WALAILAK JOURNAL
http://wjst.wu.ac.th Agriculture Technology and Biological Sciences

Influence of Dietary Dinamune® on Growth Performance and Lysozyme Activity in Rainbow Trout (*Oncorhynchus mykiss*) Fry

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Received: 17 December 2012, Revised: 28 April 2014, Accepted: 1 June 2014

Abstract

This study was conducted to evaluate the effect of a commercial β-glucan based immunostimulant preparation, Dinamune®, in the form of a feed supplement, on the growth performance and lysozyme activity of rainbow trout fry (*Oncorhynchus mykiss*) weighing 1 g. Fish were fed diets containing 0 (control), 0.5, 1 and 1.5 g Dinamune® kg^{-1} of dry diet for a period of 11 weeks. Body weight was generally increased in fish which were fed diets supplemented with concentrations (1.5 Dinamune®/kg feed) (23.75 ± 7.48), compared with the control (21.38 ± 7.04) ($p < 0.05$). Specific growth rate was significantly ($p < 0.05$) the highest in 1.5 g Dinamune® kg^{-1} of dry diet (4.27 ± 0.40) and the lowest in the control group (4.13 ± 0.41). FCR value was found to be the best in the 1g kg^{-1} group (0.67 ± 0.17), followed by the 1.5g kg^{-1} group (0.73 ± 0.23), the 0.5 g kg^{-1} group (0.80 ± 0.36) and the control group (1.00 ± 0.25). Hepatosomatic index (HSI) showed a significant decrease in 1.5 g Dinamune® kg^{-1} of dry diet (0.88 ± 0.18), compared with the control (1.15 ± 0.19). Condition factor (CF) in the control, 0.5 g kg^{-1} diet, 1 g kg^{-1} diet and 1.5 g kg^{-1} diet was (1.30 ± 0.16, 1.34 ± 0.15, 1.19 ± 0.10 and 1.18 ± 0.10) respectively. Lysozyme activity of the liver, kidney, and serum were significantly stimulated in the 0.5, 1 and 1.5 g Dinamune® supplemented groups at the end of experiment, compared with the control ($p < 0.05$).

Keywords: Dinamune, immunostimulant, rainbow trout, Lysozyme

Introduction

The success of intensive aquaculture is due to improved genetics, nutrition, and management, as well as the increasing efficiency of disease control. A commercial production system can only be economically viable if these factors are optimized. Growth rate, feed conversion ratio (FCR), and mortality, which are influenced primarily by the quality of feed and feed intake, have key roles in the evaluation of an aquaculture system. In recent years, much of the research has focused on the nutritional requirements of fish and the benefits of supplementing feed with pro-nutrients [1].

Pro-nutrients are not essential to fish; however, they can benefit animal health and performance through improving availability or utilization of nutrients in a variety of ways. Fish are constantly under threat from a large number of pathogens, consisting of bacteria, viruses, fungi, and parasites [2].

The non-specific immune system consists of several components that provide innate protection against infection, regardless of the pathogen type [3]. A proper immune system is a sensitive indicator of the health in an organism, and is frequently employed in aquaculture to supervise fish health. As aquaculture improves, we require greater knowledge of medicinal treatments, both for the prevention of and treatment of disease [4].

Non-specific immunity of fish is considered to be the first line of defense against a broad spectrum of pathogens, and is more important for fish as compared with mammals [5]. This type of immunity includes constitutive physical and chemical factors, which are always present, and inducible factors, such as lysozyme. It can be found in peripheral blood, cutaneus mucus, and certain tissues of marine and freshwater fishes [5].

Dinamune® is the trade name for a combination of a highly purified yeast extract chemically known as beta 1-3-D-glucan. 1-3 β Glucanase has a GRAS (generally considered as safe) classification by the FDA.

In this work we aimed to investigate the effect of the pro-nutrient, Dinamune®, whose main active ingredient is 1-3 β Glucan, on growth performance and lysozyme level in the non-specific (innate) immune system of rainbow trout.

Materials and methods

Fish

In the trial, 2000 healthy rainbow trout, averaging 1 g, were randomly divided into 4 raceways at water temperature 16 ± 1 °C and dissoluble oxygen 8 mg/L. Water supply during fry rearing at the start, about 0.25 liters/min; at the end, about 3.5 - 4.5 liters/min water.

Dinamune® provided from Nutri-Agri Technologies Inc., dinatec, Jordan was mixed directly with commercial fish feed (Biomar, Bio-optimal Start) to achieve 0.5 g and 1.0 g and 1.5 g 1000 g^{-1} of feed. The feeding rate was 4 - 5 times per day according to weight and water temperature (**Table 1**). Mixture-feed were prepared for each day. The fish were maintained on this diet for 11 weeks; then, fish in all groups were counted and group weights were taken for estimation of weight gain and growth parameters, 11 weeks following the initiation of the experiment. A control group was also included.

Calculation formula for relative index

The initial and final weights of fish in each group were measured individually. Specific growth rates (SGRs), condition factors (CFs), FCR, HSI and CF were calculated according to Laird and Needham (1988) as follows: Feed conversion rate (FCR) = Weight gain / Feed consumption
Specific growth rate (SGR %) = 100 (Ln(Average terminal BW)-Ln(Average initial BW)/Test days); Hepatosomatic index (HSI %): liver weight/total weight×100; Percent body weight gain = ((final weight-initial weight)/initial weight)×100; Total weight gain = final weight-initial weight;
Condition factor (CF) = weight (g)/[length (cm)]

Lysozyme assay

The lysoplate assay [6], with modifications outlined by Yousif *et al*. [7] was used to determine lysozyme activity in serum, liver, and kidney samples. Briefly, this assay involved preparing agar plates (lysoagar) containing 0.60 mg/ml micrococcus lysodeikticus (Sigma), 0.02 M NaCl, 0.50 % Agarose (Sigma) in phosphate buffer (PB, 0.06 M, pH 6.0). Wells (with approximately 3 mm diameters) were punched into the lysoagar, which was air dried. Hen egg white lysozyme (HEWL, Sigma) standards were used. The activity of the HEWL standard (under the assay conditions described) was measured using the turbidimetric method described by the supplier of the HEWL (Sigma), with modifications by Grinde [8].
2.4 Data statistics and analysis.

The results were presented as mean ± SD, all data were subjected to ANOVA, and comparison of the mean values was done by using Bonferroni tests, at the 5 % level of significance. The software program SPSS (Version 16.0) for Windows was used.

Results and discussion

FCR, SGR, HSI, CF, and weight gain of fish in each group were calculated by using the growth parameters, and are shown in **Table 1**. After the 11 week feeding period in the feeding trial, enhanced weight gain was generally observed in fish fed the supplemented diets (1.5 g Dinamune/kg feed) ($p <$

0.05). SGR was significantly higher in concentrations (1 and 1.5 g Dinamune/kg feed) ($p < 0.05$). FCR was significantly lower in treatment groups compared with the control. HSI showed significant decrease in concentrations (1.5 g Dinamune/kg feed) compared with the control ($p < 0.05$). We didn't observe any difference in BW and Lysozyme activity in liver, kidney and serum showed a significant increase compared with control.

Table 1 Feed conversion rate (FCR), Specific growth rate (SGR), Body weight (BW), Hepatosomatic index (HSI), Condition factor (CF), and Survival rate of rainbow trout fed with 0 (Control), 0.5 and 1.5 Dinamune after 11 weeks.

	Dinamune concentrations (g/1 kg diet)			
Variable	**Control**	**0.5**	**1**	**1.5**
Body weight gain (%)	21.38±7.04[a]	23.63±9.15[a]	23.10±5.75[a]	23.75±7.48[b]
Specific growth rate (%)	4.13±0.41[a]	4.21±0.52[a]	4.24±0.33[b]	4.27±0.40[b]
Feed conversion rate	1.00±0.25[a]	0.80±0.36[b]	0.67±0.17[b]	0.73±0.23[b]
Condition factor	1.30±0.16[a]	1.34±0.15[a]	1.19±0.10[b]	1.18±0.10[b]
Hepatosomatic index (%)	1.15±0.19[a]	1.27±0.31[a]	1.01±0.22[a]	0.88±0.18[b]
Survival rate (%)	99.2[a]	99.78[b]	99.80[b]	99.80[b]

Values are mean ± S.E. Values within the same row, not sharing common superscript letters, are significantly different $p < 0.05$, $n = 100$.

Table 2 Lysozyme (mm) of kidney liver and serum (mean ± SD, n = 10) in *Oncorhynchus mykiss* fed with 0 (Control), 0.5 and 1.5 Dinamune after 11 weeks.

	0.5 g/kg diet	**1 g/kg diet**	**1.5 g/kg diet**	**control**
Liver	10.50±0.52[*]	10.50±0.52[*]	11.50±0.52[*]	00.00±00.00
kidney	10.40±0.51[*]	10.60±0.51[*]	11.10±0.87[*]	00.00±00.00
serum	10.50±0.52[*]	10.50±0.52[*]	8.90±3.38[*]	00.00±00.00

Values within the same row, not sharing common superscript letters, are significantly different $p < 0.05$, $n = 10$.

The results from this study show that Dinamune is able to increase the growth parameters and lysozyme activity of rainbow trout larvae. Weight gain in the experimental period was significantly higher in fish fed a diet containing 1.5 g Dinamune by week 11.

The SGR data shows that dietary Dinamune supplementation (1 and 1.5 g Dinamune kg^{-1} of diet) significantly enhances the SGR of rainbow trout when compared with the fish fed on the control diet. Dinamune® is combination of a highly purified yeast extract chemically known as beta 1-3-D-glucan; the physical and physiological properties of β-glucan are of commercial and nutritional importance. During the last two decades, there has been increasing interest in β-glucan, due to its acceptance as a functional and bio-active ingredient [9]. Kumara and Kumari [10] showed that glucan administration in Asian catfish, *Clarias batrachus*, at 0.1 % in feed, significantly enhanced lysozyme levels, irrespective of length of exposure. These results were in accordance with our study. However, the SGR were not affected by the dietary supplementation of yeast glucan. Moreover, dietary application of other commercial β-glucan preparations, such as EcoActiva (Melbourne, Australia) and MacroGard (Trosmø, Norway), have also

been shown to enhance weight gain respectively in snapper (*Pagrus auratus*) and turbot (*Scopthalamus maximus L.*) [11,12]. In contrast, there was no enhancement in the growth rate of dentex fed diets containing a variety of different commercial β-glucan preparations [13].

However, fish fed the 0.5,1 and 1.5 g Dinamune diets had significantly lower FCR values than that of fish fed the 0 g Dinamune diets. Whittington *et al*. [14] reported that the relatively low inclusion levels of β-glucan could interfere with the digestion and absorption of dietary nutrients.

Lysozyme contributes to the innate immunity of animals by its bactericidal and anti-inflammatory properties [15]. Recent studies show that lysozyme activity in Atlantic salmon, rainbow trout, and turbot, can be up regulated by treatment with particulate yeast cell wall β-glucan [16]. Elevated levels of plasma lysozyme activity have been observed in several fish species as a response to intraparitoneal injected particulate yeast β-glucan. The lysozyme activity peaked at day 14 after injection in Atlantic salmon (*Salmo salar L.*), while a stable elevated lysozyme activity was still present at day 21 in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Scophthalmus maximus L.*) [17,18]. In accordance, our results showed that lysozyme activity in liver, kidney and serum was enhanced by dietary inclusion of Dinamune (0.5, 1 and 1.5 g kg^{-1} fish feed) after 11 weeks.

Conclusions

In conclusion, the results of this study show that the continuous oral administration of β-glucan preparations, like Dinamune, to rainbow trout over an 11 week period may be beneficial. Lysozyme activity is enhanced, thus providing for a potential increase in disease resistance at a time when the fish are more susceptible to infection. Furthermore, the addition of Dinamune to the diet of these fish increased growth rates.

Acknowledgements

This work was financially supported by the Research Council of the University of Tehran Center of Excellence of Aquatic Animal Health, University of Tehran.

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