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Effects of Dietary Supplementation with Immunogen® on Growth, Hematology and Gut Microbiota of Fingerling Common Carp *Cyprinus carpio*

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Abstract

We studied the effects of the proprietary prebiotic Immunogen® on the growth, hematology and gut microbiota of common carp fingerlings. A basal diet was formulated using common feed ingredients and supplemented with Immunogen® at concentrations of 0, 5, 10, 20 and 40 g kg^{-1}, each of which was tested experimentally on replicated groups of fish. The trials ran for 8 weeks. Common carp fingerlings with an initial weight of 4.82 ± 0.05 g were randomly distributed among the experimental tanks at a stocking density of 25 fish per tank. The experimental diets were provided thrice per day; on each occasion the fingerlings were given a weight of feed that amounted to 4% of fish biomass. At the end of the experimental period, we determined the growth performance, feed conversion ratio, hematological parameters, body composition and gut micro-flora parameters of the test fish. Inclusion of 5 g kg^{-1}Immunogen® in the diet significantly improved growth performance and feed utilization in comparison with controls. However, the whole-body composition of the fish was not significantly influenced by prebiotic inclusion. Inclusion of 5 g kg^{-1} Immunogen® significantly increased the total bacterial and *Lactobacillus* counts in fish intestines, but these bacterial parameters were significantly negatively impacted by higher concentrations of the prebiotic. Red blood cells counts were increased by prebiotic dietary supplementation at concentrations of 5 and 10 g kg^{-1} prebiotic. Glucose and cholesterol levels were elevated by administration of Immunogen®. Thus, dietary supplementation with 5 g kg^{-1} Immunogen® improved fingerling common carp growth performance and feed utilization, and beneficially influenced the gut microflora.

Key words: *Cyprinus carpio* Common carp, Immunogen®, Prebiotic, Growth, Feed utilization, Intestinal microflora

Introduction

A protracted era of research on prebiotics has promoted the general belief that dietary supplements containing these compounds have health benefits for humans (Burr et al., 2005; Gatlin et al., 2006; Merrifield et al., 2010; Ringo et al., 2010). Prebiotics are non-digestible food ingredients that beneficially affect the consumer by selectively stimulating the growth and/or the activities of specific health-promoting gut bacteria (Gibson and Roberfroid, 1995).

The recent rapid expansion of aquaculture has been limited by the restricted availability of natural resources, such as fresh water and land, which in turn has led to the intensification of fish farming (Klinger and Naylor, 2012). Agricultural intensification frequently increases physiological stress and the incidence of disease in cultured fish. Stressed fish have a reduced ability to resist negative biological and physicochemical factors in their immediate environment. Stress is considered to

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be a primary contributory factor that leads to impaired health in cultured fish (Iwama et al., 1997). Antibiotics have been used at sub-therapeutic levels to prevent disease and enhance growth performance in fish farms. However, negative collateral effects, such as the evolution of antibiotic-resistant bacteria, makes the use of antibiotic growth promoters relatively undesirable (Cabello, 2006), and the use of these compounds is now limited by regulatory restrictions.

Since the early years of the present century, prebiotics have been extensively tested for potentially beneficial effects on fish health, growth and survival (Mussatto and Mancilha, 2007; Grisdale-Helland et al., 2008; Yousefian and Amiri, 2009; Ebrahimi et al., 2012; Amirkolaie et al., 2013). This research has demonstrated that elevating the levels of these non-digestible dietary ingredients can have wide-ranging beneficial effects on the growth and survival of the host fish via alterations in the bacterial composition of the gut flora (Gibson and Roberfroid, 1995; Mei et al., 2011). Furthermore, prebiotics can improve growth parameters, disease resistance, gut villous surface area and microvillus length (Genc et al., 2007; Li et al., 2007; Staykov et al., 2007; Zhou et al., 2007; Torrecillas et al., 2007; Burr et al., 2008; Salzeet al., 2008). Immunogen®, which is a commercial prebiotic, contains diverse stimulatory components, such as mannan-oligosaccharide and β-glucans, which have been used as feed additives in diverse animals. Supplementation with Immunogen® improved the growth performance of the common carp *Cyprinus carpio* (Ebrahimi et al., 2012) and the rainbow trout *Oncorhynchus mykiss* (Amirkolaie et al., 2013), and the reproductive performance of the platy *Xiphophorus maculates* (Hajibeglou and Sudagar, 2011).

Available literature describes the effects of prebiotics on general fish performance and health-related parameters, but there is little information on the effects of Immunogen® inclusion on the performance of common carp or its gut micro-flora. Previous studies on prebiotics have focused largely on the effects of single oligosaccharides; however, little is known of the efficacy of carbohydrate mixtures. Thus, our main goal was to assess the effects of the Immunogen® mixed prebiotic supplement on the performance of the common carp and its gut micro-flora.

Materials and Methods

Experimental fish and design of the trials

This study was carried out at the experimental facility in the Jahade-Daneshgahi Aquaculture Complex, Sari, Iran. Common carp fingerlings were bred in the reproduction facility in the complex and acclimated to the experimental conditions for several days before the start of the trials. After acclimation, the fingerlings (average weight: 4.82 ± 0.05 g) were divided randomly among 15 160-L tanks at a stocking density of 25 per tank.

We formulated a basal diet using locally grown feed ingredients (Table 1). Five experimental diets were prepared. Each contained a different dose of Immunogen®: 0, 5, 10, 20 and 40 g kg^{-1}.The trials ran for 8 weeks. The Immunogen® prebiotic used in this study comprised mannan-oligosaccharide (18%), β-glucans (1-3, 1-6) (30%), protein (33%), ash (9%), moisture (8%) and fiber (2%) (components provided by Soroush Radian Co., Tehran, Iran).

Table 1. Formulation and proximate composition of experimental diets (g kg^{-1})

Experimental diet	Control	Immunogen 5	Immunogen 10	Immunogen 20	Immunogen 40
Soybean meal	210	210	210	210	210
Fish meal	340	340	340	340	340
Wheat meal	100	100	100	95	95
Corn meal	60	60	60	60	60
Wheat gluten	25	25	25	30	30
Meat meal	120	120	120	120	120
Cotton seed meal	40	40	40	40	40
Cellulose	40	35	30	20	0
Molasses	20	20	20	20	20
Salt (NaCl)	5	5	5	5	5
Immunogen	0	5	10	20	40
Dicalcium phosphate	10	10	10	10	10
Vit& Min. Premix	30	30	30	30	30
Nutrient composition of the experimental diets diet in g kg^{-1}					
Dry matter	915.2	910.4	912.6	918.4	909.8
Crude protein	375.3	375.4	379.4	378.3	377.9
Crude fat	107.4	107.6	108.1	106.9	107.8
Crude ash	98.2	99.2	97.2	99.3	97.9

Experimental procedure

The fish were fed experimental diets (4% of fish biomass on each occasion; Takeuchi et al., 2002) three times per day (08.00, 13.00 and 18.00). Each of the different diets was randomly assigned to one of 15 tanks; each diet was replicated thrice. Water quality parameters were monitored daily to ensure that they were in an appropriate range for the fish to thrive. The ranges of water temperature and pH were 25–26℃ and 7.3–7.9, respectively, during the experiment. Oxygen concentrations were measured in a randomly selected tank using a digital oxygen detector; O_2 concentrations were always > 6.1 mg L^{-1}.

On day 56, all fish were weighed. Five specimens were then randomly selected from each tank and sacrificed using an overdose of clove essence solution. We measured the body compositions of these individuals. Three fish were also randomly selected from each tank and immediately anesthetized with clove essence solution (20 mg L^{-1}), then subjected to blood parameter measurements. We collected 1.5 mL of blood from the caudal blood vessels of each selected fish using a heparinized syringe containing 3 mg Na_2-EDTA. The samples were shaken gently and kept at 4°C. For plasma measurements, we transferred *ca*. 1 mL of each sample into cooled 1.5 mL plastic tubes, mixed the contents and centrifuged them at 6,000 *g* for 5 min at 4°C. After centrifugation, we collected the plasma for storage at −20°C until further analyses were performed. Total plasma protein content was determined following the procedures of Henry (1964). Albumin content was estimated colorimetrically following the procedures of Wotton and Freeman (1982). Albumin values were subtracted from total protein values to estimate the globulin concentrations. The plasma glucose concentration was measured colorimetrically following the procedures of Trinder (1969). We used 0.5 mL of each fresh blood sample to count leucocytes and erythrocytes and measure hematocrit and hemoglobin concentrations. Red and white blood cells were counted following the procedures of Schalmet al. (1975). Hemoglobin (Hb) and hematocrit (Ht) concentrations were determined following the procedures of Barros et al. (2002).

Chemical analyses and bacterial counts

We analyzed collected fish and feed samples for measurements of dry matter, ash, crude protein and lipid contents. Dry weights were obtained after drying for 24 h at 103ºC, when constant weight had been reached (ISO 6496 1983). Ash content was determined by incineration in a muffle furnace for 4 h at 550ºC (ISO 5984, 1978). Crude protein (N × 6.25) was measured by the Kjeldahl method after acid digestion, in accordance with the ISO 5983 (1979) protocol. Lipid was extracted with petroleum ether in a Soxhlet apparatus (ISO 6492, 1999).

At the end of the experimental period, we collected three fingerlings from each treatment tank and counted bacteria in intestinal samples taken from them (total and *Lactobacillus* counts). Prior to dissection and homogenization, the fry were rinsed in sterilized distilled water, cleaned with ethanol (70.0%) and then washed again with sterilized distilled water to eliminate all superficial bacteria. The intestinal samples were dissected out under sterile conditions. Subsequently, three samples from the mid portion of each intestine were collected and diluted with sterilized normal saline solution (0.85% NaCl w/v). The diluted samples were transferred to nutrient agar and Lactobacilli MRS plates, on which we counted total and *Lactobacillus* bacteria, respectively (Ebrahimi et al., 2012).

Statistical analysis

Data are presented here as means ± SD for each treatment. Proportional data (as %) were arcsine transformed and tested for normality (Kolmogorov-Smirnov test).We used one-way ANOVA to detected significant effects of the Immunogen treatments on fish performance parameters and bacterial counts. Tukey's test was used for multiple pairwise comparisons between means. Significance effects were identified when $P < 0.05$ in each of the statistical tests applied. Individual tanks were treated as experimental units in all analyses.

Results

Details of fish growth performances are listed in Table 2. Highest weights were attained when fish were treated with 5 g kg^{-1} Immunogen® ($P < 0.05$). The feed conversion ratio (FCR) and specific growth rate (SGR) were also significantly improved in comparison with the controls at this concentration of prebiotic diet ($P < 0.05$). However, growth-related parameters were similar to control group fish in treatments supplied with 10, 20 and 40 g kg^{-1} Immunogen®. Fish survival rates were not affected by prebiotic supplementation.

The inclusion of dietary prebiotic did not affect the body chemical composition of *C. carpio* (Table 3), but it did influence the intestinal microflora (Table 4). Total counts of bacteria and *Lactobacillus* bacterial counts were elevated by the addition of 5g kg^{-1} Immunogen®, but the counts were lower in fish fed prebiotics at concentrations of 20 and 40 g kg^{-1} ($P < 0.05$) than in control fish.

Fish blood parameters were also influenced by the inclusion of Immunogen® in the diet ($P < 0.05$; Table 5). Red blood cell counts increased with Immunogen® supplementation at concentrations of 5 and 10 g kg^{-1}, but not in treatments supplied with 20 or 40 g kg^{-1} Immunogen®, in which counts were similar to the controls. Hemoglobin and hematocrit values showed similar trends. Fish fed 20 g kg^{-1} Immunogen® had significantly lower white blood cell counts than the controls. Glucose and cholesterol levels significantly increased with the

Table 2. Growth performance and feed utilization of common carp fed different levels of Immunogen® for 56 days experimental period

Parameters	Diets				
	Control	Immunogen 5	Immunogen 10	Immunogen 20	Immunogen 40
Initial weight (g)	4.81 ± 0.04	4.82 ± 0.01	4.82 ± 0.05	4.82 ± 0.06	4.81 ± 0.05
Final weight (g)	10.31 ± 0.16[b]	12.28 ± 0.10[a]	9.7 ± 0.12[b]	9.35 ± 0.12[b]	8.8 ± 0.04[b]
SGR (%/day)[1]	0.55 ± 0.01[b]	0.67 ± 0.01[a]	0.50 ± 0.01[ab]	0.47 ± 0.01[ab]	0.44 ± 0.02[ab]
FCR[2]	2.67 ± 0.14[b]	2.5 ± 0.09[a]	2.73 ± 0.1[a]	2.94 ± 0.08[b]	3.19 ± 0.28[b]
PER[3]	0.98 ± 0.03[b]	1.09 ± 0.02[a]	0.97 ± 0.04[b]	0.90 ± 0.03[c]	0.85 ± 0.04[c]
Survival rate (%)[4]	100	100	100	100	100

All values are means ± standard deviation of triplicate tanks/treatment (N = 3). Different superscript letters in same row show significant differences ($P < 0.05$)

[1]Specific growth rate (SGR) = 100 × (Ln $Weight_{final}$ - Ln $Weight_{initial}$) $/days^{-1}$

[2]Feed conversion ratio (FCR) = dry feed consumed (g) / wet body weight gain (g)

[3]Protein efficiency ratio (PER) = protein consumed (g) / weight gain (g)

[4]Survival rate (SR) = number of fish at the end of the experiment / number of fish at the beginning

Table 3. Body composition in common carpfeeding on different levels of Immunogen® for 56 days experimental period

Proximate composition	Diets				
	Control	Immunogen 5	Immunogen 10	Immunogen 20	Immunogen 40
Dry mater	28.66 ± 0.77	29.1 ± 0.48	29.55 ± 0.49	29.15 ± 0.18	29.63 ± 0.27
Protein	14.2 ± 1.1	15.33 ± 0.78	15.35 ± 1.15	15.65 ± 0.86	15.53 ± 0.43
Fat	9.21 ± 0.22	9.6 ± 0.26	9.44 ± 0.56	9.36 ± 0.6	9.44 ± 0.17
Ash	3.3 ± 0.49	2.91 ± 0.13	3.43 ± 0.94	3.21 ± 0.26	3.07 ± 0.74

All values are means ±standard deviation of triplicate tanks/treatment (N = 3).

Table 4. Bacterial counts in the intestine of common carp feeding on different levels of Immunogen® for 56 days experimental period

Bacterial community	Diet				
	Control	Immunogen 5	Immunogen 10	Immunogen 20	Immunogen 40
Total count (Log CFU/g)	5.22 ± 0.07[c]	5.70 ± 0.1[d]	5.15 ± 0.1[c]	4.94 ± 0.14[b]	4.46 ± 0.15[a]
Lactobacillus (Log CFU/g)	2.62 ± 0.10[c]	2.83 ± 0.13[d]	2.56 ± 0.10[c]	2.3 ± 0.05[b]	2.06 ± 0.15[a]

All values are means ±standard deviation of triplicate tanks/treatment (N=3). Different superscrip letters in same row show significant differences ($P<0.05$)

Table 5. Blood parameters and plasma analyses in common carp feeding on different levels of Immunogen® for 56 days experimental period

Blood parameters	Diets				
	Control	Immunogen 5	Immunogen 10	Immunogen 20	Immunogen 40
Red blood cells (10^6 μL)	1.36 ± 0.05[a]	1.71 ± 0.18[bc]	1.88 ± 0.1[c]	1.56 ± 0.15[ab]	1.43 ± 0.18[ab]
Haemoglobin (g dL^{-1})	7.03 ± 0.04[a]	7.99 ± 0.86[bc]	8.27 ± 0.11[c]	7.39 ± 0.37[ab]	7.09 ± 0.08[a]
Haematocrit (%)	42.33 ± 1.52[a]	48.66 ± 2.51[bc]	51.07 ± 1.7[c]	46.53 ± 1.0[ab]	43.53 ± 2.51[a]
White blood cells (10^3 μL)	14650 ± 132[b]	14333 ± 152[ab]	14500 ± 300[ab]	13966 ± 321[a]	14100 ± 500[ab]
Glucose (mg. dL^{-1})	78.16 ± 9.14[a]	89.93 ± 2.92[a]	119.34 ± 9.7[b]	107.62 ± 4.9[b]	107.98 ± 2.54[b]
Cholesterol (mg. dL^{-1})	65.64 ± 0.72[a]	73.57 ± 1.32[b]	74.61 ± 1.86[b]	73.66 ± 0.21[b]	63.45 ± 2.9[a]
Triglyceride (mg. dL^{-1})	147.65 ± 0.72[b]	103.07 ± 10.64[a]	111.88 ± 1.5[a]	116.04 ± 2.21[a]	104.21 ± 19.26[a]
Albumin (g. dL^{-1})	0.35 ± 0.01[a]	0.42 ± 0.06[ab]	0.58 ± 0.08[c]	0.51 ± 0.02[b]	0.56 ± 0.04[c]
Plasma protein (g. dL^{-1})	2.39 ± 0.05	2.65 ± 0.14	2.58 ± 0.23	2.49 ± 0.06	2.56 ± 0.25

All values are means of three replicates (tanks)/treatment ± standard deviation. Means with the different letters are significantly different ($P < 0.05$).

administration of prebiotics in the concentration range of 5–15 g kg^{-1} ($P < 0.05$). However, blood triglycerides were reduced in fish fed diets supplemented with Immunogen®.

Discussion

Supplementation with the prebiotic Immunogen® at a concentration of 5 g kg^{-1} improved growth performance and feed utilization of common carp fingerlings. Many previous studies have demonstrated similar effects in aquatic animals (Staykov et al., 2007; Torrecillas et al., 2007; Burr et al., 2008; Taati et al., 2011; Amirkolaie et al., 2013). Colonization by beneficial bacteria that were induced by the consumption of dietary Immunogen® may account for these positive impacts. The stimulation of beneficial bacterial activity in fish guts through the provision of prebiotics in the diet leads to the secretion of a wide range of exo-enzymes (Moriarty 1996, 1998; Suzer et al., 2008) and increases digestive enzyme activity (Askarian et al., 2011). Thus, nutrient digestion is improved and better growth is achieved. A balanced production of essential nutrients, especially the fatty acids produced by micro-organisms, may also contribute to the improved growth performance of prebiotic-fed fish (Irianto and Austin, 2002).

We identified a threshold effect for prebiotic inclusion (at a concentration of 5 g kg^{-1}). Additional supplementation with Immunogen® did not further improve growth and/or *Lactobacillus* counts in common carp, in agreement with the findings of Hoseinifar et al. (2011), who detected a gradual increase in both lactic acid bacterial counts and body growth in *Huso huso* fed diets containing 10 and 20 g kg^{-1} oligofructose, but found that further increases in prebiotic concentration depressed bacterial counts and fish growth. Similarly, Olsen et al. (2001) found that supplementation with inulin at a high dose (15% of total diet) had a negative impact on micro-villous organization (disarray, absent in some gut sections, microvilli not straight) in the hindgut of Arctic charr *Salvelinus alpinus*. We found that inclusion of Immunogen® in the concentration range of 5–10 g kg^{-1} provided sufficient substrate for *Lactobacillus* colonization, thereby promoting the growth of the fish host, but concentrations 10 g kg^{-1} can negatively impact fish performance.

Our fish body proximate analyses showed that the administration of prebiotic Immunogen® did not affect the composition of the tissues (Table 3), which was not the case for Atlantic salmon *Salmo salar* (Grisdale-Helland et al., 2008) or rainbow trout *Oncorhynchus mykis* (Dimitroglou et al., 2009) fed diets containing 10 and 20 g kg^{-1} mannan oligosaccharide, respectively. The disparity between our results and those of other studies may relate to species, prebiotic dosages, fermentability of the prebiotics and the different intestinal morphology and microbiota of the respective fish guts (Hoseinifar et al., 2010). Furthermore, the effect of prebiotic supplementation on fish is likely dependent on diet composition, especially the carbohydrate fraction, and on environmental conditions, which may interact with the chosen prebiotic. Future work should focus on the gut bacterial community in trials that test different types/concentrations of carbohydrates and a range of environmental conditions.

We found that prebiotic supplementation influenced fish hematological parameters, in agreement with earlier studies (Merrifield et al., 2010; Akrami et al., 2012; Ebrahimi et al., 2012). However we detected no effect of dietary Immunogen® on white blood cell counts, which we had expected to rise in response to treatment. In contrast, Ebrahimi et al. (2012) found that white blood cell counts increased slightly with increasing Immunogen® levels; their findings may reflect negative impacts of high doses of Immunogen® on white blood cells, but no explanatory mechanism has yet been identified.

In conclusion, we demonstrated that the inclusion of 5 g kg^{-1} Immunogen® in the diet improved nutrient utilization and the growth performance of common carp fingerlings. These effects probably related to the elevation of *Lactobacillus* counts in the fish guts, which improved digestive performance. Improved fish growth in the treatment group was unrelated to the immune response index (white blood cell count) in common carp.

References

Amirkolaie AK, Karimzadeh S and Jafary AM. 2013. The effects of dietary supplement of immunogen on growth performance, and visceral and hepatic somatic indices of juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792). Asian Fish Sci 26, 232-242.

Akrami R, Razeghi-Mansour M, Chitsaz H, Ziaee R and Ahmadi Z. 2012. Effect of dietary mannan oligosaccharide on growth performance, survival, body composition and some hematological parameters of carp juvenile (*Cyprinus Carpio*). J Ani Sci Adv 2, 879-885.

Askarian F, Kousha A, Salma W and Ringo E. 2011. The effect of lactic acid bacteria administration on growth, digestive enzymes activity and gut microbiota in Persian sturgeon (*Acipenser persicus*) and beluga (*Huso huso*) fry. Aquacult Nutr 17, 488–497.

BarrosMM, Lim C and Klesius PH. 2002. Effect of soybean meal replacement by cotton seed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri* challenge. Aquaculture 207, 263-279.

Burr G, Gatlin D and Ricke S. 2005. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. J World Aquacult Soc 36, 425-436.

Burr G, Hume M, Neill WH and Gatlin DM. 2008. Effects of prebiotics on nutrient digestibility of soybean-meal-based diet by red drum *Sciaenopso cellatus* (Linnaeus). Aquacult Res 39, 1680-1686.

Cabello FC. 2006. Heavy use of prophylactic antibiotics in aquaculture:

a growing problem for human and animal health and for the environment. Environ Microbiol 8, 1137-1144.

Dimitroglou A, Merrifeld DL, Moate R, Davies SJ, Spring P, Sweetman J and Bradley G. 2009. Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Animal Sci 87, 3226-3234.

Ebrahimi G, Ouraji H, Khalesi MK, Sudagar M, Barari A, Zarei Dangesaraki M and Jani Khalili K. 2012. Effects of a prebiotic, Immunogen, on feed utilization, body composition, immunity and resistance to *Aeromonas hydrophila*i infection in the common carp *Cyprinus carpio* (Linnaeus) fingerlings. J Animal Physiol Animal Nutr 96, 591-599.

Hoseinifar SH, Zare P and Merrifield DL. 2010. The effects of inulin on growth factors and survival of the Indian white shrimp larvae and postlarvae (*Fenneropenaeus indicus*). Aquacult Res 41, 348-352.

Irianto A and Austin B. 2002. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 25, 333-342.

ISO.1978. Animal feeding stuffs. Determination of crude ash. ISO 5984. International Organization for Standardization .

ISO. 1979. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. ISO 5983. International Organization for Standardization.

ISO. 1999. Animal feeding stuffs. Determination of crude fat. ISO 6492. International Organization for Standardization.

ISO. 1983. Animal feeding stuffs. Determination of moisture content. ISO 6496.International Organization for Standardization.

Gatlin DM, Li P, Wang X, Burr GS and Lawrence AL. 2006. Potential applications of prebiotics in aquaculture. In: Elizabeth Cruz Suarez, L., Marie, Denis Ricque, Salazar, Mireya Tapia, López, Martha G., Villarreal, David A., Ana, C. (Eds.), Puello Cruz y Armando Garcia Ortega. Avances en NutriciónAcuícola VIII.VIII SimposiumInternacional de NutriciónAcuícola. 15–17 Noviembre. Universidad Autónoma de Nuevo León,Monterrey, Nuevo León, Méico.

Genc M, Yilmaz E and Genc E. 2007. Effects of dietary mannan oligosaccharides (MOS) on growth, body composition, and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Israeli J Aquaculte-Bamidgeh 59, 10-16.

Gibson GR and Roberfroid MB. 1995. Dietary modulation of the human colon microbiota: introducing the concept of prebiotics. J Nutr 125, 1401-1412.

Grisdale-Helland B, Helland SJ and Gatlin DM. 2008.The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). Aquaculture 283, 163-167.

Hajibeglou A and Sudagar M. 2011.Effect of Dietary Probiotic Level on the Reproductive Performance ofFemale Platy *Xiphophorus maculatus*.Veterinary Res 4, 66-70.

Henry RJ. 1964. Clinical Chemistry, Harper and Row Publishers, New York, U.S.A., pp 181.

Iwama GK, Pickering AD, Sumpter JP and Schreck CB. 1997. Fish stress and health in aquaculture society for experimental biology seminar series 62, Cambridge University Press, UK, p 278.

Klinger D and Naylor R. 2012. Searching for Solutionsin Aquaculture: Chartinga Sustainable Course. Annu Rev Environ Resourc 37, 247-276.

Li P, Burr GS, Gatlin DM, Hume ME, PatnaikS, Castille FL and Lawrence AL. 2007. Dietary supplementation of short-chain fructooligosaccharides influences gastrointestinal microbiota composition and immunity characteristics of pacific white shrimp, *Litopenaeus vannamei*, Cultured in a Recirculating System. J Nutr 137, 2763-2768.

Mei G-Y, Carey CM, ToshS and Kostrzynska M. 2011. Utilization of different types of dietary fibers by potential probiotics. Canadian J Microbiol 57, 857-865.

Merrifield DL, Dimitroglou A, Foey A, Davies SJ, Baker RTM, Bogwald J and Castex

M Ringo E. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302, 1-18.

Moriarty DJW. 1996. Microbial biotechnology: a key ingredient for sustainable aquaculture. Infofish International 4, 29-33.

Moriarty DJW. 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164, 351-358.

Mussatto SI and Mancilha IM. 2007. Non-digestible oligosaccharides: A review. Carbohydrate Polymers 68, 587-597.

Olsen RE, Myklebust R, Kryvi H, Mayhew TM and Ringo E. 2001. Damaging effect of

dietary inulin on intestinal enterocytes in Arctic charr (*Salvelinus alpinus* L.). Aquacult Res 32, 931-934.

Ringo E, Olsen RE, Gifstad TG, Dalmo RA, Amlund H, Hemre G-I and Bakke AM. 2010. Prebiotics in aquaculture: a review. Aqua Nutr 16, 117-136.

Salze G, McLean E and Schwarz MH. 2008. Craig SR. Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. Aquaculture 274, 148-152.

Schalm OW, Jain NC and Carrol EJ. 1975 Veterinary Haematology (3rd edn), Lea and Fibiger, Philadelphia, PA.

Staykov Y, Spring P, Denev S and Sweetman J. 2007. Effect of mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture International 15, 153-161.

Suze C, Coban D, Kamacı HO, Saka S, Fırat K, Otgucuoglu O and Kucuksari H. 2008. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture 280, 140-145.

Takeuchi T, Satoh S and KironV. 2002. Common carp, In: Webster, C.D., Lim, C. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture, CABI Publishing, 245-261.

Taati T, Soltani M, Bahmaniand M and Zamini AA. 2011. Effects of the prebiotics Immunoster and Immunowall on growth performance of juvenile beluga (*Huso huso*). J Appl Ichthyol 27, 796-798.

Torrecillas S, Makol A, Caballero MJ, Montero D, Robaina L, Real F,Sweetman J, Tort L and Izquierdo MS. 2007. Immune stimulation and improved infection resistance in European sea bass (*Di-*

centrarchus labrax) fed mannan oligosaccharides. Fish Shellfish Immunol 23, 969-981.

Trinder P. 1969. Determination of glucose concentration in the blood. Ann Clin Biochem 6, 24-27.

Wotton ID and Freeman H. 1982. Microanalysis in Medical Biochemistry, Churchill, New York, USA.

Yousefian M and Amiri MS. 2009. A review of the use of prebiotic in aquaculture for fish and shrimp. African J Biotechnol 8, 7313-7318.

Zhou Z, Ding Z and Huiyuan LV. 2007. Effects of dietary short-chain fructooligosaccharide on intestinal microflora, survival and growth performance of juvenile white shrimp *Litopenaeusb vannamei*. J World Aquacult Soc 38, 296-301.