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# The Effects of Commercial Probiotic and Prebiotic Usage on Growth Performance, Body Composition and Digestive Enzyme Activities in Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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**Abstract:** Two commercial Aqualase probiotic and GroBiotic®-A prebiotic were carried out on rainbow trout fry during 12 weeks of feeding trial. Probiotic and prebiotic were introduced in four different levels of diets (G-A1: 1, G-A2: 2, AQ1: 0.1, AQ2: 0.2 percents, respectively) and their effects compared with those of control diet containing no pre-probiotic. Survival in treatments was significantly ($P<0.05$) higher than control and a slight increment of mortality rate was observed during the first week of experiment. The counts of bacteria associated with trout intestine in all treatments were significantly ($P<0.05$) higher than controls and Lactic acid Bacillus (LAB) was not detected in controls. Total bacteria counts were significantly different among treatments and controls. It could be suggested that the colonization rate of digestive tracts of rainbow trout fry with bacteria was affected by dietary bacteria level. Specific growth rate, condition factor, protein efficiency ratio were significantly ($P<0.05$) higher and feed conversion ratio was no significantly ($P>0.05$) in groups received probiotic and prebiotic via diets than controls. It may show that probiotic and prebiotic stimulates digestive development and enzymatic activity in fish. The best results were shown in treatment of growth performance with G-A2 percents.

**Key words:** Probiotic · Prebiotic · GroBiotic®-A · Aqualase · Digestive enzyme activity

## INTRODUCTION

Rainbow trout culture is very important as economic point of view in Iran. Also, bacterial infectious disease in trout farming seems to be the major cause of decline in production level of the most farms. The main success and failure of fish culture programs are determined by early life stage conditions [1, 2]. The preparation of diets not only provide the essential nutrients that are required for normal physiological function but may also serve as the medium by which fish receive other components that could be affected their health [3, 4]. Although, vaccines are being developed and marketed, they can not be used as a universal disease control measure in aquaculture. During the last decades, an antibiotic which was previously used as a conventional strategy for fish disease management has also been used for the improvement of growth and efficiency of feed conversion. However, over the years, pathogens which are resistant to antimicrobial agents developed and well documented [5-9]. Currently probiotics and prebiotics have been used to improve the health of their host and increment of growth rate. A probiotic is defined as "a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract" [10]. Nowadays, probiotics have also been widely and incorrectly applied

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in aquaculture to include the use of live microbes to beneficially alter the microbial balance in the culture system itself. On the other hand, Prebiotics have been described as "non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria" and the usage of probiotic and prebiotic in aquaculture were positive [11]. But lacking evaluation of biological influence of bacteria in natural environment and cost of the material are the restrictions of probiotics and prebiotics at this time. The prebiotics are natural increments and makes their preferable combination, so the main advantage of prebiotics over probiotics are the natural feed ingredients. Their incorporation in the diet does not require particular safeguard and their usage as feed additives may be more easily achieved, in spite of some concerns about their safety and efficiency. A scientific report [12] shown that the prebiotics were chosen to stimulate bifidobacteria and lactobacilli in human microbiota. A study [13] shown that the some of injections of a ß(1,3) and ß(1,6) linked glucan from cell walls of the yeast *Saccharomyces cerevisiae* into Atlantic salmon resulted in increased resistance to several bacterial pathogens and decreased fish mortality. A numbers of reports [14, 15] shown that the haematological parameters and biochemical compounds in fish can be used to develop the health situation of organism. Further, blood parameters in fish are considered as mirror for any changes occurred on organism due to injuries in organs, which related to infectious detect diseases similar to warm-blooded animals [16].

Therefore, the present study was designed to investigate the effect of dietary GroBiotic®-A as prebiotic and Aqualase as probiotic levels on growth performance, body composition and digestive enzyme activities of juvenile rainbow trout.

## MATERIALS AND METHODS

**Experiment Diets and Fish Rearing:** Rainbow trout, *Oncorhynchus mykiss* (Walbaum) (14 weeks old, 11.30 ±0.21g) were obtained from a well-known fish farm (Haraz Fish Company) in north of Iran. Totally 300 pieces of fish were randomly assigned into 5groups and each groups were maintained in 3 rounded concrete tanks (with 3m diameter, 0.8 m depth and 3.5$m^3$ capacity) and a flow-through with continuous freshwater supplied from river (mean temperature 15.20 ± 3.6 S.D). During 84 days. Five experimental diets were formulated with increment levels of GroBiotic®-A (1 and 2%), Aqualase (0.1 and 0.2%), control and designated as Diets 1, 2, 3, 4 and 5. In these experiments, two types of basal diets such as FFT (Fingerling Feed Rainbow Trout, commercial Rainbow Trout food, Chine) and GFT1 (Grow out Feed Rainbow Trout, commercial Rainbow Trout food, Chine) were used which containing 43.53 ± 0.23% and 41.06 ± 0.06% protein, 13.57 ± 0.15% and 15.66 ± 0.10% lipid and an estimated gross energy level of 20.31 ± 0.03 and 20.61 ± 0.04 kj.g-1, respectively. The proximate analyses of experimental diets are shown in Table 1 [17].The proper amounts of probiotic and prebiotic (mixed with sunflower oil, 1:1) were sprayed into the feed slowly. Then, the feed was air dried under sterile conditions for 2 h and stored at 20°C. The treatments were, T-1 (G-A1), T-2 (G-A2); T-3 (AQ1), T-4 (AQ2) and T-5 (C) where G-A, AQ and C refers respectively to the GroBiotic®-A, Aqualase and control feeding regime. The numerical values 1, 2, 3, 4 and 5 refers to the number of diet contained 1, 2, 0.1, 0.2 and 0% supplements, respectively [18,19]. The sampling for nutritional effects wascarried out once in 2- weeks.

**Sample Collection and Analysis:** Fish in each tank were counted and weighed at the beginning and the end of feeding trial. For determination of initial and final carcasses proximate composition, 3 fish were randomly selected in each tank before start and at the end of feeding trial. The proximate composition of the experimental diets and fish carcass were analysed for proximate composition of dry matter, crude protein, crude lipid, fibre and ash using standard [17]. Briefly, dry matter was determined by drying at 100°C 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°C 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.

**Preparation of Gastrointestinal Gland for Digestive Enzyme Assays:** After 15 h of last feeding, 9 fish were collected from each group (3 fish in a tank). When all bodies were weighted, individual rainbow trout was anaesthetized in ice water for approximately 5 minutes. To minimize the risk of contaminating samples with any microbial enzymes, which may have been present in

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

| Experimental Diets | Proximate composition | | | | | | |
|---|---|---|---|---|---|---|---|
| | Moisture (%) | Protein (%) | Lipid (%) | Fiber (%) | Ash (%) | NFE[8] (%)[8] | GE[9] (kj.g$^{-1}$,[9] |
| FFT[2] (Control) | 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] (Control) | 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 |
| FFT+G-A1[4] | 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+G-A1 | 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 |
| FFT+G-A2[5] | 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+G-A2 | 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 |
| FFT+AQ1[6] | 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+AQ1 | 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 |
| FFT+AQ2[7] | 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+AQ2 | 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 |

[1] Values are mean ± SD (n=3).

[2] Fingerling Feed Rainbow Trout (commercial Rainbow Trout food, Chine)

[3] Grow out Feed Rainbow Trout (commercial Rainbow Trout food, Chine)

[4] GroBiotic®-A 1% of diet

[5] GroBiotic®-A 2% of diet

[6] Aqualase 0.1% of diet

[7] Aqualase 0.2% of diet

[8] Nitrogen free extract (100 - (protein + lipid + ash + fiber))

[9] Gross energy content [20].

the gut, the gastrointestinal gland was dissected out, stomach and pyloric caeca and intestinal were separated of each other. The samples separately cut into small pieces and washed thoroughly three times in homogenate buffer solution (10 mM sodium citrate/0.1 M NaCl; pH 7.0). After washing, gastrointestinal gland tissue was weighed, mixed with equal amounts of homogenate buffer solution then homogenized in an Ultraturrax homogenizer for 1 min at the full speed. Finally, the homogenate was centrifuged in a Microphage set at 10, 000 × g for 10 minutes at room temperature, after which the supernatant was stored at -20$^{oC}$ until required [21]. Total soluble protein was measured by Lowry method [22] using bovine serum albumin as a standard.

**Amylase Assay:** Amylase activity was assayed by a method [23] briefly, 50µl of properly diluted enzyme (10µl supernatant and 90µl Tris buffer with pH 7.5) were added into a tube containing 250 µl of 1% (w/v) of potato starch solution and 1 ml of 0.05 M acetate buffer, pH 5.0. The reaction mixture was incubated at 37$^{OC}$ for 15 minutes. Then 300 µl of the mixture was transferred into a new tube containing 300 µl of 3, 5-dinitrosalicylic acid (DNS) and boiled (90°C) for 15 minutes. The color density was determined spectrophotometrically at 520 nm. One unit was defined as 1 µmol of glucose released per minute by 1 ml of enzyme.

**Protease Assay:** Protease activity was determined by a method [24] briefly add 1 ml of 1.5% casein solution, pH 7.0 was placed at 37°C and then 1 ml of properly diluted enzyme sample (100 µl supernatant, 900 µl Tris buffer with pH 7.8 and 11mM $CaCl_2$). The reaction was incubated for 10 minutes prior to the addition of 2 ml of 0.4 M trichloroacetic acid. The solution with precipitates was filtered and to 0.5 ml of the clear filtrate 2.5 ml of 0.4 $MNa_2CO_3$ and 0.5 ml of Folin reagent were added. After further 10 minutes of incubation, the color density developed was determined at 660 nm. One unit was defined as 1 µmol of tyrosine released per minute by 1 ml of enzyme.

**Lipase Assay:** Lipase catalyzes the hydrolysis of ester bonds on the glycerol backbone of a lipid substrate. Lipolytic activity was determined by the colorimetric method based on the activity in cleavage of p-nitrophenyl palmitate (p-NPP) at pH 8.0 [25]. The reaction mixture contained 180 µl of solution A (0.062 g of p-NPP in 10 ml of 2-propanol, sonicated for two minutes before use), 1620 µl of solution B (0.4% triton X-100 and 0.1% gum Arabic in 50 mM Tris-HCl, pH 8.0) and 200 µl of properly diluted enzyme sample (100 µl supernatant and 100 µl buffer Tris buffer-HCL). The product was detected at 405 nm wavelength after incubation for 15 minutes at 37°C. Under this condition, the molar extinction coefficient (ϵ405) of

p-nitrophenol (p-NP) released from p-NPP was 18 000 $M^{-1}$. One unit of lipase activity was defined as 1 µmol of p-nitrophenol (p-NP) released per minute by 1 ml of enzyme.

**Body Indices:** At the end of the feeding trial, three fish from each tank (N=9 treatment$^{-1}$) were euthanized by an overdose of clove oil (3mg/l Sigma-Aldrich, St. Louis, MO, USA) and bled via caudal venipuncture for measurement of hematological parameters . Fish were measured for length, weight, weight gain and feed efficiency ratio. Survival, visceral somatic index (VSI), hepatosomatic index (HSI) and muscle ratio (MR) were calculated. Muscle and liver samples also were collected for proximate analysis, including crude protein, total lipid, dry matter and ash method [17].

HSI = [(liver weight / body weight) x 100]
VSI = [(viscera weight / body weight) x 100]
IPF = [(intraperitoneal fat weight / body weight) x 100]

**Calculation and Statistical Analysis:** From the data, the following parameters were calculated:

- Specific growth rate (SGR) = [100 × ((lnwf - lnwi) / t)]
- Condition factor (CF) = [(wf /$L^3$) × 100]
- Average Daily Growth (ADG) = [((wf- wi) / wi) × (Tf-Ti)]
- Total feed intake per fish (FI) = [total feed intake / number of fish]
- Protein intake (PI) = [feed intake (g) × % protein in the diet]
- Protein efficiency ratio (PER) = [(wt - wi) × (F × P)]
- Food efficiency (FE) = [weight gain / food intake]
- Survival rate (SR) = [(final number of fish×100) / initial number of fish]
- Where; wi = initial weight, wf= final average weight, t = number of days of feeding trial,
- F= the total amount of feed (g) per fish consumed during the feeding trial
- P= the protein content of the diets.

Therefore, data of growth, haematological parameters, immunology concentration, bacterial and special enzymes activity of rainbow trout (*O. mykiss*) analyzed by using analysis of variance (ANOVA) techniques and the mean differences between treatments were tested for significant differences (P<0.05) using a Duncan's multiple range test. All statistical analyses were performed using the SPSS software package, version 11.5.

## RESULTS

**Growth and Nutrient Utilization Parameters:** No external clinical symptoms occurred in any groups during experimental times. Growth performance and feed utilization parameters of *Oncorhynchus mykiss* during the experimental period are summarized in Table 2. Growth performance such as final weight, weight gain were significantly higher in G-A 2 (%) (P<0.05) compared to Aqualase and the control, while there was not significantly difference with G-A 1(%). There was also significant difference between Aqualase and the control (P<0.05). The highest significant weight gain percent was observed in fish fed G-A 2% (P<0.05). Fish fed GroBiotic®-A tended to have higher weight gains (P<0.05) than Aqualase supplement levels used.

A similar response was also observed in the case of specific growth rate (SGR) where GroBiotic®-A 2% showed significantly were higher than control (Table 2). Average daily growth (ADG) of fish were significantly higher in GroBiotic®-A 2% (P<0.05) than the Aqualase and basal diet, while was similar to G-A 1% (Table 2).

Survival increased significantly (P<0.05) when dietary supplements increased and the maximum survival was observed in fish fed with G-A 2% diet, which was significantly higher (P<0.05) than Aqualase 0.2% and the control.

Protein utilization efficiency, calculated in terms of protein efficiency ratio (PER) and net protein utilization (NPU) is as summarized in Table 3. PER for GroBiotic®-A 2% feedings showed significant differences (P<0.05) with control, while it was similar to other supplements diet. Apparent net protein utilization (NPU) for AQ 0.2% and G-A fed fish were significantly (P<0.05) different with the control. As also shown in Table 4.4, feed efficiency (FE) in the AQ and G-A fed fish were not influenced significantly (P>0.05) by varying supplements percentage in the diet. FI (feed intake) in G-A 2% feeding tended to be better than AQ and the control, although AQ showed significantly higher than the control (P<0.05).

**Body Composition:** Body composition data for final samples of fish from different treatments and the initial fish were compared and are as presented in Table 4. At the end of experiment, in comparison to initial fish, all fish from the experimental groups and the control exhibited higher percentage of body protein, lipid and moisture but lower percentage of ash. Fish fed the dietary supplements GroBiotic®-A 2% showed with significantly (P<0.05)

Table 2: Initial weight, final weight, percentage weight gain, specific growth rate and survival of rainbow trout (*Oncorhynchus mykiss*) fed diets containing varying level of AQ and G-A and control for 84 days[1]

| | Treatments | | | | |
|---|---|---|---|---|---|
| Parameters | G-A 1(%)[2] | G-A 2(%)[3] | AQ 0.1(%)[4] | AQ 0.2(%)[5] | Control diet |
| Wi (g)[6] | 11.50 ± 0.25 | 11.20 ± 0.16 | 11.20 ± 0.32 | 11.32 ± 0.29 | 11.06 ± 0.19 |
| Wf (g)[6] | 77.71 ± 3.96$^{ab}$ | 82.49 ± 2.33$^{a}$ | 73.96 ± 4.05$^{b}$ | 73.62 ± 2.8$^{b}$ | 66.77 ± 1.16$^{c}$ |
| WG (%)[7] | 576.16 ± 41.05$^{b}$ | 636.78 ± 29.40$^{a}$ | 552.31 ± 33.05$^{b}$ | 555.58 ± 24.23$^{b}$ | 493.32 ± 13.29$^{c}$ |
| SGR (%)[8] | 2.27 ± 0.07$^{ab}$ | 2.38 ± 0.05$^{a}$ | 2.23 ± 0.06$^{ab}$ | 2.24 ± 0.04$^{ab}$ | 2.12 ± 0.03$^{b}$ |
| ADG (%)[9] | 78.82 ± 4.82$^{ab}$ | 84.87 ± 2.93$^{a}$ | 74.55 ± 4.76$^{b}$ | 74.27 ± 3.18$^{b}$ | 66.09 ± 1.45$^{c}$ |
| Survival (%)[10] | 94.89 ± 1.02$^{ab}$ | 96.44 ± 1.26$^{a}$ | 94.22 ± 2.22$^{ab}$ | 92.78 ± 1.50$^{b}$ | 89.44 ± 1.35$^{c}$ |

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

[2]GroBiotic®-A 1% of diet

[3] GroBiotic®-A 2% of diet

[4] Aqualase 0.1% of diet

[5] Aqualase 0.2% of diet

[6]Wi = Initial weight, Wf = Final weight

[7] WG = [((Wf - Wi) / Wi) × 100]

[8] SGR% = [(LnWf - LnWi) / Total days) × 100]

[9]ADG (%) = [((Wf -Wi) / Total days) × 100]

[10] Survival rate (%) = [(Final fish number / Initial fish number) ×100]

Table 3: Feed intake, feed efficiency, protein efficiency ratio, Net protein utilization and productive protein value of rainbow trout (*Oncorhynchus mykiss*) fed diets containing varying levels of AQ and G-A and control diet for 84 days[1].

| | Treatments | | | | |
|---|---|---|---|---|---|
| Parameters | G-A 1(%)[2] | G-A 2(%)[3] | AQ 0.1(%)[4] | AQ 0.2(%)[5] | Control diet |
| Feed intake | 100.22 ± 5.27$^{ab}$ | 105.04 ± 2.00$^{a}$ | 96.19 ± 1.97$^{b}$ | 95.13 ± 2.65$^{b}$ | 87.16 ± 1.80$^{c}$ |
| FE[6] | 0.66 ± 0.03 | 0.68 ± 0.01 | 0.65 ± 0.03 | 0.66 ± 0.03 | 0.64 ± 0.02 |
| PER[7] | 1.53 ± 0.06$^{ab}$ | 1.60 ± 0.03$^{a}$ | 1.51 ± 0.08$^{ab}$ | 1.55 ± 0.08$^{ab}$ | 1.48 ± 0.04$^{b}$ |
| NPU (%)[8] | 6.55 ± 0.22$^{a}$ | 7.54 ± 0.36$^{a}$ | 4.69 ± 1.18$^{b}$ | 6.51 ± 1.13$^{a}$ | 6.16 ± 1.17$^{ab}$ |
| PPV[9] | 40.11 ± 1.93$^{b}$ | 40.11 ± 0.73$^{b}$ | 40.36 ± 1.05$^{ab}$ | 43.13 ± 2.53$^{ab}$ | 43.63 ± 1.88$^{a}$ |

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

[2]GroBiotic®-A 1% of diet

[3] GroBiotic®-A 2% of diet

[4] Aqualase 0.1% of diet

[5] Aqualase 0.2% of diet

[6] Feed efficiency = [weight gain (g) / food intake (g)]

[7] Protein efficiency ratio = [weight gain (g) / protein intake (g)]

[8] Net Protein Utilization = [(Wf × Protein Muscle Final) - (Wi × (Protein Muscle Initial / Protein Consumed))]

[9] Productive protein value = [(protein gain (g) × 100) / protein intake (g)]

Table 4: Carcass proximate compositions of rainbow trout (*Oncorhynchus mykiss*) fed control and varying level AQ and G-A and control diet for 84 days[1].

| | | At the end | | | | |
|---|---|---|---|---|---|---|
| | At the start | G-A(1%)[2] | G-A (2%)[3] | AQ (0.1%)[4] | AQ (0.2%)[5] | Control |
| Protein (%) | 14.52 ± 0.13 | 17.38 ± 0.07$^{b}$ | 17.91 ± 0.17$^{a}$ | 16.76 ± 0.15$^{b}$ | 17.31 ± 0.06$^{b}$ | 16.34 ± 0.27$^{c}$ |
| Lipid (%) | 6.23 ± 0.10 | 6.66 ± 0.29$^{b}$ | 6.26 ± 0.04$^{c}$ | 6.87 ± 0.12$^{b}$ | 6.75 ± 0.19$^{b}$ | 7.31 ± 0.16$^{a}$ |
| Ash (%) | 1.95 ± 0.02 | 1.19 ± 0.05$^{b}$ | 1.07 ± 0.04$^{b}$ | 1.33 ± 0.16$^{b}$ | 1.10 ± 0.08$^{b}$ | 1.60 ± 0.26$^{a}$ |
| Moisture (%) | 74.00 ± 0.50 | 74.20 ± 0.61 | 74.05 ± 0.47 | 74.57 ± 0.22 | 74.25 ± 0.26 | 74.01 ± 0.16 |

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

[2]GroBiotic®-A 1% of diet

[3] GroBiotic®-A 2% of diet

[4] Aqualase 0.1% of diet

[5] Aqualase 0.2% of diet

Table 5: Digestive specific enzyme activity in Intestine (I), Stomach and Pyloric ceaca (S&Pc) of rainbow trout (*Oncorhynchus mykiss*) fed diets containing varying levels of AQ and G-A and control diet[1].

| | | Treatments | | | | |
|---|---|---|---|---|---|---|
| Enzyme | Organ | G-A 1(%)[2] | G-A 2(%)[3] | AQ 0.1(%)[4] | AQ 0.2(%)[5] | Control diet |
| Amylase[6] | I | 1.51 ± 0.38$^{b}$ | 2.21 ± 0.23$^{a}$ | 1.11 ± 0.07$^{c}$ | 1.07 ± 0.06$^{c}$ | 0.87 ± 0.04$^{c}$ |
| | S&Pc | 0.86 ± 0.09$^{bc}$ | 1.42 ± 0.23$^{a}$ | 0.78 ± 0.12$^{c}$ | 1.03 ± 0.06$^{b}$ | 0.65 ± 0.06$^{c}$ |
| Protease[7] | I | 2.30 ± 0.40$^{b}$ | 4.26 ± 0.35$^{a}$ | 1.15 ± 0.08$^{c}$ | 2.71 ± 0.20$^{b}$ | 0.84 ± 0.06$^{c}$ |
| | S&Pc | 1.71 ± 0.20$^{bc}$ | 4.66 ± 1.08$^{a}$ | 1.23 ± 0.15$^{c}$ | 2.28 ± 0.07$^{b}$ | 0.92 ± 0.18$^{c}$ |
| Lipase[6] | I | 2.76 ± 0.25$^{b}$ | 3.83 ± 0.86$^{a}$ | 2.19 ± 0.46$^{b}$ | 2.24 ± 0.11$^{b}$ | 1.22 ± 0.05$^{c}$ |
| | S&Pc | 1.70 ± 0.21$^{b}$ | 3.74 ± 0.25$^{a}$ | 0.90 ± 0.12$^{c}$ | 2.06 ± 0.53$^{b}$ | 0.80 ± 0.24$^{c}$ |

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

[2]GroBiotic®-A 1% of diet

[3]GroBiotic®-A 2% of diet

[4]Aqualase 0.1% of diet

[5]Aqualase 0.2% of diet

[6]mg specific enzyme/mg protein/min

[7]µg specific enzyme /mg protein/min

higher body protein than fish from other treatments (Table 4) as well as G-A 1% and AQ 1-2 were significantly higher with the control. GroBiotic®-A and Aqualase supplemented feeds resulted in a decrease in body lipid with increase in these two supplements in the diet with the lowest value in G-A 2% (P<0.05). However, both feeding regimes tended (although statistically significant, P<0.05) to have lower body ash and lipid contents as supplements level increased in the diet. Inverse relationship was noted in the body moisture content among all the treatments. Moisture contents of fish fed with both supplement dietary regimes were not influence by the supplements levels and also did not show any notable trends (and were also statistically insignificant, P>0.05).

**Digestive Enzymes:** Data for protease, lipase and amylase activities are presented in Table 5. The mentioned intestinal specific amylase enzymes activity under added up GroBiotic®-A feeding changed significantly different (P<0.05) compared to fish that fed Aqualase and control diets and also stomach and pyloric ceaca specific amylase activity the fish fed G-A2 and AQ2 showed significantly different (P<0.05) compared to fish that fed AQ1 and control diets. However, comparatively highest specific protease activity was observed in stomach and pyloric ceaca (S & Pc) in fish fed supplement GroBiotic®-A 2% (Table 5). Furthermore, the specific enzyme activity was enhanced with increasing supplements (1 to 2%) in dietary treatment, G-A diets. Fish fed with G-A2 showed the tendency for higher protease activity than others fed dietary G-A1, AQ levels and the control. Intestinal, stomach and pyloric ceaca specific lipase activities in fish fed both supplement rations were significantly (P<0.05) promoted, with increasing supplements in the diet compared to the control while the fish fed G-A2 was found significantly (P<0.05) heighest value compared to the other treatments.

Statistically, it was noted that the enzyme concentrations in fish fed G-A and AQ treatments were different. Amylase and lipase activities in fish fed G-A and AQ diets were higher in the intestine than in the stomach and pyloric ceaca, respectively. However specific protease activity was higher in the intestine of fish fed with 1% than 2% G-A, while in AQ 0.1 and 0.2% vice versa.

## DISCUSSION

This study was planned to bring to mind the differential details of using probiotics and prebiotic in rainbow trout, *Oncorhynchus mykiss* from the growth performance point of view as well as their effect on the increasing digestive enzyme activities. Concerning the growth performance of *O. mykiss* treated with two commercial products including GroBiotic®-A and Aqualase (THEPAX). The results disclosed that both groups received prebiotic and probiotic-supplemented diets were shown higher growth rate than those kept on a basal diet. The addition of probiotics and prebiotics that suggested to improved the growth performance and reduced the effects of mortality in the tanks which is the important in aquaculture systems. In some technical report shown that the improvement in growth may however, be related to the improvement in the intestinal microbial flora balance [26]. This report mentioned that the

probiotics can adhere to an intestine surface or colonize secretions as mucin overlying the epithelial layer. A study [27] shown that the probiotics are able to adhere to epithelial layer.

The statistical analysis of different growth parameters of *O. mykiss* at the end of experimental period (Table 2, 3) indicated an increase in the body weight gain (W.G. %) between the five applied treatments. *O. mykiss* in group G-A kept on diet supplemented with (GroBiotic®-A) showed the fast grower than the group AQ (Aqualase) in comparison to control group. The specific growth rate (SGR) took almost the same pattern of W.G. in which *O. mykiss* in the group G-A have the highest SGR followed by *O. mykiss* in group AQ in comparison to control group. These were valso true for protein efficiency ratio (PER) and survival in which the *O. mykiss* in both groups treated with probiotic and prebiotic supplemented diets exceeded the amount of control group. In our study, survival of fish in the treatment which produced the highest growth performance was 96.44 ± 1.26% and declined to 89.44 ± 1.35% which was the lowest survival value. Since the feed efficiency (FE) of *O. mykiss* kept on a basal diet (control) was similar to the diets supplemented with probiotics and prebiotics, but represented somehow a positive aspect of supplemented diets. A study [28] with Biogen® as food additive containing *Bacillus subtilis* came to the conclusion that, this organism germinates in the intestine of fish, using a large numbers of sugar (carbohydrates) and produces a wide range of digestive enzymes (amylase, lipase and protease) which have a beneficial effects including higher growth rate and higher feed efficiency. Also the incorporation of *S. cerevisiae* as a probiotic in fish diet was investigated by a lot of researchers in which similar results were obtained. [18, 29, 30] supported the present result, reported fish fed the diets supplemented with brewer's yeast,commercial prebiotics GroBiotic®-A and GroBiotic®AEshowed that the prebiotic promoted the growth performance fish fed the basal diet during feeding trial and feed efficiency showed a similar trend. The oral administration of levamizole hydrochloride causes 20% increase biomass in Russian sturgeon (*Acipencerbaeri*) and Bester by [31, 32] suggested incorporation 100 mg levamizole for each kg of food to stripped bass diet showed a meaningful increase in growth compared with control group. The other experiment has shown, shrimp fed all experimental diets (fructooligosaccharides) demonstrated a high growth rate and survival. Other study [33] also reported that by adding scFOS (Short-Chain Fructo oligosaccharides) to diets did not affect feed efficiency. Our results shown from a start weight of approximately 11.5 g, fish given GroBiotic®-A and Aqualase grew to an average weight of 82.5 and 74 g vs. 66.5 g in controls and 23.5 and 10.3% higher final weight. Fish survival ranged from 89.4 to 96.4 and 94.4% in control, prebiotic and probiotic, respectively which showed 7.8 and 5.3% higher than control ($P<0.01$). Similar studies were administered at Trakia University, Stara Zagora, Bulgaria with rainbow trout (*Oncorhynchus mykiss*) with supplementation of prebiotic Bio-Mos® (0.2%) in standard commercial extruded feeds in which the rainbowtrout raised in net cages and raceways [34].

The PER and NPU (protein utilization) results indicated that supplementing diets with either the prebiotics or probiotics significantly improved protein utilization in *O. mykiss*. A research [35] shown that the same results in which the addition of probiotics and prebiotics improved feed utilization in practical terms. This means that probiotic and prebiotic used can decrease the amount of feed necessary for animal growth which could result in production cost reduction.

Body composition of fish was significantly ($P<0.05$) influenced by feeding regime or dietary pro and prebiotic level. Gram-positive bacteria, including members of the genus *Bacillus*, secret a wide range of exoenzymes [36], which might have supplied digestive enzymes and certain essential nutrients to promote better growth. *Bacillus subtilis*and and *Bacillus leicheniformis* can break down proteins and carbohydrates [37, 38]. So it can be suggested that administration of *Bacillus* bacteria to trout fry results in enhanced digestion of food and improved growth, including high food efficiency (FE) and high specific growth rate (SGR). High protein efficiency ratio (PER) as well as greater protein values of carcass in probiotic treatments may be due to proteins secreted by members of genus *Bacillus* [37]. We detected that supplementation of trout starter diet with the proper density of commercial probiotic and prebiotic could be beneficial for growth and survival of rainbow trout fry, especially in fast growing conditions, where it would be essential to stimulate the precocious maturation of digestive system [39]. Based on our present data, the beneficial influence of probiotic and prebiotic on growth was possibly due to an alteration of the intestinal microflora.

Administration of the G-A and AQ to *O. mykiss* resulted in an increase in the specific activity of Amylase, Protease and Lipases in the *O. mykiss*'s digestive tract, because gram- positive bacteria, particularly members of the genus *Saccharomyces cerevisiae* and yeast do

secrete a wide range of exoenzymes [40, 36, 41]. In the current study, specific enzyme activity Amylase, Protease and Lipases of pro and prebiotic diets were significantly higher than control group when crude intestinal enzyme extract of *O. myki*ss were used.

The observed increases in specific activities of digestive enzymes in probiotic and prebiotic treatments may have led to increase digestion and enhanced absorption of food, which in turn contributed to the improved survival and growth in *O. myki*ss [41]. The *Bacillus* species produce proteases (for example, subtilin), which helps in digestion [42]. Our finding is similar to that obtained by [43], who revealed protein digestion in juvenile *Scophthalmus maximus* and exhibited that supplementation of the diet with a potential probiont, *Vibrio proteolyticus*, resulted in increased digestion and absorption of protein, particularly in the distal portion of the gastrointestinal tract. The enhanced amylase and protease secretion in the intestine of the probiotic feed fed fishes could also be attributed to the superior maturation of their intestinal secretory cells [44].

These results were in agreement with the study of [45] who demonstrated the effects of *Bacillus* sp. probiotics on growth parameters and protease, amylase and lipase specific activities in *C. carpio* juveniles and recorded that mean digestive enzyme activities of all probiotics treatment groups were significantly different ($p<0.05$) with that of the basal.

Positive influences of Aqualase and GroBiotic®-A on growth performance and digestive enzyme activities of rainbow trout in the present study showed acceptable efficacy of probiotic and prebiotics for aquaculture.

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## REFERENCES

1. Ghosh, K., S.K. Sen and A.K. Ray, 2002. Growth and survival of Rohu, *Labeorohita* (Hamilton) spawn fed diets supplemented with fish intestinal microflora. Acta. Icthol. Piscat. 32: 83-92.
2. Farabi, S.M.V., S.H. Najafpour and G.D. Najafpour, 2009. Aspect of Osmotic-ions Regulation in Juvenile Ship, *Acipenser nudiventris* in the Southeast of Caspian Sea. World Apply Science J., 7(9): 1090-1096.
3. Gatlin, III, D.M., 2002. Nutrition and fish health, In: Halver, J.E. Hardy, R.W. (Eds.), Fish Nutrition, Academic Press, San Diego, CA, USA, pp: 671-702.
4. Khajepour, F. and S.A. Hosseini, 2010. Mineral Status of Juvenile Beluga (*Huso huso*) Fed Citric Acid Supplemented Diets. World Applied Sciences J., 11(6): 682-686.
5. SCAN, 2003. Opinion of the Scientific Committee on Animal Nutrition (SCAN) on the criteria for assessing the safety of microorganisms resistant to antibiotics of human clinical and veterinary importance, European Commission Health and Consumer Protection Directorate-General.
6. Kim, S., L. Nonaka and S. Suzuki, 2004. Occurrence of tetracycline resistance genes tet (M) and tet (S) in bacteria from marine aquaculture sites, FEMS Microbiol Letters; 237: 147-156.
7. Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbio., 8: 1137-1144.
8. Sørum, H., 2006. Antimicrobial drug resistance in fish pathogens. In: Aarestrup, F.M. (Ed.), Antimicrobial Resistance in Bacteria of Animal origin. ASM Press, Washington DC, pp: 213-238.
9. Metwally, M.A.A. and A.M. El-Gellal, 2009. Used of Some Plant Wastes for Fish Feeding with Reference on its Impact on Growth Performance and Body Composition. World Applied Sci. J., 6 (10): 1309-1313.
10. Roberfroid, M.B., 2000. Prebiotics and probiotics: are they functional foods? Amer. J. Clinic. Nutr., 71(6): 1682S-1687S.
11. Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr., 125: 1401-1412.
12. Gatesoupe, F.J., 2005. Probiotics and prebiotics for fish culture, at the parting of the ways, Aqua Feeds: Formulation & Beyond, 2(3): 3-5.
13. Dalmo, R.A. and R. Seljelid, 1995. The immunomodulatory effect of LPS, laminaran and sulphatedlaminaran [ß (1.3)-D-glucan] on Atlantic salmon, *Salmo salar* L. macrophages in vitro, J. Fish Diseas., 18: 175-185.
14. Thrall, M.A., 2004. Veterinary Haematology and Clinical Chemistry, Vol. 19, Williams and Wilkins cap. Philadelphia, pp: 277-289.
15. Pimpao, C.T., A.R. Zampronio and H.C. Silva de Assis, 2007. Affects of deltamethrin on heamatological parameters and enzymatic activity in *ancistrus multispinis* (Pisces, Teleostei). Pest Biochem. Physiol., 88(2): 122- 127.

16. Adeyemo, O.K., 2005. Hematological and histological effects of Cassava Mill Effluent in Clarias gariepinus. African J. Biomedical Res., 8: 179-183.
17. AOAC, 1994. Official Methods of Analysis, 15th edition, Association of Official Analytical Chemists, Arlington, VA.
18. Li, P., 2005. Evaluation of Various Dietary Supplements and Strategies to Enhance Growth and Disease Management of Hybrit Striped Bass Moronechrysops× *M. saxatilis*, Ph.D. Thesis, Ocean University, China, pp: 134.
19. Medne, R. and I. Savicka, 2003. Promotion of salmon rearing efficiency by including yeast extract Aqualase Two in the diet, Acta Universitatis Latviensis, 662: 45-50.
20. Brafield, A.E., 1985. Laboratories studies on energy budgets. In: Fish Energetics. New Perspective (Tytler, P. & Calow, P. eds), pp: 257- 281. Croom Helm, London and sydney.
21. Pavasovic, M., N.A. Richardson, A.J. Anderson and D. Mann, 2004. Mather P.B. Effect of pH, temperature and diet on digestive enzyme profiles in the mud crab, Scylla serrata Aquaculture, 242: 641- 654.
22. Lowry, O.H., N.J. Rosbrough, A.L. Farr and R.J. Randall, 1951. J. Biol. Chem., 193, 265.
23. Bernfeld, P., 1955. Amylase, In: Colowick, S.P. Kaplan, N.O. (Eds.), Methods in Enzymology. Academic Press, New Yourk, pp: 149-158.
24. Anson, M.L., 1938. The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. J, gen. Physiol., 22: 79-89.
25. Mongkolthanaruk, W. and S. Dharmsthiti, 2002. Biodegradation of lipid-rich wastewater by a mixed bacterial consortium, Int. Biodeterior. Biodegrad. 50: 101-105.
26. Fuller, R., 1989. Probiotic in man and animals, Journal of applied Bacteriology. 66: 365-378.
27. Tannock, G.W., 1999. Introduction. In: Probiotics: A critical review pp: 1-4. Horizon Scientific Press, Norfolk, England.
28. El-Haroun, E.R., A.S. Goda, A.M. Kabir and M.A. Chowdhurry, 2006. Effect of dietary probiotic Biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquacult. Res., 37: 1473-1480.
29. Li, P. and D.M. Gatlin III, 2004. Dietary brewer's yeast and the Prebiotic Grobiotic AE influence growth performance, immune responses and resistance of hybrid striped bass (*Moronechrysops* × *M. saxatilis*) to *Streptococcus iniae* infection, Aquaculture, 231: 445- 456.
30. Li, P. and D.M. Gatlin III, 2005. Evaluation of the prebiotic GroBiotic®-A and brewer's yeast as dietary supplements for sub-adult hybrid striped bass (*Moronechrysops M.saxatilis*) challenged in situ with Mycobacterium marinum Aquacul., 248: 197-205.
31. Vahabzadehroodsari, H., 2003. PhD thesis, Surveying oxygen peroxide and Levamizole hydrochloride application treating eggs and frys of Persian Sturgeon and Chinese Carp, 107: 30-50.
32. Li, P., X. Wany and D.M. Gatlin, 2006. Evaluation of levamisole as a feed additive for growth and health management of hybrid striped bass, Aquaculture, 251: 201-204.
33. Li, P., G.S. Burr, D. M. Gatlin. M.E. Hume, S.P. Frank, L. Castille and A.L. Lawrence, 2007. Dietary Supplementation of Short-Chain Fructoo ligosaccharides Influences Gastrointestinal Microbiota Composition and Immunity Characteristics of Pacific White Shrimp, *Litopenaeus vannamei*, Cultured in a Re-circulating System. The J. Nutrition (Nutritional Immunology).
34. Staykov, Y., P. Spring, SA. Denev and J. Sweetman, 2007. Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*), Aquacul. Int., 2: 153-161.
35. Lara-Flores, M., M. A. Olvera-Novoa, B. E. Guzman-Mendez and W. Lopez-Madrid, 2003. Use of bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *saccharomyces cerevisiae* as growth promoters in the Nile tilapia (*Oreochromis niloticus*), Aquacalture, 216: 193-201.
36. Moritary, D.J.W., 1998. Control of *Luminous vibrio* species in *panaeid* aquaculture ponds.
37. Rosovitz, M.J., M.I. Voskuil and G.H. Chambliss, 1998. Bacillus. In: A. Balows and B.I. Duerden (Eds), Systematic Bacteriology. Arnold Press, London, pp: 709-720
38. Farzanfar, A., 2006. The use of probiotics in shrimp aquaculture. FEMS Immunol Medical Microbiol., 48(2): 149-158.
39. Wache, Y., F. Auffray, F.J. Gatesoupe, J. Zambonino, V. Gayet, L. Labbe and C. Quentel, 2006. Cross effects of the strain of dietary Saccharomyces cerevisiae and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, Onchorhynchusmykiss fry, Aquaculture, 258: 470-478.
40. Moritary, D.J.W., 1996. Microbial biotechnology, a key ingredient for sustainable aquaculture. Info fish Int. 4: 29-33.

41. Ziaei-Nejad, S., M. Habibi Rezaei, G. Azari Takami, D. Lovett, A.R. Mirvaghefi and M. Shakouri, 2006. The effect of Bacillus spp. bacteria used as probiotics on digestive enzymes activity, survival and growth in the Indian white shrimp *Fennero penaeusindicus*. Aquaculture, 252: 516-524.
42. Sanders, M.E., L. Morelli and T.A. Tompkins, 2003. *Sporeformers* as human probiotics: *Bacillus*, *Sporolactobacillus* and *Brevibacillus*. Comprehensive Reviews in Food Science and Food Safety, 2: 101-110.
43. De-Schrijver, R. and F. Ollevier, 2000. Protein digestion in juvenile turbot (*Scophthalmus maximus*) and effects of dietary administration of *Vibrio proteolyticus*, Aquaculture, 186: 107-116.
44. Tovar, D., J. Zambonino, C. Cahu, F.J. Gatesoupe, R. Vazquez-Juarez and R. Lesel, 2002. Effect of yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae, Aquaculture, 204: 113-123.
45. Wang, Y.B. and Z.R. Xu, 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Technol., 127: 283-292.