Effects of Inulin, Savory and Onion Powders in Diet of Juveniles Carp
Cyprinus Carpio (Linnaeus 1758) on Gut Micro Flora, Immune Response and Blood Biochemical Parameters

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Abstract

In this experiment for evaluation the effect of inulin, savory (Satureja khuzestanica) and onion powder on common carp, a diet was formulated as a control (0) and four other diets to contain 1% inulin, 1% savory, 1% onion and 1% mixture of savory and onion powder (savory-onion). Fish with initial average weight of 20.0 ± 0.08 g were allotted to 15 circular tanks of 300 L capacity at a density of 10 fish per each tank and fed to satiation the experimental diets three times a day. At the end of 45 feeding days, the lysozyme activity and complement C3 and C4 values of fish fed 1% inulin powder were significantly higher (P<0.05) than the control. The blood protein and globulin of fish fed 1% inulin, 1% savory and 1% savory-onion powder were significantly higher (P<0.05) whereas, the triglyceride values of fish fed 1% savory and 1% onion powder were significantly lower (P<0.05) than the control. The number of total lactic acid bacteria significantly increased (P<0.05) in all treatments compared to the control group. So, 1% dietary inulin can be used as an immune additive in diet of carp juvenile.

Keywords: Inulin, Herbs, Carp, Savory, Onion.

Introduction

Different chemotherapeutic agents such as antibiotics and disinfectants have been habitually used in the treatment and prevention of numerous diseases in farmed fish. However, they cannot be suggested since unacceptable and incessant use of antibiotics may direct to potential increase of antibiotic resistant bacteria, environmental pollution and the increase of antibiotic residues in fish (Ringo, Olsen, Gifstad, Dalmo, Amlund, Hemre and Bakke. 2010). Therefore, the success of the antibiotics for treating fish diseases is no longer guaranteed. Many countries have prohibited the use of certain chemotherapeutics, and also declined importing aquaculture products treated with antibiotics and chemicals. Consequently, researchers have intensified efforts to use natural new food additives such as prebiotics, herbs and plants in progress of alternative dietary supplements that helpfully affect the host by selectively stimulate the activity of health-promoting bacteria in the intestinal tract (Gibson, 2004). In aquaculture, prebiotics have received increasing attention because of stimulating growth performances, food utilization, positive effects on gut microbiota, gut morphology, immune system, and disease resistance (Merrifield, Dimitroglou, Foey, Davies, Baker, Bogwald, Castex and Ringo. 2010; Ringo, Olsen, Gifstad, Dalmo, Amlund, Hemre and Bakke. 2010; Ringo, Dimitroglou, Hoseinifar and Davies. 2014). Regardless of some negative results (Olsen, Myklebust, Kryvi, Mayhew and Ringo. 2001; Akrami, Hajimoradloo, Matinifar and Abedian Kinari. 2009), numerous studies have reported positive effects of inulin as growth promoter (Mahious, Gatesoupe, Hervi, Metailler and Ollevier. 2006; Burr, Hume, Ricke, Nisbet and Gatlin. 2010; Ortiz, Rebole, Velasco, Rodri guez, Trevin, Tejedor and Alzueta. 2013). Inulin is one of the most studied prebiotic and consists mainly of polydisperse b-linked fructan and is naturally present in a number of common foods such as garlic, onion, artichoke and asparagus (Van Loo, Cummings and Delzenne. 1999; Akhter, Wu, Memon and Mohsin. 2015; Roberfroid, 2007).

Onion (Allium cepa L.) has a high content of free and glycosidically bonded quercetin and oxidized...
quercetin derivatives (Suh, Lee, Cho, Kim and Chung, 1999; Griffiths, Trueman, Crowther, Crowther and Smith, 2002). Onion has been known to have antibacterial, antioxidant, and anticancer effects (Ramos, Takaishi, Shirotori, Kawaguchi, Tsuchiya, Shibata, Higuti, Takeuchi, 2006; Jeong, Heo, Choi and Shim, 2009), and it reduces endogenous lipogenesis and increases catabolism of lipids (Kumari and Augusti, 2007). Onions include a large variety of micro constituents such as trace elements, vitamins, flavonoids and sulphur compounds (Breu, 1996), which may have protective effects against cancer. Additionally, a previous study discovered that onion powder was one of the most useful dietary additives that improve lysozyme activity of the Olive flounder (Paralichthys olivaceus) juvenile (Cho and Lee, 2012). However, only few studies have documented the effects of supplemental onion on farmed fish including African catfish (Bello, Olaifa, Emikpe and Ogunbanwo, 2012); brown-marbled grouper (Apines-Amar, Amar, Faisan, Rolando, Pakingking and Satoh, 2012) and olive flounder (Cho and Lee, 2012). Furthermore, medicinal plants show potential to be main sources of therapeutics in fish culture since these products provide a cheaper supply for treatment and greater accuracy without causing toxicity (Madhuri, Mandloi, Govind and Sahni, 2012). In common, plants have a variety of functions due to the existence of different active compounds similar to alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids and essential oils (Citarasu, 2010). Savory with scientific name of Satureja khuzestanica contains many essential volatile oil phenols such as thymol and carvacrol, as well as compounds like linalool, camphene, caryophyllene, terpineol, myrcene, and other terpenoids. Thymol, one of the important essential oils, has scientifically been found to have antiseptic, anti-fungal characteristics. In addition, another phenolic compound, carvacrol, inhibits the growth of several bacteria strains like Escherichia coli, and Bacillus cereus (Hajhashemi, Sadraei, Ghannadi and Mohseni, 2000). Carvacrol, therefore, has been used as healthy food additive for its antibacterial properties but little information is available on the efficacy of dietary savory in fish foods.

The Common carp (Cyprinus carpio) is one of the most important species in Iran. This species is suitable for aquaculture because of its fast growth, easy reproduction and high tolerance to poor environmental conditions. Therefore, further search for commercially available dietary additives improving performance and disease resistance, is still needed. So, the purpose of the present study was to evaluate the effects of inulin, savory and onion powder in diet on gut microflora, immune response and blood biochemical parameters of juveniles carp.

### Materials and methods

#### Diet Preparation

Ingredients and proximate composition of the basal diet are given in Table1. Experimental diets were formulated and used as control (basal diet) and four other diets to contain, 1% inulin, 1% savory, 1% onion powder, respectively.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>(% of Dry Matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal a</td>
<td>24.5</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>10</td>
</tr>
<tr>
<td>Corn gluten</td>
<td>15</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>26.3</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>10</td>
</tr>
<tr>
<td>Fish oil b</td>
<td>3</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin premix c</td>
<td>2</td>
</tr>
<tr>
<td>mineral premix d</td>
<td>2</td>
</tr>
<tr>
<td>Antioxidants e</td>
<td>0.2</td>
</tr>
<tr>
<td>mollass</td>
<td>1</td>
</tr>
<tr>
<td>Binder  f</td>
<td>2</td>
</tr>
<tr>
<td>Filler (Clay)</td>
<td>1</td>
</tr>
<tr>
<td>Proximate composition (DM %)</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>38</td>
</tr>
<tr>
<td>Lipid</td>
<td>10</td>
</tr>
<tr>
<td>Ash</td>
<td>8.8</td>
</tr>
</tbody>
</table>

a Clopeonaella meal, Iran  
b Kilka oil, Mazandaran Co, Iran  
c Vitamin premix (composition per 1kg): A=1600000 IU, D3=400000 IU, E=40000 mg, K3=20000 mg, B1=6000 mg, B6=8000 mg, B12=12000 mg, B12=4000 mg, B9=2000 mg, B2=8 mg, H2=40 mg, C=60000 mg, Inositol=20000 mg  
d Mineral premix (composition per 1kg): Iron:60000 mg, Zinc:10000 mg, Selenium:20 mg, Cobalt:100 mg, Copper:6000 mg, Manganese:5000 mg, Iodine:5000 mg, CoCl3:8000 mg  
e Antioxidant: Butylated hydroxytoluene (BHT)  
f Binder: Amet Binder (Component: Crude Protein: 71.98%, Crude Fiber: 0.9%, Ash: 17.8%, Moisture: 9.5%)
onion and 1% mixture of onion (0.5%) and savory (0.5%) powder based on preliminary experiments by replacing clay as a filler in basal diet. Dry ingredients were weighed and ground (100 µm particle sizes) and then mixed thoroughly. Fish oil, soybean oil and water were added to the dry ingredients and mixed again until dough was formed. Then prepared dough was pelleted using a pelleting machine which it was dried at room temperature for 24 h and grounded into desirable particle sizes and stored at -20°C until later usage.

Proximate analyses of ingredients and the basal diet were determined according to the method of AOAC (1995). Crude protein content was determined by Kjeldahl method using an Auto Kjeldahl System (Kjeltc™2300, Foss, Sweden). Crude lipid was analyzed by Soxtec system, moisture content by a dry oven (D-63450, Heraeus, Hanau, Germany) drying at 105°C for 24 h and ash by a furnace muffle (550°C for 4 h).

Experiment fish and feeding conditions

The experiment was done in lab of Khorramshar University of Marine Science and Technology, Khorramshar, Iran. Juveniles of carp were obtained from a commercial farm. The fish were acclimated to laboratory condition for 2 weeks before starting the feeding trial. Juvenile fish (initial mean weight, 20.0±0.08 g) were randomly distributed into 15 polyvinyl circular tanks of 300 L capacity at the density of 10 fish per tank. Each tank was supplied with tap fresh water with 25% changes of it every two days and aeration to maintain enough dissolved oxygen. Three replicate groups of fish were hand-fed to apparent satiation three times a day (9:00, 13:00 and 17:00) for 45 days. During the experimental period, mean water temperature was 28.35±0.2°C, dissolved oxygen was 6.35±0.19 mg L⁻¹ and the pH was 8.13±0.19. The photoperiod was left under natural conditions during the feeding trial.

Sample preparation

Blood sampling from juveniles was scheduled after 45 days of treatments. Blood was drawn from the caudal vein of ten fish per each tank and pooled by each other. The serum samples were separated using standard procedures and stored at -80 °C prior to analysis.

Biochemical assays

The sera total proteins were assayed using a diagnostic kit (Pars Azmon, Diagnostics Co., Iran). Albumin content was determined following the method of Doumas, Ard Watson and Biggs (1997). Globulin content (subtracting albumin from the total protein) was calculated as described by Kumar, Sahu, Pal, Choudhury, Yengkokpam and Mukherjee (2005). Blood biochemical analysis was performed in the sequence: Plasma glucose, triglycerides and total cholesterol were determined by colorimetric tests of commercial kits (Pars Azmoon, Tehran, Iran).

Immunological assays

Lysozyme activity

Lysozyme activity in serum was determined according to the method of Demers and Bayne (1997) based on the lysis of the lysozyme sensitive gram positive bacterium, Micrococcus lysodeikticus (Sigma). The dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 20 µL mL⁻¹ (in 0.1 M phosphate citrate buffer, pH 5.8) were taken as the standard. This along with the undiluted serum sample (25 µL) was placed into wells of a 96-well plate in triplicate. One hundred and seventy-five microliters of M. lysodeikticus suspension (75 mg mL⁻¹) were prepared in the same buffer then added to each well. After rapid mixing, the change in turbidity was measured every 30 s for 5 min at 450 nm at approximately 20°C using a microplate reader.

Complement amount

Complement C₃ and C₄ were assayed with ELISA kit (Pars azmon, Tehran, Iran). Based on the procedure assay of kit; a complement C₃ specific antibody was precoated onto 96-well plates and blocked. Standards or test samples were added to the wells and subsequently biotinylated complement C₃ was added and then followed by washing with wash buffer. Streptavidin peroxidase complex was added and unbound conjugates were washed away with wash buffer. Tetramethylbenzidine (TMB) was then used to visualize Streptavidin peroxidase enzymatic reaction. TMB was catalyzed by Streptavidin peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration was inversely proportional to the amount of Complement C₃ captured in plate. Also, C₄ amount was measured in the same way of C₃.

Intestinal lactic acid bacteria

The analysis of gut microbiota was conducted at the end of the nutrition trial. Three fish were sampled from each treatment after cease of feeding for 24 h. The fish were killed by immersion in high concentrations of anesthetic carnation powder and the skin washed in a solution of 0.1% benzalkonium chloride before opening the ventral surface with sterile scissor. Intestinal tract of fish were removed, weighted and suspended in sterile saline (0.85% (w/v) NaCl). Then 1cc of the solution was diluted in 9 ml of sterile saline and then 0.5 ml of it was spread in triplicates on nutrient agar (NA). DeMan, Rogosa,
and Sharpe (MRS) (general term of MRS media) were also used for counting number of lactic acid bacteria (LAB). All plates were incubated at 30°C and examined for 1 day (Rengpipat, Phianphak, Piyaratitivorakul and Menasveta. 1998 ), and then the numbers of colonies were counted. Identification of the samples was carried out according to Bergey’s method (Peter and Sneath. 1986).

Statistical Analysis

Data were subjected to one-way ANOVA to test treatments effects on fish. When significant differences were found in one-way ANOVA, Duncan’s multiple range test was used to rank the groups. All statistical analyses were performed using SPSS version 16 (SPSS, Chicago, IL USA) with a significant level of (P<0.05). The values presented are mean ± standard error (SE).

Results

The results of dietary savory, onion and inulin powder effects on some immunological and biochemical parameters of common carp juveniles are shown in Table 2 and Table 3.

Fish fed the diets supplemented with 1% inulin and 1% onion powder showed significant higher lysozyme activity (P<0.05) than the control group (Table 2). Also, fish fed on the diet to contain 1% inulin showed significant higher value of C3 from those fed on the other diets (P<0.05). Furthermore, higher value of C4 observed in fish fed diets to contain 1% inulin and 1% savory from the other groups (P<0.05).

Protein and globulin values of fish fed diets containing 1% inulin, 1% savory and1% savory-onion significantly increased (P<0.05) compared to the control group (Table 3). Also, albumin value of fish fed 1% inulin was significantly higher (P<0.05) than the other groups. Total cholesterol value of fish was not significantly different (P>0.05) among treatments. Also, significantly (P<0.05) lower triglycerides value was detected in fish fed 1% savory and 1% onion powder in diet among treatments. On the other hand, the glucose values of fish fed the 1% onion and 1% savory-onion diets were just significantly lower than the control group (P<0.05). A significant increase (P<0.05) in the calcium and phosphor values of fish was observed in treatments containing 1% inulin, 1% savory, 1% savory-onion compared to the control group. Also, total count of lactic acid bacteria (CFU) in gut of fish fed the experimental diets was significantly higher (P<0.05) than control group (Table 4).

Discussion

The prebiotics and phytoadditives improve growth performance and immune system by balancing the gut microbial community and stimulating the secretion of endogenous digestive enzymes in animals (Wenk, 2003a). So, insoluble inulin has been suggested to have adjuvant activity because it activates the alternative complement pathway (Silva, Cooper and Petrovsky. 2004). Also, herbs are rich sources of immune-enhancing substances that, not only stimulate the acquired immune response by increasing the diseases resistance, but also enhance innate, humoral and cellular defense mechanisms (Galindo-Villegas and Hosokawa. 2004). On the other hand, It was reported that carvacrol or thymol of herbal plant promote various health functions (Zheng, Tan, Liu, Zhou, Xiang and Wang. 2009; Ahmadifar, Falahatkar and Akrami. 2011), antioxidant protective capacities (Giennenas, Triantafillou, Stavrakakis, Margaroni, Mavridis, Steiner and Karagouni. 2012) and also increased disease resistance in fish (Zheng, Tan, Liu, Zhou, Xiang and Wang. 2009; Rattanachaikunsopon and Phumkhachorn. 2010; Volpatti, Chiara, Francesca and Marco. 2012).Therefore, in accordance with the stated facts, lysozyme activity, C3 and C4 values of fish affected by inulin. Also, lysozyme activity, and C4 value of fish was affected by onion and savory in diets respectively which it is similar to olive flounder fed with 0.5% onion powder (Cho and Lee, 2012)

Based on the result, the higher serum protein level and globulin was recorded in fish fed with inuline, savory and savory-onion. Similarly, the highest serum protein level was recorded in juvenile beluga fed with inulin (Akrami, Hajimoradloo, Matinfar and Abedian Kinari. 2009), and catfish fed with onion and garlic ( Al-Salahy, 2002). Since there is a close relationship between the level of protein synthesized in liver tissue and plasma protein pools, the total protein levels in plasma may be elevated due to the increased levels of protein synthesis in liver tissue of fish. Also, it was reported that the increase in the levels of serum protein, albumin and globulin in fish is thought to be associated with a stronger innate immunity response (Wiegtjtes, Stet, Parmentier and Van Muiswinkel. 1996).

Generally, the onion and savory effectiveness as hypoglycaemic agents has been scarcely investigated. Recently, it has been reported that long-term absorption of natural flavonoids as quercetin could be useful to prevent advanced glycation of collagens, which contributes to development of cardiovascular complications in diabetic patients (Urios, Grigorova-Borsos and Sternberg. 2007). Also, the bioactive constituents from onion, such as methiin and S-allyl cysteine sulphoxide (SACS), exert their anti-diabetic action by stimulating the insulin production and secretion by pancreas, interfering with dietary glucose absorption, and favoring the insulin saving (Srinivasan, 2005). Therefore, hypoglycaemic effect of onion and savory-onion was observed in this experiment. On the other hand, some investigations have demonstrated that onion also has compounds
with capacity to reduce blood triglycerides levels (Effendi, Simmons, Campbell and Campbell. 1997). Allicin and its derivative compounds are the main active substances responsible for the hypolipidemic effects of onion and garlic, as much in humans as in experimentation animals (Liu and Yeh. 2002; Yeh, Lin, Yeh and Evans. 1997). Also, it was reported that carvacrol and tymol are the main active substances responsible for the hypolipidemic effects of herbal plant such as savory (Hajhashemi, Sadraei, Ghannadi and Mohseni. 2000). So, savory and onion powder in diet showed capacity to reduce blood triglycerides levels in fish.

In this experiment, calcium and phosphor values of fish increased by inulin, savory and savory-onion powder in diet. It was reported that absorbed prebiotics in gut were fermented to fatty acid with short chain like acetate, butyrate and propionate that induced low pH in gut and consequently increase mineral solution and absorption from intestine (Scholz-Ahrens and Schrezenmeir. 2002). Also, it seems that savory through positive effect on numbers of lactic acid bacteria induces rise in mineral absorption from intestine.

It was mentioned that inulin may have interesting applications in aquaculture to motivate the good gut bacteria. The increased number of lactic acid bacteria observed in the gut of juvenile fish fed with a diet containing inulin, is in agreement with results of Akrami, Hajimoradloo, Matinfar and Abedian Kinari. (2009) investigated the effects of inulin on the intestinal microflora of Beluga (H. huso) juvenile and Mourino, Nascimento, Viera, Jatoba, Silva, Jesus, Seiffert and Martins (2012) studied the effects of inulin and Weisella cibaria on hybrid surubium (Pseudoplatystoma sp). In gut, the increase in short-chain fatty acids and lactic acid from the fermentation of inulin leads to a decrease in pH. This provides optimal condition for the growth of LAB. The increased number of LAB competes with pathogens for nutrients and receptors on the gut wall (Akrami, Hajimoradloo, Matinfar and Abedian Kinari. 2009). Also, the number of LAB increased in the gut of fish by savory and onion in diet. Onion prebiotic activity is also being investigated (Benkeblia and Shiomi. 2006; Sharma, Kainth and Gill. 2006) due to their high soluble fibre content, especially inulin and fructooligosaccharides which stimulate in the colon the growth of specific microorganisms, as bifidobacteria and lactobacilli. Moreover, it has a general positive health effects (Binaii. 2014). Furthermore, it was mentioned that phenolic compounds and flavanoids such as carvacrol and thymol extracted from savory can modulate gut bacteria through negative effect on harmful bacteria or increase the number of lactic acid bacteria in gut of

### Table 2. Immunological parameters of common carp fed experimental treatments for 45 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Inulin</th>
<th>Savory</th>
<th>Onion</th>
<th>Savory-onion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoroprotein (μg/ml)</td>
<td>1.01±0.20^a</td>
<td>1.74±0.24^b</td>
<td>1.36±0.24^ab</td>
<td>1.66±0.10^b</td>
<td>1.28±0.04^ab</td>
</tr>
<tr>
<td>C3 (mg/l)</td>
<td>5.00±0.00^a</td>
<td>8.85±0.15^b</td>
<td>5.66±0.33^a</td>
<td>5.33±0.33^a</td>
<td>5.50±0.50^a</td>
</tr>
<tr>
<td>C4 (mg/l)</td>
<td>1.00±0.2^a</td>
<td>2.00±0.10^b</td>
<td>2.05±0.05^b</td>
<td>1.06±0.21^b</td>
<td>1.4 ± 0.45^ab</td>
</tr>
</tbody>
</table>

Values are mean ± SE of three replicate groups. Mean values with different superscripts are significantly different from each other. Significance level is defined as P<0.05.

### Table 3. Blood biochemical parameters of common carp fed experimental treatments for 45 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Inulin</th>
<th>Savory</th>
<th>Onion</th>
<th>Savory-onion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>3.13±0.03^a</td>
<td>4.06±0.02^a</td>
<td>3.65±0.07^b</td>
<td>3.23±0.00^b</td>
<td>3.64±0.09^ab</td>
</tr>
<tr>
<td>Albinin (g/dl)</td>
<td>2.07±0.06^a</td>
<td>2.59±0.14^b</td>
<td>2.05±0.02^a</td>
<td>2.10±0.02^a</td>
<td>2.16±0.05^a</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>1.05± 0.03^a</td>
<td>1.56±0.20^a</td>
<td>1.54±0.10^bc</td>
<td>1.14±0.02^b</td>
<td>1.57±0.09^c</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>214.25±8.05^a</td>
<td>216.60±7.25^b</td>
<td>178.00±6.80^a</td>
<td>182.00±7.18^a</td>
<td>215.00±7.00^b</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>75.66±5.17^ns</td>
<td>83.66±3.84^a</td>
<td>76.00±3.05^a</td>
<td>74.75±2.92^a</td>
<td>84.33±2.90^a</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>82.00±4.04^a</td>
<td>78.33±4.50^ab</td>
<td>79.00±4.38^ab</td>
<td>67.50±6.35^a</td>
<td>67.33±5.50^a</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>1.86±0.24^a</td>
<td>11.76±0.55^a</td>
<td>4.56±0.40^b</td>
<td>2.16±0.27^a</td>
<td>3.45±0.85^ab</td>
</tr>
<tr>
<td>Phosphor (mg/dl)</td>
<td>3.9±0.20^a</td>
<td>8.2±0.23^c</td>
<td>4.9±0.10^a</td>
<td>3.85±0.05^a</td>
<td>4.65±0.25^b</td>
</tr>
</tbody>
</table>

Values are mean ± SE of three replicate groups. Mean values with different superscripts are significantly different from each other. Significance level is defined as P<0.05.

### Table 4. Total number of lactic acid bacteria in gut of common carp fed experimental diets for 45 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Inulin</th>
<th>Savory</th>
<th>Onion</th>
<th>Savory-onion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus (CFU)</td>
<td>1.8×10^10±21^a</td>
<td>2.48×10^10±24^b</td>
<td>2.6×10^10±29^c</td>
<td>2.57×10^10±32^bc</td>
<td>2.68×10^10±32^c</td>
</tr>
</tbody>
</table>

Values are mean ± SE of three replicate groups. Mean values with different superscripts are significantly different from each other. Significance level is defined as P<0.05.
rainbow trout (Pirbalouti, Jahanbazi, Enteshari, Malekpour and Hamedi. 2010a; Pirbalouti, Malekpour, Enteshari, Yousefi, Montaz and Hamedi. 2010b; Pirbalouti, Broujeni, Momeni, Malek and Hamedi. 2011).

Conclusion

Generally, the results of the experiment indicated that feeding fish with 1% dietary inulin as prebiotic was by far than other treatments improve the health of fish through enhancing immune response and blood biochemical parameters.

Acknowledgements

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