

2013, 1 (4), 393-406

Effect of Increasing Dietary Prebiotic GroBiotic[®]-A Concentration on Growth Performance, Body Indices and Haematological Parameters in Rainbow Trout (*Oncorhynchus mykiss*) Fingerling

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Received: 7 April 2013 / Accepted: 17 February 2014 / Published Online: 15 June 2014

ABSTRACT An 84-day feeding trial was carried out onfingerling (4.44 ± 0.06 g) rainbow trout (Oncorhynchusmykiss, Walbaum, 1792) to evaluate the effect of dietary supplementation with a commercial prebiotic GroBiotic®-A (G-A) on the growth, feed efficiency, haematology and immunlogical parameters. Ttreatments containing various inclusions of G-A (0 %, 0.5 %, 1.0 %, 1.5 %, 2.0 %, 2.5 %, and 3.0 %) were added to a commercial fish dietand were fed twice daily at 2-6 % of body weight. The highest weight gain (WG), specific growth rate (SGR) and average daily gain (ADG) were obtained in fish fed the diet containing 2.5 % G-A followed by 3.0 % inclusion (P < 0.05). The highest feed efficiency (FE), protein efficiency ratio (PER) and net protein utilization (NPU) were also recorded in the 2.5 % G-A inclusion (P<0.05). Survival was significantly higher (P < 0.05) in fish fed with 2 % and 2.5 % G-Asupplement (P < 0.05). Although higher Hb, haematocrit, RBC, WBC, MCH, MCHC, MCV, lymphocytes, and neutrophils were observed at all G-A supplemented diets, the differences among themwerenot significant (P>0.05). On the other hand, significantly higher difference (P < 0.05) in lysozyme and immunoglobulin (IgM) concentrations wereobserved in 2.5% G-A inclusion. The results of this study indicated that 2.5 % G-A inclusionhad a better performance on growth and haematoimmunological parameters in rainbow trout fingerling.

Key words: Feed efficiency, Fish, Growth performance, Oncorhynchus mykiss, Prebiotic GroBiotic[®]-A

1 INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is one of the most commonly farmed freshwater trout (FAO, 2011) and is popular in Europe, South and North American countries as well as Iran. Recently, Chile produced the largest amount of rainbow trout while Iran ranked 8th in global production (IFRO, 2011). However, production figures in Iran in recent years have been declining due to the outbreak of bacterial

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infections, particularly by Aeromonas hydrophila, Pseudomonas fluorescen and *Streptoccocus* spp. Bactrial diseases are considered to be a significant constraint to the development of the aquaculture sector over the years (Bondad-Reantaso et al., 2005; Leung and Bates, 2013). During the last two decades, traditional use of antibiotics in aquaculture has been criticized because of the potential development of antibiotic-resistant bacteria, the presence of antibiotic residues in seafood, the destruction of microbial populations in the aquacultural environmentand the suppression of the aquatic animal's immune system (Smith et al., 2003; Cabello 2004; Sørum 2006; Sapkota et al., 2008, Noga et al., 2011). Furthermore, vaccines cannot be used alone as a universal disease control measure in aquaculture (Amábile-Cuevas et al., 1995). Concerted research efforts have concentrated on optimising production with ecofriendly alternatives to the therapeutic use of antimicrobials. A new approach, that is gaining acceptance within the industry, is the use of prebiotic to control potential pathogens (Gomez-Gil et al., 2000, Sapkota et al., 2008, Noga et al., 2011). They experresed the effects of various inclusion levels of prebiotic, GroBiotic®-A (G-A) on the growth performance, body indices, parameters, and non-specific haematology immune responses of rainbow trout juveniles. GroBiotic[®]-A is now regarded as a viable alternative to manage fish health. Similarly, xylooligosaccharides (XOS), fructooligosaccharides (FOS), inulin, lactulose and lactosucrose and other carbohydrate sources have received increasing interest for fish health benefits (Sealey et al., (2007); Mussatto and Mancilha 2007). The presence of prebiotics is responsible for the enhancement of cell growth and also restricts the growth of harmful bacteria in the colon (Foolad et al., 2012). The purpose of this study was to explore growth performance, haematology and non-specific immune reactions of fingerlings rainbow trout fed varying levels of functional nutrient in the form of dietary prebiotic, GroBiotic[®]-A in order to determine an optimum inclusion level.

2 MATERIALS AND METHODS

2.1 Rearing conditions and experimental fish Trout fingerlings (4.44±0.06gaverageweight) from a hatchery in north of Iran were transported in oxygenated containers at $15.0 \pm 1.1^{\circ}$ C). Fish were acclimatised in laboratory conditions for 1 week prior to the commencement of the study. Thereafter, fish weight were randomly with similar body distributed into eighteen tanks (1.5x1.5x0.45m, $\approx 1 \text{m}^3$) at a density of 25 fish/ tank to evaluate the effect of prebiotic supplementation in the diet. The total water exchange in the tank was set at 0.2 lit/sec. The feeding trial was conducted for 84 days.

2.2 Test Diets

Commercial rainbow trout feeds (FFT and GFT1 (Table from Chineh Feed 1) ManufacturingCo., Tehran, Iran) supplemented with varying levels of G-A (International Ingredient Corporation, Fenton (St. Louis, Missouri, USA) were used in the study. Six concentrations of prebiotic (G-A), including 0.5 %, 1 %, 1.5 %, 2 %, 2.5 %, and 3 % wereadded toboth the FFT and GFT1 feeds (Table 1), plus a control treatment without adding prebiotic. The G-A was prepared according to the manufacturer's instructions and the designated doses were sprayed on the diets. The FFT diet was used when the fish weighed between 5-20g while the GFT1 was used for the fish that were above 20gapproximately 6 weeks after the commencement of feeding. The treatments were carried out in triplicates. Proximal composition of the feeds and carcass sample analyseswere based on the AOAC (1997) standard reference methods. Dietary variations in protein for the prebiotic and control diets ranged between 43.18 % and 43.30 % and between 40.35 % to 40.58 %, while lipid content averaged 13.70 % and 15.15 % in the FFT and GFT1 based diets, respectively (Table 1).

Experimental diets weremanually supplied at 2-5 % of fish size and at three daily intervas.

2.3 Determination of nutritional effects and survival

The average weight of the fish from each treatment group was measured at 2-week intervals, while the weight of individual fish in each treatment was measured for somatic growth and survival at the end of the feeding trial. At the end of 12 weeks, feed efficiency (FE), specific growth rate (SGR), survival rate (SR), net protein utilization (NPU), protein efficiency ratio (PER), protein intake (PI), total feed intake per fish (FI),condition factor (CF), were measured as follows:

2.4 Growth parameters

FE =[weight of produced trout (g)/weight of consumed food (g)] (De Silva, 1995).

PER (%) = [(wet weight of produced trout (g) \times 100)/weight of consumed food (g)] (Helland*et al.*, 1996).

SGR (%) = $[(Ln Wf - Ln Wi) \times (100/t)]$ (Heveroy*et al*,2005).

ADG Average Daily Gain (%) = [((Wf – Wi)/total days)) \times 100] (De Silva, 1995).

Where t is the time of rearing in days, lnWi is the natural logarithm of the weight of the fish individual at the start of the experiment, and lnWt is the natural logarithm of the weight of the juvenile at the end of the experiment (84 days). Wi and Wf are fish weight (g), and TL is total length (cm).

Survival rate (%) = [(final number of fish \times 100)/initial number of fish] (Heveroy*et al.*, 2005) Total feed intake per fish (FI) = [total feed intake/number of fish] (Helland*et al.*, 1996) Protein intake (PI) = [feed intake (g) \times percent

protein in the diet] (Helland *et al.*, 1996) NPU = [(protein in muscle after experiment – protein in muscle before experiment)/PI] $CF = [(Wf \times 100)/TL^3]$ (Austreng, 1978).

		Proximate composition	osition					
Treatments	Experiment Diets	Moisture (%)	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)	NFE ^{III} (%)	GE ^{II} (kj.g. ¹)
Control	FFT ²	5.83 ± 0.14	43.30 ± 0.07	13.57 ± 0.15	6.57 ± 0.09	9.12 ± 0.03	27.44 ± 0.13	20.31 ± 0.03
	GFT1 ¹	6.74 ± 0.01	$40,36 \pm 0,17$	15.16±0.05	7.24 ± 0.08	8.52 ± 0.09	28.73 ± 0.22	20.48 ± 0.03
G-A1*	FFT+G1	$5,79 \pm 0.12$	43.19 ± 0.11	13.70 ± 0.15	6.52 ± 0.11	9.12 ± 0.17	27.47 ± 0.44	20.34 ± 0.06
	GFT1+G1	6.91 ± 0.07	$40,42 \pm 0.10$	15.15 ± 0.06	7.18 ± 0.06	8.58 ± 0.12	28.67 ± 0.13	20.44 ± 0.01
G-A25	FFT+ G2	5.89 ± 0.05	43.29 ± 0.21	13.60 ± 0.21	6.48 ± 0.15	9.14 ± 0.04	27.43 ± 0.22	20.38 ± 0.07
	GFT1+G2	6.91 ± 0.03	40.35 ± 0.06	15.15 ± 0.03	7.22 ± 0.09	8.57 ± 0.18	28.70 ± 0.25	20.41 ± 0.02
G-A36	FFT+G3	5.85 ± 0.11	43.18 ± 0.04	13.65 ± 0.30	6.49 ± 0.20	9.15 ± 0.06	27.53 ± 0.42	20.43 ± 0.06
	GFT1+G3	6.92 ± 0.06	40.58 ± 0.28	15.17 ± 0.11	7.31 ± 0.05	8.50 ± 0.17	28.44 ± 0.30	20.40 ± 0.01
G-A47	FFT+ G4	5.76 ± 0.11	$43, 19 \pm 0.11$	13.69 ± 0.12	6.51 ± 0.07	9.13 ± 0.03	27.47 ± 0.19	20.49 ± 0.10
	GFT1+G4	6.83 ± 0.05	40.38 ± 0.06	15.18 ± 0.15	7.33 ± 0.10	8.51 ± 0.04	28.59 ± 0.09	20.76 ± 0.04
G-A5*	FFT+G5	5.92 ± 0.10	43.21 ± 0.20	13.68 ± 0.06	6.47 ± 0.17	9.20 ± 0.10	27.43 ± 0.10	20.67 ± 0.03
	GFT1+G5	6.80 ± 0.10	40.37 ± 0.05	15.19 ± 0.15	7.20 ± 0.11	8.51 ± 0.08	28.72 ± 0.26	20.49 ± 0.05
G-A6 ⁹	FFT+ G6	5.73 ± 0.16	43.30 ± 0.13	13.68 ± 0.03	6.56 ± 0.06	9.14 ± 0.09	27.31 ± 0.27	20.53 ± 0.03
	GFT1+G6	6.85 ± 0.15	40.56 ± 0.06	15.19 ± 0.04	6.59 ± 0.07	9.22 ± 0.02	28.44 ± 0.07	20.61 ± 0.04
Values are mean ± SD (n=3) Grow out Rambow Trout Fo GroBiotic [®] - A (A commercia GroBiotic [®] - A (A commercia	Values are mean ± SD (n=3) Grow our Rainbow Trout Feed (commercial Rainbow Trout food, Chine Co.) GroBiotic [®] -A (A commercial prebiotic) 1% of diet GroBiotic [®] -A (A commercial prebiotic) 2% of diet GroBiotic [®] -A (A commercial prebiotic) 3% of diet	nercial Rainbow Tr (c) 1% of diet (c) 2% of diet (c) 3% of diet	out food, Chine C	2 Fingerling 0.) 4 GroBiotic 6 GroBiotic 8 GroBiotic 10 Nitrogen	Rainbow Trout - A (A commer - A (A commer - A (A commer - A (A commer rec extract = [1]	2 Fingerling Rainbow Trout Feed (commercial Ruinbo 4 Großiotic [®] A (A commercial prebiotic) 0.5% of diet 6 Großiotic [®] A (A commercial prebiotic) 1.5% of diet 8 Großiotic [®] A (A commercial prebiotic) 2.5% of diet 10 Nitrogen free extract = [100 – (protein + lipid + ash	 Fingerling Rainbow Trout Feed (commercial Rainbow Trout 4 GroBiotic[®] A (A commercial prebiotic) 0.5% of diet 6 GroBiotic[®] A (A commercial prebiotic) 1.5% of diet 8 GroBiotic[®] A (A commercial prebiotic) 2.5% of diet 10 Nitrogen free extract = [100 – (protein + lipid + ash + fiber)] 	 Fingerling Rainbow Trout Feed (commercial Ruinbow Trout food, Chine Co.) 4 GroBiotic[®] - A (A commercial prebiotic) 0.5% of diet 6 GroBiotic[®] - A (A commercial prebiotic) 1.5% of diet 8 GroBiotic[®] - A (A commercial prebiotic) 2.5% of diet 100 Nitrogen free extract = [100 – (protein + lipid + ash + fiber)]

Table 1 Proximate composition of rainbow trout (Oncorhynchus mykiss) fed diets varying Concentrations of GroBiotic^a-A and control

2.5 Body indices

Body indices, hepatosomatic index (HSI), viscerosomatic weight (VSI), and intraperitoneal fat (IPF) were determined (AOAC 1997). Muscle and liver samples were also collected for proximate analysis:

Hepatosomatic index (HSI %) = [100 - (liver weight (g)/body weight (g))] (AOAC 1997).

Intraperitoneal fat (IPF %) = [100 - (intraperitoneal fat weight (g)/body weight (g))](AOAC 1997).

Viscerosomatic weight (VSI %) = [100 - (viscera weight/body weight)] (AOAC 1997).

2.6 Blood parameters

Upon completion of the experiment, (17 h after the last feeding) (Shimeno *et al.*, 1990), 3 fish from each tank (9 fish per treatment) were sampled and placed directly into a bucket filled with 10 L of freshwater mixed with 200 ppm of tricainemethanesulfonate (MS-222; Sigma– Aldrich Corporation, St. Louis, MO, USA). Then, blood was drawn from the caudal vein of the sampled fish for the determination of haematological parameters immediately after anesthetization.

2.7 Haematocrit

Haematocrit was determined according to the methods described by Schäperclaus *et al.* (1993). Blood samples were placed into standard heparinised microhematocrite capillary tubes and centrifuged immediately for 4 min at 10,000×g by using a Hawksley centrifuge (Lancing, Sussex, England). The haematocrit value was calculated according to the following formula:

PCV= [height of packed red cells (mm)/height of packed red cells and plasma (mm)] x 100Where, PCV is the packed cell volume (mm).

2.8 Haemoglobin concentration

The cyano-haemoglobin method was used to determine the haemoglobin concentration at

wavelength of 540 nm in experimental fish with a CECIL 1020, England spectrophotometer at 540 nm. The haemoglobin concentration of the blood sample was estimated using a standard curve. A cover slip was placed over a Neubauer haemocytometer, a specially designed slide that acts as a blood cell counting chamber (Blaxhall and Daisley 1973). Then were was computed mean cell volume (MCV), mean cell hemoglobin concentration (MCHC) and the amount of hemoglobin per erythrocyte (MCH) by the following formula (AOAC 1997):

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Haemoglobin concentration (g dL⁻¹)=[absorbance of sample/absorbance of standard \times concentration of standard]

 $\begin{array}{ccc} Mean & corpuscular & haemoglobin \\ concentration & (MCHC) & gdL^{-1} & = [haemoglobin \\ g\%/hematocrit volume \% \times 100] \end{array}$

Mean corpuscular haemoglobin (MCH) pg $cell^{-1} = [haemoglobin g\%/erythrocyte (10^6 mm^{-3}) \times 10]$

Mean corpuscular volume (MCV) μm^3 =[haematocrit volume/erythrocyte (10⁶mm⁻³) × 10]

2.9 Total red blood cell count (erythrocyte/ RBC 10⁶ mm⁻³)

Total red blood cell count (RBC) and white blood cells (WBC or LC) was performed according to the methods of the EWOS Technology Centre (2000) and Johnson *et al.*, (2002). The blood samples in the heparin tubes were diluted 200 times with phosphate-buffered saline (PBS), and red blood cell concentrations were calculated in a haemocytometer chamber using a microscope.

White blood cells were measured using Natt–Herrick solution as the diluent and were stained in a Neubauer haemocytometer.

2.10 Lysozyme assay

Lysozyme level in blood sera samples was estimated by turbidimetric evaluation according to the protocol by Ellis (1990), with slight modifications. The blood samples were maintained at room temperature for 1 h, centrifuged at 10,000×g for 10 min, and the separated sera were frozen at -20°C until used for the lysozyme assay within 7 d of sampling. Aliquots (175µL) of Micrococcus lysodeikticus suspension (Sigma) (0.375 mg mL⁻¹, 0.05 M PBS, pH 6.2) were mixed with 25 µLof each sample, and optical density was measured after 15 and 180s by spectrophotometer (BioPhotometer; Eppendorf, Germany) at 600 nm. PBS was used as the blank, and results were expressed in amounts of lysozyme (µg) per 1 mg of sample calibrated to a standard curve using hen egg white lysozyme (Sigma) in PBS.

2.11 IgM

IgM level in blood sera was determined by immunoturbidmetric assay with a Parsazmun kit (www.parsazmun.com) and Eurolyser with slight modifications. At the end of the 4, 8, and 12weeks feeding trials and after 4 weeks post injection, 3 healthy fish (with no obvious signs of skin injury or visceral granuloma) from each tank (9 fish per treatment) were anesthetized with tricaine methanesulfonate (MS-222). Blood samples (1mL) were obtained from the caudal vein of each specimen using a 2-mL syringe. After clotting, the sample was centrifuged (Kokusan; 5000×g) for 5 min, and the serum was removed and frozen at -20°C until used. In this test, each sample was diluted with physiological serum at a ratio of 1:10. Then, standard polyclonal antibody was added to the sample. The complex of IgM with polyclonal antibodies caused the solution to become turbid, and the degree of opacity was directly related to serum IgM concentration.

The solution was prepared in a special cuvette, and IgM was determined using an autoanalyser (Eurolyser, Austria).

2.12 Statistical analysis

Data were analyzed by SPSS ver. 15.1 (Statistical Package for Socials Sciences) and analyzed using analysis of variance (ANOVA). Comparison among treatment means was carried out using Duncan's Multiple Range Test to evaluate any significant differences at the level of 0.05. Standard deviation (\pm SD) was calculated to determine the range of means. Treatment mean differences were tested between whole feeding regimes, at each GroBiotic[®]-A levels.

3 RESULTS

3.1 Growth performance

The growth performance of the fish fed diets supplemented with different levels of G-A after 84-days feeding trial is displayed in Table 2. SGR and ADG were significantly higher (P < 0.05) in fish fed diets supplemented with G-A level above 1.5%. A significant difference (P < 0.05) in increased FE was found among the treatments. PER and NPU also were significantly higher (P < 0.05) in fish fed diets supplemented with G-A level above 1%.No significant difference (P>0.05) was found in protein indices among the treatments. As for the feed intake, the diets supplemented with G-A showed higher values than the basal diet (0% G-A.

Survival of rainbow trout was significantly improved (P<0.05) in all the G-Asupplemented diets, the highest of which was observed in 2.5% G-A.

Parameters	Treatments	ots							
	Ĩ		G-A 2	G-A 3	G-A 4	-	G-A 5	G-A 6	Control
Wi (g) ²	4.46 ± 0.07		4.48 ± 0.04	4.40 ± 0.06	4.43 ± 0.03	.03	4.40 ± 0.11	4.45 ± 0.05	4.46 ± 0.05
Wf (g) ³	$38.98 \pm 0.74^{\circ}$		$38.92 \pm 1.84^{\circ}$	$38.24 \pm 1.17^{\circ}$	41.93 ± 1.12^{b}	.12 th	$45.21 \pm 1.46^{\circ}$	43.24 ± 1.13^{ab}	$36.76 \pm 2.14^{\circ}$
Wg (%)4	$774.09 \pm 18.14^{\circ}$		$768.86 \pm 39.36^{\circ}$	$770.08 \pm 33.11^{\circ}$	00		$933.09 \pm 14.86^{\circ}$	871.51 ± 29.04^{h}	$724.04 \pm 41.60^{\circ}$
SGR (%)5	$2.58 \pm 0.02^{\circ}$		$2.57 \pm 0.05^{\circ}$	$2.57\pm0.05^\circ$			$2.78 \pm 0.02^{*}$	2.71 ± 0.04^{5}	$2.51 \pm 0.06^{\circ}$
ADG (%)6	$41.10 \pm 0.86^{\circ}$		$41.00 \pm 2.17^{\circ}$	$40.29 \pm 1.42^{\circ}$	44.64 ± 1.29^{b}	295	48.60 ± 1.62^{2}	46.18 ± 1.36^{ab}	$38,45 \pm 2.50^{\circ}$
Survival(%) ⁷	97.33 ± 2.31^{sh}		94.67 ± 2.31^{sh}	97.33 ± 2.31^{ab}	$98.67 \pm 2.31^{\circ}$.31 ⁿ	$98.67 \pm 2.31^{\circ}$	97.33 ± 2.31^{sh}	93.33 ± 2.31^{b}
Feed intake	40.24 ± 1.78^{bc}		41.20 ± 0.23 ^{ab}	41.77 ± 1.07^{ab}	41.55 ± 0.85 th	.85 th	$42.99 \pm 0.65^{*}$	41.36 ± 1.16^{ab}	$39.00 \pm 0.86^{\circ}$
FE ⁸	0.86 ± 0.03^{cd}		0.84 ± 0.04^{4e}	$0.81 \pm 0.01^{\circ}$	0.90 ± 0.01^{h}	410.	$0.95 \pm 0.02^{*}$	0.94 ± 0.00^{ab}	0.83 ± 0.04^{de}
PER9	1.99 ± 0.06^{cd}		1.97 ± 0.09^{d}	1.89 ± 0.03^{d}	2.08 ± 0.02^{bc}	.02 ^{bc}	$2.19 \pm 0.04^{*}$	2.16 ± 0.01^{sb}	1.89 ± 0.08
NPU (%)10	4.77 ± 2.61^{cd}		2.40 ± 0.01^{d}	7.46 ± 0.92^{bc}	8.27 ± 1.28^{b}	.28 ^b	13.84 ± 1.29^{a}	9.97 ± 2.79^{b}	2.24 ± 0.61^{d}
1Values are mean ± S 2 Wi = Initial weight, 3 Wf = Final weight	an ± SD (n=3), N eight, eight	4ean values wi	hin columns	with different s	uperscript lett	ers are sig	1 Values are mean \pm SD (n=3). Mean values within columns with different superscript letters are significantly different (P < 0.05) 2 Wi = Initial weight, 3 Wf = Final weight	nt (P < 0.05)	
5 ADG (%) = [(Ln									
	4 wg = 1(wt - wt/wt1 × 100 5 SGR% = [(LnWf - LnWi) / Total days (t)] × 100 6 ADG (%) = [(Wf - Wi) / Total days (t)] × 100	otal days (t)] × l days (t)] × 10	00						
7 Survival rate (3 Feed efficienc	4 wg = $[(w_1 - w_1)w_1] \times 100$ 5 SGR% = $[(LnWf - LnWi) / Total days (t)] \times 100$ 6 ADG (%) = $[(Wf - Wi) / Total days (t)] \times 100$ 7 Survival rate (%) = $[(Final fish number / Initial fish number) \times 100]$ 8 Feed efficiency = weight gain (g) / food intake (g) 8 Feed efficiency = weight gain (g) / food intake (g)	otal days (t)] × l days (t)] × 10 n number / Initi (g) / food intak	100) al fish numb c (g)	er) × 100]					
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7 Survival rate (8 Feed efficienc 9 Protein efficien 10 Nett Protein 10 Nett Protein Table 3 Carc	wr - LnWi) / To wr - Wi) / Tota \ll) = ((Final fish y = weight gain ncy ratio = weig Utilization (NPL Utilization (NPL ass proximate) At the start 14.68 \pm 0.13	tal days (t)] × t days (t)] × 10 t number / Initi (g) / food intak ht gain (g) / pn J) = [(Wf × Pro J) = [(Wf × Pro J) = [(Wf × Ard At the end G-A 1 15.50 \pm 0.43 ^d	$\frac{100}{\text{al fish number}}$ $e (g)$ $rein Muscle Findstein (g)$ $rein Muscle Findstein (g)$ $e (g)$ $e (g)$ $\text{frainbow trees (g)}$ $\frac{\text{G-A 2}}{15.10 \pm 0.00^{\circ}}$	er) \times 100] g) Final) - (Wi \times Prot trout (<i>Oncorhync</i> A and control die G-A 3 0° 15.85 \pm 0.16°	Protein Musele Protein Musele Vinchus mykis diet for 84 di	cle Initial/P (<i>iss</i>) fed co days ¹ $\overline{G-A 4}$ 7 ± 0.21^{bc}	otein Consumed) ntrol and varyin G-A 5	g Concentrations	of GroBiotic Control
7 Survival rate (8 Feed efficiene 9 Protein efficiene 10 Nett Protein Table 3 Carc Table 3 Carc Protein (%)	$wr - vrij \times roowr - wij / Totawr - wij / Totawr = ((Final fishy = weight gainney ratio = weigUtilization (NPUUtilization (NPUAt the start14.68 \pm 0.139.40 \pm 0.05$	tidays (t)] × t days (t)] × 10 t number / J × 10 t g) / food intak (g) /	100 al fish number e (g) tein Muscle F tein Muscle F of rainbow tr e (g) e (h) e (h)	er) × 100] g) Final) – (Wi × Prot trout (Oncorhync A and control die G-A 3 6° 7.44 ± 0.12 ^b	Protein Muscle Protein Muscle Nuchus mykis diet for 84 dt diet for 84 dt	Muscle Initial/P mykiss) fed co r 84 days ¹ $r 84 days^1$ $r 84 days^1$ $r 84 days^1$ $r 84 days^1$	ntrol and varyin G-A 5 $17.06 \pm 0.06^{\circ}$ $7.05 \pm 0.04^{\circ}$	g Concentrations G-A 6 16.23 ± 0.06^{b} 7.09 ± 0.05^{c}	of GroBiotic Control 15.06 ± 0.11 ^c 8.02 ± 0.07 ^d
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 Table 2 Initial weight, final weight, percentage weight gain, specific growth rate, average daily growth, survival, Feed intake, feed efficiency, protein efficiency ratio, Nett protein utilization and productive protein value of rainbow trout (Oncorhynchus mykiss) fed diets containing varying

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¹ Values are mea	Moisture (%)
are mean ± SD (n=3). N	75.10±0.36
). Mean values withi	15.16 ± 0.13
in columns with	3 ^a 74.93 ± 0.05 ^{ab}
different supersc	74.89 ± 0.63 ^m
ript letters are sig	74.49 ± 0.10 ^{pc}
nificantly differen	74.24 ± 0.09°
tt (P < 0.05)	74.51 ± 0.18 ^{ee}

ŝ 1 ŝ CHATTER. cut (r < 0.00)

3.2 Proximate composition of carcasses

A significant difference (P < 0.05) in the protein, lipid, and moisture content of carcass were found among the treatments. The concentrations of protein and ash were higher in the diet supplemented with 2.5 % G-A (17.06±0.06 % and 1.46±0.05 %, respectively), whereas those of lipid and moisture were lower (Table 3).

3.3 Body indices

The results of the body indices (HSI, IPF, and VSI) are summarized in Table 4. Although some differences in HSI, IPF and VSI were found among the tretments, they were not significant (P>0.05).

3.4 Haematological/immunological parameters

Haematological parameters of the trout fed with various leels of G-A for 12-weeks are given in Table 5. Although no significant differences (P>0.05) in haematological parameters were found among different treatments, higher values for haematocrit (PCV), haemoglobin (Hb), RBC, WBC, and neutrophils were recorded in fish fed diets supplemented with G-A levels above 1.5%. The immunoglobulin (IgM) and lysozyme valueswere significantly higher (P<0.05) in fish fed with G-A levels above 1.5%.

Table 4 Hepatosomatic index (HSI), intraperitoneal fat (IPF) ,viscerosomatic index (VSI), and condition factor of rainbow trout (*Oncorhynchus mykiss*) fed control and varying concentrations of GroBiotic[®]-A and control diet for 84 days¹

	Treatments						
Parameters	G-A 1	G-A 2	G-A 3	G-A 4	G-A 5	G-A 6	Control
$CF(\%)^2$	1.20 ± 0.04	1.20 ± 0.03	1.19 ± 0.01	1.21 ± 0.04	1.18 ± 0.06	1.20 ± 0.02	1.24 ± 0.02
HSI $(\%)^{3}$	1.15 ± 0.17	1.12 ± 0.13	1.03 ± 0.18	1.05 ± 0.25	1.04 ± 0.14	0.97 ± 0.06	1.13 ± 0.10
VSI $(\%)^4$	$13.02 \pm$	12.67 ±	$14.00 \pm$	12.07 ±	12.64 ±	11.86 ±	$14.04~\pm$
IPF $(\%)^5$	1.27 ± 0.12	1.33 ± 0.55	1.38 ± 0.15	1.39 ± 0.51	1.41 ± 0.25	1.51 ± 0.22	1.33 ± 0.69

1 Values are mean \pm SD (n=3).

2 Condition factor (CF %) = [(weight / L3) $\times 100$]

3 Hepatosomatic index (HSI%) = [100 - (liver weight / body weight)]

4Intraperitoneal fat (IPF %) = [100 - (intraperitoneal fat weight / body weight)]

5Viscerosomatic weight (VSI %) = [100 - (viscera weight / body weight)]

	Treatments						
	G-A 1	G-A 2	G-A 3	G-A 4	G-A 5	G-A 6	Control
Hematology parameters							
RBC(cells × 10 ⁶ /mm ³) ²	$0.99 \pm 0.04^{\circ}$	$0.99 \pm 0.04^{\circ}$	$1.00 \pm 0.04^{\circ}$	1.12 ± 0.03^{b}	1.18 ± 0.05^{a}	$1 14 + 0.04^{ab}$	0 07 + 0 08°
WBC(cells $\times 10^3$ /mm ³) ³	$9.25 \pm 0.82^{\circ}$	$9.33 \pm 0.68^{\circ}$	$9.50 \pm 0.77^{\circ}$	10.75 ± 0.42^{b}	12.50 ± 1.05^{a}	11.42 ± 0.66	2 0 1 1 C 0 2
Hematocrit (%)	40.17 ± 2.48^{cd}	41.83 ± 1.47^{bcd}	42.50 ± 2.07^{bc}	44.50 ± 1.87^{b}	47.83 ± 3.54^{a}	$4433 + 266^{b}$	pCS U + 11 000
Hemoglobin(g/dL)	6.57 ± 0.43^{b}	7.08 ± 1.41^{b}	7.13 ± 0.44^{b}	7.88 ± 0.22^{ab}	8.52 ± 0.72^{a}	8.30 ± 1.40^{ab}	6.58 ± 0.57^{b}
Lymphocytes (%)	98.83 ± 1.17^{a}	98.67 ± 1.47^{a}	$97.83\pm0.98^{\rm ab}$	97.33 ± 1.21^{b}	96.83 ± 0.52^{b}	97.17 ± 0.98^{b}	99.00 ± 0.63^{a}
Neutrophils (%)	1.17 ± 1.17^{b}	1.33 ± 0.52^{b}	$2.17 \pm 0.98^{\circ}$	2.67 ± 1.21^{a}	3.17 ± 1.47^{a}	2.83 ± 0.98^{4}	1.00 ± 0.63^{b}
MCV(µm ³) ⁴	408.70 ± 35.15^{ab}	424.19 ± 12.52^{a}	426.20 ± 25.00^{ab}	397.36 ± 14.03^{ab}	404.95 ± 35.47^{ab}	387.69 ± 17.52^{b}	409.51 ± 37.39 ^{ab}
MCH(pg/cell) ⁵	66.84 ± 6.31	72.05 ± 15.64	71.66 ± 6.92	70.42 ± 2.38	72.15 ± 7.38	72.68 ± 12.42	68.47 ± 7.70
MCHC(g/dL)°	16.37 ± 1.06	16.97 ± 3.56	16.81 ± 1.23	17.73 ± 0.67	17.82 ± 1.09	18.73 ± 2.90	16.74 ± 1.44
Immune parameters							
IgM(mg/ml) ⁷	37.78 ± 1.01^{d}	40.02 ± 3.08^{d}	$44.75 \pm 3.61^{\circ}$	49.38 ± 3.44^{b}	55.20 ± 2.73^{a}	50.60 ± 3.18^{b}	37.65 ± 1.82^{d}
lysozyme(mg/ml)	9.49 ± 0.60^{d}	9.49 ± 0.68^{d}	$10.80 \pm 0.48^{\circ}$	12.16 ± 0.85^{b}	12.99 ± 0.40^{a}	12.66 ± 0.61^{ab}	9.54 + 0.17 ^d

Table 5 Haematology/immune parameters of rainbow trout (Oncorhynchus mykiss) fed diets containing varying levels of GroBiotic®-A and ntrol diet for 12 pal-1

3 Leukocyte or total white blood cell counts 4 Mean corpuscular volume = [(Haematocrit × 10) / RBC] 5 Mean corpuscular volume = [(Haemoglobin × 10) / RBC]

6 Mean corpuscular hemoglobin concentration = [(Haemoglobin × 100) / Haematocrit] 7 Immunoglobulin (IgM)

4 **DISCUSSION**

In this study, growth performance of O. mykiss showed an increasing trend with increasing G-A levels and was significantly higher (23 %) in fish fed with the diet supplemented with 2.5 % G-A.Positive effects of various prebiotics on growth of hybrid red tilapia (Hanley et al., 1995) and the European catfish (Bogut et al., 2006) have also been reported. The growth improvement and enhanced protein utilization upon adding prebioticshave been attributed to the improvement in digestive enzymes activities and absorption of food (Xu et al., 2009; Burr et al., 2010). Feed utilization indices in O. mykissshowedimproved protein efficiency ratio (PER), protein utilization (NPU) and feed efficiency (FE) by addingprebiotic, which is in correspondence with several earlier works dealing with the application of prebiotics inrainbow trout (Staykov et al., 2007; Rodrigues-Estrada et al., 2009; Řehulka et al., 2011) and several other species (Li and Gatlin, 2004, 2005; Buentello et al., 2010; Grisdale-Helland et al., 2008; Lochmann, 2011); Zheng et al., 2011). Besides, the reason of enhanced growth could be related to improved stability of intestinal microbial flora (Fuller, 1989).

Beside improving the FE, SGR, and FI, prebiotic also significantly enhanced feed efficiency and resistance of O. mykiss; significantly higher (P<0.05) PER and survival rates were also recorded in fish fed G-A prebiotic above 1.5 %. (Table 2). The higher survival rates in the prebiotic-treated groups may also indicate improved response potential and improved ability to tolerate the damaging conditions likely encountered in the rearing tanks (Olsen et al., 2001). Supplementation with G-A modified body composition by decreasing the fat and increase in protein contents(Table 3), which was in correspondence with an earlier study in rainbow trout (Yilmaz et al., 2007). Values for visceral somatic indices (VSI), intraperitoneal fat (IPF) and hepatosomatic index (HSI) did not significantly differ among the treatments (Table 4), which were similar to an earlier result in rainbow trout (Yilmaz et al.,2007). However, Refstie et al., (2006) revealeda higher relative gut weight (relative to total body weight) in Atlantic salmon fed inulin, but the relative liver and stomach weights were unaffected. Furthermore, McKellep et al. (2007)showed that in the Atlantic salmon with the exception of the distal intestinal somatic index, inulin administration in the diet did not affect other gastric organosomatic indices. insignificant The difference for HSI value in the fish fed various levels of G-A in this study(Table 4) is in agreement with the previous studies in Atlantic salmon (Rosenlund, et al., 2001;Menoyoet al., 2003, 2005), Murray cod (Francis et al., 2007) and turbot (Regost et al., 2001).

Haematological characteristics have been studied in numerous fish species to determine their normal ranges, and any variation from normal is indicative of problems in fish physiological processes (Rainza-Paivaet al., 2000). In this study, higher concentrations of haemoglobin, haematocrit, RBC, WBC, MCH, MCHC, MCV, lymphocytes, and neutrophils were observed in the fish fed with various levels of G-A compared to the control diet, but the difference among the treatments was not significant (P>0.05). In contrast, lysozyme and concentrations significantly differed IgM (P<0.05) among different G-A treatments, peaking at 2.5% (Table 5). This observation indicates that fish fed with supplementary G-A are healthier, possiblydue to the increased enzymatic levels in the blood plasma. Similar results have also been reported (Carnevali et al., 2006; Rollo et al., 2006). In the present experiment, haematocrit value agreed with the findings of Li and Gatlin (2004)who reported that haematocrit value in the hybrid striped bass did not increase. Our results for HB, HC, WBC, RBC, MCH, MCHC, and MCV in rainbow

trout were not significantly different (P>0.05) among treatments and were similar to values reported by Sheikholeslami (2008). A higher immune response was stimulated when diets supplemented with G-A level above 1.5% were used (Table 5). Serum lysozyme increased in fish fed various levels of G-A (Table 5), which was in agreement with an earlier finding by Sheikholeslami (2008).

The results indicated that an inclusion level of 2.5% of GroBiotic[®]-A yielded an optimal growth performance, feed efficiency, body indices, and haematological parameters in rainbow trout (*O. mykiss*) fry.

Further studies are needed to recognize the optimum duration of prebiotic supplementation to the fish, particularly rainbow trout. In addition, it would be suggested to evaluate the long term effect of GroBiotic-A in rainbow trout and others fish species.

5 ACKNOWLEDGEMENT

This research was supported by the Iranian Research Organisation, Fisheries IIC. International Ingredient Corporation of USA farm and a private (Mr. Nouparvar, GhezelalaParvarCo., Mazandaran province, Iran.). The authors thank Dr. Abass Motalebi, Dr. Reza Pourgholam, and all other colleagues for their generous support, without which this study would not have been possible.

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بررسی اثر افزایش میزان پری بیوتیک گروبیوتیک A بر روی رشد، فاکتورهای بدن و پارامترهای خونی در بچه ماهیان قزلآلای رنگین کمان (Oncorhynchus mykiss)

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تاریخ دریافت: ۱۸ فروردین ۱۳۹۲ / تاریخ پذیرش: ۲۸ بهمن ۱۳۹۲ / تاریخ چاپ: ۲۵ خرداد ۱۳۹۳

چکیده اثر مصرف مکمل غذائی (گروبیوتیک A) بر روی رشد، ضریب تبدیل غذا، فاکتورهای خونی و ایمنی در ماهی قزل آلای انگشت قد (۴۴/۴ ± ۲۰/۶ گرم) طی مدت ۸۴ روز و در ۲ تیمار و هر تیمار در ۳ تکرار بررسی شد. تیمارهای آزمایشی شامل درصدهای صفر، ۵/۰، ۱/۰، ۵/۱، ۲، ۵/۲ و ۳ درصد از گروبیوتیک A به جیره غذای ماهی بوده است. غذادهی روزانه ۲ نوبت و به میزان ۲-۶ درصد از وزن بدن ماهی انجام شد. نتایج نشان داد ماهی تغذیه شده با جیره (۸/۲٪ گروبیوتیک A بهطور قابل ملاحظه از بالاترین افزایش وزن (WG)، نرخ رشد ویژه (SGR) و میانگین افزایش وزن روزانه (ADD) برخوردار شد (۹–۵/۰). همچنین، پارامترهایی همچون بازده خوراک (FE)، نسبت بازده پروتئین (PER)و استفاده از پروتئین خالص (NPU) در ماهی بهطور قابل توجهی از بالاترین میزان در جیره غذایی گروبیوتیک A ۵/۲٪ برخوردار شد (۹–۵/۵). بالاترین بازماندگی در جیره محتوی۲و ۵/۲٪گروبیوتیک A بود (۹–۵/۰). مقدارهموگلوبین، هماتوکریت٪، ADD در ماهی بهطور قابل توجهی از بالاترین میزان در جیره غذایی گروبیوتیک مقدارهموگلوبین، هماتوکریت٪، NCH MCH، MCH، NDCH، لنفوسیت و نوتروفیلها در ماهیهای تغذیه شده از مکمل گروبیوتیک A مشاهده شد، اما تفاوت معنیداری در میان تیمارها مشاهده نشد(۹–۵/۰). از سوی دیگر، مقدارهموگلوبین، و ایمونولیو (IgM) در اطلاه منه داور معنی داری و ایشان داد و بالاترین در رژیم غذایی گروبیوتیک شره از مکمل گروبیوتیک A مشاهده شد، اما تفاوت معنیداری در میان تیمارها مشاهده نشد(۹–۵/۰). از سوی دیگر، موراه بود (۹–۵/۰). نتایج حاصل از این مطالعه نشان داد که مکمل رژیم غذایی با گروبیوتیک A ۲/۵٪ دارای اثرمثبت در قزل آلای رنگین کمان انگشت قد بود.

كلمات كليدى: A-®Oncorhynchusmykiss, Prebiotic GroBiotic، رشد، ضريب غذا، ماهى