www.trjfas.org
ISSN 1303-2712
*Turkish Journal of Fisheries and Aquatic Sciences 16: 831-838 (2016)* DOI: 10.4194/1303-2712-v16_4_09

RESEARCH PAPER

# 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

**Elham Mousavi[1], Hamid Mohammadiazarm[1,*], Seied Mohammad Mousavi[1], Ebrahim Rajabzadeh Ghatrami[1]**

[1]Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Khouzestan, Iran.

* Corresponding Author: Tel.: +989165810642;
E-mail: azarmhamid@gmail.com

Received 07 May 2016
Accepted 12 June 2016

**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 $C_3$ and $C_4$ 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 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 increase the growth performance, health and immune system of cultured fish. The candidates to replace antibiotics are organic acids, probiotics, prebiotics and plant extracts, which have been suggested to control intestinal microbial growth (Higgins, Higgins, Wolfenden, Henderson, Torres-Rodriguez, Tellez and Hargis. 2008).

Prebiotics are non-digestible food ingredients 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 Ringø. 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, Matinfar 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, Tadokoro and Takeuchi. 2006; Jeong, Heo, Choi and Shim. 2009), and it reduces endogenous lipogenesis and increases catabolism of lipids (Kumari and Augusti. 2007). Onions includes 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%

**Table 1.** Ingredient and proximate composition of basal diet

| Ingredients | (% of Dry Matter) |
|---|---|
| Fish meal [a] | 24.5 |
| Soybean meal | 10 |
| Corn gluten | 15 |
| Wheat flour | 26.3 |
| Wheat bran | 10 |
| Fish oil [b] | 3 |
| Soybean oil | 3 |
| Vitamin premix [c] | 2 |
| mineral premix [d] | 2 |
| Antioxidants [e] | 0.2 |
| molass | 1 |
| Binder [f] | 2 |
| Filler (Clay) | 1 |
| Proximate composition (DM %) | |
| Protein | 38 |
| Lipid | 10 |
| Ash | 8.8 |

a Clopeonella meal, Iran

b Kilka oil, Mazandaran Co, Iran

c Vitamin premix (composition per 1kg): A=1600000 IU, D3=400000 IU, E=40000 mg, K3=2000 mg, $B_1$=6000 mg, $B_2$=8000 mg, $B_3$=12000 mg, $B_5$=40000 mg, $B_6$=4000 mg, $B_9$=2000 mg, $B_{12}$=8 mg, $H_2$=40 mg, C=60000 mg, Inositol=20000 mg

d Mineral premix (composition per 1kg): Iron:6000 mg, Zinc:10000 mg, Selenium:20 mg, Cobalt:100 mg, Copper:6000 mg, Manganese:5000 mg, Iodine:600 mg, $CoCl_2$:6000 mg

e Antioxidant: Butylated hydroxytoluene (BHT)

f Binder: Amet Binder (Component: Crude Protein: 71.98٪, Crude Fiber: 0.9٪, Ash: 17.8٪, Moisture: 9.55٪)

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 -20ºC 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 (Kjeltec $^{TM}$2300, Foss, Sweden). Crude lipid was analyzed by Soxtec system, moisture content by a dry oven (D-63450, Heraeus, Hanau, Germany) drying at 105℃ for 24 h and ash by a furnace muffler (550℃ 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 $C_3$ and $C_4$ were assayed with ELISA kit (Pars azmon, Tehran, Iran). Based on the procedure assay of kit; a complement $C_3$ specific antibody was precoated onto 96-well plates and blocked. Standards or test samples were added to the wells and subsequently biotinylated complement $C_3$ 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 $C_3$ captured in plate. Also, $C_4$ amount was measured in the same way of $C_3$.

**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, Piyatiratitivorakul 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 $C_3$ from those fed on the other diets ($P<0.05$). Furthermore, higher value of $C_4$ 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, $C_3$ and $C_4$ 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 (Wiegertjes, 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

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

| Parameters | Control | Inulin | Savory | Onion | Savory-onion |
|---|---|---|---|---|---|
| Lysosym(µg/ml) | $1.01\pm0.20^{a}$ | $1.74\pm0.24^{b}$ | $1.36\pm0.24^{ab}$ | $1.66\pm0.10^{b}$ | $1.28\pm0.04^{ab}$ |
| C3(mg/l) | $5.00\pm0.00^{a}$ | $8.85\pm0.15^{b}$ | $5.66\pm0.33^{a}$ | $5.33\pm0.33^{a}$ | $5.50\pm0.50^{a}$ |
| C4(mg/l) | $1.00\pm0.2^{a}$ | $2.00\pm0.10^{b}$ | $2.05\pm0.05^{b}$ | $1.06\pm0.21^{ab}$ | $1.4\pm0.45^{ab}$ |

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

| Parameters | Control | Inulin | Savory | Onion | Savory-onion |
|---|---|---|---|---|---|
| Total protein(g/dl) | $3.13\pm0.03^{a}$ | $4.06\pm0.02^{c}$ | $3.65\pm0.07^{b}$ | $3.23\pm0.00^{a}$ | $3.64\pm0.09^{b}$ |
| Albomin (g/dl) | $2.07\pm0.06^{a}$ | $2.59\pm0.14^{b}$ | $2.05\pm0.02^{a}$ | $2.10\pm0.02^{a}$ | $2.16\pm0.05^{a}$ |
| Globulin (g/dl) | $1.05\pm0.03^{a}$ | $1.56\pm0.20^{c}$ | $1.54\pm0.10^{bc}$ | $1.14\pm0.02^{ab}$ | $1.57\pm0.09^{c}$ |
| Triglyceride(mg/dl) | $214.25\pm8.05^{b}$ | $216.60\pm7.25^{b}$ | $178.00\pm6.80^{a}$ | $182.00\pm7.18^{a}$ | $215.00\pm7.00^{b}$ |
| Cholesterol (mg/dl) | $75.66\pm5.17^{n.s}$ | 83.66±3.84 | 76.00±3.05 | 74.75±2.92 | 84.33±2.90 |
| Glucose (mg/dl) | $82.00\pm4.04^{b}$ | $78.33\pm4.50^{ab}$ | $79.00\pm4.38^{ab}$ | $67.50\pm6.35^{a}$ | $67.33\pm5.50^{a}$ |
| Calcium (mg/dl) | $1.86\pm0.24^{a}$ | $11.76\pm0.55^{c}$ | $4.56\pm0.40^{b}$ | $2.16\pm0.27^{a}$ | $3.45\pm0.85^{ab}$ |
| Phosphor (mg/dl) | $3.9\pm0.20^{a}$ | $8.2\pm0.23^{c}$ | $4.9\pm0.10^{b}$ | $3.85\pm0.05^{a}$ | $4.65\pm0.25^{b}$ |

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

| Parameters | Control | Inulin | Savory | Onion | Savory-onion |
|---|---|---|---|---|---|
| Lactobacillus (CFU) | $1.8\times10^{3}\pm21^{a}$ | $2.48\times10^{3}\pm24^{b}$ | $2.6\times10^{3}\pm29^{c}$ | $2.57\times10^{3}\pm25^{bc}$ | $2.68\times10^{3}\pm32^{c}$ |

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.

with capacity to reduce blood triglycerides levels (Effendy, 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 Evens. 1997). Also, it was reported that carvocrol 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, Vieria, Jatoba, Silva, Jesus, Seiffert and Martins (2012) studied the effects of inulin and *Weissella 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

rainbow trout (Pirbalouti, Jahanbazi, Enteshari, Malekpoor and Hamedi. 2010a; Pirbalouti, Malekpoor, Enteshari, Yousefi, Momtaz 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

This work was supported by the funds of the Khorramshar University of Marine Science and Technology, Iran.

## References

Ahmadifar, E., Falahatkar, B. and Akrami, R. 2011. Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, *Oncorhynchus mykiss*. Journal of Applied Ichthyology, 27: 1057-1060. doi: 10.1111/j.1439-0426.2011.01763.x;

Akrami, R., Hajimoradloo, A.M., Matinfar, A. and Abedian Kinari, A.M. 2009. Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, *Huso huso* (Linnaeus, 1758). Journal of the World Aquaculture Society, 40: 771-779. doi: 10.1111/j.1749-7345.2009.00297.x;

Akhter, N., Wu, B., Memon, A, M. and Mohsin, M. 2015. Probiotics and prebiotics accociated in aquaculture: a review. Fish and shell fish immonology, 45(2):733-741. doi: 10.1016/j.fsi.2015.05.038;

Al-Salahy, M.B. 2002. Some physiological studies on the effect of onion and garlic juices on the fish, *Clarias lazera*. Fish Physiology and Biochemistry, 27: 129-142. doi: 10.1023/B:FISH.0000021913.60189.76;

AOAC. 1995. Official Methods of Analysis of AOAC International. In: Association of Official Analytical Chemists, (16th edn) (ed. by P. Cunniff). AOAC InternationalArlington, Virginia, USA.

Apines-Amar, M.J.S., Amar, E.C., Faisan, J.P., Rolando, V., Pakingking, R.V. and Satoh, S. 2012. Dietary onion and ginger enhance growth, hemato-immunological responses, and disease resistance in brown-marbled grouper, *Epinephelus fuscoguttatus*. Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society, 4: 231–239.

Bello, O.S., Olaifa, F.E., Emikpe, B.O. and Ogunbanwo, S.T. 2012. The effect of Walnut (*Tetracarpidium conophorum*) leaf and Onion (*Allium cepa*) bulb residues on the tissue bacteriological changes of *Clarias gariepinus* juveniles. Bulletin of Animal Health and Production in Africa, 60: 205-212.

Benkeblia, N. and Shiomi N. 2006. Hydrolysis kinetic parameters of DP 6, 7, 8, and 9-12 fructooligosaccharides (FOS) of onion bulb tissues. Effect of temperature and storage time. Journal of agricultural and food chemistry, 54: 2587-2592. doi: 10.1021/jf052848i;

Binaii, M., Ghiasi, M., Farabi, SMV., Pourgholam, R., Fazli, H., Safari, R., Alavi, SE., Taghavi, MJ. and Bankehsaz, Z. 2014. Biochemical and hemato-immunological parameters in juvenile beluga (*Huso huso*) following the diet supplemented with nettle (Urtica dioica). Fish & shellfish immunology, 36: 46-51. doi: 10.1016/j.fsi.2013.10.001;

Breu, W. 1996. Allium cepa L.(onion) Part 1: Chemistry and analysis. Phytomedicine, 3: 293-306. doi: 10.1016/S0944-7113(96)80069-9;

Burr, G., Hume, M., Ricke, S., Nisbet, D. and Gatlin, D. 2010. In vitro and in vivo evaluation of the prebiotics GroBiotic®-A, inulin, mannanoligosaccharide, and galactooligosaccharide on the digestive microbiota and performance of hybrid striped bass (*Morone chrysops*× *Morone saxatilis*). Microbial ecology, 59: 187-198. doi: 10.1007/s00248-009-9597-6;

Cho, S. H. and Lee, S. M. 2012. Onion powder in the diet of the olive flounder, *Paralichthys olivaceus*: effects on the growth, body composition, and lysozyme activity. Journal of the World Aquaculture Society, 43: 30-38. doi:10.1111/j.1749-7345.2011.00489.x;

Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International, 18: 403-414. doi:10.1007/s10499-009-9253-7;

Demers, N. E. and Bayne, C. J. 1997. The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. Developmental and Comparative Immunology, 21: 363-373. doi: 10.1016/S0145-305X(97)00009-8;

Doumas, B. T., Watson, W. A. and Biggs, H. G. 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta, 31: 87-96. doi: 10.1016/S0009-8981(96)06447-9;

Effendy, J L., Simmons, DL., Campbell, GR. and Campbell, JH. 1997. The effect of the aged garlic extract,Kyolic, on the development of experimental atherosclerosis. Atherosclerosis, 132: 37-42. doi: 10.1016/S0021-9150(97)00078-6;

Galindo-Villegas, J. and Hosokawa, H. 2004. Immunostimulants: towards temporary prevention of diseases in marine fish. Advances en Nutricion. Acuicola VII Memorias del VII Simposium Internationale de Nutricion Acuícola 16-19.

Giannenas, I., Triantafillou, El., Stavrakakis, S., Margaroni, M., Mavridis, S., Steiner, T. and Karagouni, E. 2012. Assessment of dietary supplementation with carvacrol or thymol containing feed additives on performance, intestinal microbiota and antioxidant status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 350: 26-32. doi: 10.1016/j.aquaculture.2012.04.027;

Gibson, G. R. 2004. Fibre and effects on probiotics (the prebiotic concept). Clinical Nutrition Supplements, 1: 25-31. doi: http://dx.doi.org/10.1016/j.clnu.2004.09.005;

Griffiths, G., Trueman, L., Crowther, T., Thomas, B. and Smith, B. 2002. Onions—a global benefit to health. Phytotherapy Research, 16: 603-615. doi: 10.1002/ptr.1222;

Hajhashemi, V., Sadraei, H., Ghannadi, AR. and Mohseni, M. 2000. Antispasmodic and anti-diarrhoeal effect of Satureja hortensis L. essential oil. Journal of ethnopharmacology, 71: 187-192. doi: 10.1016/S0378-8741(99)00209-3;

Higgins, S. E., Higgins, J. P., Wolfenden, A. D., Henderson,S. N., Torres-Rodriguez, A., Tellez, G. and Hargis, B. 2008. Evaluation of a Lactobacillus-based probiotic culture for the reduction of Salmonella Enteritidis in neonatal broiler chicks. Poultry Science, 87: 27-31. doi: 10.3382/ps.2007-00210;

Jeong, C., Heo, HJ., Choi, S. and Shim, K. 2009. Antioxidant and anticancer properties of methanolic extracts from different parts of white, yellow, and red onion. Food Science and Biotechnology, 18: 108-112. doi: 10.1007/s10068-013-0228-0;

Kumar, S., Sahu, N.P., Pal, A.K., Choudhury, D., Yengkokpam, S. and Mukherjee, S.C. 2005. Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *L. rohita* juveniles. Fish and shellfish immunology, 19: 331-344. doi: 10.1016/j.fsi.2005.03.001;

Kumari, K. and Augusti, KT. 2007. Lipid lowering effect of S-methyl cysteine sulfoxide from *Allium cepa* Linn in high cholesterol diet fed rats. Journal of ethnopharmacology, 109: 367-371. doi: 10.1016/j.jep.2006.07.045;

Liu, L. and Yeh, Y. Y. 2002. S-alk (en) yl cysteines of garlic inhibit cholesterol synthesis by deactivating HMG-CoA reductase in cultured rat hepatocytes. The Journal of nutrition, 132: 1129-1134.

Madhuri, S., Mandloi, A.K., Govind,P. and Sahni, Y.P. 2012. Antimicrobial activity of some medicinal plants against fish pathogens, International research journal of pharmacy, 3: 28-30.

Mahious, A.S., Gatesoupe, F.J., Hervi, M., Metailler, R. and Ollevier, F. 2006. Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). Aquaculture International, 14: 219-229. doi: 10.1007/s10499-005-9003-4;

Merrifield, D.L., Dimitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Bøgwald, J., Castex, M. and Ringo, E. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, 302: 1-18. doi:10.1016/j.aquculture.2010.02.007;

Mourino, J.L.P., Nascimento, F.D.O., Vieria, A.B., Jatoba, B.C., Silva, D.A., Jesus, G.F.A., Seiffert, W.Q. and Martins, M.L. 2012. Effect of dietary supplementation of inulin and W. cibaria on haemato-immunological parameters of hybrid surubim (*Pseudoplatystoma sp*). Aquaculture Nutrition, 18: 73-80. doi :10.1111/j.1365-2095.2011.00879.x;

Olsen, R.E., Myklebust, R., Kryvi, H., Mayhew, T.M. and RingQ, E. 2001. Damaging effect of dietary inulin on intestinal enterocytes in Arctic charr (*Salvelinus alpinus* L.) Aquaculture Research, 32: 931-934. doi: 10.1046/j.1365-2109.2001.00626.x;

Ortiz, L., Rebole , A., Velasco, S., Rodriguez, M., Trevin o, J., Tejedor, J. and Alzueta, C. 2013. Effects of inulin and fructooligosaccharides on growth performance, body chemical composition and intestinal microbiota of farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition, 19: 475-482. doi: 10.1111/j.1365-2095.2012.00981.x;

Peter, H. and Sneath, A. 1986. Bergey's manual of systematic Bacteriology, 2: 1104–1154.

Pirbalouti, A. G., Jahanbazi, P., Enteshari, S., Malekpoor, F. and Hamedi, B. 2010a. Antimicrobial activity of some of the Iranian medicinal plants. Archive Biological Scicence, 62: 633-642.

Pirbalouti, A. G., Malekpoor, F., Enteshari, S., Yousefi, M., Momtaz, H. and Hamedi, B. 2010b. Antibacterial activity of some folklore medicinal plants used by Bakhtiari tribal in Southwest Iran. International Journal of Biology, 2: 55. doi: http://dx.doi.org/10.5539/ijb.v2n2p55;

Pirbalouti, G. A., Broujeni, N. V., Momeni, M., Poor, M. F. and Hamedi, B. 2011. Antibacterial activity of Iranian medicinal plants against Streptococcus iniae isolated from rainbow trout (*Oncorhynchus mykiss*). Archives of Biological Sciences, 63: 59-66. doi: 10.2298/ABS1101059P;

Ramos, F. A., Takaishi, Y., Shirotori, M., Kawaguchi, Y., Tsuchiya, K., Shibata, H., Higuti, T., Tadokoro, T. and Takeuchi, M. 2006. Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (*Allium cepa*) skin. Journal of Agricultural and Food Chemistry, 54: 3551-3557. doi: 10.1021/jf060251c;

Rattanachaikunsopon, P. and Phumkhachorn, P. 2010. Assessment of synergistic efficacy of carvacrol and cymene against Edwardsiella tarda in vitro and in Tilapia (*Oreochromis niloticus*). African Journal of Microbiology Research, 4: 420-425. doi: 10.5897/AJMR

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S. and Menasveta, P. 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture, 167: 301-313. doi: 10.1016/S0044-8486(98)00305-6;

Ringo, E., Olsen, R.E., Gifstad, T.O., Dalmo, R.A., Amlund, H., Hemre, G.I. and Bakke, A.M. 2010. Prebiotics in aquaculture: a review. Aquaculture Nutrition 16, 117-136. doi: : 10.1111/j.1365-2095.2009.00731.x;

Roberfroid, M. 2007. Prebiotics: the concept revisited. The Journal of nutrition, 137: 830-837.

Scholz-Ahrens, K. E. and Schrezenmeir, J. 2002. Inulin, oligofructose and mineral metabolism experimental data and mechanism. British Journal of Nutrition, 87: 179-186. doi: 10.1079/BJN/2002535;

Sharma, A.D., Kainth, S. and Gill, P.K. 2006. Inulinase production using garlic (*Allium sativum*) powder as a potential substrate in Streptomyces sp. Journal of Food Engineering, 77: 486-491. doi: 10.1016/j.jfoodeng.2005.06.072;

Silva, D.G., Cooper, P.D. and Petrovsky, N. 2004. Inulin-derived adjuvants efficiently promote both Th1 and Th2 immune responses. Immunology and cell biology, 82: 611-616. doi:10.1111/j.1440-1711.2004.01290.x;

Srinivasan, K. 2005. Plant foods in the management of diabetes mellitus: spices as beneficial antidiabetic food adjuncts. International journal of food sciences and nutrition, 56: 399-414. doi: 10.1080/09637480500512872;

Suh, H.J., Lee, J.M., Cho, J.S., Kim, Y.S. and Chung, S.H. 1999. Radical scavenging compounds in onion skin. Food Research International, 32: 659-664. doi: : 10.1016/S0963-9969(99)00141-6;

Urios, P., Grigorova-Borsos, A.M. and Sternberg, M. 2007. Flavonoids inhibit the formation of the cross-linking AGE pentosidine in collagen incubated with glucose, according to their structure. European journal of nutrition, 46: 139-146. doi: 10.1007/s00394-007-0644-0;

Van Loo, J., Cummings, J., Delzenne, N., Englyst, H., Franck, A., Hopkins, M., Kok, N., Macfarlane, G.,

Newton, D. and Quigley, M. 1999. Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). British Journal of Nutrition, 81: 121-132. doi: http://dx.doi.org/10.1017/S0007114599000252;

Volpatti, D., Chiara, B., Francesca, T. and Marco G. 2012. Growth parameters, innate immune response and resistance to *Listonella* (*Vibrio*) *anguillarum* of *Dicentrarchus labrax* fed carvacrol supplemented diets. Aquaculture Research, 45: 31-44. doi: 10.1111/j.1365-2109.2012.03202.x;

Wenk, C. 2003a. Growth promoter alternatives after the ban on antibiotics. Pig News and Information, 24: 11-16.

Wiegertjes, G.F., Stet, R.J.M., Parmentier, H.K. and Van Muiswinkel, W.B. 1996. Immunogenetics of disease resistance in fish: a comparative approach. Developmental and Comparative Immunology, 20: 365-381. doi: 10.1016/S0145-305X(96)00032-8;

Yeh Y.Y., Lin R.I., Yeh S.M. and Evens S. 1997. Garlic reduces plasma cholesterol in hypercholesterolemic men maintaining habitual diets. Food Factors for Cancer Prevention. Springer.

Zheng, Z.L., Tan, J.Y.W., Liu, H.Y., Zhou, X.H., Xiang, X. and Wang, K.Y. 2009. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against Aeromonas hydrophila in channel catfish (*Ictalurus punctatus*). Aquaculture, 292: 214-218. doi: 10.1016/j.aquaculture.2009.04.025;