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Growth performances and hemato-immunological responses of common carp (*Cyprinus carpio* Linnaeus, 1758) to fermented *Aspergillus oryzae*

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Abstract

The present study investigates the effects of varying levels of dietary fermented *Aspergillus oryzae* (0 g Kg^{-1} as control group, 10, 20 and 30 g Kg^{-1}) on performance and haemato-immunological indices of common carp (*Cyprinus carpio*) fingerlings. Common carp fingerlings (n=240, w=4.56±0.17 g) were supplied, randomly stocked in 12 aquaria and fed with experimental diets for 7 weeks. The results revealed no significant differences between performance parameters of fermented *A. oryzae* fed test and control diets ($p>0.05$). Also, evaluation of haematological parameters (RBC, Hct, Hb) and white blood cell (WBC) counts showed no notable changes between experimental groups ($p>0.05$). However, respiratory burst activity was meaningfully higher in fish fed fermented *A. oryzae* compared to control group ($p<0.05$). The present results showed that possible effects of administration of fermented *A. oryzae* on immune responses in carp fingerlings.

Keywords: Diet, Fish immunology, Hemato-immunological indices, Prebiotic

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Introduction

The ultimate goal in aquaculture is raising the production efficiency to maximize commercially benefits. One of the methods for increasing the efficiency of production is raising the density or intensive culture but it might lead to disease in fish and shrimp due to reduction of water quality and increases the occurrence of stressful situations. Using feed additives such as probiotics, prebiotics and fermented products as functional feed ingredients to increase the feeding efficiency and immune response is one of the proposed ideas (Nayak, 2010).

The potential negative effects of antibiotics application in aquaculture include emergence of resistant bacteria causing increased attention toward non-pathogenic bacteria as probiotic and control agents. Fermented products and prebiotics can prevent these negative effects by improving the digestibility of feed ingredients through stimulating desirable microbial fauna of gastrointestinal tract (Yanbo and Zirong, 2006). It is well documented that fermented products have significant effects on performance and health status of fish (Mazurkiewicz *et al.*, 2008; Yarahmadi *et al.*, 2014). Hoseinifar *et al.* (2011a) indicated the positive effects of inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus,* on some growth factors and also microbial fauna of gastrointestinal tract in Beluga juveniles. In a study conducted on parrot fish (*Oplegnathus fasciatus*) it was demonstrated that a diet containing *A. orizae* increased red blood cell counts (Kim *et al.*, 2009).

Fermacto® is the commercially fermented product of *Aspergillus orizae* which is known as *Aspergillus* Meal (AM). The AM does not have any spores or live cell and is experimented for exploring to increase intestinal digestive efficiency (Torres-Rodriguez, 2005). It has been shown that administration of Fermacto® in culture media increases the growth of *Lactobacillus spp*. (Higgins, 2007). However, this feed additive has been used mainly in terrestrial animals and there is very limited information about the possible effects on fish performance and health. Therefore, this study was carried out to investigate the effects of fermented *A. oryzae* on performance and hemato-immunological indices of common carp fry.

Materials and methods

Fish maintenance and culture

Common carp fries were obtained from a private farm and transported to Aquaculture Laboratory of Gorgan University of Agricultural Science and Natural Resources. Fish were allowed to acclimatize to experimental condition for two weeks and thereafter, randomly stocked (n=240, w=4.56±0.17 g) into 12 aquaria (200 L) at a density of 20 fish per aquaria. Fish were hand-fed with experimental diets twice a day at rate of 3% of body weight during the trial (Akbary *et al.*, 2015).

Experimental diets

The experimental diets were prepared by supplementation of a basal diet

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(control diet, 0 g Kg^{-1} fermented *A. oryzae*), with different levels (10, 20 30g Kg^{-1}) (Hoseinifar *et al.*, 2015) of fermented *A. oryzae* (Fermacto®) (Table 1). The ingredients were blended and pelleted using a meat grinder (2-mm die). Thereafter diets were air-dried and stored in plastic bag at 4°C till use.

Table 1: Dietary formulations (g Kg^{-1}) and proximate composition of experimental diet.

Ingredient	Control
Fish meal	400
Wheat flour	210
Soybean meal	135
Gluten	55
Soybean oil	60
Fish oil	60
Mineral premix*	30
Vitamin premix*	20
Binder†	20
Anti-fungi‡	5
Antioxidant§	5
Proximate analysis (dry matter basis)	
Dry matter	895.0
Crude protein	382.2
Crude lipid	102.4
Ash	34.5
Fiber	112.0
Energy (MJ kg^{-1}) **	17.55

* Premix detailed by (Hoseinifar *et al*., 2015)
† Amet binder ™, Mehr Taban-e- Yazd, Iran
‡ ToxiBan antifungal (Vet-A-Mix, Shenan- doah, IA)
§ Butylated hydroxytoluene (BHT) (Merck, Germany)
Nitrogen-free extracts (NFE) = dry matter – (crude protein + crude lipid + ash + fibre)
** Gross energy (MJ kg^{-1}) calculated according to 23.6 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17.0 kJ g^{-1} for NFE

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Growth performance

The performance parameters and survival rate were calculated as follows (Castell and Tiews 1979):

Weight gain (%) = $(W_2 - W_1/W1)*100$;

SGR = $100 * (\ln W_f – \ln W_i)/T$;

FCR = Feed intake (g)/Weight gain (g);

Survival rate= $(N_f/N_i) * 100$;

Which; $\mathbf{W_f}$ is the final weight (at the end feeding trial) and $\mathbf{W_i}$ is the initial weight (at the beginning of trial); **T** is the duration of trial (7 weeks or 49 days); $\mathbf{N_f}$ and $\mathbf{N_i}$ are the final and initial number of fish, respectively.

Hemato-immunological indices

After 7 weeks of feeding, fish were euthanized and blood samples collected from the caudal vein of each fish (three fish per tank) to assess hemato-immunological parameters. The total number of red blood cells (RBCs) and white blood cells (WBCs) were enumerated according to Blaxhall and Daisley, (1973) using a Neubauer slide under the light microscope. Haematocrit was measured by the microhaematocrit method (Brown, 1988) and reported as percentage of packed cell volume (% PCV) (Chardeh Baladehi *et al.,* 2017). The levels of Hemoglobin were assessed using Sahli's method (Blaxhall and Daisley, 1973). The respiratory burst activity was evaluated in triplicates with a modified chemiluminescent assay (measurement of light emission) using an automated system for chemiluminescent analysis (Luminoskan Ascent T392; Thermo Fisher Scientific, Inc.) Khoshbavar-Rostami *et al*. (2006).

Statistical analysis

All of the analyses were carried out using SPSS (version 20) and the charts were drawn with Microsoft Office Excel (version 2010). Values are presented as the mean±SE. In order to perform statistical analysis, one-way ANOVA was used to determine the effects of various levels of fermented *A. oryzae* on performance and haemato-immunological parameters, followed by a Duncan's multiple range test, when there was a significant difference (p<0.05).

Results

Table 2 represents the effects of fermented *A. oryzae* on common carp growth performance. At the start of the trial no remarkable differences in the initial weight of the treatments (p>0.05). After 7 weeks feeding with fermented *A. oryzae* (10, 20 or 30 g Kg^{-1}), no significant change observed with regard to; final weights, WG, SGR, or FCR in common carp fry (p>0.05) (Table 2).

Table 2: Effects of dietary fermented *Aspergillus oryzae* on growth performance and survival rate of carp fry.

	Control	**10 g Kg^{-1}**	**20 g Kg^{-1}**	**30 g Kg^{-1}**
Initial weight (g)	4.60±0.25	4.54±0.21	4.61±0.30	4.50±0.28
Final weight (g)	8.04±0.39	8.14±0.42	8.33±0.35	8.34±0.21
Weight gain (%)	74.30±8.03	79.18±12.46	80.31±13.65	85.33±15.57
SGR	1.13±0.11	1.19±0.16	1.20±0.12	1.25±0.15
FCR	2.88±0.41	2.75±0.25	2.50±0.36	2.64±0.51
Survival (%)	100	100	100	100

The effects of dietary fermented *A. oryzae* on haemato-immunological parameters of carp fry are presented in Table 3.

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Table 3: The effects of dietary fermented *Aspergillus oryzae* on hemato-immunological parameters of carp fry.

Groups	Erythrocyte count (× 10^6 µL^{-1})	Leukocyte count (×10^3 µL^{-1})	Haemoglobin (g dL^{-1})	Haematocrit (%)
Control	1.65±0.26	38.66±2.8	7.50±0.20	24.10±2.30
10 g Kg^{-1}	1.70±0.20	38.26±3.6	7.90±0.50	23.86±1.80
20 g Kg^{-1}	1.62±0.45	37.33±1.7	7.30±0.30	23.40±2.70
30 g Kg^{-1}	1.74±0.30	39.30±2.4	7.20±0.50	24.60±2.60

Evaluation of the parameters showed that fermented *A. oryzae* had no remarkable effects on erythrocyte count (RBC), haemoglobin, haematocrit and leukocyte counts (p>0.05). The respiratory burst activity data are presented in Fig. 1. Common carp fed with fermented *A. oryzae* supplemented diet showed significantly increased respiratory burst activity compared to the control group (p<0.05) (Fig. 1). There were no significant differences between different levels of fermented *A. oryzae* with regard to respiratory burst activity (p>0.05).

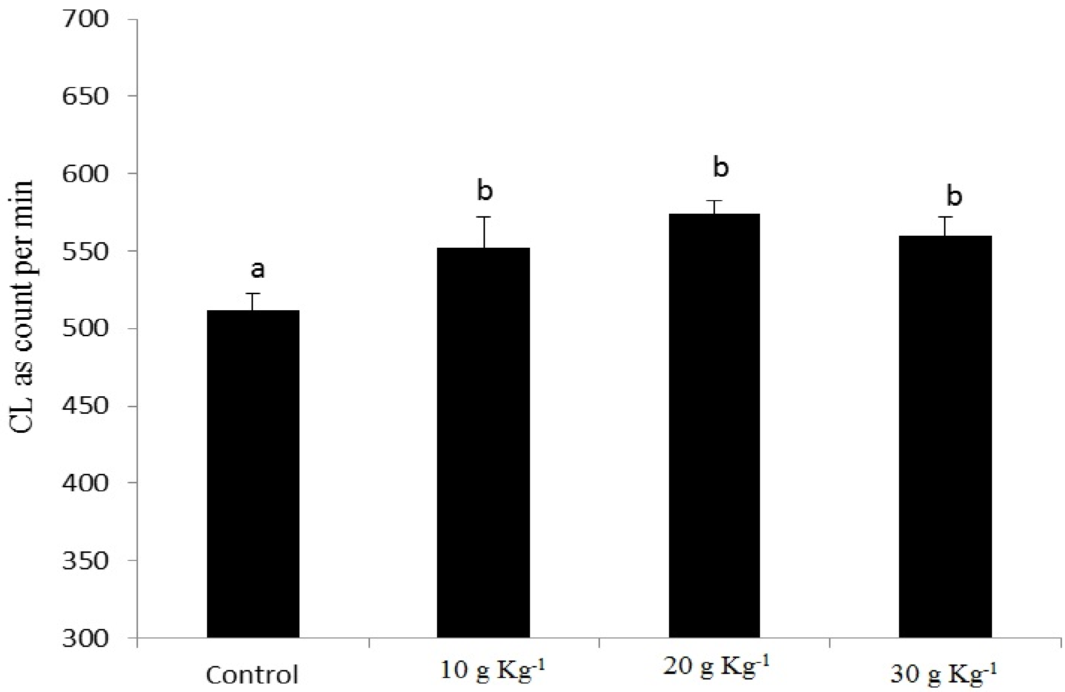

Figure 1: Respiratory burst activity (chemiluminescence response; light emission count per min) of carp fry fed different levels of dietary *Aspergillus*; control diet (0 g Kg^{-1}), 10 g Kg^{-1}, 20 g Kg^{-1} and 30 g Kg^{-1} for 7 weeks. Bars assigned with different superscripts are significantly different (p<0.05); Values are presented as the mean ± SE.

Discussion

Today a wide range of non- digestible dietary supplements such as fermentable products are using in fish nutrition in order to modulate the intestinal microbiota (Hoseinifar *et al.*, 2011b). It is now well-documented that manipulation of intestinal microbiota toward potentially beneficial communities leads to promotion of the

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immune response and overall host health. Hence, increasing researches were performed regarding the effects of feed additives like prebiotics and fermented products.

Several studies evaluated the effects of fermented products on fish health, immune responses and other aspects of the fish physiology (Bagheri *et al.*, 2008; Merrifield *et al.*, 2010; Akrami *et al.*, 2013). The present results revealed that diets supplemented with fermented *A. oryzae* had no significant effects on growth parameters on common carp fry (p<0.05). In agreement with our findings, Kim *et al.*, (2009) reported that dietary administration of *Aspergillus* had no significant effect on diet acceptability and growth performance for juvenile parrot fish (*O. fasciatus*). However, it has been reported that Fermacto® (fermented product of *A. oryzae*) fed at 30 g Kg^{-1} level increased final weight and daily gain weight of broiler chickens (Navidshad *et al.*, 2010). Furthermore, in a study on *Huso huso* feeding with 10 g Kg^{-1} and 20 g Kg^{-1} of brewer's yeast (*S. cerevisiae* var. *ellipsoideus*) (inactive form) significantly increased final weight, weight gain, SGR and FCR (Hoseinifar *et al.*, 2011a).

It has been suggested that administration of prebiotics and fermented products can modulate intestinal microbiota toward beneficial bacterial groups like LAB (Lactic Acid Bacteria) which improve growth performance. The lack of observed differences here may be due to the method of diet preparation, differences in culture condition, species and dosage.

Hematological parameters of fish are a useful parameter for monitoring physiological status and general health (Merrifield *et al.*, 2010). Total and differential leukocyte counts are important indicators of fish immune status as leukocytes are the main phagocytic and immune effector cells against pathogens. In accordance with these findings, Hoseinifar *et al.*, (2011a) observed no remarkable change in haematological factors of *H. huso* fed dietary inactive brewer's yeast *S. cerevisiae* var. *ellipsoideus*. However, increased red blood cell count was reported in *Aspergillus* fed parrot fish (Kim *et al.*, 2009). In contrast with our results with regard to haematological parameters, respiratory burst activity was remarkably higher in fish fed fermented *A. oryzae*. It seems that probably fermented *A. oryzae* modulated immune response of common carp fry. Likewise, dietary Vitacel®, a commercial fermentable fiber, beneficially affected innate immune response and resistance of rainbow trout (*Oncorhynchus mykiss*) (Yarahmadi *et al.*, 2014).

To sum up, the results of this study suggested that inclusion of fermented *A. oryzae* in carp fry diet can beneficially affects immune response. However, determination of exact effects on innate immune response and mode of action need further research.

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