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

Abdolhamid Azari[1, 2*], Roshada Hashim[1], Ghobad Azari Takami [3]and Aboulghasem Roohi[2]

[1]*Laboratory of Feeds and Feeding Management, Aquaculture Research Group, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*.
[2]*Caspian Sea Ecological Research Institute, P.O.Box 961, Khazarabad Boolvar, Sari, Iran*.
[3]*Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*.

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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 differencee (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

* *Corresponding author: Caspian Sea Research Institute of Ecology, Khazarabad Boolvar, Sari, Iran, Tel: +98 911 124 5274, E-mail: ahazaritakami@yahoo.com*

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 eco-friendly 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, haematology parameters, and non-specific 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 purpoose 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±1.1°C). Fish were acclimatised in laboratory conditions for 1 week prior to the commencement of the study. Thereafter, fish with similar body weight were randomly distributed into eighteen tanks (1.5x1.5x0.45m, ≈1m^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 1) from Chineh Feed 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 sizeand 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) × 100)/weight of consumed food (g)] (Hellande*t al.*, 1996).

SGR (%) = [(Ln Wf – Ln Wi) × (100/t)] (Heveroy*et al*,2005).

ADG Average Daily Gain (%) = [((Wf – Wi)/total days)) × 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 × 100)/initial number of fish] (Heveroy*et al.*, 2005)
Total feed intake per fish (FI) = [total feed intake/number of fish] (Hellande*t al.*, 1996)
Protein intake (PI) = [feed intake (g) × percent protein in the diet] (Helland *et al*., 1996)
NPU = [(protein in muscle after experiment – protein in muscle before experiment)/PI]
CF = [(Wf × 100)/TL^3] (Austreng, 1978).

Table 1 Proximate composition of rainbow trout (*Oncorhynchus mykiss*) fed diets varying Concentrations of GroBiotic®-A and control for 84 days[1]

Treatments	Experiment Diets	Proximate composition						
		Moisture (%)	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)	NFE[10] (%)	GE[11] (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[3]	6.74 ± 0.01	40.36 ± 0.17	15.16 ± 0.05	7.24 ± 0.08	8.52 ± 0.09	28.73 ± 0.22	20.48 ± 0.03
G-A1[4]	FFT+ G1	5.79 ± 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 ± 0.10	15.15 ± 0.06	7.18 ± 0.06	8.58 ± 0.12	28.67 ± 0.13	20.44 ± 0.01
G-A2[5]	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-A3[6]	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-A4[7]	FFT+ G4	5.76 ± 0.11	43.19 ± 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[8]	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

1 Values are mean ± SD (n=3)
2 Fingerling Rainbow Trout Feed (commercial Rainbow Trout food, Chine Co.)
3 Grow out Rainbow Trout Feed (commercial Rainbow Trout food, Chine Co.)
4 GroBiotic®-A (A commercial prebiotic) 0.5% of diet
5 GroBiotic®-A (A commercial prebiotic) 1% of diet
6 GroBiotic®-A (A commercial prebiotic) 1.5% of diet
7 GroBiotic®-A (A commercial prebiotic) 2% of diet
8 GroBiotic®-A (A commercial prebiotic) 2.5% of diet
9 GroBiotic®-A (A commercial prebiotic) 3% of diet
10 Nitrogen free extract = [100 – (protein + lipid + ash + fiber)]
11 Gross energy content (Brafield 1985)

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):

Haemoglobin concentration (g dL^{-1})=[absorbance of sample/absorbance of standard × concentration of standard]

Mean corpuscular haemoglobin concentration (MCHC) gdL^{-1} =[haemoglobin g%/hematocrit volume % × 100]

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

Mean corpuscular volume (MCV) μm^3 =[haematocrit volume/erythrocyte ($10^6 mm^{-3}$) × 10]

2.9 Total red blood cell count (erythrocyte/ RBC 10^6 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 (±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.

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 Concentrations of GroBiotic®-A and control for 84 days[1]

Parameters	Treatments						
	G-A 1	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	4.40 ± 0.11	4.45 ± 0.05	4.46 ± 0.05
Wf (g)[3]	38.98 ± 0.74^{c}	38.92 ± 1.84^{c}	38.24 ± 1.17^{c}	41.93 ± 1.12^{b}	45.21 ± 1.46^{a}	43.24 ± 1.13^{ab}	36.76 ± 2.14^{c}
Wg (%)[4]	774.09 ± 18.14^{c}	768.86 ± 39.36^{c}	770.08 ± 33.11^{c}	847.08 ± 18.41^{b}	933.09 ± 14.86^{a}	871.51 ± 29.04^{b}	724.04 ± 41.60^{c}
SGR (%)[5]	2.58 ± 0.02^{c}	2.57 ± 0.05^{c}	2.57 ± 0.05^{c}	2.68 ± 0.02^{b}	2.78 ± 0.02^{a}	2.71 ± 0.04^{b}	2.51 ± 0.06^{c}
ADG (%)[6]	41.10 ± 0.86^{c}	41.00 ± 2.17^{c}	40.29 ± 1.42^{c}	44.64 ± 1.29^{b}	48.60 ± 1.62^{a}	46.18 ± 1.36^{ab}	38.45 ± 2.50^{c}
Survival(%)[7]	97.33 ± 2.31^{ab}	94.67 ± 2.31^{ab}	97.33 ± 2.31^{ab}	98.67 ± 2.31^{a}	98.67 ± 2.31^{a}	97.33 ± 2.31^{ab}	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^{ab}	42.99 ± 0.65^{a}	41.36 ± 1.16^{ab}	39.00 ± 0.86^{c}
FE[8]	0.86 ± 0.03^{cd}	0.84 ± 0.04^{de}	0.81 ± 0.01^{e}	0.90 ± 0.01^{b}	0.95 ± 0.02^{a}	0.94 ± 0.00^{ab}	0.83 ± 0.04^{de}
PER[9]	1.99 ± 0.06^{cd}	1.97 ± 0.09^{d}	1.89 ± 0.03^{d}	2.08 ± 0.02^{bc}	2.19 ± 0.04^{a}	2.16 ± 0.01^{ab}	1.89 ± 0.08^{d}
NPU (%)[10]	4.77 ± 2.61^{cd}	2.40 ± 0.01^{d}	7.46 ± 0.92^{bc}	8.27 ± 1.28^{b}	13.84 ± 1.29^{a}	9.97 ± 2.79^{b}	2.24 ± 0.61^{d}

1Values are mean ± 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
4 Wg = [(Wf – Wi)/Wi] × 100
5 SGR% = [(LnWf – LnWi) / Total days (t)] × 100
6 ADG (%) = [(Wf – Wi) / Total days (t)] × 100
7 Survival rate (%) = [(Final fish number / Initial fish number) × 100]
8 Feed efficiency = weight gain (g) / food intake (g)
9 Protein efficiency ratio = weight gain (g) / protein intake (g)
10 Nett Protein Utilization (NPU) = [(Wf × Protein Muscle Final) – (Wi × Protein Muscle Initial/Protein Consumed)]

Table 3 Carcass proximate compositions of rainbow trout (*Oncorhynchus mykiss*) fed control and varying Concentrations of GroBiotic®-A and control diet for 84 days[1]

		At the end						
	At the start	G-A 1	G-A 2	G-A 3	G-A 4	G-A 5	G-A 6	Control
Protein (%)	14.68 ± 0.13	15.50 ± 0.43^{d}	15.10 ± 0.00^{e}	15.85 ± 0.16^{c}	16.17 ± 0.21^{bc}	17.06 ± 0.06^{a}	16.23 ± 0.06^{b}	15.06 ± 0.11^{e}
Lipid (%)	9.40 ± 0.05	7.59 ± 0.45^{b}	8.23 ± 0.06^{a}	7.44 ± 0.12^{b}	7.44 ± 0.03^{b}	7.05 ± 0.04^{c}	7.09 ± 0.05^{c}	8.02 ± 0.07^{a}
Ash (%)	1.87 ± 0.03	1.22 ± 0.02^{b}	1.21 ± 0.06^{b}	1.23 ± 0.23^{b}	1.38 ± 0.11^{ab}	1.46 ± 0.05^{a}	1.44 ± 0.05^{a}	1.21 ± 0.02^{b}
Moisture (%)	75.10 ± 0.36	75.16 ± 0.13^{a}	74.93 ± 0.05^{ab}	74.89 ± 0.63^{ab}	74.49 ± 0.10^{bc}	74.24 ± 0.09^{c}	74.51 ± 0.18^{bc}	75.01 ± 0.02^{a}

[1] Values are mean ± SD (n=3). Mean values within columns with different superscript letters are significantly different (P < 0.05)

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 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 days[1]

	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 ±	12.67 ±	14.00 ±	12.07 ±	12.64 ±	11.86 ±	14.04 ±
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 ± SD (n=3).
2 Condition factor (CF %) = [(weight / L3) ×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)]

Table 5 Haematology/immune parameters of rainbow trout (*Oncorhynchus mykiss*) fed diets containing varying levels of GroBiotic®-A and control diet for 12 weeks[1]

	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 ± 0.04^{c}	0.99 ± 0.04^{c}	1.00 ± 0.04^{c}	1.12 ± 0.03^{b}	1.18 ± 0.05^{a}	1.14 ± 0.04^{ab}	0.97 ± 0.08^{c}
WBC(cells × 10^3 /mm^3)[3]	9.25 ± 0.82^{c}	9.33 ± 0.68^{c}	9.50 ± 0.77^{c}	10.75 ± 0.42^{b}	12.50 ± 1.05^{a}	11.42 ± 0.66^{ab}	8.92 ± 0.38^{c}
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}	44.33 ± 2.66^{b}	39.33 ± 0.82^{d}
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 ± 0.98^{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 ± 0.98^{c}	2.67 ± 1.21^{a}	3.17 ± 1.47^{a}	2.83 ± 0.98^{a}	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)[6]	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 ± 3.61^{c}	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 ± 0.48^{c}	12.16 ± 0.85^{b}	12.99 ± 0.40^{a}	12.66 ± 0.61^{ab}	9.54 ± 0.17^{d}

1 Values are mean ± SD (n=3). Mean values within columns with different superscript letters are significantly different (P < 0.05)

2 Erythrocyte or total red blood cell counts

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 effeects 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. mykiss*showedimproved 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. The insignificant 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;Menoyo*et 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-Paiva*et 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 IgM concentrations significantly differed (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.

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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*)

عبدالحمید آذری[۲و۱*]، روشادا هاشیم[۱]، قباد آذری تاکامی[۳] و ابوالقاسم روحی[۲]

۱- آزمایشگاه غذا و تغذیه آبزیان، گروه تغذیه آبزیان، دانشکده علوم دانشگاه یو اس ام مالزی، پیننگ، مالزی
۲- پژوهشکده اکولوژی دریای خزر، صندوق پستی ۹۶۱، بلوار خزر، ساری، ایران
۳- دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

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چکیده اثر مصرف مکمل غذائی (گروبیوتیک A) بر روی رشد، ضریب تبدیل غذا، فاکتورهای خونی و ایمنی در ماهی قزل‌آلای انگشت قد (۴۴/۴ ± ۰/۰۶ گرم) طی مدت ۸۴ روز و در ۷ تیمار و هر تیمار در ۳ تکرار بررسی شد. تیمارهای آزمایشی شامل درصدهای صفر، ۰/۵، ۱/۰، ۱/۵، ۲، ۲/۵ 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 ۲/۵٪ گروبیوتیک A به‌طور قابل ملاحظه از بالاترین افزایش وزن (WG)، نرخ رشد ویژه (SGR) و میانگین افزایش وزن روزانه (ADG) برخوردار شد (P<۰/۰۵). 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 ۲/۵٪ برخوردار شد (P<۵/۰۵). بالاترین بازماندگی در جیره محتوی۲و ۲/۵٪گروبیوتیک A بود (P<۰/۰۵). مقدارهموگلوبین، هماتوکریت٪، RBC، WBC، MCH، MCHC، MCV، لنفوسیت و نوتروفیل‌ها در ماهی‌های تغذیه شده از مکمل گروبیوتیک A مشاهده شد، اما تفاوت معنی‌داری در میان تیمارها مشاهده نشد(P<۰/۰۵). از سوی دیگر، لیزوزیم و ایمونوگلوبولین (IgM) در غلظت مختلف تفاوت معنی‌داری P) نشان داد و بالاترین در رژیم غذایی ۲/۵٪ همراه بود (P<۰/۰۵). نتایج حاصل از این مطالعه نشان داد که مکمل رژیم غذایی با گروبیوتیک A ۲/۵٪ دارای اثرمثبت در قزل‌آلای رنگین کمان انگشت قد بود.

کلمات کلیدی: *Oncorhynchusmykiss, Prebiotic GroBiotic®-A*، رشد، ضریب غذا، ماهی