Research in Veterinary Science 101 (2015) 84–88

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/yrvsc

Dietary effects of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*)

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ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form 16 May 2015
Accepted 7 June 2015

Keywords:
Spirulina platensis
Immunostimulants
Hematology
Serum biochemistry
Rainbow trout

ABSTRACT

This study evaluated the effects of diets containing 0, 2.5, 5, 7.5 and 10% of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Fish (n = 180; 101 ± 8 g) were randomly divided into fifteen 300 L fiberglass tanks in triplicates for a period of ten weeks. The RBC, WBC, hemoglobin, total protein and albumin levels increased significantly in the groups supplemented with *S. platensis*. Dietary inclusion of *S. platensis* had no significant effects on hematocrit, cholesterol, triglyceride and lactate of the blood. HDL-cholesterol was larger in rainbow trout fed 10% *S. platensis* in comparison with the other diets, whereas LDL-cholesterol significantly decreased with increasing of *S. platensis* inclusion. Cortisol and glucose significantly decreased with increasing of *S. platensis* inclusion. The present results demonstrate that inclusion of 10% *S. platensis* can be introduced as an immunostimulant in rainbow trout diets.

1. Introduction

The limitation of natural resources such as fresh water and land has led to intensification of production systems. This overcrowding and the other stress conditions are likely to produce poor physiological environment and increase susceptibility to infectious diseases (Trenzado et al., 2008). Moreover, nutrition has an influence on health and immune responses of fish; therefore, research into dietary immunostimulant supplements such as organic, inorganic and synthetic matters has increased and many agents (such as chitosan, oligosaccharide, FK-565, etc) are currently used in the aquaculture industry (Sakai, 1999). The use of immunostimulants has been considered as an effective method to improve fish resistance against unsuitable environmental conditions. The application of immunostimulants can induce protection against pathogens, enhance non-specific defense mechanism and indirectly improve growth performance in fish (Talpur et al., 2013).

Nowadays, various natural products such as herbal extracts, microalgae and yeasts are known to have important roles in improving defense system due to their antioxidant, antistress, antimicrobial, growth promotion and immunostimulation activities. Moreover, these types of natural immunostimulants have been shown to have less negative side effects and more cost effective than the commercial ones (Kim et al., 2002; Harikrishnan et al., 2003; Supamattaya et al., 2005; Salnur et al., 2009). Among several microalgae, *Spirulina* and *Chlorella* have received particular attention due to their in vitro and/or in vivo immunostimulation effects (Miranda et al., 1998). *Spirulina platensis*, which belongs to cyanobacteria (blue–green algae) family, is plenitudinous in antioxidant compounds such as β-carotene, phycocyanin, tocopherols and superoxide dismutase (SOD) enzyme that have significant effects on scavenging free radicals. Apart from the antioxidants, it also contains high content of protein (up to 70%), polyunsaturated fatty acid (PUFA), vitamins (especially B_{12}) and minerals (zinc, magnesium, manganese, selenium and iron) (Simsek et al., 2007). *S. platensis* does not have cellulose cell wall and therefore fish can digest it (Karkos et al., 2008). It is observed that it can also upregulate the cellular immune response (interleukin 1β and tumor necrosis factor (TNF)-α genes) in carp (Watanuki et al., 2006), and build both red and white blood cells and potent inductor of γ-interferon in mice (Lisheng et al., 1991). Recently, it was found that whole *Spirulina* and/or cell extracts can enhance immunity in animals by increasing the phagocytic activity (Duncan and Klesius, 1996). In addition, Harikrishnan et al. (2003) showed that hot water extract of *Spirulina* activated the human immune system by increasing the production of interferon and cytolysis NK cells.

Preliminary study on *Spirulina* revealed that supplementing diets with this microalga did not have negative effects on growth performance of different fish species (Teimouri et al., 2013a). Similar studies have revealed that *Spirulina* improves pigmentation (Teimouri et al., 2013b), meat quality (Promya and Chitmanat, 2011; Teimouri et al., in press) and reproduction (Vasudhevan and James, 2011); however, more information is needed for the practical use of *S. platensis* in fish diets. Hematology and serum biochemistry analyses in animals are increasingly becoming a usual and important method in clinical practices. Previous studies revealed that fish blood as a suitable indicator can identify the results of nutritional status, influence of environment conditions, and the effects of stress (Lermen et al., 2004). Since *S. platensis*

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http://dx.doi.org/10.1016/j.rvsc.2015.06.002
0034-5288/

can affect several physiological, biochemical and immunological indices, the main goals of the present study were to determine the effect of dietary *S. platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*).

2. Materials and methods

2.1. Experimental diets

Five diets were formulated using the microalga *S. platensis* (Sinamicroalgae Co., Qeshm, Iran). Since the proximate composition of *S. platensis* and fish meal was nearly similar (Teimouri et al., 2013a), the alga was only substituted for 0, 2.5, 5, 7.5 and 10% of the dietary fishmeal. Zero percentage was designated as a control diet. The formulation and proximate composition of control diet are given in Table 1.

Dietary feed ingredients were ground using a laboratory grinder, and then blended into a homogenous doughy matter by adding water, which were pelleted by pressing through a 4 mm die in a grinding machine. The pellets were then stored in plastic containers at −30 °C until use. All fish were fed the control diet during the first 7 days after stocking to adapt them to feeding and handling practices. After that, the fish were fed with the experimental diets.

2.2. Fish rearing

Fish (n = 180; 101 ± 8 g) were randomly divided into fifteen 300 L fiberglass tanks in triplicates (12 fish per replication). The whole water of the tanks was exchanged with fresh water every day. Water quality parameters were checked three times per week after the first feeding. Those parameters were kept within optimal range for rainbow trout; temperature 13 ± 2 °C, salinity 0.6 ± 0.1 ppt, pH 7.6 ± 0.2 and dissolved oxygen (DO) 8.6 ± 1 mg L^{-1}. All tanks were maintained under a constant photoperiod (12 h dark:12 h light) created by fluorescent lamps. Fish were fed by hand, twice per day at a rate of 2% of body weight during the experiment. The experiment lasted for ten weeks.

Table 1
Feed ingredients (% dry weight) of the basal experimental diet.

Ingredients (g kg^{-1})	
Soybean meal	160
Fish meal[a]	420
Wheat gluten	40
Wheat flour	150
Meat meal	80
Fish oil[b]	60
Soybean oil[b]	60
Mineral premix[c]	5
Vitamin premix[d]	5
Binder	20
Proximate composition (%)	
Dry matter	90
Crude protein	42
Crude lipid	17
Crude fiber	1.3
Ash	10
Energy (kcal g^{-1})	3.8

[a] Herring meal, produced by Mirood, Mazandaran province, Iran.
[b] Produced by Mirood, Mazandaran province, Iran.
[c] Mineral premix consisted of (mg kg^{-1} premix): 2600 mg Mn, 600 mg Cu, 6000 mg Fe, 4600 mg Zn, 50 mg Se, 100 mg IU, 50 mg Co, 100,000 mg cholin chloride, up to 1 kg carrier.
[d] Vitamin premix consisted of (mg kg^{-1} premix): 1,200,000 IU Vitamin A, 400,000 IU Vitamin D3, 3000 IU Vitamin E, 1200 mg K3, 5400 mg C, 200 mg H2, 200 mg B1, 3360 mg B2, 7200 mg B3, 9000 mg B5, 2400 mg B6, 600 mg B9, 4 mg B12.

2.3. Blood sampling and analytical procedures

At the end of the trial, rainbow trout were anesthetized with clove essence solution following a 20 h fasting. Blood sample of three fish from each tank (9 fish per treatment) was drawn gently from the caudal vein by 5-mL plastic syringe, for hematological and biochemical tests. Collected blood was divided to two sections. The first part was transferred to an eppendorf tube coated with heparin as anticoagulant and was used for hematological indices. The second part was transferred to an eppendorf tube without heparin for serum biochemical indices. The second part was left at room temperature to clot for 2 h and centrifuged at 5000 rpm for 10 min to collect serum (Rehulka, 2000). Serum was stored at −20 °C for later analysis.

Red blood cell (RBC) and white blood cell (WBC) were determined with a Neubauer hemocytometer by using a Natt and Herrick solution. Hemoglobin concentration was determined with Drabkin's reagent and read at absorbance at 540 nm with a Unico S-2150UV spectrophotometer. Hematocrit was determined by the microhematocrit heparinized capillaries, using a microhematocrit centrifuge (600 rpm for 10 min) (Rehulka, 2000).

Triglyceride, cholesterol, HDL-cholesterol, LDL-cholesterol, cortisol, total protein, glucose, albumin and lactate were analyzed using commercial kits, according to the manufacturer instructions (Pars Azmoon, Tehran, Iran).

2.4. Statistical analysis

All data were expressed as the mean ± SD. All data were verified for normality after transformation (ASIN). One-way ANOVA was used to determine the effects of *S. platensis* on hematological and serum biochemical parameters using SPSS (version 17). Duncan's multiple range test was used to compare differences between the means at 5% probability.

3. Results

At the end of experiment, fish grew normally and no specific signs of disease were observed. The impact of *S. platensis* as a feed additive on growth performance of rainbow trout was fully demonstrated in Teimouri et al. (2013a). Briefly, final weight was between 217.7 and 235.8 g in different treatments. No differences ($P > 0.05$) were observed in the final weight and SGR (1.26–1.39). The study also showed that FCR (1.03–1.13) was unaffected ($P > 0.05$) by the supplementation.

The effect of *S. platensis* as a feed additive on hematological parameters is shown in Table 2. The RBC, WBC and hemoglobin levels increased significantly ($P < 0.05$) in the groups supplemented with *S. platensis*. Maximum values of RBC and WBC were observed in rainbow trout fed SP 10 diet. Similarly, 7.5 and 10% dietary *S. platensis* elevated hemoglobin concentration in the blood ($P < 0.05$). However, dietary inclusion of *S. platensis* had no significant ($P > 0.05$) effect on hematocrit levels.

As shown in Table 3, the cholesterol and triglyceride levels were not affected ($P > 0.05$) by dietary *S. platensis*. However, there was a tendency to decrease these values with increasing of *S. platensis* level. HDL-cholesterol was larger ($P < 0.05$) in rainbow trout fed on the SP 10 diet in comparison with the other diets, whereas LDL-cholesterol significantly ($P < 0.05$) decreased with increasing of *S. platensis* inclusion.

Serum biochemical parameters in fish fed *S. platensis* are shown in Fig. 1. Significant decreases ($P < 0.05$) in the biochemical profiles such as cortisol and glucose were found with increasing of *S. platensis* inclusion, whereas total protein and albumin concentrations in the blood of test groups were significantly higher ($P < 0.05$) than those of the control group. No differences ($P > 0.05$) were observed in lactate concentration among the dietary treatments.