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Effects of Prebiotic Supplementation on Survival, Growth Performance and Feed Utilization of Kutum, *Rutilus frisii kutum* (Kamenskii 1901), Fingerlings

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Abstract: A feeding trial was conducted to evaluate the effects of a commercial prebiotic, immunogen on growth performance and survival of the kutum *Rutilus frisii kutum* fingerlings. The fingerlings were adopted for 2 weeks and then reared as triplicate groups in 250-l tanks (n = 50 per tank with average initial weights of 499.1±5 mg). Four incremental levels (0.5, 1.0, 1.5 and 2.5 g kg^{-1} prebiotic diet) of a commercial prebiotic, immunogen (Provided by Soroush radian Co., Tehran, Iran) were added to the basal diet A (control) to prepare diets B, C, D and E as the treatments. The fish fed on the experimental diets *ad libitum* thrice a day for 8 weeks. Weight gain and specific growth rates showed significantly differences among the groups fed different immunogen levels. Feed conversion ratio showed a decrease with increasing immunogen level in diet. Both feed and protein efficiency ratios enhanced significantly with the highest prebiotic level of 2.5 g kg^{-1} diet ($p<0.05$). Feed Intake (FI) and Protein Intake (PI) didn't significantly affect by the prebiotic immunogen level ($p>0.05$). The lowest mean of total bacterial counts was observed in the fish fed a dietary immunogen level of 2.5 g kg^{-1} diet ($p<0.05$). The present study shows that a dietary immunogen supplementation of 1.5 g kg^{-1} is capable to improve the feed efficacy and growth performance of *R. frisii kutum* fingerlings.

Key words: Kutum, *Rutilus frisii kutum* (Kamenskii, 1901), growth performance, feed utilization, prebiotic suplementation, survival

INTRODUCTION

Fishing in Caspian sea concentrates mainly on two fish species: Kutum and common carp, both being appropriate growth rate. But kutum, *Rutilus frisii kutum* (Kamenskii, 1901) is a very popular food fish because of its good meat quality and good consumer acceptance especially in north of Iran. The chief population of kutum is found in Iranian waters (Abdoli, 1990). Now a days, annually to restock this valuable species, the Iranian fisheries organization produces and releases >150 million fingerlings into rivers which carry them toward the Caspian sea. The annual landing of kutum rose from 563 mt in 1982-16118 mt in 2006 (Salehi, 2008).

Improving the health conditions and growth performance in aquatic animal is a topic of strenuous interest. Hence, such components as antibiotics, hormones, ionophores and some salts have been frequently used to enhance fish health and growth (Fuller, 1992; Klaenhammer and Kullen, 1999). Because of the development of antibiotic-resistant bacterial strains, there is a growing trend towards exploring alternative techniques to provide different functional attributes. Probiotics, prebiotics, synbiotics and immunostimulants are dietary supplements that have been revealed to raise feed efficiency, growth performance and survival of some fishes which may help reduce their susceptibility to diseases. The term prebiotic has been defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria (Gibson and Roberfroid, 1995). Effects of different prebiotics and their levels on growth rate, feed efficiency, digestibility, immune responses and survival have been investigated in a number of studies (Burr *et al*., 2008; Dimitroglou *et al*., 2010).

In this study, a commercial prebiotic, immunogen, was used. This prebiotic is a natural product containing various stimulating compounds such as β-glucan and Mannan Oligosaccharide (MOS) which have been used as feed additives in various animals. Several studies indicate that supplements such as MOS and β-glucun could induce an improvement in the growth and an enhancement in health status of fish (Staykov *et al*., 2007; Torrecillas *et al*., 2007; Burr *et al*., 2008). Likewise, appropriate or low levels of β-glucans were stated to be efficient stimulators of non-specific immune functions in fish (Robertsen *et al*., 1994; Santarem *et al*., 1997). The present trial was conducted to evaluate the effects of graded levels of the prebiotic Immunogen® on survival, growth performance and feed utilization of *R. frisii kutum* fingerlings.

MATERIALS AND METHODS

Kutum and facilities: Kutum fingerlings, *Rutilus frisii kutum* (Kamenskii, 1901) employed in this study was acquired from the Shahid Rajaee Hatchery, Sari, Iran. The healthy and disease-free fish (n = 750, average weight 499.1±5 mg) were randomly distributed in 15 circular tanks of 250 l and acclimatized to the experimental conditions for 15 days feeding on a basal diet. During the study, the fish were hand-fed with experimental diets *ad libitum* three times for 8 weeks. Water quality was checked periodically (pH = 7.9-8.2, temperature = 23.2-26.2°C and dissolved oxygen >6.8 mg L^{-1}).

Diet preparation: Basal practical diet was formulated with estimated gross energy and protein levels of 19.5 MJ kg^{-1} and 410 g kg^{-1}, respectively. Four incremental levels (0.5, 1.0, 1.5 and 2.5 g kg^{-1} prebiotic diet) of a commercial prebiotic, Immunogen (Provided by Soroush radian Co., Tehran, Iran) were added to the basal diet A (control) to prepare diets B-E as the treatments (Table 1). The formulation and proximate analysis of experimental diets are shown in Table 1.

Growth and feed efficiency parameters: Average final body weight of each experimental group was determined by dividing total fish weight of each tank by the number of fish. Weight gain, growth performance and survival percentage were calculated for each experimental group as follows:

$$\text{Feed conversion} = \left[\frac{\text{Total feed intake (g)}}{\text{Total wet weight gain (g)}}\right]$$

$$\text{Protein efficiency ratio} = \left[\frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}}\right]$$

$$\text{Feed intake} = \left[\frac{\text{Total feed intake}}{\text{Number of fish}}\right]$$

$$\text{Protein intake} = [\text{Feed intake (g)} \times \text{Percent protein in the diet}]$$

$$\text{Protein or energy utilization} = \left[\frac{\text{Protein or energy gain}}{\text{Protein or energy intake}}\right] \times 100$$

where, W_F and W_I are mean final and initial body weights (g), respectively and T is the experimental duration in days.

Table 1: Composition and proximate composition of diets containing different levels of the probiotic Immunogen®

	Diets				
Composition	A	B	C	D	E
Ingredient (g kg^{-1})					
Fish meal	400.00	400.00	400.00	400.00	400.00
Soybean meal	110.00	110.00	110.00	110.00	110.00
Gluten	55.00	55.00	55.00	55.00	55.00
Casein	65.00	65.00	65.00	65.00	65.00
Rice bran	80.00	80.00	80.00	80.00	80.00
Wheat flour	130.00	130.00	130.00	130.00	130.00
Sunflower oil	60.00	60.00	60.00	60.00	60.00
Fish oil	20.00	20.00	20.00	20.00	20.00
Cellulose	40.00	39.50	39.00	38.50	37.50
Vitamin premix[a]	20.00	20.00	20.00	20.00	20.00
Mineral premix[b]	20.00	20.00	20.00	20.00	20.00
Prebiotic immunogen[c]	0.00	0.50	1.00	1.50	2.50
Proximate composition[d]					
Dry matter	915.60	911.90	920.20	916.80	917.50
Crude protein	410.40	414.30	409.70	409.20	412.80
Crude lipid	125.60	131.20	135.60	130.40	129.10
Ash	89.70	91.80	88.10	88.90	91.50
Moisture	84.40	88.10	79.80	83.20	82.50
Gross energy[e]	19.51	19.56	19.83	19.64	19.60

[a] Vitamin premix contained the following vitamins (each kg^{-1} diet): vitamin A, 10 000 IU; vitamin D_3 2000 IU; vitamin E, 100 mg; vitamin K, 20 mg; vitamin B_1, 400 mg; vitamin B_2, 40 mg. vitamin B_6 20 mg; vitamin B_{12}, 0.04 mg; biotin, 0.2 mg; choline chloride, 1200 mg; folic acid, 10 mg; inositol, 200 mg; niacin, 200 mg; pantothenic calcium, 100mg. [b] Contained (g kg^{-1} mix): $MgSO_4.2H_2O$, 127.5; KCl, 50.0; NaCl, 60.0; $CaHPO_4.2H_2O$, 727.8; $FeSO_4$. $7H_2O$, 25.0; $ZnSO_4.7H_2O$, 5.5; $CuSO.5H_2O$, 0.785; $MnSO_4.4H_2O$, 2.54; $CoSO_4.4H_2O$, 0.478; $Ca(IO_3)_2 . 6H_2O$, 0.295; $CrCl_3$, $6H_2O$, 0.128.[c] Sigma, St. Louis, MO, USA. [c]Provided by Soroush radian Co, Tehran, Iran, [d] g kg^{-1} diets. [e]MJ gross energy kg^{-1} diets, based on 23.4 kJ g^{-1} protein, 39.2 kJ g^{-1} lipid and 17.2 kJ g^{-1} NFE

Total bacterial count: At the end of experiment, 12 fish were assembled from each treatment. Their intestinal samples were tested to conclude counts of total bacteria. The intestines were dissected out in sterile conditions. Three intestine samples from each fish were taken for microbiological analysis. All samples were diluted successively using sterilized normal saline solution (0.85% NaCl w/v) and then placed onto nutrient agar plates for total counts of bacteria.

Statistical analysis: The data were subjected to one-way ANOVA and correlation analysis where appropriate. Multiple comparisons between the means of individual treatments were made by Duncan's multiple range tests. The significance level was at $p<0.05$. The results are presented as means±SEM.

RESULTS AND DISCUSSION

Growth performance and survival data for kutum fingerlings are shown in Table 2. Survival rates were generally satisfactory, from 77.1-87.6% over the 8 weeks trial and it was affected by the differing dietary prebiotic Immunogen ($p<0.05$). Feeding *R. frisii kutum* fingerlings

Table 2: Feed utilization of the kutum fingerlings fed diets containing different levels of the prebiotic Immunogen® for 8 weeks

	Treatments				
Parameters	A (control)	B	C	D	E
IW(g)	0.49 ± 0.04^{a}	0.49 ± 0.03^{a}	0.50 ± 0.02^{a}	0.50 ± 0.04^{a}	0.51 ± 0.02^{a}
WGP	175.3 ± 15.30^{a}	233.1 ± 13.60^{bc}	196.4 ± 20.30^{ab}	213.9 ± 18.40^{abc}	255.9 ± 11.30^{c}
S (%)	77.1 ± 1.650^{c}	82.8 ± 1.640^{ab}	86.6 ± 0.950^{a}	87.6 ± 1.320^{a}	78.8 ± 1.160^{cb}
SGR	1.80 ± 0.10^{a}	2.14 ± 0.07^{bc}	1.93 ± 0.12^{ab}	2.03 ± 0.10^{abc}	2.26 ± 0.05^{c}
FI	42.41 ± 1.03^{a}	40.77 ± 0.77^{a}	39.88 ± 1.28^{a}	38.98 ± 1.20^{a}	39.18 ± 1.03^{a}
PI	17.40 ± 0.42^{a}	16.89 ± 0.32^{a}	16.33 ± 0.52^{a}	15.95 ± 0.49^{a}	16.17 ± 0.42^{a}
FER	0.37 ± 0.04^{a}	0.50 ± 0.01^{bc}	0.44 ± 0.03^{ab}	0.49 ± 0.02^{bc}	0.57 ± 0.01^{c}
PER	0.90 ± 0.09^{a}	1.21 ± 0.04^{bc}	1.09 ± 0.07^{ab}	1.19 ± 0.06^{bc}	1.38 ± 0.03^{c}
PU (%)	13.19 ± 1.52^{a}	17.64 ± 0.86^{b}	16.23 ± 1.10^{ab}	18.23 ± 1.09^{bc}	21.38 ± 0.63^{c}
EU (%)	11.87 ± 1.55^{a}	16.00 ± 0.58^{bc}	14.31 ± 1.11^{ab}	15.91 ± 0.87^{bc}	18.96 ± 0.62^{c}

Means±SD having various superscript letters in the same row are significantly different ($p<0.05$). IW: Initial Weight, PWG: Weight Gain percent, FC: Feed Conversion, FI: Feed Intake, PI: Protein Intake, FER: Feed Efficiency Ratio, PER: Protein Efficiency Ratio, PU: Protein Utilization and EU: Energy Utilization

on the prebiotic immunogen at level 1.5 g kg^{-1} diet revealed the highest survival mean (87.6±1.32%) throughout the experiment. The prebiotic immunogen levels significantly affected the growth performance and feed conversion. Feed Intake (FI) and Protein Intake (PI) didn't significantly affect by the prebiotic immunogen level ($p>0.05$). Feed efficiency was significantly affected by the dietary levels of the prebiotic immunogen ($p<0.05$) and improved with increasing prebiotic levels. The data on feed efficiency indices indicates that the kutum fingerlings fed diet E (containing 2.5 g kg^{-1} prebiotic diet) registered highest FER, PER, PU%, EU% and the lowest FC of the groups.

In the present study also showed that weight gain and SGR improved with extra Immunogen levels in the diets. The highest SGR of kutum (500 mg initial weight) was 2.26±0.05% day^{-1} which is appropriate score compared to other researches. For instance, Haghighi (2006) registered maximum SGR of 2.78% day^{-1} for kutum fry (0.2 g initial weight) fed a diet containing 468 g kg^{-1} protein and 80 g kg^{-1} lipid. Results of the previous studies showed that different prebiotic as MOS can improve growth performance in various aquatic species such as rainbow trout, European sea bass (*Dicentrarchus labrax*) and green tiger prawn (*Penaeus semisulcatus*) (Genc *et al*., 2007; Staykov *et al*., 2007; Torrecillas *et al*., 2007).

The best results on feed utilization were obtained in the fish fed 2.5 g immunogen kg^{-1} diet ($p<0.05$). Calculated FER and PER in all treatments were: 0.37±0.04-0.57±0.01 and 0.90±0.09-1.38±0.03, respectively. These values are similar to those reported in various studies on the same species (Neverian *et al*., 2005; Haghighi, 2006). The improved FC of the kutum fingerlings in this study is in agreement with earlier findings in such species as rainbow trout (Staykov *et al*., 2007), Persian sturgeon *Acipenser persicus* (Nodeh, 2010) and Atlantic salmon (Grisdale-Helland *et al*., 2008), all of

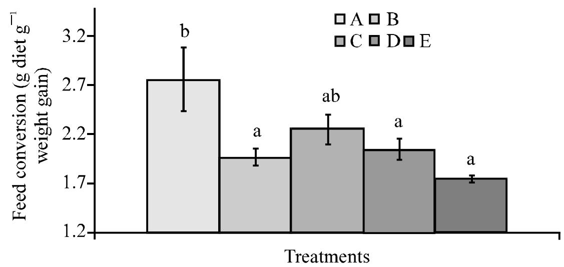

Fig. 1: Feed conversion of the kutum fingerlings fed diets containing different levels of the prebiotic immunogen®

which supplemented with a variety of dietary prebiotics (Fig. 1). It has recently been shown by Nodeh (2010) that supplementing the diet of the Persian sturgeon fingerlings with the commercial prebiotic Immunogen considerably modified the intestinal microflora. He also reported that Lactobacillus (lactic acid bacteria) population in the intestine of the fish fed a diet containing 2 g prebiotic/kg was higher than that of other supplemented groups while total counts of bacteria steadily declined with an increase in the prebiotic levels up to 2 g kg^{-1} diet ($p<0.05$). Furthermore, it has been confirmed in a number of studies that lactic acid bacteria as one of the common probiotics have beneficial effects on improvement of host nutrition through the production of supplemental digestive enzymes; they also produce bacteriocins that inhibit specific fish pathogens. These bacteria as a result, efficaciously influence the host's microflora (Moriarty, 1998; Ringa and Gatesoupe, 1998; Verschuere *et al*., 2000). Total bacterial counts in kutum intestine which fed the commertial prebiotic immunogen levels were significantly different and ranged from 5.51±0.19- 6.84±0.19 log cfu g^{-1} intestine (Fig. 2). Fish fed diet A (without the prebiotic immunogen) had the highest mean of total bacterial counts on the contrary the lowest mean of total bacterial counts was observed in the fish fed

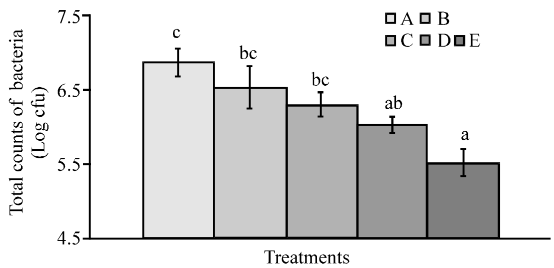

Fig. 2: Total counts of bacteria of the kutum fingerlings fed diets containing different levels of the prebiotic Immunogen®

the diet E containing 2.5 g kg^{-1} prebiotic immunogen diets ($p<0.05$) (Fig. 2). Altogether, the relatively enhanced growth performance and feed efficiency in the kutum fingerlings fed the supplemented diets could be related to the improvement of intestinal microbiota. Nonetheless, more research in this area is needed in the future. On the other hand, Salze *et al*. (2008) deduced that a 0.2% dietary MOS supplementation increased intestinal microvilli length of cobia larvae, *Rachycentron canadum*. Likewise, Dimitroglou *et al*. (2010) also reported that MOS supplementation increased microvilli densities in both the anterior and posterior intestinal regions of gilthead sea bream (*Sparus aurata*).

CONCLUSION

It accordingly can be speculated that changes in the villi morphology and density in the *R. frisii* fingerlings fed the prebiotic Immunogen could be another likely cause for the increased potential of nutrient absorption leading to elevated feed efficiencies. Though, experimentation of the intestinal microvilli morphology is required. Conversely, high levels of prebiotics may yield harmful influences on the performance and health status of fish. Olsen *et al*. (2001) observed destructive effects (disarray, lacking in some areas and less straight) on microvillous organization in the hindgut of Arctic charr *Salvelinus alpinus* L. fed a high concentration of inulin (15% of the diet).

Based on the findings of this study, supplementation of immunogen at levels of 1-1.5 g kg^{-1} diet could be appropriate for feeding *R. frisii kutum* fingerlings.

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