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Effects of Synbiotic (*Biomin Imbo*) on Growth Performance, Survival Rate, Reproductive Parameters of Angelfish (*Pterophyllum scalare*)

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Abstract

This study was carried out to evaluate the effect of synbiotic (*Biomin imbo*) on growth performance, survival rate and reproductive parameters of Angelfish (*Pterophyllum scalare*) via supplementation with Biomar. Four levels of Biomar experimental diets (54 % protein and 14 % lipid) were prepared by adding synbiotic (0.15, 0.5, 0.75, 1 g/Kg) at a basal diet (Biomar) and the Angelfish larvae in experimental treatments were fed four levels of synbiotic with 5 percent body weight (3 times a day). The larvae in the control treatment were fed without supplemented Biomar. The results showed that larvae fed on the synbiotic had significantly increased final body weight in comparison with the control treatment. After 90 days, 5 adult female and male Angelfish were divided from each treatment. The results showed that fecundity in experimental treatments increased in comparison with the control treatment. The synbiotic also had positive effects on hatching rate in comparison with those in the control treatment but, there were no significant differences ($P > 0.05$) among treatments. The synbiotic also had significant positive effects on the specific growth rate (SGR) and feed conversation efficiency (FCE) in comparison to those fed the control treatment, however treatment T3 was more than T4 (T3 > T4 > T2 > T1 > control treatment).

Keywords: *Pterophyllum scalare*, growth performance, survival rate, hatching rate, fecundity

Introduction

The Angelfish (*Pterophyllum scalare*) is one of the most popular aquarium species, as this species commands a higher price compared with most freshwater food species and other ornamental fish. In spite of the importance of Angelfish in ornamental fish culture, there has been neither research nor development of cost-effective feed for the intensive culture of this species. All ornamental fish feeds are 10 - 60 times higher in price than aquaculture feeds for food species. Second, the price of the feed targeted for a single ornamental species varies dramatically compared to the price of fish food feeds, each of which is targeted for a specific species [1]. For this reason, formulation feed rations for ornamental fish carry importance for the aquaria sector [2].

Synbiotics affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria, and thus improving the host “welfare”. In humans, probiotics are mainly active in the small intestine while prebiotics are only effective in the large intestine, so the combination of the two may give a synergistic effect [3]. The first application of synbiotics in fish was reported by Rodriguez-Estrada *et al*. [4].

The appropriate use of probiotics in the aquaculture industry was shown to improve intestinal microbial balance, and also to improve feed absorption, thus leading to increased growth rate [5,6], and also reduced feed conversion ratio (FCR) during the cultural period [7]. Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathenogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes [8]. As *Bacillus* bacteria secrete many exoenzymes [9], these bacteria have been used widely as putative probiotics.

In Angelfish, as in all vertebrates, reproduction is regulated by the hypothalamus-pituitary-gonadal axis. The hypothalamus, integrating internal and external stimuli, releases Gonadotropin-releasing hormone (GnRH) [10]. In recent years, it has been established that GnRH transcription and secretion are gated by the state of energy reserves in the organism [11]. The impact of energy status on the reproductive axis is conveyed through a number of neuropeptide hormones and metabolic signals, such as kiss1, kiss2, and leptin, whose nature and mechanisms of action have begun to be deciphered only in recent years in mammals and, to a lesser extent, in fish [12]. Under the influence of GnRH, the pituitary secretes follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which act upon the gonads controlling follicle growth and maturation [13]. In particular at ovarian level, LH, through its receptor (LHcgr), stimulates the production of 17a-hydroxyprogesterone that is converted (by the action of cbr1l) into 17a, 20b-dihydroxy- 4-pregnen-3-one, the maturation-inducing hormone (MIH) [13]. The binding of MIH to its receptors (paqr7b and paqr8) activates the maturation processes [14].

This study investigated for the first time the effects of synbiotic (*Biomin imbo*) on growth performance, survival rate, fecundity and reproductive factors in female Angelfish via supplementation with Biomar.

Materials and methods

Fish

Larvae of Angelfish with an initial weight, 0.57 ± 0.1 g were obtained from the Institute of Commercial Supplier the Ornamental Fish Hatchery in Gorgan, Iran. They were kept in glass aquaria (each with a dimension of 30 × 40 × 60 cm^3). This experiment was conducted in a completely randomized design with 5 treatments (4 synbiotic levels and a control), and 3 replicates per treatment for a total of 15 Angelfish larvae. The density of fish larvae per aquarium were 5 fish. At the end of rearing, adult female and male zebra fish were divided from each treatment. The animals were kept in 50 L glass tanks under controlled conditions (28 ± 0.5 °C and 14 h light: 10 h darkness).

Experimental diet

The synbiotic (*Biomin imbo*) was prepared from the commercial product Protexin aquatic (Iran-Nikotak). Biomar was provided by an aquatic foods company. Four levels of Biomar experimental diets (54 % protein and 14 % lipid) were prepared by adding the synbiotic (0.15, 0.5, 0.75, 1 g/Kg) to the basal diet (Biomar) and the Angelfish larvae in experimental treatments were fed at four levels of synbiotic with 5 percent body weight for 3 times a day (6.00, 14.00 and 22.00). The control treatment was fed without supplemented Biomar.

Feed analysis

Nutrient compositions of experimental diets (Biomar) are given in **Table 1**. Proximate composition of diets was carried out using the Association of Analytical Chemists [15] methods. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method; Crude fat was determined using petroleum ether (40 - 60 Bp) for Soxhlet extraction and ash by combustion at 550 °C.

Table 1 Nutrient composition of experimental diets (%).

Ingredients	Percent %
Protein	54
Lipid	18
Fiber	1.5
Ash	10
Vitamins	2

Determination of growth parameters

Growth parameters were calculated as follows: final body weight (FBW) = W2 (final body weight (g)) – W1 (initial body weight (g)). Specific growth rate (SGR) (% BW day-1) = Ln final weight (g) – Ln initial weight (g) / (experimental period) × 100. Feed conversion ratio (FCR) (%) = (total fed/body weight increase (g)) × 100. Daily growth rate (DGR) = W2 – W1*100/ (experimental period × W1.

Determination of reproductive parameters

Reproductive performances were calculated as follows: relative fecundity = total larvae production throughout the experimental period / mean weight of female (g). Total larvae production per female = Total harvested throughout the experimental period per number of females. Gonadosomatic index (%) = (Ovary weight/body weight) × 100. Survival (%) = (Total live fish after production / initial fish throughout the experimental period) × 100 where it is the day of the experiment.

Reproductive parameters

Reproductive parameters were investigated for one pair of each replication. Feeding was continued during the spawning period. Spawning, hatching and survival for each pair of fish were investigated.

Statistical analysis

In order to determine significant differences among treatments, results were analyzed by one-way Analysis of variance (ANOVA) and Duncan's multiple range tests using the SPSS program.

Results and discussion

Synbiotic effects on Angelfish growth performance and survival rate

The results of feeding, growth performance and survival rate are presented in **Table 2** and clearly show that the synbiotic have beneficial effects on the growth performance of Angelfish larvae. The larvae fed on the synbiotic have significantly increased growth performance in comparison to the control treatment ($P < 0.05$). The four different treatments of synbiotic were significantly different for all growth parameters. The greatest effect appears to be obtained in treatments T3 (supplemented with 0.75 g/kg) and Angelfish larvae in treatment T3 clearly showed the best growth in all of the growth performance indicators for all treatments. Interestingly, the concentration of synbiotic in treatment T4 was greater than treatment T3 but result was poorer (T3 > T4 > T2 > T1 > control treatment). This is particularly true for the specific growth rate, where the highest result was obtained in treatment T3. The food conversion ratio (FCR) in the experimental treatments was significantly decreased in comparison with the control treatment ($P < 0.05$). The effects of commercial probiotic on aquaculture has been investigated by researchers, and some of this research has not shown any positive effects on growth parameters or survival rate or any promising results on cultural conditions. For instance, Shariff *et al.* [16] found that treatment of *Penaeus monodon* with a commercial *Bacillus* probiotic did not significantly increase survival or El-Dakar *et al*. [17] who found that treatment of *Siganus rivulatus* with commercial probiotic/prebiotic did not significantly increase survival rate but it had a positive effect on growth performance. These results disagree with our findings, although fish and crustaceans may respond differently to probiotics.

Table 2 Growth parameters and survival rate of Angelfish (*Pterophyllum scalare*) larvae in experimental treatments (trial 1 - 4) and control.

Growth Indices	Control Unsupplemented Biomar	T1 supplemented Biomar with 0.15 g/kg	T2 supplemented Biomar with 0.5 g/kg	T3 supplemented Biomar with 0.75 g/kg	T4 supplemented Biomar with 1 g/kg
Initial weight (g)	4.2 ± 0.01	4.2 ± 0.01	4.2 ± 0.01	4.2 ± 0.01	4.2 ± 0.01
Final body weight (g)	10.7 ± 0.2[e]	11.17 ± 0.15[d]	13.2 ± 0.1[c]	15.87 ± 0.15[a]	14.17 ± 0.06[b]
Body weight increased (g)	6.5 ± 0.2[e]	6.97 ± 0.15[d]	9.0 ± 0.1[c]	11.67 ± 0.15[a]	9.97 ± 0.58[b]
Specific growth rate for weight (% BW day^{-1})	1.04 ± 0.02[e]	1.09 ± 0.01[d]	1.27 ± 0.01[c]	1.48 ± 0.01[a]	1.35 ± 0.01[b]
Feed Conversion Ratio (%)	1.3 ± 0.1[e]	1.2 ± 0.0[d]	1.14 ± 0.1[c]	1 ± 0.0[a]	1.1 ± 0.0[b]
Feed Conversion efficiency (%)	0.77 ± 0.0[e]	0.82 ± 0.0[d]	0.87 ± 0.0[c]	0.94 ± 0.0[a]	0.9 ± 0.01[b]
Daily growth rate (DGR)	1.72 ± 0.05[e]	1.84 ± 0.04[d]	2.39 ± 0.03[c]	3.09 ± 0.04[a]	2.64 ± 0.01[b]
Survival rate (%)	92.23 ± 5.71[a]	94.28 ± 4.28[a]	96.18 ± 2.18[a]	98.79 ± 2.18[a]	98.09 ± 2.18[a]

Groups with different alphabetic superscripts in the same row differ significantly at $P < 0.05$ (ANOVA).

The maximum final body weight (FBW) was obtained in treatment T3 (15.87 ± 0.15 g). The specific growth rate (SGR) for this treatment was 1.48 ± 0.01 % body weight/day. The maximum of feed conversion efficiency (FCE) (0.94 ± 0.0 %) was also observed in treatment T3. The lowest growth parameters were obtained in the control treatment, while the highest food conversion ratio (FCR) (1.3 ± 0.1), was obtained in this treatment where the fish larvae were fed by unsupplemented Biomar. Supplemented Biomar with synbiotic had a positive effect on survival rate. The lowest survival rate observed in control treatment (92.23 ± 5.71) and was not significantly different to other treatments. Gomez-gill *et al*. [18] found selection of probiotic bacteria had beneficial effects for larva aquatic organisms. Bagheri *et al*. [19] found that supplementation of trout starter diet with the proper density of commercial bacillus probiotic could be beneficial for growth and survival of rainbow trout fry and our results support these previous studies. Ghosh *et al*. [20] indicate that the *B. circulans*, *B. subtilis* and *B. pamilus*, isolated from the gut of Rohu, have extracellular protease, amylase, and cellulose and play an important role in the nutrition of Rohu fingerlings.

Synbiotic effects on Angelfish reproductive parameters

There was no fecundity and hatching differences among treatments (**Table 3**).

Table 3 Reproductive parameters in experimental treatments.

Parameters	Control Unsupplemented Biomar	T1 supplemented Biomar with 0.15 g/kg	T2 supplemented Biomar with 0.5 g/kg	T3 supplemented Biomar with 0.75 g/kg	T4 supplemented Biomar with 1 g/kg
Fecundity	400.32 ± 18.2	427.29 ± 35.12	458.32 ± 60.21	530.14 ± 98.16	488.15 ± 72.18
Hatching (%)	80.32 ± 11.12	82.35 ± 7.14	82.41 ± 9.12	85.22 ± 8.44	82.11 ± 11.2

The four different treatments of synbiotic were not significantly different for any of the reproductive indices. Among the three different concentrations of synbiotic supplemented with Biomar fed to Angelfish, the greatest effect appears to be obtained in the T3 experimental treatment. Reproduction is gated by the state of body energy reserves and is sensitive to different metabolic cues; the neuroendocrine mechanisms responsible for the tight coupling between energy homeostasis and fertility are represented by metabolic hormones and neuropeptides that integrate the hypothalamic center governing reproduction, controlling the expression and release of GnRH [10,21-23].

Thus, full activation of the hypothalamic-pituitary-gonadal axis at puberty and its proper functioning in adulthood critically depends on adequate body energy stores [11]. The identification of the adipose hormone leptin, which signals the magnitude of energy stores to the hypothalamic centers governing reproduction [12,24], represents an important step towards understanding the mechanisms controlling this interplay.

Conclusions

The results of this study may provide guidance to achieved better growth performance and reproductive performance for Angelfish but determination of optimal dosage of synbiotic is so important also last studies showed dosage of synbiotic is different for different types of ornamental fishes.

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References

[1] CS Tamaru and H Ako. *Using Commercial Feeds for the Culture of Freshwater Ornamental Fishes in Hawaii*. UJNR Technical Report No. 28, 2000, p. 109-20.

[2] J Sales and GPJ Janssens. Methods to determine metabolizable energy and digestibility of feed ingredients in the domestic pigeon (*Columba livia domestica*). *Poultry Science* 2003; **82**, 1457-61.

[3] GR Gibson and MB Roberfroid. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 1995; **125**, 1401-12.

[4] U Rodriguez-Estrada, S Satoh, Y Haga, H Fushimi and J Sweetman. Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydrobutyric acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. *Aquacult. Sci.* 2009; **57**, 609-17.

[5] R Fuller. Probiotics in man and animals. *J. Appl. Bacteriology* 1989; **66**, 365-78.

[6] S Rengpipat, W Phianphak, S Piyatiratitvorakul and P Menasveta. Effects of probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture* 1998; **167**, 301-13.

[7] YB Wang, ZR Xu and MS Xia. The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. *Fisheries Science* 2005; **71**, 1036-44.

[8] L Verschuere, G Rombout, P Sorgeloos and W Verstraete. Probiotic bacteria as biological control agents in aquaculture. *Microbiol. Mol. Biol. Rev.* 2000; **64**, 655-71.

[9] DJW Moriarty. Control of luminous *Vibrio* species in penaeid aquaculture pond. *Aquaculture* 1998; **164**, 351-8

[10] Y Zohar, JA Munoz-Cueto, A Elizur and O Kah. Neuroendocrinology of reproduction in teleost fish. *Gen. Comp. Endocrinol.* 2010; **165**, 438-55.

[11] JW Hill, JK Elmquist and CF Elias. Hypothalamic pathways linking energy balance and reproduction. *Am. J. Physiol. Endocrinol. Metabol.* 2008; **294**, 827-32.

[12] F Casanueva and C Dieguez. Neuroendocrine regulation and actions of leptin. *Front. Neuroendocrinol.* 1999; **20**, 317-63.

[13] R Patino, G Yoshizaki, P Thomas and H Kagawa. Gonadotropic control of ovarian follicle maturation: the two-stage concept and its mechanisms. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 2001; **129**, 427-39.

[14] RN Hanna and Y Zhu. Expression of membrane progestin receptors in zebra fish (*Danio rerio*) oocytes, testis and pituitary. *Gen. Comp. Endocrinol.* 2009; **161**, 153-7.

[15] AOAC. Official methods of analysis. Association of official analytical chemist. EUA, 2000.

[16] M Shariff, FM Yusoff, TN Devaraja, S Srinivasa and P Rao. The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* (Fabricius), ponds. *Aquac. Res.* 2001; **32**, 181-7.

[17] AY El-Dakar, SM Shalaby and IP Saoud. Assessing the use of a dietary probiotic/prebiotic as an enhancer of spine foot rabbit fish *Siganus rivulatus* survival and growth. *Aquaculture Nutrition* 2007; **13**, 407-12.

[18] B Gomez-Gill, A Rouque and JF Turnbull. The use and selection of probiotic bacteria for use in the culture of larva aquatic organisms. *Aquaculture* 2000; **191**, 259-70.

[19] T Bagheri, A Hedayati, V Yavari, M Alizade and A Farzanfar. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. *Turk. J. Fish. Aquat. Sci.* 2008; **8**, 43-8.

[20] K Ghosh, SK Sen and AK Ray. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in Formulated diets for Rohu, *Labeo rohita*, Fingerlings. *Israeli J. Aquaculture - Bamidgeh* 2003; **55**, 13-21.

[21] JM Castellano, J Roa, RM Luque, C Dieguez, E Aguilar, L Pinilla and M Tena-Sempere. Kiss-1/kisspeptins and the metabolic control of reproduction: physiologic roles and putative physiopathological implications. *Peptides* 2009; **30**, 57-66.

[22] R Fernandez, AC Martini, VM Navarro, JM Castellano, C Dieguez, E Aguilar, L Pinilla and M Tena-Sempere. Novel signals for the integration of energy balance and reproduction. *Mol. Cell. Endocrinol.* 2006; **254**, 127-32.

[23] T Kitahashi, S Ogawa and IS Parhar. Cloning and expression of Kiss 2 in the zebra fish and Medaka. *Neuroendocrinology* 2009; **150**, 821-31.

[24] AG Goumenou, IM Matalliotakis, GE Koumantakis and DK Panidis. The role of leptin in fertility. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2003; **106**, 118-24.