 2005; Yousif *et al*., 2006; Badawy *et al*., 2008; Ungsethaphand *et al*., 2009) which they found that the addition of algae in fish diets improved growth performance of *O. niloticus*. Also, Zeinhom (2004) found that, Inclusion of algae in fish diets significantly ($p<0.05$) increased the body weight (39.69 g), body weight gain (26.46 g).

Also Nandeesha *et al*. (1998) and Guroy *et al*. (2012) reported that body weight gain of *O. niloticus* increased linearly with increasing the level of algae in fish diet at levels less than 20%.

From the result of the present observations, conducted with the fingerlings of *Catla catla* fed on different

Table 1: Structure of experimental diet

Item	100%				
Spirulina	0	5	10	15	20
Fishmeal	24.2	21.7	19.2	16.8	14.2
wheat bran	35	35	35	35	35
Soybean	20	20	20	20	20
Broken rice	20.3	17.8	15.3	12.7	10.3
Vitamin	0.5	0.5	0.5	0.5	0.5
Protein (%)	31	31	31	31	31

Table 2: Structure of Spirulina used as labeled: Suitable for all herbivorous fish such as pleco's and catfish as well as shrimps and snails

Composition	Percent
Crude protein	34
Crude fat and oils	6
Fiber	5
Ash	10
Vitamin A	24000 IU/kg
Vitamin D	2600 IU
Vitamin E	280 IU
Vitamin C	550 mg/kg

Table 3: Effect of replacing fishmeal with Spirulina on carp weight gain and survival rates

Treatments	Initials weight	Weight gain (gm)	Survival (%)
T1	37	8.375^{b}	64.286^{b}
T2	37.25	12.663^{ab}	92.857^{a}
T3	34	16.593^{a}	85.714^{a}
T4	36.25	13.000^{a}	92.857^{a}
T5	37.25	15.033^{a}	78.571^{ab}

Mean values with different superscripts within a column differ significantly ($p<0.05$)

Table 4: Effect of replacing fishmeal with *Spirulina* on fish meat

Treatment	Protein (%)	Lipids (%)	Ash (%)	Moisture (%)
T1	20.910^{b}	1.623^{a}	1.057^{a}	73.503^{b}
T2	20.935^{b}	0.737^{b}	1.193^{a}	71.663^{c}
T3	24.005^{a}	1.527^{a}	1.110^{a}	74.397^{b}
T4	17.865^{c}	1.047^{b}	0.967^{a}	73.530^{b}
T5	25.145^{a}	1.483^{a}	1.177^{a}	76.400^{a}

Mean values with different superscripts within a column differ significantly ($p<0.05$)

Table 5: Effect of replacing fishmeal with some sensitive

Treat-ment	Color	Freshness	Juiciness	Flavor	Complete acceptable
T1	2.000^{ab}	2.143^{a}	2.000^{a}	3.143^{a}	2.286^{a}
T2	2.571^{a}	2.143^{a}	2.286^{a}	2.857^{a}	2.143^{a}
T3	2.286^{ab}	2.857^{a}	2.571^{a}	3.000^{a}	2.714^{a}
T4	1.857^{b}	2.571^{a}	2.571^{a}	3.000^{a}	2.714^{a}
T5	2.143^{ab}	2.286^{a}	2.143^{a}	3.143^{a}	3.000^{a}

Mean values with different superscripts within a column differ significantly ($p<0.05$)

percentage of *Spirulina* fortified feeds, it is clear that incorporation of *Spirulina* in the feed of *Catla catla* considerably enhanced the growth in terms of specific growth rate, weight gain and survival rate of the fish (Holman and Malau-Aduli, 2012; Saroch *et al*., 2012). Similarly, Abu-Zead (2001) showed that the addition of water hyacinth, water primrose and algae in fish diets improved growth performance of Nile tilapia and common carp.

These results agree with those found by Kiriratnikom *et al*. (2005), James (2010) and Burr *et al*. (2012) who reported that feeding *Spirulina* to fish improved survival and growth rates.

Ahmadzade-Nia *et al*. (2011), believe that because of nutritional benefits of *Spirulina* it could improve performance of fishes and increased retention in the body, due to increase carcass crude protein. Additionally, caused to increase body length and carcass mean weight and decrease carcass crude protein, suggested *Spirulina* can be used as a high quality protein source in rainbow trout industry, the increase in the world's population is accepted as the most important factor accelerating the development of the aquaculture industry. Thus, it seems possible to use of *Spirulina* as a protein source in aquaculture industry (Promya and Chitmanat, 2011).

Nandeesha *et al*. (1998) recorded no difference in the moisture and protein content in carcasses of common carp fed on diets incorporated with up to 55% *Spirulina* powder. However, the fat content decreased concomitant with an increased *Spirulina* supplementation level. In contrast, the feeding of algae was reported to elevate the body lipid in red sea bream. For *O. niloticus*, similar values of moisture (78.1 and 79.0%), crude protein (19.8 and 18.2%), lipid (1.8 and 1.1%) and ash (1.0 and 1.36%, respectively). For *Oreochromis* sp., different results obtained from those present in this study for moisture (79.50%), crude protein (12.67%), lipid (2.05%) and ash (3.89%) (Ungsethaphand *et al*., 2009)

The supplementation of live *Spirulina* significantly affected the protein and lipid contents in whole-fish body. The better feed intake in *Spirulina*-enriched diets may have been due to the increased fish appetite, resulting in a higher feed intake and improved growth. On the other hand, changes in the protein and lipid contents in the fish body could be linked to changes in their synthesis, deposition rate in muscle and different growth rates (Smith, 1981; Fauconneau, 1984; Soivio *et al*., 1989; Kyewalyanga, 2003; Abdel-Tawwab *et al*., 2006; Karakatsouli, 2012), they demonstrated that there were no significant differences in protein and crude fat in Mekong Giant Catfish flesh for all diets.

The obtained data of Allam (2007) showed that there are no significant ($p>0.05$) differences in moisture and dry matter between fish treatments fed Pronifer, algae and yeast and the control treatment. Olvera-Novoa *et al*. (1998) showed that the dietary algae had no significant effect on moisture content of the fish.

The results of Allam (2007) showed that the fish treatments fed 5% algae had insignificant ($p<0.05$) increase in total proteins. These findings are confirmed by Mustafa *et al*., (1994) who reported that the muscle protein of red sea bream was increased as Ascophyllum meal supplementation increased, the role of algae on fat content are in full agreement with the findings of Burr *et al*. (2012); Palmegiano *et al*. (2009) who reported that 5% dietary Ascophyllum meal had insignificant increase in muscle fat of red sea bream. Also, dietary macroalge led to significant ($p<0.05$) increase in the crude protein of grey mullet (Chelonlabrows), in the same trend.

Appler and Jauncey (1983) showed a decrease in lipid contents with increasing levels of algae in the diet of *S. niloticus*. In addition, Olvera-Novoa *et al.* (1998) mentioned that body lipids of *O. mossambicus* showed no consistent relation to the microalgae in the diet.

For the role of algae in reduction of ash content of the fish, this explained by some researchers such as Olvera-Novoa *et al.* (1998) who mentioned that the highest ($p<0.05$) ash value was obtained with the control diet as compared with the lowest values with the fish treatments fed microalgae, they concluded that body ash tended to decrease with increasing levels of vegetable protein in the diet.

Feed additives supplementation significantly affected whole-fish body composition except for dry matter, which show did not difference, fish fed with the control diet had the lowest protein content; however, all feed additives supplementation appeared to improved protein content without significant difference, carcass lipid content was also affected by dietary protein content, with the highest values in the control treatment, which were statistically different from the supplemented treatments, the lowest overall lipid content was recorded with *A. fusiformis* treatment, which was not statistically different with all other treatments. Ash content increased significantly with the increase of feed additives and the highest ash content was obtained in fish fed with 1% *S. pacifica*, whereas the lowest was obtained in fish fed with the control diet (Belal *et al.*, 2012).

Lu *et al.* (2002) reported that using live *Spirulina* as the sole diet for tilapia resulted in lipid body content depression although the lipid content in raw *Spirulina* was almost the same as that in the commercial diets. The diets Alga 75 and Alga 100 produced significantly lower lipid levels which resulted in lower lipid deposition in fish fed 100% algae replacement.

Hussein *et al.* (2012) concluded that the variation in lipid levels in his study was not the confounding factor in diet utilization and fish growth. To the contrary, he has been observed that the inclusion of 5% *Spirulina* in diets fed to striped jack *Pseudocaranx dentex* resulted in depression of body lipid and improved growth rates (Watanabe *et al.*, 1990).

These results are in agreement with those obtained by Ibrahim (2001), Dawah *et al.* (2002), Stander (2004), Zhou *et al.* (2005), Yousif *et al.* (2006), Badawy *et al.* (2008) and Ungsethaphand *et al.* (2009) who found that the addition of algae in fish diets improved growth performance of Nile tilapia (*O. niloticus*). Also, Zeinhom (2004) found that, Inclusion of algae in fish diets significantly ($p<0.05$) increased the live body weight (39.69 g), body weight gain (26.46 g), daily weight gain (0.29g) and specific growth rate (1.22), also Nandeesha *et al.* (1998) and (Guroy *et al.*, 2012) reported that body weight gain of Nile tilapia (*O. niloticus*) increased linearly with increasing the level of algae in fish diet at levels less than 20%.

Olvera-Novoa *et al.* (1998) stated that the daily body weight gain did not differ significantly ($p<0.05$) affecting by different addition of microalgae *Spirulina* in fish diets for *Tilapia mossambicus* less than 30 and 50%, respectively. Deficiencies or excess of one or more of essential amino acids are known to limit protein synthesis and/or growth, EAA deficiency has been widely demonstrated to reduce feed intake (Martin, 2000; Attalla and Mikhail, 2008; Diraman *et al.*, 2009).

The average survival for *Cyprinus carpio* remained as 92.857, 92.857, 85.714, 78.571 and 64.286 under the treatments (T2) 5% Spirulina, (T4) 15% Spirulina, (T3) 10% Spirulina, (T5) 20% Spirulina and (T1) 0% Spirulina, respectively observed (T2) and (T4) were higher and all treatments had significant ($p<0.05$) difference compare to control.

Coutinho *et al.* (2006) found that supplementing feeds for goldfish fry with freeze dried biomass of *Isochrysis galbana*, as a substitute for fishmeal protein, had a negative effect on growth and survival (Coutinho *et al.*, 2006). From the result of the present observations, conducted with the fingerlings of *Catla catla* fed on different percentage of *Spirulina* fortified feeds, it is clear that incorporation of *Spirulina* in the feed of *Catla catla* considerably enhanced the growth in terms of specific growth rate, weight gain and survival rate of the fish (Holman and Malau-Aduli, 2012; Saroch *et al.*, 2012). Similarly, Abu-Zead (2001) showed that the addition of water hyacinth, water primrose and algae in fish diets improved growth performance of Nile tilapia and common carp.

ANOVA revealed that combinations of *Spirulina* and vitamin C levels significantly ($p<0.05$) enhanced the coloration in muscle, skin and fins of *C. auratus*, among the combinations, T3 diet produced the maximum coloration in tested tissues and, therefore, T3 diet is considered as suitable for producing color in *C. auratus*. Mustafa *et al.* (1994) observed that the co-feeding of *Spirulina* and vitamin C improved the coloration in red seabream *Pagrus major*, which supports the observation made in the study of Yuangsoi *et al.* (2010).

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