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Effects of dietary β-glucan on maternal immunity and fry quality of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The effects of dietary β-glucan on fry quality of rainbow trout were studied. Two feeding trials were conducted with rainbow trout broodstock (trial 1) and fry (trial 2). The experimental diets contained 0 (control), 0.1% and 0.2% β-1,3/1,6 yeast glucan. In trial 1, 45 female rainbow trout were randomly divided into three experimental groups (15 fish per group) and were fed with one of the three experimental diets over three months prior to spawning. In trial 2, fry descending from the control fed broodstock were allocated to three different treatment groups: control fed (L1, considered as the control), 0.1% β-glucan fed (L2), and 0.2% β-glucan fed (L3). The fry from the 0.1% β-glucan fed mothers were divided into two dietary groups: control fed (L4) and 0.1% β-glucan fed (L5), and the fry from 0.2% β-glucan fed females were also allocated to two treatment groups: control fed (L6) and 0.2% β-glucan fed (L7). In trial 2, the fry were fed with one of the experimental diets for two months. Brood fish fed the diet with 0.2% β-glucan exhibited the highest white blood cell (WBC) value, ACH_{50} and lysozyme activity ($P < 0.05$). Total Ig and IgM levels were significantly higher ($P < 0.05$) in all β-glucan fed females compared to broodstock fed the control diet. The ACH_{50} level was also significantly ($P < 0.05$) higher in oocytes from females fed 0.2% β-glucan diet compared to those of other groups. No significant differences ($P > 0.05$) in survival rates (from fertilization to swim-up stages) were found among dietary treatments. In trial 2, dietary β-glucan resulted in better final weight, WG and SGR in groups L2, L3, L5 and L7. ACH_{50}, lysozyme, total Ig and IgM levels were significantly higher in group L3 compared to the control (L1), and comparable with those in group L7. The challenge experiment with *Yersinia ruckeri* showed that relative percent survival (RPS) was highest in L3, L5 and L7. The results indicated that administration of 0.2% β-glucan to rainbow trout fry only instead of feeding both brood fish and fry could have the same positive effects in promoting the immunity of fry and resistance to *Y. ruckeri*.

1. Introduction

Fish offspring that develop externally are exposed to an environment full of microbial pathogens, which can have a serious impact on the survival and performance of offspring (Swain and Nayak, 2009). The production of fish larvae is routinely hampered by significant mortality rates (Zhang et al., 2013). Either direct immunization on fish offspring or maternal immunity may be considered as possible strategies to promote the immunity of fish larvae.

Maternal transfer of immunity is defined as the immunity transferred from mother to offspring, which is supposed to play a key role in protecting the vulnerable offspring against pathogenic attacks at early stages of life (Yue et al., 2013). Both innate and adaptive types of immune factors are transferred from mother to offspring in fishes. Immunoglobulin, as the most important maternal immune factor in vertebrates (Picchietti et al., 2006), and other innate immune factors such as the complement components (Huttenhuis et al., 2006), lectins (Hasan et al., 2009), lysozymes (Magnadóttir et al., 2005), and antimicrobial peptides (Seppola et al., 2009) have been found to be transferred maternally by protein or mRNA in several fish species.

The physiological condition of broodstock influences larval quality of fish by providing non-genetic factors, such as hormones and nutrients (Izquierdo et al., 2001; Watanabe and Vassallo-Agius, 2003). Thus, transfer of maternal immune factors from mother to offspring is particularly sensitive to the availability of specific nutrients or minerals that are needed as materials or precursors for the synthetic process of immune factors (Zhang et al., 2013). Immunostimulants are available tools in aquaculture that help in enhancing resistance against infectious diseases by improving the innate humoral and cellular defense mechanisms. β-Glucans are polysaccharides consisting of a backbone of repetitive nn-glucose monomer units linked by β-(1,3) glycosidic bonds with β-(1,6) branching glucose side-chains. These carbohydrates are mostly found in algae, plants, fungi and some bacteria where they represent a major component of the cell wall (Pionnier et al., 2013). In fish, a number of studies have demonstrated an immunostimulatory effect of orally

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Table 1
Fry performance of rainbow trout broodstock fed different levels of β-glucan.

Experimental diets			
Survival (%)	0	0.1%	0.2%
Fertilization	95.2 ± 1.7	95.6 ± 1.6	97.5 ± 1.2
Eyed stage	89.4 ± 0.9	90.6 ± 0.5	90.5 ± 1.0
Hatching	72.2 ± 0.5	71.7 ± 1.5	73.7 ± 1.6
Swim-up	67.5 ± 1.3	68.9 ± 1.5	70.0 ± 1.3

Values are mean ± SD of three replicate groups. Mean values with different superscripts are significantly different from each other. Significance level is defined as $P < 0.05$.

administered β-glucan resulting in both increased innate and adaptive responses as well as increased resistance to experimental infections (reviewed in Dalmo and Bøgwald, 2008 and Skov et al., 2012). However failure to induce β-glucan mediated immunostimulation and pathogen resistance has also been reported (reviewed in Dalmo and Bøgwald, 2008 and Skov et al., 2012). Growth promotion by oral administration of β-glucan is also reported (Misra et al., 2006).

Due to rapid growth and easy accommodation to environmental conditions, rainbow trout (*Oncorhynchus mykiss*) is one of the most commercially important species grown in Iran. Current annual production of farmed rainbow trout in Iran is more than 100,000 tons. In recent years, there have been growing concerns about the adverse effects of the bacterium *Yersinia ruckeri* in the aquaculture of salmonids. Numerous outbreaks of enteric red mouth disease (ERM) or yersiniosis in rainbow trout farms are currently being recorded despite established vaccination procedures against this disease (Soltani et al., 2014).

The immunonutrition may be of great promise in the prevention of infectious diseases of rainbow trout such as yersiniosis and thus warrants investigation. The objective of the present study was hence to investigate the effects of dietary β-glucan supplementation on fry quality of rainbow trout. Both immune stimulation to mother and direct immunization of offspring strategies were applied to study growth and immune parameters of rainbow trout fry and resistance to *Y. ruckeri* infection.

2. Materials and methods

2.1. Experimental diets

β-1,3/1,6 yeast glucan (MacroGard®, Biotec-Mackzymal, TromsØ, Norway) derived from the cell wall of *Saccharomyces cerevisiae* was included in feed before extrusion. A commercial rainbow trout diet lacking β-glucan (Beyza Feed Mill, Fars province, Iran) and containing 52% protein, 12.5% fat, 10% humidity and 4300 kcal kg^{-1} diet digestible energy was used. The basal diet was crushed and mixed with water and the supplements were added to obtain diets containing 0.1% and 0.2% β-1,3/1,6 yeast glucan. Control feed was prepared in the same way without the addition of β-glucan. Extruded diets were air-dried at room temperature and stored at 4 °C in sealed plastic bags until used.

2.2. Fish and experimental conditions

Two feeding trials were conducted with rainbow trout broodstock (trial 1) and fry (trial 2) in a commercial rainbow trout farm (Sheshpir Breeding Farm, Sepidan, Fars Province, Iran). In trial 1, 45 female rainbow trout of about 4 kg were randomly divided into three experimental groups (15 fish per group). The fish of each dietary group were held in three separate raceways (5 fish per raceway) supplied with natural spring water and acclimated for 2 weeks, during which they were fed control feed lacking β-glucan. The mean water quality parameters were as follows: temperature 10.5 °C, dissolved oxygen 7.9 mg L^{-1} and pH 7.8. At the end of the acclimation period, the female broodstock were fed by hand twice a day to apparent satiation with one of the three experimental diets over three months prior to spawning. Three batches of pooled oocytes from nine ovulated females of each dietary group were prepared and fertilized with milt from males from the same farm. Triplicate batches of eggs from each dietary group were incubated in separate small trays to obtain hatching data.

In trial 2, hatched fry descending from the control of broodstock were allocated to three different treatment groups: control fed (L1, considered as the control), 0.1% β-glucan fed (L2), and 0.2% β-glucan fed (L3). The fry from the 0.1% β-glucan fed mothers were divided into two dietary groups: control fed (L4) and 0.1% β-glucan fed (L5). The fry originating from 0.2% β-glucan fed females were also allocated to two different treatment groups: control fed (L6) and 0.2% β-glucan fed (L7). The fish were randomly stocked into 21 tanks at a density of 300 fish per tank (3 tanks per treatment). Fish were fed to apparent satiation 9 times per day with their respective diets over two months.

2.3. Fry performance

The fertilization rates (total number of developing eggs/total number of eggs) of each batch of eggs were assessed one day post-fertilization (dpf). Dead embryos or fry were removed at the eyed stage (22 dpf), hatching (34 dpf) and swim-up stage (52 dpf) and survival rates were calculated.

2.4. Growth measurements of fry

The fish in the different experimental groups were weighed at the end of the 2-month feeding trial for estimation of growth. Based on recording the weight of each fish, specific growth rate (SGR), percentage of body weight gain (WG) and feed conversion ratio (FCR) were calculated for each group as follows: SGR = 100 × [ln final weight − ln initial weight] ÷ total duration of the experiment, WG = [100 × (final body weight − initial body weight) / initial body weight], and FCR = feed given (dry weight) ÷ weight gain (wet gain). In addition, survival rate was calculated at the end of the experiment: survival = (N_f / N_0) × 100; where N_0 is the initial number of fish and N_f is the final number of fish.

Table 2
Growth performance of rainbow trout fry in different treatments of trial 2.

Experimental groups							
Parameters	L1	L2	L3	L4	L5	L6	L7
Initial weight (mg)	181.05 ± 3.57	184.7 ± 2.87	186.50 ± 3.54	179.92 ± 1.89	187.47 ± 3.49	183.5 ± 2.12	187.22 ± 2.91
Final weight (mg)	884.2 ± 38.8^{ab}	996.5 ± 56.1^{bc}	1060.2 ± 84.9^{c}	757.1 ± 82.2^{a}	1009.7 ± 84.4^{bc}	880.0 ± 55.0^{ab}	1033.3 ± 86.2^{c}
WG%	388.7 ± 28.6^{abc}	438.3 ± 18.8^{bcd}	493.3 ± 9.0^{d}	321.2 ± 50.3^{a}	438.3 ± 35.0^{bcd}	366.6 ± 33.1^{ab}	451.6 ± 39.8^{cd}
FCR	0.87 ± 0.06^{abc}	0.76 ± 0.05^{a}	0.72 ± 0.03^{a}	1.01 ± 0.14^{b}	0.75 ± 0.08^{a}	0.87 ± 0.06^{ab}	0.78 ± 0.08^{a}
SGR (% day^{-1})	2.64 ± 0.10^{ab}	2.80 ± 0.06^{bcd}	2.97 ± 0.03^{d}	2.39 ± 0.19^{a}	2.80 ± 0.11^{bcd}	2.57 ± 0.12^{ab}	2.84 ± 0.12^{cd}
Survival	97.3 ± 0.7	98.7 ± 1.0	98.4 ± 0.5	97.4 ± 0.5	97.8 ± 0.7	97.1 ± 0.9	98.9 ± 1.2

Values are mean ± SD of three replicate groups. Mean values with different superscripts are significantly different from each other. Significance level is defined as $P < 0.05$. Experimental groups: fry and broodstock without glucan as control group (L1), fry fed 0.1% glucan and broodstock 0% (L2), fry fed 0.2% glucan and broodstock 0% (L3), fry fed 0% glucan and broodstock 0.1% (L4), fry fed 0.1% glucan and broodstock 0.1% (L5), fry fed 0% glucan and broodstock 0.2% (L6), and fry fed 0.2% glucan and broodstock 0.2% (L7).