Research Article

Evaluation of feed efficiency, growth and biochemical parameters of rainbow trout (*Oncorhynchus mikyss*) juveniles fed with different levels of Alphamune prebiotic

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Abstract

This research was conducted to evaluate the effect of different levels of Alphamune prebiotic on growth performance and blood chemistry of rainbow trout (*Oncorhynchus mykiss*) juvenile. Rainbow trout with an initial weight of 16.46±0.09 g were randomly assigned to four dietary treatments for eight weeks. The dietary treatment was: 0 (ALP0), 1 (ALP1), 1.5 (ALP1.5), and 2 (ALP2) g of Alphamune prebiotic/kg of basal diet. The results showed that there was no significant difference in survival rate and SGR at the end of the trial. A significant difference was observed in the final weight and weight gain, and the highest one was obtained in ALP1.5 treatment (*p<0.05*). Food conversion rate (FCR) was affected by Alphamune levels and the lowest FCR was observed in ALP1.5 treatment. Blood biochemistry assay revealed that glucose, triglyceride, and cholesterol were not influenced by Alphamune's different levels. There were significant differences in IgM and total protein and the highest value was obtained in ALP2. These results showed that 1.5% Alphamune prebiotic (ALP1.5) had a positive effect on growth performance and biochemical parameters of rainbow trout juveniles.

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Introduction

In recent years, aquaculture has been one of the fastest-growing sectors of food production and has rapidly become a dynamic and growing industry (Piazzon *et al*., 2017). The use of functional feed additives has increased due to public awareness of the antibiotic disadvantages for human health and the banning of antibiotics used in aquaculture. (Zhongzhen *et al*., 2019). Prebiotics are the indigestible ingredient that has beneficial effects on the host by improving the health, by

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stimulating the growth or activity of several bacterial species residents in the gut (Hoseinifar *et al*., 2014a; Gibson *et al*., 2017). Prebiotics improve the balance of the intestinal microbiota and increase the defense mechanism of the body (Li and Gatlin, 2004). Nutrients that are classified as prebiotics should have properties such as being indigestible in the upper gastrointestinal tract, selective fermentation by one or more beneficial intestinal bacteria, and stimulating intestinal microbiota to produce healthier compounds (Fooks and Gibson, 2002). Besides, the most important products of prebiotic metabolism are short-chain fatty acids (SCFAs) which are absorbed through the intestinal epithelium and strengthen enterocytes and improve nutrition (Mahious and Ollevier, 2006b). The most important compounds used as prebiotics are inulin, fructooligosaccharides, glucooligosaccharides, Galactooligosaccharide, mannan oligosaccharides, isomaltooligosaccharides, and xylooligosaccharides (Ringo *et al*., 2010). Alphamune is a mixture of β glucans (24%) and mannan oligosaccharide (15%) and it is produced as a by- product of Saccharomyces cerevisiae (Huff *et al*., 2006). Numerous studies indicate dietary supplementations of mannan oligosaccharides improve growth performance and immune parameters of rainbow trout (Staykov *et al*., 2007; Aimrkolaie *et al*., 2016). Also, several studies showed that immune activity in the different fish species improves by supplementation of β-glucan (Ganguly *et al*., 2013; Hoseinifar *et al*., 2014, 2015). Previous studies on prebiotic mostly focused on different types of oligosaccharides alone as a source of prebiotic, but little is known about the efficiency of a mixture of carbohydrate sources. Therefore, this study aimed to investigate the effect of different levels of Alphamune on the hematological parameters of rainbow trout.

Material and methods

Fish and experimental condition

A total of 300 rainbow trout juveniles (16.46 ± 0.09 g) were obtained from a commercial fishery center and were randomly assigned to 12 tanks at a density of 25 fish per tank (300 L). The fish were acclimated to the experimental condition and fed with the commercial diet for 2 weeks. After acclimation, fish were fed with experimental diets at the rate of 2% body weight, three times a day for 56 days. Water quality parameters were regularly checked periodically and the flow rate was found 200 L/h, dissolved oxygen was 7.31 ± 0.60 mg/L, pH was 7.65 ± 0.2, water temperature ranged from 12.2 ± 1.98 °C. The water volume was renewed one-third of the total before the first feeding time in the morning. Any mortality was removed daily and recorded survival rate.

Experimental diet

Diet components were purchased from the Mazandaran Animal & Aquatic Feed (Sari, Mazandaran, Iran). A basal diet with different levels of Alphamune ALP0 (0 g/kg Alphamune diet prebiotic), ALP1 (1 g/kg Alphamune diet prebiotic), ALP1.5 (1.5 g/kg Alphamune diet prebiotic) andALP2 (2 g/kg

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Alphamune diet prebiotic), (Provided by Alpharma Co, Sao Paulo, Brazil) were formulated based on rainbow trout nutritional needs (Table 1). The mixed dough was extruded through an electric meat grinder (Electrokar EC-1, Tehran, Iran) to form pellets with a 3 mm diameter. Then, the pellet was air-dried at 50 °C.

Table 1. The ingredient used in the experimental diet

Alphamune level	**0**	**1**	**1.5**	**2**
Ingredient (g/kg diet)				
Fish meal	430	430	430	430
Soybean meal	200	200	200	200
Wheat gluten	50	50	50	50
corn	40	40	40	40
Fish oil	65	65	65	65
Sunflower oil	65	65	65	65
Mineral and Vitamin premix	15	15	15	15
Alphamune	0	1	1.5	2
binder	15	15	15	15
Proximate composition (g/kg dry matter)				
Crude protein	360	360	360	360
Crude lipid	220	220	220	220
Ash	140	142	140	140
Carbohydrate	192	192	192	192

1 kg Mineral Supplementation contained: co, 100; I, 400; se, 20; Zn, 10,000; Fe, 6,000; Cu, 600; Mn, 5,000

5 kg Vitamin Supplementation 0.5% contained: vitamin A 80,000 IU/ kg;vitamin D3 2,000 IU/kg; vitamin k 20 mg/kg; thiamin 60 mg/kg; riboflavin 60 mg/ kg; pyridoxine 100 mg/kg; pantothenic acid 150 mg/kg; niacin 300 mg/kg; biothin 2 mg/kg; folic acid 20 mg/kg; vitamin B12 0.1 mg/kg; inositol 300 mg/kg; ascorbic acid 600 mg/kg; choline chloride 3000 mg/kg.

Carbohydrate =100 - (crude protein + crude lipid + ash + moisture).

Growth performance

At the end of the experiment, feeding was stopped for 24 hours, after that the fish in each tank were separately weighed and the growth performance was calculated as below (Mohammadzadeh *et al*., 2017):

Weight gain: final weight (g) –initial weight (g)

Body weight increase (BWI, %) = 100 × [final weight (g) –initial weight (g)] / initial weight (g)

Feed conversion ratio (FCR) = dry weight of feed given (g) / WG (g)

Specific growth rate (SGR) = Ln final weight- Ln initial weight×100/ days

Condition factor (CF) = body weight/body length^3 × 100

Sample collection

At the end of the feeding trial, seven fish from each tank were sampled for hematological and blood biochemistry test. For preventive stress, fish feeding was stopped for 24 hours then were anesthetized by a stock solution of clove oil (Kralicin) solution (50-70 ppm) (Esmaeili *et al*., 2017b). Blood samples were collected by venipuncture of the caudal vein using a sterile 2-ml syringe and introduced to both heparinized and nonheparinized tubes. Non-heparinized blood was centrifuged (1,600 × g for 10 min) to obtain the serum. Supernatant was separated and stored at -20°C for later analysis.

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Blood biochemistry

Levels of glucose, triglyceride (TG), total cholesterol, total protein, and IgM were measured by a colorimetric method using Pars Azmoon kit with an autoanalyzer (Hitachi 902, Boehringer Mannheim Germany).

Statistical analysis

SPSS software (version 16, Chicago, IL, USA) was used to analyze data. Shapiro-Wilk and Levene's tests were applied to check the data normality and homogeneity of variances, respectively. The effect of the treatments on growth performance and blood biochemistry was examined by one-way analysis of variance (ANOVA). Duncan's multiple range tests were used to assess differences among four treatments in growth performance factors and blood biochemistry.

Results

The growth indices of rainbow trout juveniles fed by different levels of Alphamune are shown in Table 2. There was no significant survival rate and SGR at the end of the trial ($p>0.05$). After 8 weeks, a significant difference was observed in the final weight and WG, and the highest one was obtained in ALP1.5 treatment ($p<0.05$). FCR was affected by various dietary Alphamune levels and the lowest FCR was observed in ALP1.5 treatment ($p<0.05$).

Effect of Alphamune different levels on blood biochemistry indices of rainbow trout juveniles was presented in Table 3. Blood biochemistry assay revealed that glucose, triglyceride and cholesterol were not influenced by Alphamune different levels ($p>0.05$). There were significant differences in IgM and total protein and the highest value was obtained in ALP2.

Table 2. Growth performance of rainbow trout fed experimental diets containing different levels of Alphamune for eight weeks

Growth indices	Diet			
	ALP0	**ALP1**	**ALP1.5**	**ALP2**
Initial weight (g)	16.12 ± 0.1	16.75 ± 0.36	16.5 ± 0.8	16.49 ± 0.1
Final weight (g)	73.47 ± 2.39^{b}	74.61 ± 1.23^{b}	77.44 ± 1.36^{a}	75.23 ± 1.66^{ab}
WG (g)	57.35 ± 1.96^{b}	57.86 ± 1.15^{b}	60.94 ± 1.62^{a}	58.74 ± 1.71^{ab}
SGR	2.70 ± 0.48	2.66 ± 0.14	2.76 ± 0.05	2.71 ± 0.32
FCR	1.08 ± 0.11^{a}	1.08 ± 0.10^{a}	1.03 ± 0.12^{b}	1.07 ± 0.06^{ab}
Survival rate (%)	100	100	100	100

Values are represented by means ± SDM of triplicate tanks; means without letter labels are not significantly different. The letters a, and b indicate significant differences in the treatments according to Duncan's multiple range tests ($p<0.05$).

Table 3. Blood biochemistry indices of rainbow trout fed experimental diets containing different levels of Alphamune for eight weeks

Blood biochemistry indices	Diet			
	ALP0	**ALP1**	**ALP1.5**	**ALP2**
Glucose (mg/dL)	22.02 ± 1.75	25.3 ± 1.32	27.75 ± 1.16	27.19 ± 1.7
Triglyceride (mg/dL)	76.17 ± 4.39	82.14 ± 4.16	79.61 ± 3.23	84.83 ± 19.26
Cholesterol (mg/dL)	58.65 ± 4.26	58.24 ± 4.32	62.46 ± 4.15	64.94 ± 4.71
IgM (mg/ml)	2.70 ± 0.48	2.76 ± 0.05	2.66 ± 0.14	2.71 ± 0.32
Total protein (g/dL)	3.05 ± 0.206^{b}	4.10 ± 0.584^{ab}	4.56 ± 0.441^{ab}	5.01 ± 0.24^{a}

Values are represented by means ± SDM of triplicate tanks; means without letter labels are not significantly different. The letters a, and b indicate significant differences in the treatments according to Duncan's multiple range tests ($p<0.05$).

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Discussion

Many studies have been performed in association with adding dietary supplements (prebiotics and probiotics) to improve fish growth performance and immunity. In the present study, the result showed that the addition of 1, 1.5 and 2 g/kg Alphamune in rainbow trout diet has led to a significant difference in final weight and weight gain, and the highest final weight and weight gain were observed in fish fed with 1.5 g/kg Alphamune. The results of the present study are consistent with the results of Li and Gatlin (2005) and Torrecillas *et al*. (2007) who reported higher final weight in hybrid striped bass (*Morone chrysops* × *M. saxatilis)*, rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*) fed with different prebiotic. Miandare *et al*. (2016) reported the significant effect of galactooligosaccharide on the growth performance of goldfish (*Carassius auratus gibelio*). Besides, Hoseinifar *et al.* (2013) reported the effect of dietary galactooligosaccharide on increasing some growth parameters in Caspian roach (*Rutilus rutilus*). Positive effects of Alphamune on fish growth found in this study may be due to the composition of this prebiotic. The mannan oligosaccharide in the Alphamune is a good source of nutrients for the growth and activity of gastrointestinal flora bacteria such as lactic acid bacteria, lactobacilli and bifidobacteria (Ringo *et al*., 1998). Mannan oligosaccharides are nondigestible compounds that provide the location of mannose (the main compound of mannan oligosaccharide) on the intestine and prevent the binding of pathogenic bacteria to the intestinal epithelial cells and also prevent the formation of colonies (Pryor *et al*., 2003; Newman, 2007). These properties improve intestinal function and absorb more nutrients, thereby improving nutritional efficiency and enhancing growth (Bolu *et al*., 2009). β glucan, another component of Alphamune, also has positive effects on growth performance (Misra *et al*., 2006; Zhou *et al*., 2009) as well as it has beneficial effects on fish immune systems and their resistance to bacterial and viral infections (Sang and Fotedar, 2010). Therefore, the positive effect of Alphamune on growth performance may be due to the improvement of intestinal morphological features, alteration of the gastrointestinal microbial population by mannan oligosaccharides and improvement of the immune system by β-glucan.

In the current study, the best FCR was observed in fish fed with 1.5 g/kg Alphamune and had a significant difference with the control group. This result is similar to the result of Staykov *et al*. (2007) on rainbow trout who reported that mannan oligosaccharide improves FCR. MOS can produce glucose, which provides energy for the metabolism of body tissues and promotes growth and improves FCR (Torrecillas *et al*., 2011).

The results of the present study showed that a lower level of Alphamune (1.5 gr/kg) can improve growth indices better than a higher level (2 gr/kg). Therefore, it seems that

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Alphamune at lower levels has better effects on the population of beneficial microorganisms in the gastrointestinal tract or gastrointestinal morphology, while in high levels probably due to lack of fermentation and decomposition leads to the accumulation of these carbohydrates and have adverse effects on intestinal enterocytes (Olsen *et al*., 2001).

In this study, glucose, triglyceride and cholesterol were not influenced by Alphamune different levels. A similar result was reported by Akrami *et al*. (2010) in rainbow trout fed with mannan oligosaccharides and β-glucan. IgM and total protein levels in fish fed with different Alphamune levels were higher than in the control group. Yousefian *et al*. (2012) reported that levels of 0.5 and 2% galactooligosaccharide increased total Ig and total protein levels compared to the control group in zebrafish (*Danio rerio*). A similar result was reported by Hoseinifar *et al*. (2013) in Caspian roach fed with galactooligosaccharide. Miandare *et al*. (2016) reported that supplemented diet with 2% of galactooligosaccharides increased total mucus protein in goldfish. Total protein and immunoglobulin increased in the treated groups, indicating that enhancement of nonspecific immunity. However, a definite statement in this regard requires careful consideration of other immunity indicators.

The results of this study showed that the addition of different levels of Alphamune prebiotics per kg of diet caused a significant change in growth parameters and some blood biochemical factors. Therefore, it seems that the 1.5% Alphamune prebiotics has better effects on the population of beneficial microorganisms in the gastrointestinal tract or gastrointestinal morphology, while in high amounts probably due to lack of fermentation and decomposition leads to accumulation of these carbohydrates and adverse effects on fish growth performance.

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Conflict of interest

Authors have no conflict of interest on this work.

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