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Effect of prebiotic (GroBiotic®-A) on the growth performance and intestinal microflora on rainbow trout (*Oncorhynchus mykiss* Walbaum)

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ABSTRACT:

The use of prebiotics as feed supplements that improve efficiency of intestinal bacteria is becoming de rigueur in animal husbandry in many regions worldwide. We tested the effects of a commercial prebiotic (GroBiotic®-A) including yeast and dairy fractions in different levels on survival, growth, carcass composition and intestinal microflora on rainbow trout (*Oncorhynchus mykiss* Walbaum). In the present study, we were able to detect high amounts of lactic acid bacteria (LAB) in the intestine after 12 weeks of prebiotic supplementation, conducted. Ultimately, when all supplementation diets are considered, GroBiotic®-A inclusion to diets for appearing on increase on growth, survival, and carcass composition on *O. mykiss*.

Keywords:
Rainbow trout, prebiotics, GroBiotic®-A and lactic acid bacteria (LAB).

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INTRODUCTION:

The culture of salmonids particularly Atlantic salmon, Salmo salar and rainbow trout (Oncorhynchus mykiss) is one of the most important examples of commercially successful intensive aquaculture in the world. Rainbow trout is a large economic fish in Iran and artificial breeding farms of this fish species are increasing apparently. It is also the second cultured fish species in Iran (FAO, 2006). The increasing economic and social concerns by decreasing the use of antibiotics and other chemicals used in fish farming have encouraged more environment friendly approaches for increasing growth (Verschuere *et al*., 2000). Prebiotic, unlike probiotic, is not an organism and has less influence in natural environment. These so-called prebiotic carbohydrates are defined as ''nondigestible food ingredient(s) that beneficially affect host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon'' (Gibson and Roberfroid 1995). Therefore, the need for alternative techniques is increasing, and the contribution of prebiotics such as xylooligosaccharides (XOS) may be considerable. XOS, fructooligosaccharides (FOS), inulin, and other related carbohydrates have received considerable attention because of the health benefits they are believed to confer on the host (Mussatto and Mancilha 2007; Cerezuela *et al*., 2008). However, it is evident that they play an important role in generating a colonic microflora that comprises predominantly bifidobacteria in young mammals (Houdijk *et al*., 1998; Erney et al. 2000; Costalos *et al*., 2008).

A 'functional nutrient' can be further defined as a dietary ingredient that employs possible positive effects on health in addition to its direct role as a nutrient. Therefore, the present study was conducted to explore growth performance, survival and microflora into intestine of juvenile rainbow trout, that feed the variety levels of dietary prebiotic, GroBiotic®-A which is a commercial mixture of partially autolyzed brewer's yeast, dairy ingredient components and dried fermentation products (with 5, 10, 15, 20, 25 and 30g kg^{-1} GroBiotic®-A).

MATERIAL AND METHODS:

Source and maintenance of fish

The experimental system was used here for an experiment to optimize the feeding regime of rainbow trout, *Oncorhynchus mykiss*. Apparently healthy *O. mykiss* with an average body weight of 4.5g were obtained from private farm at Haraz, Niyak Ghezel. The fish were acclimated for one week in four fiberglass tanks (4×4×1 m, 8 m^3) to fish house conditions before the start of the trial. Fish were kept in fiberglass tanks in dimension of 1.5×1.5×0.4 m, 1 m^3 with an open system of supply located in the north of Iran. Fishes randomly were divided into 7 groups which were placed in fiberglass tanks as described in above and in fresh water-flow at 9-12^oC for 84 days prior to use in experiments.

Experimental Procedure

The present work was performed at the Department of Aquaculture, Ecological Academy of Caspian Sea, Sari, Iran. The trial was performed for 12-weeks using fish in 3 replicates per treatment. Before the start of the feeding trial, three fish were randomly sacrificed with an overdose of clove oil, and triplicate pooled samples were taken for the determination of initial whole body composition. At the end of the trial, all fish were weighed and counted and three fishes from each fiberglass tank were collected for resolution of whole carcass composition.

Experimental diets

The fish were fed with a standard commercial food (Chine Co.) at a rate of 2-5 % of the biomass per day. Commercial dry food served as a basis in which the various concentrations of prebiotic, GroBiotic®-A were added, in 20 ml of sunflower oil, to commercial dry feed followed by mixing with a blender or by handy. Six experimental diets were formulated with increasing levels of GroBiotic®-A (1, 1.5, 2, 2.5 and 3% of basal diet) and non-supplemented diet (control): as diet 1, 2, 3, 4, 5, 6 and 7 containing 43.30 ± 0.07% and 40.56 ± 0.06% protein, 13.57 ± 0.15% and 15.19 ± 0.04% lipid and an estimated gross energy level of 20.31 ± 0.03 and 20.61 ± 0.04 kj.g-1 (FFT, Fingerling Feed Rainbow Trout and GFT1, Growth Feed Rainbow Trout), respectively.

Analytical methods

Chemical analyses

The food, experimental diets and fish carcass were analysed for proximate composition of dry matter, crude protein, crude lipid, fibre and ash using standard AOAC methods (1997). Briefly, dry matter was determined by drying at 100 ^0C to constant weight; crude protein was determined by the Kjeldahl procedure (Nitrogen×6.25); crude fat by chloroform methanol extraction (2:1, v/v); crude ash content by determining the residue after heating in a muffle furnace at 550 ^0C for 5 h and crude fibre

by loss on ignition of dried residue after successive digestion with 5% H2SO4. Nitrogen free extract (NFE) was calculated by subtracting the sum of crude protein, crude fat, ash, and crude fibre from the total dry matter content. Proximate analyses of the trial diets are described in Table 1.

Bacterial analysis

The microbial analyses were done twice; before the trial and at the end of the fourth week. Ten fish from each group were sacrificed by immersion in a tank containing MS-222 at a concentration of 150 mg L^{-1} of water for 15 min, and they were eviscerated aseptically and the whole intestine was removed. The intestine was dissected and the contents were collected by carefully scraping with a rubber spatula and weighed. The microbial analyses were performed by spreading appropriate dilutions in PBS from 10^1 to 10^6 on tryptic soy agar (TSA, Merck, Darmstadt, Germany), a general media for facultative aerobic bacteria. The plates were incubated aerobically at 30°C for 2 days. For determination of anaerobic bacteria was used from anaerobic jars with disposable Anaerocult A bags (Merck, Darmstadt, Germany) for the generation of an anaerobic medium. For lactic acid bacteria (LAB) determination, diluted samples were plated on deMan, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany) and incubated at 30°C for 2–3 days in an anaerobic jars with disposable Anaerocult C bags (Merck, Darmstadt, Germany) (Jones *et al.,* 2008).

Calculation and Statistical Analysis

The following formulae were applied to the data:

Feed intake (FI) = [total feed intake / number of fish (g)]

Specific growth rate (SGR %) = [((ln Wf – ln Wi) / T) × 100]

Feed efficiency (FE) = [total wet weight gain (g) / total feed intake (g)]

Protein Efficiency Ratio (PER) = [wet weight gain (g) / total protein intake]

Average Daily Growth (ADG = [((wf– wi) / wi) × (Tf-Ti)]

Survival rate (%) = [(Number of fish which survived / initial number of fish) × 100]

Where Wf refers to the mean final weight, Wi is the mean initial weight of fish, T is the feeding trial period in days.

All data are expressed as mean ±SD, where *n* represents the number of fish. The differences in parameters were tested for significance using a one-way analysis of variance (ANOVA) which was performed using SPSS.V11.5 Subsequent significance between groups was delineated by Duncan's test. A value of $P<0.05$ were taken for significance in all statistical tests.

RESULTS:

Growth, Survival and Feed Performance

No external clinical sign occurred in any treatment during the period of this experiment. The statistical analysis of different growth parameters of *Oncorhynchus mykiss* at the end of experimental period (Table 2) indicated a significant increase in body weight gain percent (WG %) between the six different groups and control diet ($P<0.05$). *O. mykiss* in group G-A5 kept on diet supplemented with (GroBiotic®-A2.5%) were the fast grower followed by the *O. mykiss* in the groups G-A4 and G-A6 received diet supplemented with (GroBiotic®-A 2 and 3% respectively) in comparison to other groups. The specific growth rate (SGR) takes almost the same pattern of WG% in which *O. mykiss* in the group G-A5 (2.5%) have the highest SGR followed by *O. mykiss* in groups G-A4 (2%) and G-A6 (3%) in comparison to other groups.

Average daily growth (ADG) of fish under G-A (2, 2.5 and 3%) feeding were significantly greater ($P<0.05$) than the basal diet and other groups.

Survival also was affected significantly ($P<0.05$) by dietary treatments as the fish fed control diet had lower survival 93.33 ± 2.31compared to an overall survival rate of 98.67 ± 2.31 among G-A5 and G-A6 fed the remaining diets (Table 2).

Apparent Protein Utilization

Protein utilization efficiency, measured in term of protein efficiency ratio (PER) and net protein utilization (NPU) is summarized in Table 3. These were the same for protein efficiency ratio (PER) in which the *O. mykiss* in the groups treated with prebiotic 2, 2.5 and 3% supplemented diets exceeded the value of other groups and even control. The feed efficiency (FE) of *O. mykiss* takes almost the same patterns of PER in which in the group G-A5 (2.5%) have the highest FE followed by *O. mykiss* in group G-A2 and G-A3% in comparison to other groups and basal diet. Apparent net protein utilization (NPU) the groups

Table 1 Proximate analyses of the diets used in varying levels -response experiments in rainbow trout (*Oncorhynchus mykiss*) during 84 days trial[1].

		Proximate composition						
Treatments	**Experiment Diets**	**Moisture (%)**	**Protein (%)**	**Lipid (%)**	**Fiber (%)**	**Ash (%)**	**NFE[9] (%)**	**GE[10] (kj.g^{-1})**
Control	FFT[1]	5.83 ± 0.14^{b}	43.30 ± 0.07^{a}	13.57 ± 0.15^{b}	6.57 ± 0.09^{b}	9.12 ± 0.03^{a}	27.44 ± 0.13^{b}	20.31 ± 0.03^{g}
	GFT1[2]	6.85 ± 0.15^{a}	40.56 ± 0.06^{b}	15.19 ± 0.04^{a}	6.59 ± 0.07^{a}	9.22 ± 0.02^{b}	28.44 ± 0.07^{a}	20.61 ± 0.04^{b}
G-A1[3]	FFT+G1	5.79 ± 0.12^{b}	43.19 ± 0.11^{a}	13.70 ± 0.15^{b}	6.52 ± 0.11^{b}	9.12 ± 0.17^{a}	27.47 ± 0.44^{b}	20.34 ± 0.06^{fg}
	GFT1+G1	6.91 ± 0.07^{a}	40.42 ± 0.10^{b}	15.15 ± 0.06^{a}	7.18 ± 0.06^{a}	8.58 ± 0.12^{b}	28.67 ± 0.13^{a}	20.44 ± 0.01^{de}
G-A2[4]	FFT+G2	5.89 ± 0.05^{b}	43.29 ± 0.21^{a}	13.60 ± 0.21^{b}	6.48 ± 0.15^{b}	9.14 ± 0.04^{b}	27.43 ± 0.22^{b}	20.38 ± 0.07^{efg}
	GFT1+G2	6.91 ± 0.03^{a}	40.35 ± 0.06^{b}	15.15 ± 0.03^{a}	7.22 ± 0.09^{a}	8.57 ± 0.18^{a}	28.70 ± 0.25^{a}	20.41 ± 0.02^{def}
G-A3[5]	FFT+G3	5.85 ± 0.11^{b}	43.18 ± 0.04^{a}	13.65 ± 0.30^{b}	6.49 ± 0.20^{b}	9.15 ± 0.06^{b}	27.53 ± 0.42^{b}	20.43 ± 0.06^{de}
	GFT1+G3	6.92 ± 0.06^{a}	40.58 ± 0.28^{b}	15.17 ± 0.11^{a}	7.31 ± 0.05^{a}	8.50 ± 0.17^{a}	28.44 ± 0.30^{a}	20.40 ± 0.01^{def}
G-A4[6]	FFT+G4	5.76 ± 0.11^{b}	43.19 ± 0.11^{a}	13.69 ± 0.12^{b}	6.51 ± 0.07^{b}	9.13 ± 0.03^{b}	27.47 ± 0.19^{b}	20.49 ± 0.10^{cd}
	GFT1+G4	6.83 ± 0.05^{a}	40.38 ± 0.06^{b}	15.18 ± 0.15^{a}	7.33 ± 0.10^{a}	8.51 ± 0.04^{a}	28.59 ± 0.09^{a}	20.76 ± 0.04^{a}
G-A5[7]	FFT+G5	5.92 ± 0.10^{b}	43.21 ± 0.20^{a}	13.68 ± 0.06^{b}	6.47 ± 0.17^{b}	9.20 ± 0.10^{b}	27.43 ± 0.10^{b}	20.67 ± 0.03^{b}
	GFT1+G5	6.80 ± 0.10^{a}	40.37 ± 0.05^{b}	15.19 ± 0.15^{a}	7.20 ± 0.11^{a}	8.51 ± 0.08^{a}	28.72 ± 0.26^{a}	20.49 ± 0.05^{cd}
G-A6[8]	FFT+G6	5.73 ± 0.16^{b}	43.30 ± 0.13^{a}	13.68 ± 0.03^{b}	6.56 ± 0.06^{b}	9.14 ± 0.09^{b}	27.31 ± 0.27^{b}	20.53 ± 0.03^{c}
	GFT1+G6	6.74 ± 0.01^{a}	40.36 ± 0.17^{b}	15.16 ± 0.05^{a}	7.24 ± 0.08^{a}	8.52 ± 0.09^{a}	28.73 ± 0.22^{a}	20.48 ± 0.03^{cd}

[1]Fingerling Feed Rainbow Trout (commercial Rainbow Trout food, Chinne)
[2] Growth Feed Rainbow Trout (commercial Rainbow Trout food, Chinne)
[3] GroBiotic®-A (A commercial prebiotic) 0.5% of diet
[4] GroBiotic®-A (A commercial prebiotic) 1% of diet
[5] GroBiotic®-A (A commercial prebiotic) 1.5% of diet
[6] GroBiotic®-A (A commercial prebiotic) 2% of diet
[7] GroBiotic®-A (A commercial prebiotic) 2.5% of diet
[8] GroBiotic®-A (A commercial prebiotic) 3% of diet
[9] Nitrogen free extract {100 – (protein + lipid + ash + fiber)}
[10] Gross energy content (Brafield 1985).

Table 2 Initial weight, final weight, percentage weight gain, specific growth rate, average daily growth and survival of rainbow trout(*Oncorhynchus mykiss*) fed diets containing varying Concentrations of GroBiotic®-A and control for 84 days[1].

	Treatments						
Parameters	**G-A 1[2]**	**G-A 2[3]**	**G-A 3[4]**	**G-A 4[5]**	**G-A 5[6]**	**G-A 6[7]**	**Control**
Wi (g)[8]	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)[9]	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 (%)[10]	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 (%)[11]	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 (%)[12]	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(%)[13]	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}

[1]Values are mean ± SD (n=3). Mean values within columns not sharing the same superscript are significantly different (P<0.05)
[2] GroBiotic®-A (A commercial prebiotic) 0.5% of diet
[3] GroBiotic®-A (A commercial prebiotic) 1% of diet
[4] GroBiotic®-A (A commercial prebiotic) 1.5% of diet
[5] GroBiotic®-A (A commercial prebiotic) 2% of diet
[6] GroBiotic®-A (A commercial prebiotic) 2.5% of diet
[7] GroBiotic®-A (A commercial prebiotic) 3% of diet
[8] Wi = Initial weight,
[9] Wf = Final weight
[10] Wg = {(Wf – Wi)/Wi} × 100
[11] SGR% = {(LnWf – LnWi) / Total days} × 100
[12] ADG (%) = {(Wf – Wi) / Total days} × 100
[13] Survival rate (%) = (Final fish number / Initial fish number) × 100

which receiving G-A feeding were vary significantly ($P < 0.05$) with these supplements level in the diets, similarly to PER. FI (feed intake) for G-A feeding tended to have better values than control diet with increasing supplements level and also feeding to G-A produced a better productive. The protein productive values (PPV) of the fingerlings fed with different experimental diets are presented in Table 3. Fish fed 25 g G-A kg^{-1} diet (G -A5) had maximum PPV and there was no improvement ($P>0.05$) in PPV beyond this level in comparison to basal diet.

Body Composition

In order to determine the nutritional effects of administered prebiotic on rainbow trout fry, the biochemical composition of carcass was analyzed. The results are presented in Table 4. At the end of the experiment, in comparison to initial values, all the experimental groups within control showed higher percentage of protein and ash but a lower percentage lipid and moisture. Protein values of carcass in all treatments were significantly higher ($P<0.05$) than controls. The best result was obtained from G-A5 (17.06 ± 0.06%). Significantly ($P<0.05$) different lipid values of carcass in prebiotic groups, compared to the controls, were indicated. GroBiotic®-A feeding resulted in a decrease in body lipid (fat) with increase in dietary supplements. Moisture values of G-A2, G-A3, G-A4, G-A5 and G-A6 indicated a significant ($P<0.05$) difference as well (Table 4). Fish fed the diet (commercial rainbow trout food, CHINE) under feeding received G-A 2, 2.5 and 3% showed significant ($P<0.05$) difference that had higher body ash than fish from the control and other treatments (Table 4).

Bacterial Analysis

In all treatments, lactic acid bacteria (LAB) successfully colonized the *O. mykiss* digestive tract on the end of trials (12-weeks). At the beginning of the study, we observed that all the fish were not significantly different on the aerobic, anaerobic and LAB media count ($P>0.05$; Table 5). Total bacterial counts among prebiotic supplemented groups were significantly different from total bacterial counts in controls in digestive tract of fish (Table 5; $P<0.05$). The mean of total bacterial counts of intestine with control diet in aerobic and anaerobic condition in TSA media were 4.46 ± 0.01 CFU/g and 4.80 ± 0.01 CFU/g, respectively increased exponentially from the experimental groups in intestine ($P<0.05$). On the other hand, LAB colonization was detected artificially dominant in experimental groups,

Table 3 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 diet for 84 days[1].

	Parameters				
Treatments	Feed intake	FE[8]	PER[9]	NPU (%)[10]	PPV[11]
G-A 1[2]	40.24 ± 1.78^{bc}	0.86 ± 0.03^{cd}	1.99 ± 0.06^{cd}	4.77 ± 2.61^{cd}	89.50 ± 6.14^{a}
G-A 2[3]	41.20 ± 0.23^{ab}	0.84 ± 0.04^{de}	1.97 ± 0.09^{d}	2.40 ± 0.01^{d}	86.21 ± 0.48^{a}
G-A 3[4]	41.77 ± 1.07^{ab}	0.81 ± 0.01^{e}	1.89 ± 0.03^{d}	7.46 ± 0.92^{bc}	88.46 ± 1.61^{a}
G-A 4[5]	41.55 ± 0.85^{ab}	0.90 ± 0.01^{b}	2.08 ± 0.02^{bc}	8.27 ± 1.28^{b}	89.88 ± 2.73^{a}
G-A 5[6]	42.99 ± 0.65^{a}	0.95 ± 0.02^{a}	2.19 ± 0.04^{a}	13.84 ± 1.29^{a}	91.44 ± 1.17^{a}
G-A 6[7]	41.36 ± 1.16^{ab}	0.94 ± 0.00^{ab}	2.16 ± 0.01^{ab}	9.97 ± 2.79^{b}	90.33 ± 2.73^{a}
Control	39.00 ± 0.86^{c}	0.83 ± 0.04^{de}	1.89 ± 0.08^{d}	2.24 ± 0.61^{d}	88.23 ± 1.69^{a}

[1] Values are mean ± SD (n=3). Mean values within columns not sharing the same superscript are significantly different (P<0.05)
[2] GroBiotic®-A (A commercial prebiotic) 0.5% of diet **[3] GroBiotic®-A (A commercial prebiotic) 1% of diet**
[4] GroBiotic®-A (A commercial prebiotic) 1.5% of diet **[5] GroBiotic®-A (A commercial prebiotic) 2% of diet**
[6] GroBiotic®-A (A commercial prebiotic) 2.5% of diet **[7] GroBiotic®-A (A commercial prebiotic) 3% of diet**
[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)
[11] Productive protein value = protein gain (g) × 100 / protein intake (g).

however, the opposite pattern was observed for the digestive tracts of *O. mykiss*, in which the mean of total LAB counts among prebiotics administered groups were more than control (P<0.05). The results of identification according to media on to aerobic, anaerobic and lactic acid bacteria condition, the highest count revealed the experiment that was under G-A5 (4.92 ± 0.01, 7.21 ± 0.02 and 6.60 ± 0.01 CFU/g, respectively; Table 5).

DISCUSSION:

Growth Performance and Protein utilization

Fish growth performance is affected by different factors including water quality, stresses and diseases, diet quality and quantity etc. Fish under intensive culture conditions will be badly affected and often fall target to different microbial pathogens that have been conducted with chemotherapeutic substances of which antibiotics were used. The use of biological products namely the probiotic either alone or in combination with prebiotics is recently the aim of the disease biocontrol program in aquaculture as they improve the fish health and alter the fish associated microbial population (Gibson and Roberfroid, 1995). While Gibson and Roberfroid (1995) defined, prebiotic term as "A nondigestible foods ingredient (s) that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health. Besides FAO experts "A prebiotic is a non-viable food component that presents a health benefit on the host associated with modulation of the microbiota" (FAO 2007). The data of prebiotic research is still young, yet the progress made in elucidating the useful health effects of specific prebiotics is significant not only for humans and animals, but for fish species also as well. In the present study, growth performance was increased about 23 % in fish fed prebiotic (GroBiotic®-A) diet compared to fish fed control diet; growth performance was significantly higher (P<0.05) exhibited in *O. mykiss* juvenile maintained on the G -A diet supplemented with (prebiotic diet). The specific growth rate (SGR) illustrated almost the same pattern of W.G% in which *O. mykiss* in the group that received G-A5 have the highest SGR followed by *O. mykiss* in group G-A4 and G-A6 content in comparison to control group, G-A1, G-A2 and G-A3. These were also true for protein efficiency ratio (PER), NPU (protein utilization) and survival in which the *O. mykiss* in groups treated with prebiotic supplemented diets was more than the amount of control group. In our study, survival of fish in the treatment which produced the highest growth performance was 5.7 % than the

lowest survival value. Since the feed efficiency (FE) of *O. mykiss* kept on a basal diet (control) was lower than the diets supplemented with prebiotics, while the highest FE was belonged to group which had G-A 2.5 % and a 14.5 % increased on feed efficiency, corroborating results of other previous studies. The PER results indicated that supplementing diets with prebiotics significantly improved protein utilization in rainbow trout. Similar results have been reported by Li *et al.,* (2005) who observed increased weight gain and feed efficiency which were generally observed in hybrid striped bass fed diets supplemented with partially autolyzed brewer's yeast and GroBiotic®-A at each sampling time (4, 8, 12 and 16 weeks). Staykov *et al.,* (2007) have conducted rainbow trout (*O. mykiss*) with supplementation of prebiotic Bio Mos® (0.2%) in standard commercial extruded feeds which were raised in net cages and raceways, at the end of the six weeks trial period, the mean body weight of fish receiving prebiotic was 13.7% and 9.97%, respectively higher, compared to the control groups ($P<0.01$). The supplementation of prebiotic in the diet significantly decreased FCR ($P<0.05$) and mortality ($P<0.01$). This result was in agreement with the results expressed by Bogut *et al.,* (2006) which demonstrated improvements in growth with the use of prebiotic MOS (Bio-Mos®) to the diet of other freshwater species, European catfish (*Silurus glanis)* juveniles from 22 to 76 g in the control groups and 83 g in the Bio-Mos® groups, a 9.7% higher average body weight ($P<0.01$). Here the FCR was also lower to 11.6% ($P<0.01$) and mortality decreased from 28.33 to 16.67% ($P<0.01$). These data supported the findings of Hanley *et al.,* (1995) who also have shown that hybrid red tilapia juveniles, fed 0.6% prebiotic (Aqua-Mos™, Alltech Inc., KY, USA) in their hatchery diets, that have had a 22.5% improved survival with 27.2% increase in weight gain. The results revealed that both groups received prebiotic-supplemented diets showed higher growth rate than those kept on a basal diet, suggesting that the addition of prebiotics enhanced the growth have performance and feed utilization.

In our study observed proximate analysis at the end of the experiment indicated significant ($P<0.05$) differences in muscle protein, lipid, ash and moisture contents of all the treatments. Although the diets included containing different levels of G-A, had increased effect on total muscle protein and ash (13.3% and 20.6%), respectively however decreased influence on muscle lipid and moisture (20.6% and 1.3%), respectively content of the fish. This study has found that supplementation with G-A may help to positively change body composition by increasing lean body mass (which includes muscle mass) and decreasing fat mass.

The fillets (MR%) of the groups of rainbow trout differed with regard to the received of G-A and non- G-A; the groups that fed G-A supplemented diets exhibited higher values than of basal diet ($P< 0.05$). Results demonstrated that prebiotic G-A can be an influence device for the enhancement of growth of performance, health status and feed efficiency of rainbow trout grown in

Table 4 Carcass proximate compositions of rainbow trout (*Oncorhynchus mykiss*) fed control and varying Concentrations of GroBiotic®-A and control diet for 84 days[1].

		Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
At the start		14.68 ± 0.13	9.40 ± 0.05	1.87 ± 0.03	75.10 ± 0.36
At the end	G-A 1[2]	15.50 ± 0.43^{d}	8.23 ± 0.06^{a}	1.22 ± 0.02^{b}	75.01 ± 0.02^{a}
	G-A 2[3]	15.10 ± 0.00^{e}	7.59 ± 0.45^{b}	1.21 ± 0.06^{b}	74.93 ± 0.05^{ab}
	G-A 3[4]	15.85 ± 0.16^{c}	7.44 ± 0.12^{b}	1.23 ± 0.23^{b}	74.89 ± 0.63^{ab}
	G-A 4[5]	16.17 ± 0.21^{bc}	7.44 ± 0.03^{b}	1.38 ± 0.11^{ab}	74.49 ± 0.10^{bc}
	G-A 5[6]	17.06 ± 0.06^{a}	7.05 ± 0.04^{c}	1.46 ± 0.05^{a}	74.24 ± 0.09^{c}
	G-A 6[7]	16.23 ± 0.06^{b}	7.09 ± 0.05^{c}	1.44 ± 0.05^{a}	74.51 ± 0.18^{bc}
	Control	15.06 ± 0.11^{e}	8.02 ± 0.07^{a}	1.21 ± 0.02^{b}	75.16 ± 0.13^{a}

[1] Values are mean ± SD (n=3). Mean values within columns not sharing the same superscript are significantly different (P<0.05)

[2] GroBiotic®-A (A commercial prebiotic) 0.5% of diet
[3] GroBiotic®-A (A commercial prebiotic) 1% of diet
[4] GroBiotic®-A (A commercial prebiotic) 1.5% of diet
[5] GroBiotic®-A (A commercial prebiotic) 2% of diet
[6] GroBiotic®-A (A commercial prebiotic) 2.5% of diet
[7] GroBiotic®-A (A commercial prebiotic) 3% of diet

Table 5 Total bacterial levels (log CFU/mg intestinal contents) in TSA medium (Aerobic, Anaerobic) and LAB (Lactic Acid Bacteria) condition in the juvenile *O.mykiss* fed with test diets for 12 weeks[1].

	Initial			Final		
Source	Aerobic (TSA)	Anaerobic (TSA)	LAB	Aerobic (TSA)	Anaerobic (TSA)	LAB
G-A1[2]	4.49 ± 0.02	4.80 ± 0.01	0.00 ± 0.00	4.49 ± 0.02^{e}	4.81 ± 0.01^{e}	3.63 ± 0.01^{f}
G-A2[3]	4.49 ± 0.02	4.80 ± 0.01	0.00 ± 0.00	4.65 ± 0.02^{d}	5.22 ± 0.01^{d}	3.90 ± 0.03^{e}
G-A3[4]	4.48 ± 0.02	4.80 ± 0.01	0.00 ± 0.00	4.49 ± 0.02^{c}	6.82 ± 0.01^{c}	6.06 ± 0.05^{d}
G-A4[5]	4.48 ± 0.03	4.81 ± 0.01	0.00 ± 0.00	4.86 ± 0.02^{b}	7.20 ± 0.02^{a}	6.46 ± 0.01^{c}
G-A5[6]	4.46 ± 0.02	4.80 ± 0.01	0.00 ± 0.00	4.92 ± 0.01^{a}	7.21 ± 0.02^{a}	6.60 ± 0.01^{a}
G-A6[7]	4.47 ± 0.01	4.81 ± 0.02	0.00 ± 0.00	4.87 ± 0.01^{b}	7.15 ± 0.01^{b}	6.55 ± 0.03^{b}
Control	4.46 ± 0.02	4.80 ± 0.00	0.00 ± 0.00	4.46 ± 0.01^{e}	4.80 ± 0.01^{e}	0.00 ± 0.00^{j}

[1] Values are mean ± SD (n=3). Mean values within columns not sharing the same superscript are significantly different (P<0.05)

[2] GroBiotic®-A (A commercial prebiotic) 0.5% of diet
[3] GroBiotic®-A (A commercial prebiotic) 1% of diet
[4] GroBiotic®-A (A commercial prebiotic) 1.5% of diet
[5] GroBiotic®-A (A commercial prebiotic) 2% of diet
[6] GroBiotic®-A (A commercial prebiotic) 2.5% of diet
[7] GroBiotic®-A (A commercial prebiotic) 3% of diet.

a variety of production systems. On other hand, the beneficial influence of G-A supplemented on growth may be due to a change of the intestinal microflora by mannanoligofructose, lactose or other carbohydrates from the dairy ingredient, partially autolyzed yeast and/or dried fermentation products. In the present study, it is given the evidence of improvement in the health and growth performance of fish in spite of the differences in methods and species used.

Intestinal microflora

At the end of trial, it was noticed that the prebiotic supplementation into diet (extruded pellet food) in these studies resulted significant (P<0.05) increases in total bacterial counts among prebiotic supplemented groups, which were significantly different in compared to total bacterial counts in controls at the digestive tract of *O. mykiss* (P<0.05). In the present study, we were able to detect high amounts of lactic acid bacteria (LAB) in the intestine after 12 weeks of prebiotic supplementation. However the values noted on different media such as: (Aerobic; 4.92 vs. 4.46 CFU/g), (Anaerobic; 7.21 vs. 4.80 CFU/g) and (LAB; 6.60 vs. 0 CFU/g) G-A group treatments and basal which these present data the beneficial influence of prebiotic on growth was perhaps due to an alteration of the intestinal microflora. After 12 weeks of G-A-feeding in the present study, we were able to detect high amounts of LAB and total bacterial counts on aerobic and anaerobic media in the intestine, which demonstrates that G-A supplemented diets and basal group, isolated from the inner digestive tract bacteria of rainbow trout, have a big capacity to adhere to and survive in the intestinal tract. LAB supplementation, however, demonstrated an ability to challenge the resident microbiota, since the increase in LAB observed was possibly the result of a fall in intestinal pH motive by lactic acid or other fermented products produced by LAB strains. The previous studies (Ringø *et al.,* 1998; Ringø and Olsen 1999) indicated that dietary fatty acids and carbohydrates changed the bacterial flora of the gastrointestinal tract of fish. Similar findings were pointed out by Li and Gatlin (2004, 2005), who investigated the effect of commercial prebiotics GrobioticTM AE and GrobioticTM supplemented in diets of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) growth and exhibited that the prebiotic promoted the growth performance. The results of this present study clearly indicated that diet supplemented with G-A at a suitable concentration (20, 25 and 30 g kg^{-1}) could improve the total bacterial counts and LAB of all intestine *O. mykiss* (Table 5). Lactic acid bacteria had been considered beneficial residents of the fish intestinal function because of producing bacteriocins and hence positively affecting the host's microflora (Ringø *et al.,* 1998; Irianto and Austin 2002; Ringø *et al.,* 2006). Prebiotic oligosaccharides such as inulin and oligofructose are fermented in the colon where they promote the growth of bacterial populations associated with a healthy, well functioning colon. This selective

stimulation occurs because oligosaccharides are readily fermented by beneficial types of colonic bacteria and are not used effectively by potentially pathogenic bacteria species (Flickinger *et al.,* 2003). Our results have demonstrated that G-A, as well as other prebiotic ingredients, may promote the maintenance of lactic acid-production as some reports have also supported the point (Mussatto and Mancilha 2007; Moura *et al.,* 2007). Based on our data, the beneficial influence of prebiotic on growth was possible due to an alteration of the intestinal microflora.

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REFERENCE:

AOAC. 1997. Official Methods of Analysis of AOAC International, 16th edn, . Association of Official Analytical Chemists, Arlington, VA, USA. 1(4):1–3

Bogut I, Milakovic Z, Pavlicevic J, Petrovic D. 2006. Effect of Bio-Mos® on performance and health of European catfish (*Silirus glanis*). In: Nutritional Biotechnology in the Feed and Food Industries: Proceedings of Alltech's 22nd Annual Symposium (Suppl., Abstracts of Posters presented), April 23-26, Lexington, KY, USA. 90.

Brafield AE. 1985. Laboratories studies on energy budgets. In: Fish Energetics. New Perspective (Tytler, P. & Calow, P. eds), Croom Helm, London and sydney. 257-281.

Cerezuela R, Cuesta A, Meseguer J, Esteban M. A. 2008. Effects of inulin on gilthead seabream (*Sparus aurata L.*) innate immune parameters. Fish Shellfish Immunol., 24:663-668.

Costalos C, Kapiki A, Apostolou M, Papathoma E. 2008. The effect of a prebiotic supplemented formula on growth and stool microbiology of term infants. Early Hum Dev., 84:45-49.

Erney RM, Malone WT, Skelding MB, Marcon AA, Kleman-Leyer KM. 2000. Variability of human milk neutral oligosaccharides in a diverse population. J. Pediatr, Gastroenterol Nutr., 30:131-133.

FAO. 2006. The state of world fisheries and aquaculture 2004. Rome, Italy. pp 14-17.

FAO. 2007. Technical Meeting on Prebiotics. September 15-16, FAO, Rome, Italy. 11.

Flickinger EA, Van Loo J, Fahey GC. 2003. Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals, a review. Crit. Rev. Food Sci. Nutr., 43:19-60.

Gibson GR and Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota introducing the concept of prebiotics. J, nutr., 125: 1401-12.

Hanley F, Brown H and Carberry J. 1995. First observations on the effects of mannan oligosaccharide added to the hatchery diets for warmwater Hybrid Red Tilapia. In: Nutritional Biotechnology in the Feed and Food Industries: Proceedings of Alltech's 11th Annual Symposium (Suppl. 1, Abstracts of Posters presented), May 20-23, Lexington, KY, USA. 11.

Houdijk JGM, Bosch MW, Verstegen MWA. and Berenpas HJ. 1998. Effects of dietary oligosaccharides on the growth performance and faecal characteristics of young growing pigs. Anim, Feed Sci, Technol., 71, 5-48.

Irianto A and Austin B. 2002. Probiotics in aquaculture. J. Fish Dis **25**, 633–642.

Jones R, Hussein HM and Zagorec M. 2008. Food Microbiology 25:228-234.

Li P and Gatlin DM. 2004. Dietary brewer's yeast and the prebiotic Grobiotic® AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* ×*M. saxatilis*) to *Streptococcus iniae* infection. Aquaculture 231:445-456.

Li P, Gatlin DM. 2005. Evaluation of the prebiotic Grobiotic® AE and brewer's yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops* × *M. saxatilis*) challenged in situ

with *Mycobacterium marinum*. Aquaculture 248: 197-205.

Moura P, Marques S, Alves L, Freire JPB, Cunha LF, Esteves MP. 2007. Effect of xylo oligosaccharides from corn cobs autohydrolysis on the intestinal microbiota of piglets after weaning. Livest. Sci., 108:244-248.

Mussatto SI, Mancilha IM. 2007. Non-digestible oligosaccharides: a review. Carbohydr Polym., 68:587-597.

Ringø E and Gatesoupe FJ. 1998. Lactic acid bacteria in fish: a review. Aquaculture 160:177-203.

Ringø E and Olsen RE. 1999. The effect of diet on aerobic bacterial flora associated with intestine of Arctic charr, *Salvelinus alpinus* L. J. Appl. Microbiol., 86:22-28.

Ringø E, Sperstad S, Myklebust R, Refstie S and Krogdahl A. 2006. Characterisation of the microbiota associated with intestine of Atlantic cod (*Gadus morhua L.*). Aquaculture 261:829-841.

Staykov Y, Spring P, Denev SA and Sweetman J. 2007. Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*), Aquacul. Int., 2:153-161.

Verschuere L, Rombaut G, Sorgeloos P and Verstraete W. 2000. Probiotic bacteria as biological control agents in aquaculture, Microbiol, Mol, Biol, Rev, 64:655-671.