Short Communication

Effects of dietary inulin on growth performance, survival, body composition, stress resistance and some hematological parameters of Gibel carp juveniles (*Carassius auratus gibelio*)

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Improving and protecting fish health in commercial production practices is a major factor in the aquaculture industry. This has prompted new initiatives toward the development/appraisal of novel agents or functional dietary supplements in commercial feeds for fish and crustacean species. Prebiotics are nondigestible carbohydrates that selectively stimulate the growth and metabolism of health-promoting bacteria already present in the host gut. This leads to increased growth rate and better health of the host (Ahmadifar *et al*., 2011). Dietary supplements including probiotics and prebiotics such as inulin and immunostimulants have shown promise as preventive and environmentally friendly alternatives to antibiotics in aquaculture, especially for fishes (Genc *et al*., 2007). Criteria which allow the classification of a food ingredient as a prebiotic include: (1) it must be neither hydrolyzed, nor absorbed in the upper part of the gastrointestinal tract. (2) Selective fermentation by one or a limited number of potentially beneficial bacteria in the colon. (3) Alteration of the composition of the colonic microbiota towards a healthier composition (Fooks and Gibson, 2002). Inulin, is a polydisperse carbohydrate consisting mainly of β (2→1) fructosyl-fructose links, generally referred to as fructan and is found in a variety of

edible fruits and vegetables such as wheat, onions, leeks, garlic, asparagus, artichokes and bananas. Although inulin is not a natural fibre in fish diets, the prebiotic potential of inulin and other dietary fibres may have interesting application in aquaculture by stimulating beneficial gut bacteria and suppressing the potentially deleterious bacteria (Ringø *et al*., 2006). Dietary supplementation of the polysaccharide prebiotic inulin has been shown to enhance the growth rate of Siberian sturgeon (*Acipenser baerii*) (Mahious and Ollevier, 2005), African catfish (*Clarias gariepinus*) (Mahious and Ollevier, 2005), Turbot larvae (*Psetta maxima*) (Mahious *et al*., 2006), Nile tilapia (*Oreochromis niloticus*) (Ibrahem *et al*., 2010) and Kutum (*Rutilus frisii*) fry (Mira *et al*., 2011). It has been reported that dietary inulin affects the microbiota of Beluga (*Huso huso*) juvenile (Akrami *et al*., 2009) and Hybrid surubium (*Pseudoplatystoma* sp) (Mourino *et al*., 2012). Additionally, several studies have indicated that dietary inulin may influence hematological and/or serum biochemical parameters (Sheikholeslami Amiri, 2008; Ahmadifar *et al*., 2011; Mourino *et al*., 2012), which are valuable indicators of fish health and wellbeing (Hoseinifar *et al*., 2011). *Carassius auratus gibelio* is an improved strain of carp and suitable fish model for carrying out *in vivo* aquaculture experiments. The purpose of the present study was to determine the effects of inulin as a prebiotic on growth performance, survival, body composition, stress resistance and some hematological parameters of gibel carp (*C.auratus gibelio*) juvenile.

The fish were acclimated to 500 liter tank (1 m×1 m×0.5 m) and fed on a control diet (BioMar) for 7 days before the experiment began and then 240 Gibel carp juveniles with an initial mean body weight of 3.5±0.2 g were randomly distributed in 12 aquaria (110-cm length× 42-cm width× 50-cm height), with 20 fish per aquaria, with three replicates per diet. Continuous aeration was provided to each aquarium through an air stone connected to a central air compressor. Water quality parameters such as dissolved oxygen (6.5±0.2 mgL^{-1}), temperature (25.7±2.5 °C) and pH value (7.3±0.2) were measured every day during the experimental period.

A commercial pellet diet (BioMar, France; containing 38.87% protein, 15% lipid, 11.1% ash, 0.6% fiber and 20. 3 MJ/Kg GE) supplemented with 0 (control), 0.5, 1.0 and 1.5 g inulin kg^{-1} (ORAFTI, Belgium) in triplicate replication for each group. To prepare the diets, a commercial pellet diet was crushed, mixed with the appropriate inulin concentration and water. The pelleted diets were air-dried, ground and sieved to produce a suitable crumble (0.5 mm). Then the feed was stored at 4 °C until feeding trial. Control diet was prepared adding only water (Cerezuela *et al*., 2008). During the 8-week trial, the fish were fed with the amount of feed equal to 5% body

weight to apparent satiation 3 times per day at 8:00, 14:00 and 20:00. Uneaten food and feces were siphoned out before next feeding.

Fish in each aquarium were weighed once every 2 weeks and counted to record growth and determine the daily ration. At the end of trial, growth performance (weight gain (WG), body weight index (BWI), specific growth rate (SGR), condition factor (CF)) and feed utilization (feed conversion ratio (FCR) and feed intake) were calculated.

At the end of the experiment, proximate analysis of the fish carcasses from each treatment were determined according to standard method (AOAC, 1996). Crude protein content was determined by Kjeldahl method using auto Kjeldahl system, crude lipid content by soxhlet extraction method, ash content by a furnace muffler (550 $^{\circ}C$ for 4 h), and moisture content by a dry oven (105 °C for 24 h).

The analysis of intestinal microbiota was conducted at the end of the nutrition trial. Three fish were sampled in each treatment after cease of feeding for 48 h. The fish were killed by physical destruction of the brain 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]. The suspension, serially diluted to 10^{-6} and 0.1 ml of the solution, was spread in triplicates on nutrient agar (NA). DeMan, Rogosa, and Sharpe (MRS) (general term of MRS media) were also used to detect lactic acid bacteria (LAB). All of the plates were incubated at room temperature (25°C) and examined for 5 days (Rengpipat *et al*., 1998; Mahious and Ollevier, 2005), and then the numbers of colonies were counted. Identification of the samples was carried out according to Bergey's method (Peter and Sneath, 1986).

At the end of the feeding trial, about 2 ml blood sample was drawn from the caudal vein of three fish from each aquarium after they were starved for 24 h. In order to study the hematological parameters, the blood samples were suspended in heparinized tube and then values of red blood cell (RBC), white blood cell (WBC), hematocrit (Hct), hemoglobin (Hb), were measured (Feldman *et al*., 2000). Differential white blood cell counts, including neutrophil, lymphocyte, monocyte and eosinophil were also identified (Borges *et al*., 2004).

In order to carry out stress challenge, ten fish per treatment were randomly starved for 24 h prior to challenge. Then, they were exposed to high temperature (33 °C), acidity (pH=2, HCl), alkalinity (pH=12, NaOH) and salinity stress (35 gL^{-1}) according to suggested method by Jafaryan *et al*. (2011). Sulfuric acid (H_2SO_4) and sodium-silicate added into water to decrease and increase pH. The death time of last fish in these solutions were recorded based on second and their survival times were evaluated.

The normality and homogeneity of data were explored by examining the residual plots. The data were subjected

to one-way analysis of variance (ANOVA) and if significant differences (p<0.05) were found, Duncan's multiple range test was used to rank the groups using SPSS (version 18) software.

At the end of the study, fish fed 1.5 g inulin kg^{-1} supplemented diet displayed improved growth performance and feed utilization, including final weight, WG, BWI, SGR, (p<0.05). There were no significant differences (p>0.05) in FCR and CF among control group with other groups (Table 1).

Table 1: Growth and feed utilization of Gibel carp fed with different dietary levels of inulin during 60 days.

Levels of prebiotic	Control	0.5 g Inulin kg^{-1}	1.0 g Inulin kg^{-1}	1.5 g Inulin kg^{-1}
Parameters				
Initial weight (g)	5.2 ± 0.05	5.27 ± 0.03	5.27 ± 0.03	5.22 ± 0.07
Final weight (g)	13.7 ± 0.25 ^{a}	15.34 ± 0.37 ^{b}	16.6 ± 0.75 ^{bc}	17.24 ± 1.13 ^{c}
WG (g)[1]	8.51 ± 0.19 ^{a}	10.06 ± 0.4 ^{b}	11.32 ± 0.74 ^{bc}	12.02 ± 1.16 ^{c}
BWI (%)[2]	163.9 ± 2.16 ^{a}	191.03 ± 8.42 ^{ab}	215.00 ± 14.00 ^{bc}	230.46 ± 23.83 ^{c}
SGR (%/day)[3]	1.61 ± 0.01 ^{a}	1.78 ± 0.05 ^{b}	1.91 ± 0.07 ^{bc}	1.99 ± 0.12 ^{c}
FCR[4]	2.75 ± 0.06	2.61 ± 0.1	2.6 ± 0.41	2.3 ± 0.22
CF [5]	1.08 ± 0.08	1.05 ± 0.08	1.06 ± 0.18	0.98 ± 0.08
Survival (%)	100.00 ± 00.00	100.00 ± 00.00	93.3 ± 11.5	100.00 ± 00.00
Feed intake (%/day)	4.63 ± 0.07 ^{a}	4.87 ± 0.05 ^{b}	4.66 ± 0.09 ^{a}	4.81 ± 0.14 ^{ab}

Data in the same row with different superscripts are significantly different (p<0.05).
WG: Weight gain, BWI: Body weight index, SGR: Specific growth rate, FCR: Feed conversion ratio, CF: Condition factor.

Whole body crude lipid of fish fed 1.0 g inulin kg^{-1} was significantly higher than other groups (p<0.05). Whole body crude protein was not different among dietary treatments. But fish fed with 1.5 g inulin kg^{-1} had higher protein content compared to other groups (Table 2).

Table 2: Body proximate composition (% dry matter) of Gibel carp fed with different dietary levels of inulin during 60 days.

Levels of prebiotic / Composition (%) body	Control	0.5 g Inulin kg^{-1}	1.0 g Inulin kg^{-1}	1.5 g Inulin kg^{-1}
Crude protein	20.36 ± 0.55	20.22 ± 0.62	20.17 ± 0.7	20.36 ± 0.02
Crude lipid	12.76 ± 0.14 ^{bc}	12.32 ± 0.35 ^{b}	14.29 ± 0.04 ^{a}	12.36 ± 0.44 ^{c}
Dry matter	32.43 ± 0.6	32.81 ± 0.74	33.35 ± 0.8	32.06 ± 0.46

Data in the same row with different superscripts are significantly different (p<0.05).

The results showed that with increased supplementation level of inulin, the total heterotrophic autochthonous bacteria and lactic acid bacteria count levels (LAB) increased (p<0.05) (Table 4).

Table 3: Bacteria counts of the intestinal tract of Gibel carp fed with different dietary levels of inulin during 60 days.

Levels of prebiotic / Bacteria counts log (CFU g^{-1})	Control	0.5 g Inulin kg^{-1}	1.0 g Inulin kg^{-1}	1.5 g Inulin kg^{-1}
Total bacteria	6.86 ± 1.94 [ab]	6.88 ± 1.2 [ab]	8.1 ± 0.79 [a]	8.95 ± 0.7 [a]
Lactic acid	4.32 ± 0.04 [b]	4.73 ± 0.5 [b]	6.13 ± 0.62 [a]	6.83 ± 0.03 [a]

Data in the same row with different superscripts are significantly different (p<0.05).

Resistance rate to alkalinity and thermal stress increased significantly in groups treated with 0.5 and 1.0 g inulin kg^{-1} respectively, compared with control group (p<0.05). Also a non significant elevation of acidity and salinity stress were observed in the fish fed diet 1.5 g inulin kg^{-1} (p>0.05) (Table 4).

Table 4: Resistance to environmental stress in Gibel carp fed with different dietary levels of inulin during 60 days based on second.

Levels of prebiotic / Challenge test	Control	0.5 g Inulin kg^{-1}	1.0 g Inulin kg^{-1}	1.5 g Inulin kg^{-1}
Alkalinity stress	29.5 [a] ± 386.00	12.00 [b] ± 583.6	53.9 [b] ± 555.6	178.4 [b] ± 553.3
Acidity stress	257.3 ± 1325.00	176.4 ± 1393.3	279.4 ± 1480.3	46.9 ± 1506.3
Salinity stress	274.1 ± 3466.3	363.8 ± 3628.7	188.7 ± 3527.7	311.9 ± 3876.7
Thermal stress	81.3 ± 9.7 [a]	127.00 ± 31.00 [b]	149.00 ± 16.5 [b]	145.00 ± 14.1 [b]

Data in the same row with different superscripts are significantly different (p<0.05).

The effects of dietary supplementation of inulin on hematological parameters are presented in Table 5. Statistical analysis of data showed that there were no significant differences in hematological parameters such as WBC, RBC, haemoglobin, haematocrit, neutrophil, lymphocyte, monocyte and eosinophil between treatment groups (p>0.05).

Table 5: Hematological parameters of Gibel carp fed with different dietary levels of inulin during 60 days.

Levels of prebiotic / Parameters	Control	0.5 g Inulin kg^{-1}	1.0 g Inulin kg^{-1}	1.5 g Inulin kg^{-1}
WBC (per/mm^3)	14.80 ± 1.68	16.10 ± 0.85	14.66 ± 0.75	15.36 ± 1.04
RBC (per/mm^6)	1.66 ± 0.01	1.60 ± 0.03	1.58 ± 0.15	1.60 ± 0.10
Hb (g/dL)	11.26 ± 0.32	10.73 ± 0.45	11.16 ± 0.66	11.06 ± 0.64
Hct (%)	40.03 ± 0.65	38.60 ± 1.05	39.66 ± 1.97	39.33 ± 2.02
Neutrophil (%)	14.00 ± 2.64	14.33 ± 4.04	13.66 ± 4.04	15.33 ± 1.52
Lymphocyte (%)	81.33 ± 3.05	80.00 ± 3.60	81.33 ± 3.21	78.66 ± 2.08
Monocyte (%)	3.00 ± 1.73	2.66 ± 1.52	2.33 ± 0.57	3.00 ± 1.00
Eosinophil (%)	1.66 ± 0.57	2.66 ± 0.57	2.33 ± 0.57	2.66 ± 1.52

Data are presented as mean±S.D.
WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, Hct: hematocrit.

In the present study, continuous oral administration of inulin caused the improve growth of Gibel carp. This is in agreement with the results from previous studies in some fish species. Mira *et al*. (2011) used 0, 0.5, 1.0 and

1.5 % inulin to evaluate the effect on growth performance of kutum (*Rutilus frisii*) fry and found significantly improved growth performance and feeding efficiency in fish fed the diet supplemented with 0.5 % inulin compared with controls, and also like turbot, (*Psetta maxima*) (Mahious *et al*., 2006). Mahious and Ollevier (2005) investigated the effect of inulin and oligofructose on growth performance of the Siberian sturgeon (*A. baerii*) and the African catfish (*C. gariepinus*) and found that dietary oligofructose improved the final mean weight, SGR and FCR between treatment groups. Ibrahem *et al*. (2010) investigated the effect of different levels of inulin and vitamin C on Nile tilapia (*O. niloticus*) and showed that the body weight gain, specific growth rate and survival were significantly increased (p<0.05) in group supplemented with inulin and vitamin C. Improved growth performance is likely to be brought about by elevated digestive enzyme activities, possible improvements of intestine morphology or via prebiotic fermentation by endogenous gut microbes to produce short chain fatty acid (SCFAs).

In contrast to positive effects of inulin, studies by Sheikholeslami Amiri (2008) on rainbow trout (*Oncorhynchus mykiss*), Akrami *et al*. (2009) on Beluga (*Huso huso*) juvenile and Khosravi (2010) on Caspian roach (*R. rutilus caspicus*) showed different levels of inulin made no significant difference on growth and survival between fish fed control and inulin supplementation diets. This contradictory result may be attributable to the low dosage, different duration of prebiotic administration, life stage and/or different fish species (Soleimani *et al*., 2012). Measurements of the carcass chemical composition have been used as a reliable index to estimate nutritional conditions and growth of fish larvae (Pooramini *et al*., 2009). Our study indicated that no significant differences were found in protein in the carcass composition of Gibel carp juveniles among treatment groups, but the group treated with 1 g inulin kg^{-1} showed significant difference in crude lipid carcass. Higher body protein content in the group treated with 1.5 g inulin kg^{-1} stresses on this fact that by application of inulin, the ingested food was converted more effectively into structural protein and subsequently resulted in more muscle production which is a desirable aspect in fish farming (Mehrabi *et al*., 2012). Also it seems that supplementing diets with the inulin improved fatty acid utilization in Gibel carp. Unlike this study, Akrami *et al*. (2009), Khosravi (2010) and Mira *et al*. (2011) explained that inulin had no effects on the body composition in beluga (*H. huso*), Caspian roach (*R. rutilus caspicus*) and Kutum (*R. frisii*) fry, respectively.

The immunostimulatory nature of prebiotics may be attributed to stimulation of the growth of beneficial bacteria such as lactic acid bacteria. In the present study, the fish fed with 1.5 g inulin kg^{-1} displayed a significant

increase of total aerobic bacteria and LAB levels compared to the control group (p<0.05). Increased lactic acid bacteria were observed in the intestinal tract of Gibel carp fed with a diet containing inulin is in agreement with results of Akrami *et al*. (2009) investigating the effects of inulin on the intestinal microflora of Beluga (*H. huso)* juvenile and results of Mourino *et al*. (2012) on the effects of inulin and *Weissella cibaria* on hybrid surubium (*Pseudoplatystoma* sp). The inulin used as a prebiotic in this study is from fructan-based ingredients and is not fully digestible. It can contribute to the growth of the animals by increasing the lactic acid bacteria population in the intestinal tract. In large intestines, the increase in short-chain fatty acids and lactic acid from the fermentation of inulin lead to a decrease in pH, which 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 *et al*., 2009). Unlike this study, Olsen *et al*. (2001) observed that high level of dietary inulin (15% dietary inclusion) had negative effects on the gastrointestinal tract of Arctic char (*Salvelinus alpinus*). According to Ringø *et al*. (2006) substituting dextrin with 15% inulin reduces the bacterial population in the hindgut of Arctic charr (*S. alpinus*).

Environmental stress has often been used as a final indicator of fish quality after nutrition trials. The results of present study showed that resistance rate to environmental stress increased in fish fed with diet supplemented with inulin than those fish fed with the control diet. In a study with Grass carp (*Ctenopharyngodon idella*) and tilapia (*Tilapia aureus*), Wang and Wang (1997) showed that although survival rates against *Aeromonas hydrophila* and *Edwardsiella tarda* were higher, the values were not significantly different from that of the control fish. Sheikholeslami Amiri (2008) explained that rainbow trout fed with 0.5% and 2.0% inulin had significantly higher survival to bacteria (*streptococcus*) challenge compared to the control group.

The analysis of blood can reveal internal biochemical changes, physiological condition, and living situation of fish. Also it can point up for us the pollutants, nutrition, stress and ecological conditions and in general the status of fish (Yousefian *et al*., 2012). In the present study, supplementation with inulin had no effects on hematological parameters such as WBC, RBC, hemoglobin, hematocrit, neutrophil, lymphocyte, monocyte and eosinophil between experimental groups (p>0.05). Opposite to our results, application of inulin in beluga (*H. huso*) juvenile (Ahmadifar *et al*., 2011) and hybrid surubim (Mourino *et al*., 2012) diet had significant effect on hematological parameters. Based on the Cerezuela *et al*. (2008) results, inulin does not seem to be good immunostimulant for seabream (*Sparus aurata*). Sheikholeslami Amiri (2008)

found that administration of inulin has a positive effect on rainbow trout (*O. mykiss*) hematological parameters. The lack of hematological response to inulin in Gibel carp probably be due to the gut system which has the mechanisms to increase absorptive surface area including intestine wall folding with long length. Fluctuation of lymphocyte and eosinophil counts in the present study hint at a possible immunomodulatory effect; however, additional studies are required to assess the effects of inulin on the immune response of Gibel carp. The reasons for different results are not clear yet. It appears that the different basal diet, level of inulin supplementation, type of inulin, adaptation period, chemical structure, origin of inulin, animal characteristics (species, age, stage of production), period and hygienic conditions of the experiment can be causing these differences.

This study corroborates the functionality of inulin in the diet of Gibel carp which positively affects growth performance, beneficial intestinal microbiota and stress resistance.

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