Effects of a probiotic, protexin, on the growth performance and hematological parameters in the Oscar (*Astronotus ocellatus*) fingerlings

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Abstract This study examined the effects of a probiotic, protexin, on the growth performance and hematological parameters in an ornamental fish, the Oscar *Astronotus ocellatus* fingerlings. A completely randomized experimental design was applied with three experimental diets each with three replicates. A commercial food, BioMar, was supplemented with protexin at levels of 0.15, 0.5, and 1.0 g kg^{-1} dry food and fed three times a day for 60 days. The control diet was prepared with no protexin supplementation. The experimental fish were biometried every 15 days to compare their growth rates at each treatment. For hematological assays, blood samples were prepared every 30 days to measure such parameters as red and white blood cells, hemoglobin, hematocrit, and percentages of lymphocytes, monocytes, neutrophiles, basophiles, and eosinophiles. Based on the results, the fingerlings fed a 0.15 g kg^{-1} supplemented food were significantly different from the fish in the other treatments and in the control, with the highest mean of both final weight (35.07 ± 1.19) and body weight gain (30.17 ± 1.08). Significant differences in both hemoglobin concentration and mean red and white blood cells were found between the experimental groups and the control within 2 months. The highest hemoglobin concentration and also red and white blood cells was observed in the fish-fed 0.15 dietary protexin in both months. The results of this study show that the probiotic, protexin, at a level of 0.15 g kg^{-1} dry food could have measurable effects on the growth and hematological parameters in the Oscar *A. ocellatus* fingerlings.

Introduction

Cultivation of ornamental fish species, in addition to the aquaculture of food fish species, has gained ground nowadays, and research projects in this field have become of great interest. Since the economic importance of aquarium fish is not less than that of the food fish, it is, therefore, important to investigate various aspects of their cultivation including growth and survival as well as ways of increased resistance of ornamental fish against diseases. One important aspect of aquaculture is nutrition, to which special attention should be paid by fish farmers as it accounts for a high portion of cultivation expenses. By applying the knowledge of nutrition, aquaculturists try to enhance such factors as reduction in feed conversion ratio, increase in growth rate, immunology level and

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disease resistance, and fish survival, all of which will ultimately result in a high economic efficiency.

Science of nutrition has always been in progress and research in this field presents at times new and valuable findings, which are beneficial for consumers and also contribute to the public health. With respect to this, an important scientific finding is achievement of the probiotics. The first definition of the word "probiotic" by Parker (1974) describes them as organisms and substances that establish a balance in the intestine microbial flora. Other refined interpretations of probiotics include: live, food-additive microbes which are useful to the host because of a balance in the intestinal microbial flora (Fuller 1989) and functional bacteria causing a stability in the intestinal microbial flora (Wang et al. 2008a). Moreover, probiotics produce digestive enzymes that, by promoting food assimilation, cause a higher utilization of ingested food and eventually result in an enhanced growth and production (Bairagi et al. 2002; Ramiez and Dixon 2003). Hence, they can be appropriately substituted for antibiotic growth accelerators, the consumption of which is being limited (Irianto and Austin 2002a; Wang et al. 2008a). In teleost fish, morphological and functional analyses of alimentary canal have revealed the role of gut microbiota in the health and growth of the fish (Dimitroglou et al. 2011).

Besides recognition of the physiology of a fish species, identification of the hematological parameters is a specific, distinguishing characteristic of any species. This is not only important in species identification but can also be practical in disease recognition and as a determining tool for health status of fish. Blood is an important biological fluid tissue, the composition of which is affected by a variety of physiological and pathological situations. Accordingly, data on natural levels and the quality of changes in hematological parameters are important for recognizing a number of diseases (Jain 1986).

The application of probiotics as growth accelerators in the farmed food fish as well as for the improvement of disease resistance of these fishes has been frequently investigated (Wang et al. 2008a; Gatesoupe 1994; Gildberg et al. 1997; Abd El-Rhman et al. 2009; Avella et al. 2010a; Dimitroglou et al. 2011). The ornamental fish, on the other hand, have been rarely studied in that regard (Gosh et al. 2007, 2008; Avella et al. 2010b). The Oscar *A. ocellatus*

(family Cichlidae) has been turned out as a popular ornamental fish due to its unique beauty and colorfulness. Being a carnivore, this species is a predator hunting small fish, insects, and aquatic invertebrates in nature (Firouzbakhsh and Aliasghari 2009). Oscar is considered as an ever-hungry fish showing interest in food uptake even at satiation. Therefore, reductions in feed intake as well as the farming period to attain desired weight and ultimately decreasing the fish-farming costs were the incentives in the study of this species.

The use of multi-species probiotics as feed complements is probably more influential than mono-species probiotics in terms of more diversity in antimicrobial compounds and higher adhesion on the gut mucus (Lahtinen and Ouwehand 2009; Nayak 2010). It has recently been shown that a mixture of *Bacillus* probiotics has significantly increased growth rate and body weight in the sea bream *Sparus aurata* (Avella et al. 2010a). Protexin, a commercial probiotic used in this study, is a multi-species probiotic, consisting mainly of a variety of lactic acid bacteria and also yeast and fungi species. This investigation aims at study of the effects of different dietary levels of the probiotic protexin on growth response and likely changes in hematology and blood cell indices as well as on differential count of white blood cells in an ornamental fish, the Oscar *A. ocellatus* fingerling.

Materials and methods

The fish

The Oscar *A. ocellatus* fingerlings with mean initial weights of 4.92 ± 0.12 g were obtained from a local center for ornamental fish propagation located in the study area (Sari, Iran). The fingerlings, packed in water-filled, aerated plastic bags, were transferred to an aquarium room at the Sari Agricultural Sciences and Natural Resources University, Sari, Iran. For adaptation purposes, the fingerlings were reared in the new aquarium conditions for 2 weeks.

Water parameters

The rearing water was supplied from a well. Water temperature $(28 \pm 0.5°C)$ was provided by an

Table 1 Averages of qualitative water parameters measured during the experiment

Dissolved oxygen (ppm)	Temperature	pH	Hardness	Ammonia (mg l^{-1})	Nitrite (mg l^{-1})
6.5–7	28 ± 0.5°C	7.3–7.8	150 ± 0.5	0.05	0.02

automatic heater in each aquarium (55 (L) × 40 (W) × 50 (H) cm). The aquaria were aerated by a central pump. To control water quality during the experiment, water temperature and dissolved oxygen were measured daily, and ammonia, hardness, and pH were determined weekly (Table 1). To maintain water quality throughout the study, an under-gravel filter was used, and 25% of the water was replaced once every 3 days.

The probiotic

In this study, a probiotic with the trade name, protexin (Probiotics International Ltd., Lopen Head, Somerset, TA13 5JH, UK) was used. Protexin is a multi-strain probiotic, consisting of seven bacteria species and two species of yeasts and fungi (Table 2).

The experimental design

The fish were divided into four experimental groups with three replicates for 60 days. The Oscar fingerlings were completely random partitioned into the aquaria (12 fingerlings per aquarium). A commercial food, BioMar (BioMar SAS, 60, Rue Pierre-Georges Debouchaud, Zone Industrielle, FR-16440 Nersac, France) was fed to the fingerlings based on 4% of their biomass (Ghosh et al. 2008) at three intervals (6 a.m., 12, and 6

Table 2 Numbers and types of microorganisms exist in the Protexin probiotic (based on the producer's protocol)

Kind of microorganisms	CFU[a] g^{-1}
Lactobacillus plantarum	1.28×10^8
L. delbrueckii	2.22×10^8
L. acidophilus	2.14×10^8
L. rhamnosus	2.28×10^8
Bifidobacterium bifidum	2.10×10^8
Streptococcus silivarius	4.18×10^8
Enterococcus faecium	5.60×10^8
Aspergillus oryzae	5.60×10^7
Candida pintolopesii	5.68×10^7
Total	2.09×10^9

[a] Colony-forming unit

p.m.). After the 2-week adaptation period, all groups were fed daily lasting for 60 days. The dietary protexin was supplemented at levels of 0.15 (T_1), 0.5 (T_2), and 1.0 (T_3) g kg^{-1} dry food for the three experimental groups. The control (C) group received no protexin supplement. Different levels of protexin sprayed into the diets (10 ml of sterile distilled water kg^{-1}); the diets were air-dried under a sterile hood for 1 h and stored at 4° C until use. The control diet did not receive any protexin during spraying.

Estimation of growth criteria

In order to analyze the growth indices of the Oscar fingerlings and to compare effects of the different treatments, the fingerlings in all groups were biometried once every 15 days during the 60-day experiment. The fingerlings were weighed by a digital scale (bearing: 0.01 mg) after they had been anesthetized using Tricaine Methanesulfonate (0.1 ppm). The average weight of the fingerlings was then calculated. Based on the results of the biometry, the daily ration of the fish in the supplemented groups and in the control was determined. Some of the growth parameters were also measured by the following equations:

$$\text{Weight gain } (\text{g fish}^{-1}) = \text{Final mean body weight}$$
$$(\text{g fish}^{-1}) - \text{Initial mean body weight}$$
$$(\text{g fish}^{-1}) \text{ (Jafarian et al. 2007)}$$

$$\text{Specific growth rate (SGR \%)} = [\text{Ln final weight (g)}$$
$$- \text{Ln initial weight (g)}/t] \times 100$$
$$(\text{Hevroy et al. 2005})$$
$$(t \text{ is the number of days in the feeding period})$$

$$\text{Feed conversion ratio (FCR)} = \text{total feed intake (g)}/$$
$$\text{body weight gain (g) (De Silva and Anderson 1995)}$$

Measuring hematological parameters

Blood samples were prepared at two times, in the end of the 1st and 2nd month of the experiment. For each preparation, 16 fingerlings were randomly selected

from each group and anesthetized, and then, blood samples were taken through dissecting their peduncles. Red and white blood cells (RBC & WBC) diluted using the Natt & Herrick's stain solution were counted under a Neubaur hemocytometer (Bullis 1993). Hemoglobin was assessed by a spectrophotometer (unico UV-2150) at 540 nm through the method cyanmethemoglobin (Drobkin 1945). Hematocrit was measured by the procedure microcentrifuge with heparinated tubes (Svetina et al. 2002). Differential recognition of the WBC was based on blood films preparation and Gimsa staining. Blood cell indices (MCH, MCV, and MCHC) were assessed according to the following standard calculations (Campbell and Ellia 2007):

$$MCV(\mu m^3) = [Hct/RBC(million)] \times 10$$

$$MCH\,(pg\,cell^{-1}) = [Hb/RBC(million)] \times 10$$

$$MCHC\,(g\,dL^{-1}) = (Hb/Hct) \times 100$$

Statistical analysis

One-way ANOVA was applied to analyze the data by the SPSS (Ver. 17) software. The average values were compared using the LSD test at 5% confidence.

Results

Growth parameters

Table 3 shows the measured growth parameters of the Oscar fingerlings fed the dietary protexin for 60 days. The experimental groups (T_1, T_2, and T_3) were significantly different ($P < 0.05$) in both average final weights and weight gains from the control fish. The highest averages of final weight and weight gain were observed in the T_1; it was statistically different ($P < 0.05$) from T_2, T_3, and the control fingerlings. The T_1 also showed significant difference ($P < 0.05$) in reduction in FCR compared to T_2, T_3, and the control, whereas this parameter was not significantly different ($P > 0.05$) between T_2 and T_3, and also in comparison with the control. Specific growth rate was not different ($P > 0.05$) between the experimental groups T_2 and T_3, but the differences between the T_2 and T_3 and between each of these groups versus the control were statistically significant ($P < 0.05$). The T_1 showed the highest SGR, which was significant ($P < 0.05$) compared to the control and also to the dietary-supplemented groups.

Hematological factors

Tables 4 and 5 present the hematological factors and differential counting of leukocytes, respectively, found in the Oscar fingerlings supplemented various levels of dietary protexin. All protexin-supplemented groups were different from the control ($P < 0.05$) in hemoglobin density and numbers of both RBC and WBC within the 2 months of the study period. The highest hemoglobin density within months 1 and 2, RBC and WBC was observed in the T_1.

In the 1st month, hematocrit increased in the T_1, and in the 2nd month, both T_1 and T_2 showed an increased hematocrit, all of which differed statistically from the control ($P < 0.05$). The T_1 was the only group in the 1st month that showed a significant rise ($P < 0.05$) in MCV compared to the control and even to the T_2 and T_3; the latter groups were not different from the control in the 1st month. In the 2nd month, all groups were different in MCV content from each other and also

Table 3 Growth performance of the Oscar *A. ocellatus* fingerlings after 2 months of dietary protexin supplementation

Parameters	C	T_1	T_2	T_3
Initial body weight (g fish^{-1})	4.88 ± 0.12	4.89 ± 0.67	5.01 ± 0.32	4.92 ± 0.12
Final body weight (g fish^{-1})	23.00 ± 0.14^c	35.07 ± 1.19^a	26.12 ± 0.92^b	25.31 ± 0.76^b
Weight gain (g fish^{-1})	18.12 ± 0.05^c	30.17 ± 1.08^a	21.11 ± 0.76^b	20.39 ± 0.69^b
Feed conversion ratio (g g^{-1})	1.23 ± 0.01^a	1.13 ± 0.04^b	1.21 ± 0.03^a	1.23 ± 0.02^a
Specific growth rate (% day^{-1})	2.58 ± 0.02^c	3.27 ± 0.01^a	2.75 ± 0.09^b	2.73 ± 0.04^b

Dietary codes: *C* Control; $T_1 = 0.15$ mg kg^{-1}, $T_2 = 0.5$ mg kg^{-1}, and $T_3 = 1$ mg kg^{-1} dry food. Values are expressed as means ± standard deviation

Significant differences among the experimental groups are indicated by difference in superscript letters

Table 4 Blood parameters in the Oscar *A. ocellatus* fingerlings after 2 months of dietary protexin supplementation

Parameters	Month 1				Month 2			
	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Hemoglobin (g dl^{-1})	8.40 ± 0.00[d]	12.30 ± 0.00[a]	11.00 ± 0.00[b]	9.80 ± 0.00[c]	8.20 ± 0.10[d]	12.75 ± 0.16[a]	11.17 ± 0.08[b]	10.18 ± 0.18[c]
Hematocrit (%)	24.00 ± 0.00[b]	29.00 ± 1.00[a]	24.67 ± 0.57[b]	25.00 ± 1.00[b]	24.67 ± 0.57[c]	35.00 ± 1.00[a]	29.67 ± 1.52[b]	26.33 ± 0.57[c]
RBC (106 µl)	0.91 ± 0.00[c]	1.40 ± 0.02[a]	0.96 ± 0.01[b]	0.95 ± 0.01[b]	0.97 ± 0.01[d]	3.22 ± 0.11[a]	1.72 ± 0.07[b]	1.35 ± 0.05[c]
WBC (103 µl)	11.00 ± 0.05[d]	15.86 ± 0.2[a]	13.06 ± 0.20[b]	12.03 ± 0.25[c]	12.06 ± 0.07[d]	18.85 ± 0.45[a]	14.76 ± 0.51[b]	13.43 ± 0.25[c]
MCV (fL)	263.70 ± 1.45[a]	206.60 ± 3.48[b]	256.04 ± 3.97[a]	261.71 ± 6.36[a]	254.18 ± 6.04[a]	108.75 ± 3.99[d]	169.46 ± 8.05[c]	195.14 ± 4.20[b]
MCH (pg)	92.30 ± 0.50[c]	87.66 ± 1.56[d]	114.20 ± 1.80[a]	102.63 ± 1.61[b]	84.57 ± 1.71[a]	39.07 ± 1.02[d]	64.75 ± 2.26[c]	75.54 ± 3.67[b]
MCHC (g dl^{-1})	35.00 ± 0.00[c]	42.44 ± 1.46[b]	44.61 ± 1.05[a]	39.24 ± 1.57[b]	33.25 ± 0.47[b]	36.05 ± 1.47[a]	37.66 ± 1.79[a]	38.67 ± 1.47[a]

Dietary codes: *C* Control; T_1 = 0.15 mg kg^{-1}, T_2 = 0.5 mg kg^{-1}, and T_3 = 1 mg kg^{-1} dry food. Values are expressed as means ± standard deviation

Significant differences among the experimental groups are indicated by different superscript letters

Table 5 Leukocyte differential count in the Oscar *A. ocellatus* fingerlings after 2 months of dietary protexin supplementation

Parameters	Month 1				Month 2			
	C	T_1	T_2	T_3	C	T_1	T_2	T_3
Lymphocyte%	57.63 ± 0.57[c]	71.67 ± 0.57[a]	65.33 ± 2.51[b]	64.67 ± 2.51[b]	61.33 ± 1.52[d]	83.33 ± 1.52[a]	74.67 ± 1.52[b]	71.33 ± 1.00[c]
Monocyte%	6.00 ± 0.00[a]	3.33 ± 0.57[b]	4.00 ± 0.00[b]	5.33 ± 0.57[a]	7.00 ± 0.00[a]	2.67 ± 0.57[c]	4.67 ± 0.57[b]	6.00 ± 1.00[b]
Neutrophil%	35.67 ± 0.57[a]	25.00 ± 1.00[c]	27.33 ± 4.04[bc]	29.67 ± 1.52[b]	31.33 ± 1.15[a]	14.00 ± 2.00[c]	20.67 ± 1.15[b]	22.67 ± 1.15[b]
Basophil%	0.33 ± 0.57[a]	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.57[a]	0.33 ± 0.57[a]	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.57[a]
Eosinophil%	0.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Dietary codes: *C* Control; T_1 = 0.15 mg kg^{-1}, T_2 = 0.5 mg kg^{-1}, and T_3 = 1 mg kg^{-1} dry food. Values are expressed as means ± standard deviation

Significant differences among the experimental groups are indicated by different superscript letters

from the control ($P < 0.05$). The results of differential counting of leukocytes (Table 5) showed significant rise ($P < 0.05$) in lymphocyte numbers in all groups compared to the control. Among the experimental groups, the T_1 presented the highest count of lymphocytes in comparison with the control in months 1 and 2. However, the percentages of both monocytes and neutrophils showed a marked decrease ($P < 0.05$) in the experimental fingerlings compared to the control within months 1 and 2.

Discussion

The effects of probiotics on growth parameters have been studied in a variety of farmed fish and other aquatic species (Douillet and Langdon 1994; Ghosh et al. 2003; Carnevali et al. 2004; Wang et al. 2008b; Abdel-tawwab et al. 2008; Abd El-Rhman et al. 2009; Avella et al. 2010a; Dimitroglou et al. 2011). Nonetheless, research on the effects of probiotics upon growth and hematology parameters of ornamental fish is very scarce (Gosh et al. 2008; Avella et al. 2010b). A relatively large body of literature is concerned with the effects of various probiotic microorganisms on both terrestrial and aquatic animals; yet, lactic acid bacteria (LAB) and bacilli have received the highest number of scientific documents (Dimitroglou et al. 2011). It has been shown that *Lactobacillus* probiotics enhance growth and survival, for instance, in the farmed rainbow trout *Oncorhynchus mykiss* (Kesarcodi-Watson et al. 2008). Similarly, Carnevali et al. (2004) found that *Lactobacillus fructiorance* and *L. plantarum* could considerably increase growth of the sea bream *S. aurata*. Carnevali et al. (2006) also noticed that, when the European sea bass juveniles (*Dicentrarchus labrax* L.) were fed on *Lactobacillus delbrueckii* for 59 days, it showed elevated body weight; the weight gain appeared after 25 days when compared with the control.

The impact of lactic acid from a bacterial probiotic on the Atlantic cod *Gadus morhua* fingerlings was investigated by Gildberg et al. (1997); they reported a significant decrease in FCR. In the present study, a protexin supplement of 0.15 g kg^{-1} dry food (group T_1) resulted in a significant decrease in FCR in comparison with other treatments and also the control. In the same way, use of the probiotic *Bacillus subtilis* efficiently raised growth and survival rate with

significantly diminished FCR in live-bearing ornamental fishes (Gosh et al. 2008). Avella et al. (2010a) also demonstrated that a combination of three *Bacillus* species (*B. subtilis*, *B. licheniformis*, and *B. pumilus*) fed both via rotifer and *Artemia* nauplii and through addition to the water could significantly influence the growth rate and body weight at larval and juvenile stages of the sea bream *S. aurata*. The findings of this study confirm a growth preference together with reduced FCR in the Oscar fingerlings-fed dietary protexin at 0.15 g kg^{-1} dry food than at 0.5 and 1.0 g kg^{-1} dry food.

The influence of probiotics on enzymatic activities and, as a result, on increased digestive process is emphasized in some studies (Jafarian et al. 2007; De Silva and Anderson 1995). Many probiotic bacteria contain extracellular enzymes such as amylase, lipase, and protease that, through stimulating appetite and enhancing microbial metabolism, promote host feeding (Gildberg et al. 1997). By increasing digestibility and enhanced absorption of ingested food, these bacteria increase feeding efficiencies leading to a high growth in fish (Gatesoupe 1999). Through provision of essential nutrients (such as vitamins and short-chain fatty acids) and enzymes, probiotics can promote food digestion and absorption (Gatesoupe 1999). Elevated digestibility, therefore, together with antibacterial properties of probiotics may entrain survival and increased fish weights (Gosh et al. 2008). Accordingly, it can be concluded that the improved values of body weight, FCR, and SGR of the protexin-fed fish in this study may have arisen from the useful effects mentioned above.

Considerable increase in fish performance and durability due to supplementation of probiotics (Ghosh et al. 2003; Hevroy et al. 2005) might be because of either killing harmful bacteria by useful (probiotic) bacteria, or secretion of compounds such as bacteriocins, which prevent growth of other microorganisms (Kesarcodi-Watson et al. 2008). Altogether, according to the results of this study, it can be concluded that addition of the probiotic protexin as a dietary complement with an effective dose of 0.15 g kg^{-1} dry food efficaciously improves growth performance in *A. ocellatus* fingerlings.

It has been noted that benefiting from appropriate probiotic species with adequate dosage to be of great importance in order to avoid either overdose or lower dose ultimately leading to economic loss (Lee 2009);

these consequences may also depend on the fish species (Nayak 2010). Although most of related studies have utilized different doses of probiotics with contrasting consequences (e.g., 10^9 & 10^{12} cfu/g/feed: Nikoskelainen et al. 2001; 10^6, 10^8 & 10^{10} cfu/kg/feed: Son et al. 2009), the rationale for which are not definitely provided (Gosh et al. 2008). The present study also applied different concentrations of protexin with the lowest dose showing the best growth performance in the Oscar fingerlings. Altogether, it seems to be the only point to note that the highest doses consumed would not necessarily result in the best performances. And the required concentrations of probiotics in order to induce favorable effects and to prevent diseases in various fish species at differing situations remain to be further questioned.

Hematology parameters are considered as proper indices for tracking health status of fish and their response to environmental stresses (Schuett et al. 1997). The impacts of nutrition and food additives on blood factors have been questioned in a number of studies (Rawling et al. 2009; Merrifield et al. 2010). In this study, the hematological analyses of *A. ocellatus* fingerlings fed a dietary protexin complement of 0.15 g kg^{-1} dry food signified marked changes ($P < 0.05$) in hematological parameters as opposed to the control fish. The dietary supplementations of 0.5 and 1.0 g protexin kg^{-1} dry food also represented non-significant differences compared to the control ($P > 0.05$). In the same way, Abd El-Rhman (2009) observed a rise in the number of RBC in Nile tilapia following the use of *Micrococcus luteus* showing no significant difference as opposed to the control. On the other hand, the use of probiotic in rainbow trout caused significant increase in the number of RBC (Irianto and Austin 2002b).

Major hematological changes in the protexin-supplemented group with 0.15 g kg^{-1} dry food include statistically noticeable rise of total numbers of RBC, WBC, and hemoglobin during the 60-day experimental period. The observed hematological changes in the 2nd month might be because of relatively long-term influence of the probiotic. This means that with the extension of protexin consumption, the above changes became discernible; even some minor changes in the 1st month appeared markedly in the 2nd month. On account of the identical experimental conditions for all groups in this study, the observed hematological changes are probably as a result of protexin

supplementation for feeding the Oscar fingerlings. And no role could be depicted for such factors as the fish habitat and the governing conditions including temperature, feeds, and pollutants (Bullis 1993). The distinguished total number of RBC in the fingerlings fed 0.15 g protexin kg^{-1} dry food compared to the control apparently caused by a high, probiotic-driven metabolism with consequent increase in oxygen requirements. A rise in the number of RBC, therefore, intensifies the concentration of hemoglobin and eventually leads to a high oxygen-carrying capacity in the probiotic-fed fish. Such fishes, hence, may be more capable of supplying oxygen to tissues in situations where oxygen is highly required.

The contribution of probiotics in stimulating fish immune system in addition to their effects on fish survival and growth has greatly been studied in various fish species. Probiotics are able to multiply RBC, granulocytes, lymphocytes, and macrophages in fish similar to higher vertebrates (Irianto and Austin 2002b; Kumar et al. 2008). Also, probiotics show interactions with immune cells such as monocytes, macrophages, neutrophils, and lymphocytes in order to improve innate immune response (Nayak 2010). Irianto and Austin (2002b) presented evidence that use of probiotic could result in raised RBC and WBC, especially lymphocytes, in rainbow trout. The use of *B. subtilis* as a probiotic significantly increased leukocytes count in the Major carp (Nayak et al. 2007) and rainbow trout (Newaj-Fyzul et al. 2009) compared to the control.

Similarly, the current study revealed significant rise of average total number of WBC together with raised number of lymphocytes in the Oscar fingerlings fed a dietary protexin of 0.15 g kg^{-1} dry food. The lymphocytes outnumbered in WBC followed by neutrophils. Elevated number of WBC can be seen as a reinforcement of non-specific immune system resulting from probiotic consumption (Nayak 2010).

Effective rise of phagocytic cells leading to increased phagocytosis (Nayak 2010) has been investigated in a number of fish species as a result of probiotic supplementation (Salinas et al. 2005, 2006, 2008; Picchietti et al. 2007, 2009). Picchietti et al. (2009) showed that early bacterial colonization in the intestine of the sea bass (*D. labrax*) using probiotic bacteria (*L. delbrueckii*) isolated from the mature fish could stimulate the gut immune system and increase T cells. Salinas et al. (2005) also noticed significant increase in phagocytic activity of

leukocytes in gilthead sea bream; after 2 weeks, the fish had been fed by the bacteria *B. subtilis* and *L. delbrueckii*. Similarly, elevated leukocyte phagocytic ability was found in gilthead seabream (*S. aurata* L.) fed with a mix of two inactivated bacteria (Díaz-Rosales et al. 2006).

Given that the protexin-supplemented *A. ocellatus* at 0.15 g kg^{-1} dry food have acquired a rather higher level of immunity, they are expected to be more resistant against stressors and diseases, for which further investigation is required. As lymphocytes were the most numerous in WBC, and because interactions of lymphocytes B and T as well as macrophages is necessary for an immune response to occur (Borges et al. 2004), it can, accordingly, be concluded that fish immune system can be substantially stimulated by elevation of lymphocytes following an increase in WBC. In other words, an appropriate dose of probiotic microorganisms entering the digestive tract of fish is considered as aliens, provoking the defense system and multiplying WBC and other immunizing compounds.

Hematological parameters, in general, are considerably affected by fish physiological conditions, pollution and disease, sex (Ghosh et al. 2003, 2007), sexual maturity and behavior, age (Speckner et al. 1989) season and environment (Nikoskelainen et al. 2003), food quantity and quality, diet differences (Rawling et al. 2009; Merrifield et al. 2010), diet formulation and type, purity and dose of consumed probiotic. The overall results of this study demonstrate that a dietary protexin supplement of 0.15 g kg^{-1} dry food would give rise to positive effects on hematological factors of the Oscar fingerlings, ultimately leading to increased growth and reduction in feed conversion ratio. Considering the fact that resources and practical information regarding hematological parameters of fish in general and ornamental fish in particular are limited, this field is apparently in its infancy demanding further research.

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