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Effect of replacing different levels of Soybean meal with *Spirulina* on performance in Rainbow Trout

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ABSTRACT

The aim of this study was to investigate effects of 5 levels of Phytoplankton Spirulina additive to commercial feed on body weight gain (BWG), feed conversation ratio (FCR) and feed intake in rainbow trout during first 50 days of feeding. Four hundred-fifty Rainbow trout mean body weight 30 ± 3 gr were randomly allocated into fifty 1.5×1.5 meter fiberglass tanks (30 fish in each tank). The tanks received aerated well water with a flow rate of 5–7 Lit. min $^{-1}$. The temperature, pH and dissolved oxygen values of the water were 12.2 ± 0.1ºC, 6.9, and 10.2 ± 0.3 mg/Lit., respectively and monitored daily. Experimental diet was formulated according to nutrients requirements of rainbow trout using suggested standards. Spirulina was replaced with soybean meal of diets in five different levels, T1: kept for control and 0% Soybean meal with Spirulina, T2: 20% Soybean meal with Spirulina, T3: 40% Soybean meal with Spirulina, T4: 60% Soybean meal with Spirulina, T5: 80% Soybean meal with Spirulina which fed the experimental diets twice daily for 50 days. Data were processed in excel and statistical analysis was performed using SAS (ver/ 9.1). Our results indicated that fish which fed 2, 3, 4 and 5 diets had a significantly better FCR than group 1($P<0.01$). Also, BWG in treatment 2, 3, 4 and 5 were significant different compare to the control group ($P<0.01$). Additionally, feed intake in treatments 2, 3, 4 and 5 were significantly higher than compare to control ($P<0.01$). According to our result Spirulina is substitute protein source for replacing with soybean meal in Rainbow trout breeding systems.

Key words: *Soybean meal, Spirulina, Performance, Rainbow trout*

INTRODUCTION

Trout has been farmed for over a hundred years in Europe and America. Because all salmonids spawn in fresh water such as brown trout, rainbow trout and land locked salmon, they can complete their life in fresh water [1]. *Spirulina* is a microscopic blue-green aquatic plant and it is the nature's richest and most complete source of organic nutrition. The concentrated nutritional profile of *spirulina* occurs naturally, so it is ideal for those preferring a whole food supplement to artificial nutrient sources. *Spirulina*, the blue-green alga, has a unique blend of nutrients that no single source can provide. It contains a wide spectrum of nutrients that include e.g. B-complex vitamins, minerals, good quality proteins, gamma-linolenic acid (GLA) and the super antioxidants, beta-carotene, vitamin E and trace elements. *Spirulina* is fast emerging as a whole answer to the varied demands due to its impressive nutrient composition which can be used for therapeutic uses [2]. Nowadays, *spirulina* (blue-green alga) is becoming a health food worldwide. It is a multicultural, filamentous Cyan bacterium belonging to algae of the class *Cyanophyta*. The United Nations (UN) world food conference declared *spirulina* as "the best for tomorrow", and it is gaining popularity in recent years as a food supplement [3]. The *spirulina* ability has a potent anti-viral [4-5], anti–cancer [6-7], hypercholesterolemia [8] and health improvement [9] agent is gaining attention as a nutraceutical and as a source of potential pharmaceutical. Hence, the protein contents of *spirulina* various between 50 to 70 percent per dry mater weight. These levels are quite exceptional, even among microorganisms. When *Spirulina alga* is used as feed for young prawns and fingerlings, the fish exhibit good coloring, as well as maintain a low death rate and a high growth rate. Previous studies demonstrated using the raw *Spirulina alga* as the primary feed given to the Nile tilapia feed have found an increase in reproductive, birth and survival rates, compared with conventional fish feed. These studies have also found an increase in the amounts of linoleic acid, GLA, protein and an improved color in the meat of the fish compared with fish fed on standard instant feeds [10]. The main propose of this study was to investigate effect of replacing different levels of soybean meal with *Spirulina* on BWG, FRC and feed intake in Rainbow Trout.

MATERIALS AND METHODS

Four hundred-fifty Rainbow trout mean body weight 30 ± 3 gr were randomly allocated into fifty 1.5×1.5 meter fiberglass tanks (30 fish in each tank). The tanks received aerated well water with a flow rate of 5-7 Lit. min^{-1}. The temperature, pH and dissolved oxygen values of the water were 12.2 ± 0.1ºC, 6.9, and 10.2 ± 0.3 mg/Lit., respectively and monitored daily. *Spirulina* prepared in Agriculture Biotechnology Research Institute, Northwest and West Branch, Tabriz, Iran. Five different diets were prepared and fed to each group. T_1: 0% replacing Soybean meal with *Spirulina* as a control diet, T_2: 20% replacing Soybean meal with *Spirulina*, T_3: 40% replacing Soybean meal with *Spirulina*, T_4: 60% replacing Soybean meal with *Spirulina*, T_5: 80% replacing Soybean meal with *Spirulina* in commercial rainbow trout diet was formulated according to nutrients requirements of rainbow trout (NRC, 1981) [11] in Niro Sahand factory, East azerbayjan, Tabriz, Iran. The proper amounts of *Spirulina* were added into the basic diets, mixing part with part using mixer. Then, the feed was air dried under sterile conditions for 12 h and stored at 20°C. Fish were not fed in the weighting days. Fishes were weighted in five interval periods: 1-10, 11-20, 21-30, 31-40 and 41-50 days. In each tank, all fish were individually weighed once every ten days, and daily rations were determined after each weighing. The feed conversion ratio was calculated using the following equation: FCR = diet amount (gr per dry) / live weight gain (gr).

Data were statistically analyzed in GLM procedure using SAS statistical software (ver/ 9.1) and differences among groups were determined using Duncan's multiple-range test [12].

RESULTS

Biochemical composition of *Spirulina* used in present study was shown in table 1. Also, Ingredients and chemical composition of the basic diets used during the study was shown in table 2. Effects of *Spirulina* replacing on BWG of treatment groups is presented in table 3. Effects of *Spirulina* replacing on feed intake of the rainbow trout was shown in table 4 and finally, effects of different levels of *Spirulina* on feed conversion ratio in rainbow trout is presented in table 5. Our results indicated that, there was a significant difference in BWG levels between the treatment groups compare to control which all treats had higher BWG compare to control treat during all the study *(P <0.01).* Also, there was a significant difference in feed intake between replacements of *Spirulina* compare to the control diet which replacing different levels of Soybean meal with *Spirulina* was increased feed intake in all treatments *(P <0.01).* Finally, there was a significant difference in FCR levels between the groups which different levels of *Spirulina* decreased FCR levels compare to those fed with control diet *(P <0.01).*

Table1. Biochemical composition of *Spirulina*

Component	Percent (%)
Crude Protein	*58-73*
Carbohydrate	*8-19*
Fat	*5-11*
Ash	*5-8*
Moisture	*3-7*
Fiber	*4-7*
Digestible Energy(Kcal/kg)	*3500*

Table2. Ingredients and chemical composition of the basic diets used during the study

Ingredients of diets per gram	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Spirulina	0	23	46	69	92
Fish meal	270	268.5	267	265.5	274
Soybean meal	180	144	108	72	36
Corn	105	119.5	134	148.5	153
Wheat	117	117	117	117	117
Meat meal	150	150	150	150	150
Yeast	50	50	50	50	50
Molasses	30	30	30	30	30
Soybean oil	30	30	30	30	30
Fat	10	10	10	10	10
DCP	7	7	7	7	7
Enzyme	7	7	7	7	7
Vitamin premix	30	30	30	30	30
Mineral premix	7	7	7	7	7
NaCl	3	3	3	3	3
Met	2	2	2	2	2
Lys	2	2	2	2	2
CP (%)	38.86	38.79	38.81	38.82	38.87
Digestible Energy (kcal/kg)	3558	3553	3559	3561	3563

T_1: 0% replacing Soybean meal with Spirulina as the control diet, T_2: 20% replacing Soybean meal with Spirulina, T_3: 40% replacing Soybean meal with Spirulina, T_4: 60% replacing Soybean meal with Spirulina, T_5: 80% replacing Soybean meal with Spirulina in commercial rainbow trout diet. DCP: Di calcium phosphate, Met: Methionine, Lys: Lysine, CP: Crude Protein.

Table3. Effects of Spirulina replacing on BWG in rainbow trout

	Period (Days)				
Treatment	1-10	11-20	21-30	31-40	41-50
T_1	166.6^{e}	216.6^{c}	240^{c}	268.3^{c}	275^{c}
T_2	190^{d}	244.6^{b}	248.2^{bc}	275.6^{ab}	291.3^{b}
T_3	213.3^{c}	249.3^{ab}	253.3^{ab}	277.3^{b}	294.6^{ab}
T_4	236.6^{a}	254^{a}	258^{a}	282^{a}	300.3^{a}
T_5	225^{b}	252^{ab}	255.3^{ab}	280^{a}	297.6^{ab}

T_1: 0% replacing Soybean meal with Spirulina as the control diet, T_2: 20% replacing Soybean meal with Spirulina, T_3: 40% replacing Soybean meal with Spirulina, T_4: 60% replacing Soybean meal with Spirulina, T_5: 80% replacing Soybean meal with Spirulina in commercial rainbow trout diet.

** There are significant differences between groups with different codes in a column (superscript letters a, b, c, d and e; P <0.01).*

Table4. Effects of Spirulina replacing on feed intake in rainbow trout

	Period (Days)				
Treatment	1-10	11-20	21-30	31-40	41-50
T_1	204^{a}	218.2^{e}	258.7^{e}	303.6^{e}	353.8^{e}
T_2	204^{a}	225.5^{d}	268.3^{d}	314.8^{d}	366.3^{d}
T_3	204^{a}	226.9^{c}	273.5^{c}	320.9^{c}	372.8^{c}
T_4	204^{a}	231.3^{a}	278.9^{a}	327.1^{a}	380^{a}
T_5	201^{a}	229.1^{a}	275.9^{b}	323.9^{b}	376.3^{b}

T_1: 0% replacing Soybean meal with Spirulina as the control diet, T_2: 20% replacing Soybean meal with Spirulina, T_3: 40% replacing Soybean meal with Spirulina, T_4: 60% replacing Soybean meal with Spirulina, T_5: 80% replacing Soybean meal with Spirulina in commercial rainbow trout diet.

** There are significant differences between groups with different codes in a column (superscript letters a, b, c, d and e; P <0.01).*

Table5. Effects of replacing different levels of Spirulina on feed conversion ratio in rainbow trout

	Period (Days)				
Treatment	1-10	11-20	21-30	31-40	41-50
T_1	1.22^{e}	1.007^{b}	1.078^{a}	1.131^{a}	1.286^{b}
T_2	1.07^{d}	0.910^{a}	1.080^{a}	1.142^{ab}	1.257^{a}
T_3	0.95^{c}	0.910^{a}	1.080^{a}	1.157^{b}	1.265^{a}
T_4	0.86^{a}	0.909^{a}	1.081^{a}	1.157^{b}	1.268^{b}
T_5	0.907^{b}	0. 909^{a}	1.081^{a}	1.157^{a}	1.264^{a}

T_1: 0% replacing Soybean meal with Spirulina as the control diet, T_2: 20% replacing Soybean meal with Spirulina, T_3: 40% replacing Soybean meal with Spirulina, T_4: 60% replacing Soybean meal with Spirulina, T_5: 80% replacing Soybean meal with Spirulina in commercial rainbow trout diet.

** There are significant differences between groups with different codes in a column (superscript letters a, b, c, d and e; P <0.01).*

DISCUSSION

The study showed that feeding and growth parameters significantly improved in fish fed *Spirulina* supplemented diets. *Spirulina* reduced FCR in fish fed *Spirulina* supplemented diets. Previous research has shown that *Spirulina* can be used as a protein source in feeding two important fish in India, the Cata and the Rohu. It was found that the Rohu fish increased its growth, protein efficiency ratio, digestibility of dry matter, and both protein and lipid content in correlation with the amount of *Spirulina* consumed. They concluded that it was suitable to use *Spirulina* as a protein supplement source for both fish [13]. These results showed that *Spirulina* could improve growth, reduction of mortality; overall elements of fish quality, firmness of flesh, brightness of skin color as well as improving the cost/performance ratio of the fish feed. Similar results were reported by previous researchers [14]. Use of plant products as protein sources in

fish feeds shows considerable application potential for aquaculture worldwide. *Spirulina* is multicellular and filamentous blue-green algae that has gained considerable popularly in the health food industry and increasingly diets [15]. In this study use of *Spirulina* in Rainbow Trout diets was evaluated and it was found that increasing level of it in diet provide better growth performance comparing to the commercial feeds. Previous researchers demonstrated the effect of dietary *Spirulina* level on growth performance and feed intake in red swordtail and they reported that intake and mean body weight increased with increasing level of *Spirulina* [16]. Growth in fish is primarily due to muscle protein deposition and it therefore follows that the flow of amino acids (A.A) from food to growing biomass. Fish require some main nutrients such as protein, fat, carbohydrate, vitamins and minerals, but these requirements vary by species. Proteins are the most required nutrients for the animal. Not only it is needed for growth but also it is used in energy requirements. Like rainbow trout, all carnivore fish have HCl that encourages digestive enzymes to be produced. Pepsin makes proteins to transform into amino acids and short-chained polypeptides [17]. Protein digestibility also depends on the processing methods [18]. In this study we didn't work on digestibility parameters but according to our result, we believe that because of nutritional benefits of *Spirulina* it could improve performance of fishes and we suggested it 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.

CONCLUSION

In conclusion, replacing different levels of soybean meal with *Spirulina* were significantly increased body weight, feed intake and decreased feed conversation ratio in Rainbow Trout. In conclusion, *Spirulina* may be used as a supplement at 20, 40, 60 and 80% of soybean meal in experimental feed for the rainbow trout. These concentrations had improved the growth performance, FCR and feed intake.

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