World Journal of Fish and Marine Sciences 4 (1): 65-67, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.01.6242

Effects of Synbiotic (*Biomin imbo*) on Fecundity and Reproductive Factors of Zebrafish (*Danio rerio*)

Hamed Nekoubin, Susan Javaheri and Mohammad Reza Imanpour

Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: This study was conducted to evaluate the effect of synbiotic (*Biomin imbo*) on reproductive parameters of Zebrafish (*Danio rerio*) via supplementation with Biomar. The synbiotic were used in three concentrations of 0.5, 1, 1.5 g/Kg of diet (Biomar). The Zebrafish in experimental treatments were fed of the three levels of synbiotic with 4 percent body weight (3 times a day). The larvae in control treatment were fed without supplemented Biomar. After 90 days, 10 Adult female and male zebrafish (*Danio rerio*) were divided from each treatment. The results showed that fecundity in experimental treatments had significantly increased in comparison to control treatment. The synbiotic also had significant positive effects on hatching rate and rate of percentage of germinal vesicle breakdown (GVBD) in comparison to those in control treatment. The hatching time were also significantly decreased in comparison with the control treatment ($p<0.05$).

Key word: Zebrafish % *Danio rerio* % Synbiotic % Female % Hatching rate % Fecundity

INTRODUCTION

Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism, hence synbiotics, enhancing their isolated beneficial effects. When two nutritional ingredients or supplements are given together; the resulting positive effect generally follows one of three patterns: additivity, synergism or potentiation. Additive effect occurs when the effect of two ingredients used together approximates to the sum of the individual ingredient effects. In case of synergism, it is said to occur when the combined effect of the two products is significantly greater than the sum of the effects of each agent administered alone. The term potentiation is used differently, some pharmacologists use potentiation interchangeably with synergism to describe a greater than additive effect and others use it to describe the effect that is only present when two compounds are concurrently [1].

Synbiotics affects 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 [2]. The first application of synbiotics in fish is that of Rodriguez-Estrada *et al.* [3].

In zebrafish, 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) [4]. In recent years, it has been established that GnRH transcription and secretion are gated by the state of energy reserves in the organism [5]. 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 [6]. 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 [7]. 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) in zebrafish [7]. The binding of MIH to its receptors (paqr7b and paqr8) activates the maturation processes [8].

This study investigated for the first time the effects of synbiotic (*Biomin imbo*) on fecundity and reproductive factors in female zebrafish via supplementation with Biomar.

Corresponding Author: H. Nekoubin, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Table 1: Nutrient composition of experimental diets (%)

Ingredients	%
Protein	54
Lipid	18
Fiber	1.5
Ash	10
Vitamin	2

MATERIALS AND METHODS

The synbiotic (*Biomin imbo*) was prepared from the commercial product Protexin aquatic (Iran-Nikotak). Also Biomar was provided by aquatic foods company. Nutrient compositions of experimental diets (Biomar) are given in table 1. Proximate composition of diets was carried out using the Association of Analytical Chemists [9] methods. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method; Crude fat was determined using petroleum ether (40-60 Bp) extraction method with Soxhlet apparatus and ash by combustion at 550 °C.

This experiment was conducted in a completely randomized design with four treatments (three synbiotic levels and a control) and three replicates per treatment for a total of twelve fiberglass tanks (each with a capacity of 60 liters). Larvae of zebrafish (initial weight: 0.21±0.09 g) were obtained from the Institute of Ornamental Fish Hatchery in Gorgan, Iran. The density of fish larvae per tank were 50 fish. Zebrafish larvae in control and experimental treatments were fed 4 percent of their body weight for 3 times a day (8.00, 16.00 and 24.00). The fish in experimental treatments were fed three concentrations of 0.5, 1, 1.5 g/Kg of diet (Biomar) (treatment 1, 2 and 3 respectively). The control treatment was fed without supplemented Biomar.

After 90 days, adult female and male zebrafish 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).

One-way ANOVA and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by using the SPSS program.

RESULTS AND DISCUSSION

The results clearly showed that the synbiotic had beneficial effects on reproductive indices of zebrafish. The reproductive indices of zebrafish are presented in Table 2. Effects of synbiotic treatments on reproductive indices of zebrafish resulted better than control treatment ($p<0.05$). Also, the three different treatments of synbiotic were significantly different for any of reproductive indices that, among the three different concentrations of synbiotic supplemented with Biomar fed to zebrafish, the greatest effect appeared to be obtained in experimental treatments. This is particularly true for fecundity and hatching rate (%), where the highest was obtained in the experimental treatment. The hatching time in the experimental treatments was significantly decreased in comparison with control treatment ($p<0.05$).

Reproductive indices in among treated groups (T1,T2 and T3) were not significantly differences ($p>0.05$), but were significantly improved this factors in comparison with control treatment ($p<0.05$).

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 [4, 6, 10,11].

Thus, full activation of the hypothalamic-pituitary-gonadal axis at puberty and its proper functioning in adulthood critically depend on adequate body energy stores [5]. The identification of the adipose hormone leptin, which signals the magnitude of energy stores to

Table 2: Reproductive indices of Zebrafish (*Danio rerio*) in experimental treatments (trial 1-3) and control

Treatments ----------------------------- Reproductive Indices	Control Unsupplemented Biomar	T1 supplemented Biomar with 0.5 g/kg	T2 supplemented Biomar with 1 g/kg	T3 supplemented Biomar with 1.5 g/kg
Weight of fish (g)	$0.41±0.01^{c}$	$0.44±0.01^{c}$	$0.48±0.01^{b}$	$0.59±0.17^{a}$
Fecundity	$60.21±5.83^{b}$	$247.21±14.09^{a}$	$259.21±12.69^{a}$	$286.21±19.26^{a}$
Hatching rate (%)	$56.41±5.81^{b}$	$90.26±3.48^{a}$	$91.48±4.01^{a}$	$90.92±6/15^{a}$
Rate of GVBD (%)	$18/09±1/01^{b}$	$67/36±2/45^{a}$	$68/37±1/02^{a}$	$65.38±2.17^{a}$
Hatching time	$59±2^{a}$	$44±2^{b}$	$43±1^{b}$	$44±2^{b}$

Groups with different alphabetic superscripts in the same row differ significantly at $p<0.05$ (ANOVA) the hypothalamic centers governing reproduction [12, 13], represented an important step toward understanding the mechanisms controlling this interplay.

Zebrafish have asynchronous ovaries, containing follicles at all stages of development [14] as well as mature eggs. The growth and maturation of the oocyte occur over a period of about 10 days [15, 16] and in laboratory conditions, eggs are spawned throughout the year.

REFERENCES

1. Chou, T.C., D. Rideout, J. Chou and J.R. Bertino, 1991. Chemotherapeutic synergism, potentiation and antagonism. In: Dulbecco R (Ed), Encyclopedia of human biology, vol. Academic Press, San Diego, California, pp: 371-379.
2. Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr., 125: 1401-1412.
3. Rodriguez-Estrada, U., S. Satoh, Y. Haga, H. Fushimi and J. Sweetman, 2009. 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., 57: 609-617.
4. Zohar, Y., J.A. Mun˜oz-Cueto, A. Elizur and O. Kah, 2010. Neuroendocrinology of reproduction in teleost fish. General and Comparative Endocrinology, 165: 438-455.
5. Hill, J.W., J.K. Elmquist and C.F. Elias, 2008. Hypothalamic pathways linking energy balance and reproduction. American Journal of Physiology. Endocrinology and Metabolism, 294: 827-832.s
6. Castellano, J.M., J. Roa, R.M. Luque, C. Dieguez, E. Aguilar, L. Pinilla and M. Tena-Sempere, 2009. Kiss-1/kisspeptins and the metabolic control of reproduction: physiologic roles and putative physiopathological implications. Peptides., 30: 57-66.
7. Patino, R., G. Yoshizaki, P. Thomas and H. Kagawa, 2001. Gonadotropic control of ovarian follicle maturation: the two-stage concept and its mechanisms. Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology, 129: 427-439.
8. Hanna, R.N. and Y. Zhu, 2009. Expression of membrane progestin receptors in zebrafish (*Danio rerio*) oocytes, testis and pituitary. General and Comparative Endocrinology, 161: 153-157.
9. AOAC (Association of Official Analytical), 2000. Official methods of analysis. EUA.
10. Fernandez, R., A.C. Martini, V.M. Navarro, J.M. Castellano, C. Dieguez, E. Aguilar, L. Pinilla and M. Tena-Sempere, 2006. Novel signals for the integration of energy balance and reproduction. Molecular and Cellular Endocrinology, 254: 127-132.
11. Kitahashi, T., S. Ogawa and I.S. Parhar, 2009. Cloning and expression of Kiss 2 in the zebrafish and Medaka. Neuroendocrinology, 150: 821-831.
12. Casanueva, F. and C. Dieguez, 1999. Neuroendocrine regulation and actions of leptin. Frontiers in Neuroendocrinology, 20: 317-363.
13. Goumenou, A.G., I.M. Matalliotakis, G.E. Koumantakis and D.K. Panidis, 2003. The role of leptin in fertility. European Journal of Obstetrics, Gynecology and Reproductive Biology, 106: 118-124.
14. Selman, K., R.A. Wallace, A. Sarka and X. Qi, 1993. Stages of oocyte development in the zebrafish, *Brachydanio rerio*. Journal of Morphology, 218: 203-224.
15. Wang, Y. and W. Ge, 2003a. Involvement of cyclic adenosine 30,50-monophosphate in the differential regulation of activin betaA and betaB expression by gonadotropin in the zebrafish ovarian follicle cells. Endocrinology, 144: 491-499.
16. Wang, Y. and W. Ge, 2003b. Spatial expression patterns of activin and its signaling system in the zebrafish ovarian follicle: evidence for paracrine action of activin on the oocytes. Biology of Reproduction, 69: 1998-2006.