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PROOF

Influence of Primalac Probiotic on Growth Performance, Blood Biochemical Parameters, Survival and Stress Resistance in the Caspian Roach (*Rutilus Rutilus*) Fry

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Abstract

The effects of probiotic Primalac on the growth performance, serum biochemical performance, survival and stress resistance of the Caspian roach (*Rutilus rutilus*) fry (1.29 ± 0.17 g) was investigated in the present study Four practical diets containing 0 (control), 0.05, 0.1 and 0.15 % probiotic Primalac were used to feed fish. Fish (15 fish/ replicate) were fed on the tested diets at rate of 3 % of body weight for 45 days. Salinity stress test was carried out after 45 days of feeding, in order to determine the effect of Primalac on resistance to salinity stress. Blood samples were obtained in four times after challenge (days 1, 3, 5 and 7) evaluating hematocrit levels. Results showed that final weight, body weight gain and specific growth rate were significantly (P<0.05) increased in treatment groups with Primalac and the highest levels related to fish fed 0.15%. Also, fish fed 0.15 % diet had a significantly highest condition factor compared to the control group and other treatments (P<0.05). Feed conversion ratio was decreased significantly in treatment groups with Primalac (P<0.05). Glucose and cholesterol levels were decreased significantly (P<0.05) in fish fed with diets containing Primalac. In addition, supplementation of Primalac significantly (P<0.05) increased the total protein concentration of probiotic fed groups. On the first day after stress, the hematocrit levels were significantly increased in all groups (P<0.05). The hematocrit levels were decreased in all groups since third day. These results indicate that addition of probiotic Primalac to fish diet exerted positive effects on fish growth and blood biochemical parameters in the Caspian roach.

Keywords: Probiotic, Growth, Salinity, Supplement, Caspian roach

Introduction

The Caspian roach (*Rutilus rutilus*) is a commercially valuable species in the Caspian Sea and important prey item for sturgeons (Keyvanshokooh and Kalbassi 2006). This species is now considered a threatened species because of overfishing and destruction of spawning ground (Soleimani *et al.* 2012). Restocking and artificial culture up to marketable size have been developed by the Iranian Shilat Organization to reduce pressure on natural Caspian Sea populations (Keyvanshokooh *et al.* 2007). The elevation of disease resistance, stimulation of immune responses and improvement of growth performance through administration of feed additives such as minerals, pro- and prebiotics are of great importance in commercial aquaculture of the Caspian roach, especially in sensitive period (i.e., larvae and fry culture) (Imanpoor and Roohi 2015b).

Using probiotic is one of the positive achievements in this field (Makridis *et al.* 2002). As reported by several authors, probiotic has good effects on health and growth in aquaculture (Doos Ali Vand *et al.* 2014). For example, *Lactobacillus acidophilus* in *Oncorhynchus mykiss* (Faramarzi *et al.* 2011), Primalac in *Acipenser persicus* (Salaghi *et al.* 2013) and *Pediococcus acidilactici* in *Aequidens rivulatus* (Neissi *et al.* 2013).

The probiotic is a live, dead or component of a microbial cell that when administered via the feed or to the rearing water benefits the host by improving its microbial balance or microbial balance of the ambient environment (Merrifield *et al.* 2010; Balakrishna and Keerthi 2012). Probiotics in aquaculture have been shown to have several modes of action, including (1) competitive exclusion of pathogenic bacteria through habitat competition, nutrient competition and alternation of enzymatic activities of pathogens; (2) enhancement of immune response of host species and (3) enhancement of nutrition of host species through the production of supplemental digestive enzymes (Verschuere *et al.* 2000; Balcazar 2006; Kesarcodi-Waston *et al.* 2008). Furthermore, probiotics are also useful for improving soil and water quality (Tuan *et* *al.* 2013).

Primalac is containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidium* (Imanpoor and Roohi 2015a). *Lactobacillus* and *Bifidobacterium* are the most frequently genera used as probiotics (Isolauri *et al.* 2001) and different strains may affect their efficiency. Oral administration of *Lactobacilli* exerts a strong adjuvant activity, which is responsible for the enhanced host responses (Salaghi *et al.* 2013). Different strains of *Lactobacillus* induce distinct mucosal cytokine profiles showing different intrinsic adjuvant capacity (Maassen *et al.* 2000; Perdigo *et al.* 2002). Previous studies exhibit the positive effect of Primalac on growth performance in Persian sturgeon (*Acipenser persicus*), rainbow trout (*Oncorhynchus mykiss*), Caspian kutum (*Rutilus kutum*) and common carp (*Cyprinus carpio*) (Salaghi *et al.* 2013; Jafari *et al.* 2013; Imanpoor and Roohi 2015a; Imanpoor *et al.* 2016).

To our knowledge, despite these advances, there is no available information on the efficacy of dietary Primalac as a probiotic for the Caspian roach. Therefore, the aim of the current study was to assess the effects of dietary Primalac on the growth performance, blood biochemical parameters, survival and stress resistance of the Caspian roach fry.

Materials and methods

Fish

The Caspian roach fry were obtained from Sijaval Aquaculture Center (Turkeman Seapot, Iran). Fish were allowed to acclimatize for 2 weeks prior to the experiment and during this period were hand-fed a commercial diets three times a day. After the acclimation period, fish (1.29 ± 0.17 g) were randomly distributed into 12 aquaria (30 L), each containing 15 fish/ replicate. Water temperature, dissolved oxygen, pH and salinity were monitored daily and maintained at 24.38 ± 3℃, 5.7-7-7 mg L^{-1}, 6.9-7.7 units and 0.1 ppt, respectively. Continuous aeration was provided to each aquarium through an air stone connected to a central air compressor. At the end of the experiment, fish from each aquarium were individually weighed and measured.

Experimental Diets

A commercial probiotic Primalac, a mixture of equal proportions of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidium* was obtained from the Nikandishan Farjad Commerce Corporation, Tehran, Iran. The commercial basal diets (composition of diet shown in Table 1) were supplemented with different levels of Primalac (0, 0. 5, 1 and 1.5 g kg^{-1} diet) (Imanpoor and Roohi 2015a). To prepare the diets, firstly, ingredients were blended thoroughly with additional water and 1% binder to make a past. Experimental diets were kept in plastic bags at -4℃ until used. During the feeding trial (45 days), fish were hand-fed (3% of day weight) three times a day. The feeding ration was corrected every 2 weeks following a 24-h starvation period and batch weighing.

Growth Performance

All fish were weighed at the end of the feeding trial (day 45) for estimation of growth. Growth performance and survival rate of the Caspian roach fry were calculated using the following formula:

Weight gain (WG) = $W_f - W_i$; Feed conversion rate (FCR) = feed intake / weight gain; Specific

Table 1. Commercial diet composition

Components (%)	%
Fish meal	43.47
Meat meal	13
Wheat meal	27.53
Soybean oil	3
Fish oil	6
Methionine	1.5
Lysine	1.5
Vitamin mix	4
Chemical composition	
Moisture	9.4
Crude protein	38.5
Crude lipid	10.3
Ash	3.5
Fiber	11.30
NEF	27
Energy (MJ kg^{-1})†	17.74

† Gross energy (MJ kg^{-1}) calculated according to 23.6 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17 kJ g^{-1} for NFE (Brett and Groves 1979)

growth rate (SGR) = 100 × [(ln W_f – ln W_i) / days]; Condition factor (CF) = [(body weight (g) / (standard length)^3] × 100. Where W_f is final body weight and W_i is initial body weight. In addition, survival rate was calculated at the end of the experiment: survival = (N_f / N_0) × 100; where N_0 is initial number of fish and N_f is final number of fish.

Blood Parameters Assays

At the end of trial, fish were fasted for 24 hours immediately prior to blood sampling and five fish per aquarium were randomly chosen and anaesthetized with clove powder (5 mg L^{-1}). The blood samples were collected through a syringe by caudal vein and stored in non-heparinized tubes. For biochemical assays, blood samples were immediately centrifuged (3000 g for 10 min) at room temperature and then serum separated and stored at -20℃ until analysis. The concentration of serum glucose, cholesterol and total protein were measured by spectrophotometer at 456 nm (Model WPA-S2000-UV/VIS, Cambridge-UK) using commercial kits (Pars Azmun Co. Ltd., Tehran, Iran).

Salinity Stress Challenge

At the end the 45 days feeding trial, 10 fish of each group were transferred directly to salinity [13 part per thousand (ppt)] and survival rate was calculated at 168 h post challenge (Imanpoor and Roohi 2015b). Haematocrit levels were also measured at 24, 72, 120 and 168 h post challenge. Haematocrit was determined by the microhaematocrit method as described by Brown (1988) and reported as percentage.

Statistical Analysis

Statistical analyses were conducted using SPSS statistical package version 17.0 (SPSS Inc., Chicago IL, USA). One-way ANOVA followed by Duncan's test was used for data analysis after checking the normality of data and homogeneity of variance. Mean values were considered significantly different at P<0.05. Data are expressed as mean values ± SD.

Results

The growth performance of the Caspian roach fed diets supplemented with varying levels of dietary Primalac is present in Table 2. Compared to the control treatment, roach fed different levels of Primalac displayed improved (P<0.05) growth performance, including final weight (4.846±0.118), weight gain (3.656±0.087) and specific growth rate (3.12±0.004). Furthermore, the Caspian roach fed 0.15% Primalac had significantly improved condition factor (1.204±0.009) and food conversion rate (1.426±0.058) compared to the other groups. The survival rates were 100 % in all treatments, and no mortality was recorded during the feeding trial.

The effects of different levels of dietary Primalac on glucose, total protein and cholesterol levels are shown in Table 3. There were significant decrease in glucose and cholesterol levels in fish fed Primalac compared to the control group (P<0.05). In addition, total protein level in Primalac (0.1and 0.15%)-fed fish showed a significant increase compared to the control treatment (P<0.05).

Results of the salinity challenge are presented in Table 4. On the first day after stress, hematocrit levels significantly were decreased in all groups (P<0.05). No differences were found in the hematocrit levels between groups (P>0.05). On the other hand, hematocrit levels had tendency to decrease in all group since third day (Table 3). No mortality occurred during 7 days of exposure of the tested salinity.

Discussion

To our knowledge, this study is the first study to investigate the effects of Primalac as a probiotic on the Caspian roach (*Rutilus rutilus*) fry. Probiotics are used as dietary supplementations in aquaculture and their role are already established in intestinal microbial balance, growth, nutrition, health status and resistance against infectious agents (Ghomi *et al.* 2010; Dhama *et al.* 2011). The positive effect of probiotics depends on both the action mechanisms and the capacity of colonization, that is to say, its ability to reach, remain or reproduce in the place where the effect is required (Planas *et al.* 2004).

In the present study, better growth performance

Table 2. Growth performance of the Caspian roach fed with probiotic Primalac after 45 days

Growth index	Control	0.05 %	0.1 %	0.15 %
Initial weight (g)	2.157±0.04^a	1.174±0.06^a	1.265±0.01^a	1.19±0.05^a
Final weight (g)	3.462±0.12^c	4.005±0.00^b	4.03±0.09^b	4.846±0.12^a
Weight gain (g)	2.072±0.10^c	2.831±0.03^b	2.765±0.09^b	3.656±0.09^a
SGR (%)	1.867±0.04^d	2.729±0.06^b	2.583±0.04^c	3.12±0.00^a
CF	0.942±0.27^b	0.946±0.04^b	0.959±0.02^b	1.204±0.01^a
FCR (%)	2.031±0.12^a	1.807±0.05^a	1.509±0.05^b	1.426±0.06^b
Survival (%)	100±0^a	100±0^a	100±0^a	100±0^a

Means in the same row with different superscripts are significantly different (P<0.05); values are presented as the mean ± SD.

Table 3. Effect of different levels of Primalac on biochemical parameters in the Caspian roach

Biochemical parameters	Control	0.05 %	0.1 %	0.15 %
Glucose (mg dl^{-1})	56.8±0.53^{a}	50.2±0.1^{b}	37.6±0.5^{d}	47.8±0.32^{c}
Total protein (g dl^{-1})	9.06±0.01^{c}	9.06±0.31^{c}	9.735±0.21^{b}	10.647±0.01^{a}
Cholesterol (mg dl^{-1})	251.88±3.71^{a}	238.38±3.16^{b}	193.14±1.47^{d}	217.64±2.69^{c}

Means in the same row with different superscripts are significantly different (P<0.05); values are presented as the mean ± SD.

Table 4. Level of hematocrit in Caspian roach fed Primalac after salinity stress (13 ppt)

Primalac levels	24 h	72 h	120 h	168 h
Control	54.5±1^{aA}	54.25±0.25^{abA}	50.25±0.75^{abB}	38.925±0.82^{aC}
0.05 %	56.19±2.06^{aA}	53.125±1.12^{bAB}	49±1^{bcB}	43.25±1.25^{aC}
0.1 %	58.45±0.95^{aA}	57.25±0.75^{aAB}	54.38±1.12^{aB}	39.6±1.1^{aC}
0.15 %	53.875±1.88^{aA}	48.31±1.32^{cB}	45.3±1.3^{cBC}	41.375±0.88^{aC}

Lowercase letters in each column and uppercase letters in each row with different superscripts are significantly differences (P<0.05); values are presented as the mean ± SD.

was observed in the Caspian roach fry fed Primalac probiotic with a trend towards best results at a 0.15% inclusion level. This growth improvement can cause by positive effect this probiotic on digestive system flora and increase digestive ratio and absorption used food. In the fish fed Primalac, the lower FCR indicate the positive role of probiotics on improving food digestion. These results are in agreement with those obtained by Salaghi *et al.* (2013) who used Primalac as probiotic in Persian sturgeon (*Acipeser persicus*) diet for 105 days. They found that the use of Primalac improved the fish performance. Similarly, Imanpoor and Roohi (2015a) showed that the Caspian kutum fed of 0.1% Primalac for 45 days had increased final weight, weight gain and specific growth rate. Imanpoor *et al.* (2016) reported that administration of 0.05 % Primalac for 45 days enhanced the growth of common carp.

Growth enhancement as a result of probiotic administration has been reported in several previous studies on a variation of fish and shellfish species fed dietary probiotics (Ferguson *et al.* 2010; Faramarzi *et al.* 2011b; Mohapatra *et al.* 2012; Seenivasan *et al.* 2012; Neissi *et al.* 2013; Naseri *et al.* 2013). The function of probiotics in the improvement of growth and feed utilization in fish was noted as related to the improvement of nutrient digestibility (Faramarzi *et al.* 2011). Probiotics induce useful microflora into larval intestine and cause high growth performance (Adineh *et al.* 2013).

Blood parameters considered as valuable tools for assessing fish health (Neissi *et al.* 2013) and could be affected by dietary probiotic (Brunt and Austin 2005; Ferguson *et al.* 2010). Plasma glucose levels are considered appropriate indicator of stress as it increase as a secondary response during periods of stress to cover high energy requirements induced by this situation (Cruz *et al.* 2012). The results of present study showed that dietary inclusion of probiotic Primalac significantly decreased glucose and cholesterol levels of the Caspian roach fry (Table 3). As shown in Table 3, fish fed Primalac has significantly greater total protein compared to the control group. In according to our finding, Pouralimotlagh *et al.* (2010) reported that glucose and cholesterol concentrations were decreased in the Caspian roach fed with diet containing vegetable oils. Increased concentration of cholesterol in serum can be result of damages to liver or kidney syndrome (Yamawaki *et al.* 1986; Sancho *et al.* 1997). Also, Nayak *et al.* (2004) reported that the increase in serum total protein indicates that fish are immunologically strong.

Many studies utilize stress challenges to replay information of an organisms fitness or quality (Dhert *et al.* 1992; Taoka *et al.* 2006; Salze *et al.* 2008). Indeed, salinity stress challenge has been frequently used for determination of fry quality in nutritional studies (Smith *et al.* 2004; Taoka *et al.* 2006; Salze *et al.* 2008) and this the reason for developing this assay in the present study. Our results demonstrate that Primalac had no effect on survival of the Caspian roach. This result is agreement whit report of Imanpoor and Roohi (2015b) in the Caspian roach fed Sangrovit under salinity stress.

The hematocrit percentage, hemoglobin rate and erythrocyte count are good indicators for oxygen transportation capacity of fish thus making possible to establish relationship with oxygen concentration available and health status of these fish (Lamas *et al.* 1994). On the first day after stress, hematocrit levels significantly were decreased in all groups (P<0.05). On the other hand, hematocrit levels had tendency to decrease in all group since third day. These results agree with report of Malakpour Kolbadinezhad *et al.* (2012) in the Caspian roach.

Finally, it can be concluded that usage of Primalac can beneficially enhance growth performance and blood parameters of *Rutilus rutilus* fry. This preliminary study encourages further research on administration of probiotics in roach culture as well as the determination of the effects on immune response, digestive enzyme activities and indigenous gut microbiota.

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