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Original Article

The effects of dietary lysozyme on growth performance and haematological indices of rainbow trout (*Oncorhynchus mykiss*) fingerling

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Abstract: The present study investigates the effects of dietary lysozyme on growth performance and haematological indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings. One hundred and twenty Juvenile rainbow trout fish (initial average weight 5.46±0.05 g) were fed on varying levels of dietary lysozyme (0, 0.5, 1 and 1.5 g kg^{-1}) for 8 weeks. Thereafter, growth performance as well as haematological indices including WBC, RBC and Hct were measured. Evaluation of growth performance showed no significant increase in fishes fed on different levels of dietary lysozyme (P>0.05). Also, feeding on dietary lysozyme significantly increased WBC compared control (P<0.05); the highest level was detected in 1.5 g kg^{-1} treatment. Furthermore, RBC of fish fed 1 or 1.5 g kg^{-1} lysozyme were significantly higher than other treatments. The same results were noticed in case of Hct which was higher in fish fed treated diet. These results indicated that dietary lysozyme affect haematological parameters rather than growth performance.

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Introduction

Fish and Shellfish produced in aquaculture industry meet the market demands of increasing world population for healthy protein (FAO, 2016). The main issues aquaculture industry facing with, are disease outbreak (Azimirad et al., 2016), therefore, researches regarding fish health status and immunostimulants are of high importance (Nawaz et al., 2018). Considering the cross talk between nutrition and health status, modulation of fish immune response can be taken into account through dietary approaches (Yarahmadi et al., 2016). Besides, a major concern in aquaculture is the use of chemical therapeutics, such as antibiotics, because of their impact on the environment as well as on the fish product (Hoseinifar et al., 2017). This concern can be resolved by using environment friendly feed additive. On the other hand, determination of the mode of action of immune-stimulant will help in selection of the optimum kind and doses (Wang et al., 2017). Among various types of immunostimulant, one strategy for improving the fish feed safety and deprivation diseases is adding antimicrobial enzymes in diet (Deng et al., 2012; Chen et al., 2014; Abdel-Latif et al., 2017).

In fish, lysozyme can be found in different organs and body fluids including mucus, serum, gills, alimentary tract etc. (Chen et al., 2014). The main function of lysozyme is removing the pathogens through bacteriolytic activity (Modanloo et al., 2017). This occurs by cleave of the glycosidic linkage in the peptidoglycan component of gram-positive bacterial cell walls (Van Doan et al., 2017a). It has been suggested that the abovementioned function will finally results in improved growth performance (Abdel-Latif et al., 2017). Moreover, lysozyme along with other factors, including immunoglobulins, lactoferrin in antimicrobial activity and with lactoperoxidase, may limit the migration of neutrophils into a damaged tissue by behaving as an anti-inflammatory agent (Chen et al., 2014). However, in spite of these benefits, there is limited information available about possible effect of dietary lysozyme on fish growth performance and health (Deng et al., 2012, Chen *et al.*, 2014). Therefore, the present work aimed

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to study the effects of different levels of dietary lysozyme on growth performance and haematological parameters of rainbow trout.

Materials and Methods

Experimental design: One hundred and twenty Juvenile rainbow trout purchased from an aquaculture farms and transferred to the laboratory in Gorgan University of Agricultural and Natural Resources, then were acclimatized to the experimental condition for 2 week before the start of the main experiment. Ten juvenile fish (with initial average weight of 5.46±0.05 g) were randomly released into 12 tanks. The capacity of each tank was 200L and the exchange of 50% water occurred every day. Water temperature and dissolved oxygen were 16±3.1 and 7.5 mg l^{-1}, respectively.

Preparation of experimental diets: A commercial trout feed was used in this study as control diet (Coppens Co., Netherland). The chemical composition are shown in Table 1. Four experimental diets was prepared with different levels (0, 500, 1000, 1500 g kg^{-1}) of dietary lysozyme (Zhejiang Aegis Biotech Co., Ltd) in a dry basis. Thereafter, the prepared diets were kept in plastic bag at -4°C. Fish were hand-fed thrice (8:00 and 12:00 and 16:00) daily for 8 weeks with 3% of body weight.

Sampling: Fish were not fed for 24 hrs prior to sampling at the end of the feeding trial. Total number and mean body of fish in each tank were measured to calculate the growth, feed utilization and nutrient retention. For haematological parameters, six specimens were randomly sampled from each tank then blood was collected from the caudal vein with a heparinized syringe and transferred into a heparinized tube.

Grow performance: Growth performance and survival rate of juvenile rainbow trout were enumerated using following equations:

Weight gain (WG) = Final Weight (g) - initial Weight (g)

Specific growth rate (SGR) = 100 (ln final weight-ln initial weight) / Duration of experiment

Feed conversion ratio (FCR) = feed intake (g) / weight gain (g)

Table 1. Analysis of the commercial feed for rainbow trout (Coppens, Netherlands ultra).

Analyses	Composition
Protein	48%
Fat	22%
Crude fiber	0.9%
Ash	8.8%
Total P	1.45%

Protein efficiency ratio = weight gain (g)/protein fed (g).

Grow rate = final weight – initial weight / day

Survival rate = (Final number / Initial number) × 100

Haematological analyses: Red blood cells and white blood cells were counted using a neubauer hemocytometer according to Martins et al. (2004). Haematocrit was measured with microcentrifuge method, using standard heparinized microhaematocrit capillary tubes (75 mm at 7000 g for 10 min) (Blaxhall and Daisley, 1973).

Statistical analysis: After checking the normality of the data, statistical analysis was performed using One way ANOVA followed by Duncan's multiple-range test. The significant difference levels was considered when $P<0.05$. The statistical analysis was performed by SPSS software 19 (SPSS, USA).

Results

Grow performance: The growth indices of *O. mykiss* fed diets supplemented with different levels of dietary lysozyme shown in Table 2. The diet with 1.5 g per kg dietary lysozyme has better growth performance but no significant differences were observed in the growth parameters of fish in the different treatments. After 60 days of feeding, the variation range of weight gain, SGR and GR also FCR and feed protein rate efficiency and survival rate summarized in Table 2. According to the results, changes that was measured has not significant effect between different treatments.

Heamatological indices: The present results shown dietary lysozyme supplementation exerted a certain influence on the blood indices studied (Table 3). Respect to the values, number of white blood cells increased significantly in group with 1 and 1.5 g kg^{-1} dietary lysozyme compare to control diet group. Number of total red blood cells showed a significant

Table 2. Growth performance of rainbow trout fed with different dietary lysozyme levels (g kg^{-1}) for 8 weeks experimental period (means±SD).

Index	Control	0.5 mg kg^{-1}	1 mg kg^{-1}	1.5 mg kg^{-1}
Initial weight (g)	5.44 ± 01^{a}	5.42 ± 03^{a}	5.50 ± 04^{a}	5.48 ± 07^{a}
Final weight (g)	27.62 ± 1.75^{a}	29.19 ± 0.49^{a}	29.58 ± 0.40^{a}	30.07 ± 0.75^{a}
Feed intake (g per fish)	1.22 ± 76.6^{a}	1.32 ± 57.5^{a}	1.66 ± 0.00^{a}	1.66 ± 0.00^{a}
Weight gain (g)	25.81 ± 3.56^{a}	28.10 ± 0.46^{a}	28.83 ± 0.38^{a}	29.43 ± 0.79^{a}
FCR	0.65 ± 0.09^{a}	0.59 ± 0.01^{a}	0.57 ± 0.007^{a}	0.56 ± 01^{a}
SGR (% day)	2.70 ± 0.10^{a}	2.80 ± 0.0^{a}	2.80 ± 0.02^{a}	2.83 ± 0.03^{a}
PER	0.74 ± 0.10^{a}	0.81 ± 0.01^{a}	0.83 ± 0.01^{a}	0.84 ± 0.01^{a}
GR	0.43 ± 0.05^{a}	0.46 ± 0.02^{a}	0.48 ± 0.02^{a}	0.49 ± 0.01^{a}
Survival rate (%)	73.33 ± 46.1^{a}	80 ± 34.6^{a}	100 ± 0.00^{a}	90.21 ± 21.2^{a}

Table 3. Effect of dietary lysozyme levels on hematological indices of rainbow trout (g kg^{-1}).

Index	Control	0.5	1	1.5
WBC	13.53 ± 1.10^{a}	18.26 ± 0.70^{b}	20.53 ± 0.35^{bc}	24.36 ± 3.17^{c}
RBC	1.80 ± 0.05^{a}	1.90 ± 0.09^{a}	2.07 ± 0.06^{b}	2.01 ± 0.04^{b}
Hct	26.83 ± 3.21^{a}	39.63 ± 6.80^{b}	44.5 ± 2.17^{b}	47.5 ± 1.32^{b}

Means in the same row with different superscripts are significantly different (P<0.05)
WBC white blood cell ($\times10^{3}$ μL)
RBC red blood cell ($\times10^{6}$ μL)
Hct hematocrit (percent %)

increase in group with 1 g kg^{-1} dietary lysozyme compare to experimental treatment also control group diet. Hematocrit value show a significant increase in experimental diet compare to control diet.

Discussion

Lysozyme is an antibiotic peptide and growth enhancer with positive bacteriolytic ability that can used in aquaculture and poultry industry (Abdel-Latif et al., 2017; Gong et al., 2017). In this study, dietary lysozyme supplementation generally increased the growth performance parameters and survival rate of fish although no significant different was observed between treatment groups. In contrast with our study, previous researches shown antibacterial agents that used in growth promoting experiment has a significant effect in channel catfish (Sanchez-Martínez et al., 2008) dietary apidaecin (apidaecintype peptides) on common carp (*Cyprinus carpio*). In addition, dietary lysozyme (500 mg kg^{-1}) significantly increased the growth performance of broiler chickens Zhong-ke et al. (2008). Likewise, administration of 100-300 mg kg^{-1} lysozyme broiler chickens diet resulted in improved growth parameters (Yi-nan, 2010; Lu et al., 2009; Ding, 2010). Similar results obtained in case of 70 mg kg^{-1} lysozyme in duck diets (Gu and Zhang, 2008), 100 mg kg^{-1} lysozyme in meat rabbit diets (Guo et al., 2010) and 300-500 mg kg^{-1} lysozyme in weaned piglet diets (Wang et al., 2008; Lu et al., 2010; Wu et al., 2010). Based on the above results, it can be assumed that lysozyme is a growth promoter. However, the mode of action remained unclear. In chick, the antimicrobial activity of lysozyme could result in better intestinal health and improved digestion and it is a complex and integrated interaction between microbes and immune system, which is responsible for the development and maturation of the fishes immune system. Chen et al. (2013) found that the absence of intestine microbiota could lead to depression of the host immune response status. In terrestrial animal research, it has long been established that dietary antibacterial material include lysozyme could result in morphology changes in intestinal (Deng et al., 2012). When all this actions occurred in body, animals could save extra energy in intestine also can use instead for growth, or increasing the absorption of other nutrients (Chen et al., 2014). However, some factors like stress, season, sex ,water temperature and quality, low pH and sedimentation can change lysozyme activities in fish (Saurabh and Sahoo, 2008). The relationship between nutrition with immunity has been recognized a long years ago for researchers but to explaining this mechanism, we need more experiment (Hoseinifar et al., 2016). However,

possible reason for contradictory findings may be was effect of this agents on lysozyme activity that cause decreasing enzymes functions in intestine microbiota.

Generally, blood parameters are an important indicator of the physiological state of the internal organs. In other words, the haematology is an importance part of analysis the diagnosis in many diseases, metabolic studies as well as biological control of living organisms, such as aquatic organisms. Also, haematological parameters of fish are useful tool to assess the health status and as well providing information on nutrient status, digestive function and routine metabolic level of fish (Yarahmadi et al., 2016). Blood composition changes can be result of changes occurred in diet quality and nutrient compounds (Hoseinifar et al., 2014). Hematological parameters are important health indicators whose study reveals the health conditions of fish regarding diseases and immune system conditions before and after an experiment. According to this study, dietary lysozyme supplementation shown a significantly effect on hematological indices between treatment group and control group. Number of white blood cells was counted has increased significantly in group with 1 and 1.5 g kg^{-1} dietary lysozyme compare to control diet group. Blood leucocyte phagocytic activity and serum lysozyme deceased with the reduction in intestine microbiota. It indicated that the absence of the micro-organisms reduce the conjunction between pathogen associated molecular patterns (PAMP) and pattern recognition receptors (PRR) in the gut-associated lymphoid tissue (GALT) (Magnadottir, 2010). Also Rymuszka et al. (2005) found that lysozyme may enhance the fish immune functions by the non-specific modulation of leucocyte activities. Possibly lysozyme activity in blood and fishes' organs such as gut, liver and kidney is a reason for increasing white blood cell due to increasing phagocytosis performance with presence lysozyme on treatment diet and finally stimulating the production of white blood cells increases the ability of the body to remove pathogens such as bacteria, fungi and viruses as well as gastrointestinal tract function. Red blood cells, hematocrit and hemoglobin are responsible to carried oxygen in body. Lysosomal enzymes such as ALP plays an important role in the mineralization of skeleton of aquatic animals and in membrane transport activities (Van Doan et al., 2017b). It is often employed to assess the integrity of plasma membrane, and the decrease in activity may be taken as an index of disturbance of membrane transport system (Öner et al., 2008). In this study red blood cells count increase in diet with 1 and 1.5 g kg^{-1} dietary lysozyme also hematocrit levels has a significant difference between treatment groups and control diet. However dietary lysozyme in rainbow trout diet is associated with an increase in RBC number and hematocrit level in plasma. Suggestion reason for this influence may be attributed to the increased ALP enzyme activity in liver, that cause improve immune system function as well as transport activity. In conclusion, the present study revealed beneficial effects of dietary lysozyme on growth performance and haematological parameters of rainbow trout.

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چکیده فارسی

اثرات لیزوزیم خوراکی بر عملکرد رشد و شاخص‌های خونی بچه ماهی قزل‌آلای رنگین کمان (*Oncorhynchus mykiss*)

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چکیده:

مطالعه حاضر به بررسی اثرات لیزوزیم خوراکی در جیره بر عملکرد رشد و شاخص‌های خونی در بچه ماهی قزل آلای رنگین کمان می‌پردازد. تعداد ۱۲۰قطعه بچه ماهی قزل‌آلای رنگین کمان با میانگین وزنی ۰/۰۵±۵/۴۶ گرم به مدت هشت هفته با جیره‌های آزمایشی حاوی سطوح مختلف (صفر (شاهد)، ۰/۵، ۱ و ۱/۵ گرم بر کیلوگرم) لیزوزیم خوراکی تغذیه شدند. در پایان دوره شاخص‌های رشد و شاخص‌های خونی (شامل تعداد گلبول سفید، گلبول قرمز و هماتوکریت) اندازه‌گیری شد. بررسی شاخص‌های رشد افزایش معنی‌داری در ماهی های تغذیه شده با سطوح مختلف لیزوزیم خوراکی در مقایسه با تیمار شاهد نشان نداد ($P>0/05$). اگرچه، تغذیه با جیره حاوی لیزوزیم به‌طور معنی‌داری سبب افزایش تعداد گلبول سفید در مقایسه با گروه شاهد شد ($P<0/05$)، بیشترین میزان در تیمار ۱/۵ گرم در کیلوگرم لیزوزیم مشاهده گردید. به‌علاوه، تعداد گلبول‌های قرمز ماهی تغذیه شده با ۱ یا ۱/۵ گرم به ازای کیلوگرم لیزوزیم به‌طور معنی‌داری بیشتر از سایر تیمارها بود ($P<0/05$). نتایج مشابهی در خصوص سطوح هماتوکریت در ماهی‌های تحت تیمار مشاهده گردید. نتایج این مطالعه حاکی از اثرگذاری لیزوزیم جیره بر شاخص‌های خونی است اگرچه بر شاخص‌های رشد اثری نداشت.

کلمات کلیدی: لیزوزیم خوراکی، قزل‌آلای رنگین کمان، کارایی رشد، خون‌شناسی.