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- REVIEW ARTICLE –

Effect of Probiotics on Reproductive Performance of Fish

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Abstract

Probiotics are characterized as live microorganisms which when administered in adequate amounts, confer a health benefit on the host. Nowadays, Probiotics are used for eco-friendly and sustainable production in many sectors. They have also been common organisms that used in aquaculture as growth promoter, stress tolerance, pathogen inhibition, and nutrient digestibility, improving water quality. Probiotics use in aquaculture comprehensively reviewed as an alternative to antibiotic treatment. However, very few studies have addressed the effects of probiotics on reproductive performance and gamete quality of fish. Thus, this review summarizes most current studies on the effects of probiotics on reproductive performance and gamete quality in fish and evaluates further applications of probiotics in reproduction of fish.

Keywords:
Probiotics, broodstock management, reproduction performance, fecundity.

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Introduction

Probiotics have been characterized as live microorganisms which, when added in adequate amounts, confer a health benefit to the host (Who & Fao, 2006, Giannenas et al., 2015; Egerton, 2018). They have the ability to survive the passage through the intestinal tract. The benefits of probiotics include fish disease control (Sharifuzzaman & Austin, 2017), immune system enhancement (Nayak, 2010; Kasmani et al., 2018) feed efficiency, enzymatic contribution to digestion (Egerton et al., 2018; Mehrabi et al., 2018), inhibition of pathogenic microorganisms

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(Zapata & Lara-Flores, 2013), reproduction (Mehdinejad et al., 2018), survival and growth promoter (El-Haroun et al., 2006; Yin et al., 2011; Zapata & Lara-Flores, 2013; Cienfuegos et al., 2018; Goda et al., 2018). These benefits reduce the antibiotic and chemical use in aquaculture. Most probiotics are applied through feeding, injection, encapsulation and immersion (Gatesoupe 1994; Planas et al., 2006; Chandrakla & Soundharanayaki, 2017).

In the last decade, the use of chemicals and antibiotics for disease control in aquaculture has reduced. Whereas probiotics application increased rapidly. The initial use of probiotics in aquaculture was on growth promoters and fish health, then, new areas of research have been found, such as their effect on reproduction, maturation and fecundity, although these requires a more comprehensive development.

The recent attempt in aquaculture is to improve broodstock gamete quality by using probiotics. Ghosh et al. (2007), carried out the pioneer investigation on the effect of probiotic administration on reproduction of fish. Rahman et al. (2018), investigated the effects of probiotics on the reproduction of butter catfish, *Ompok pabda*. The results demonstrated the beneficial effects of probiotics on the reproductive performance; the GSI, fecundity, and larval survival were significantly enhanced by probiotic administration. Dash et al. (2018), studied on *Cyprinus carpio* and applied probiotic bacteria to microbial floc. The authors observed prevalence of gonadal maturation. However, their conclusion was based on their personal observation and data was not published.

Further studies have emphasized probiotics ability to stimulate gonadal development, maturation, gamete quality. Carnevali et al. (2017), reviewed the effects of probiotics on the integrated control of fish metabolism. In the last section of their assessment, condenses recent findings regrading to the positive effects of probiotic administration on fish gonads, which deeply support their role towards reproduction and gamete quality mainly achieved on zebrafish.

Nevertheless, a review paper is not available regarding to the effects of probiotics on gonadal development, gamete quality, fecundity and reproduction of fish. Therefore, the goal of this paper is to present an updated use of probiotics for fish reproduction, gamete quality and fecundity improvement.

Effects of probiotics on the reproductive performance of fish

Nutrition is known to have a considerable effect upon gonadal development and reproductive performance of fish (Watanabe, 1995; Bromage, 1995). Watanabe proved that diets lacking in supplementary trace elements or with reduced levels of lipids, proteins, fatty acids, vitamins C and E, and carotenoids produced eggs of poorer quality than those produced by fish receiving more balanced formulations (Watanabe, 1995). Furthermore, imbalanced of these components influences reproduction in various processes such as egg quality, fertility, hatching rate, survival rate of larvae (Watanabe, 1995; Bromage, 1995). Currently, for most propagated fish species, there are commercially available broodfish diets. In order to improve nutrition status of their broodstock, most fish farm use fresh organisms, like squid, tubifex, daphnia, mussels, krill, cuttlefish, and small crustaceans. However, these organisms do not provide sufficient level of nutrients that are required it by the broodstock. They increase the risk of pathogens transmission to the parents and offspring. Therefore, probiotics applied to food or water in order to hinder infections and to expand their effects on reproduction, gamete quality, hatching and fertilization rate. Very few studies have been

performed on the effects of probiotics on fish reproduction (Abasali & Mohamad, 2010; Giorgini et al., 2010; Gioacchini et al., 2010a,b and c; Gioacchini et al.,2011; Gioacchini et al.,2012; Gioacchini et al., 2013; Miccoli et al., 2015; Ariole, 2012; Carnevali et al., 2017).

A commercial probiotic, primalac was supplemented with the artificial feeds at different concentrations and fed to Swordtail, *Xiphophorus helleri* for a period of 26 weeks (Abasali & Mohamad, 2010). Based on their data, it was concluded that, supplementation of feed with probiotic primalac significantly increased the Gonadosomatic indices, fecundity and larval quality of female broodstock.

Gioacchini et al. (2010a, b and c), investigated the effects of the probiotic *Lactobacillus rhamnosus* on zebrafish fecundity as a feed supplement. In, their study, ten days of probiotic application modulated the gene expression of neuropeptide hormones and metabolic signals, such as kiss1, kiss2 and leptin both at the CNS level and at the peripheral level. The higher number of ovulated eggs in vivo demonstrated the increase in fecundity and by the higher germinal vesicle breakdown rate was obtained with the in vitro maturation assay. The authors summarized that, *L. rhamnosus* as a feed supplement has the great potency on ovarian physiology and presumably on embryo development.

The effects of probiotic *L. rhamnosus* on zebrafish oocytes maturation using FPA (focal plane array imaging together with specific biochemical assay, real-time PCR and enzymatic assay) was studied by Gioacchini et al. (2010a, 2012). Based on FPA and PCR analysis molecular changes were detected in probiotics applied groups. These molecular changes were attributed to higher fecundity in the probiotic –treated group (Gioacchini et al., 2010a). The authors concluded that, probiotics have a positive effect on the reproduction of zebrafish. Gioacchini and her colleques' s research is remarkable. Because of the zebrafish and human genomes share extensive conserved synthetic fragment. In addition, zebrafish genes and their human homologues display structural and functional similarities and are therefore seen as a powerful model system for elucidating molecular mechanism involved in human reproduction. Their results support the potentiality of probiotics, largely used in human diet, on improvement of reproductive technology.

In zebrafish, Carnevali et al. (2013), investigated the effects of *L. rhamnosus* on the reproductive performance of zebrafish as a biomedical fish model. They recorded that long-term administration of L. *rhamnosus* has a positive effects on the physiology of reproductive system. Sexual differentiation of gonocytes was detected at six weeks of post fertilization. Gonadotropin releasing hormone was also detectable at the larval stage. In addition, better ovulation detected in broodstock feed with *L. rhamnosus* supplemented diet.

Based on the PCR-DGGE analysis in the ovaries, *L. rhamnosus* was found to be absent and bacterial rRNA were not delectable in the gonads of zebrafish. Therefore Gioacchini et al. (2012), concluded that the probiotic did not affect directly the ovary but operated by systemic way.

Gioacchini and her team summarized their results in a book chapter, thus the positive effects of probiotic administration on commercial and ornamental fish species demonstrated clearly. The importance of their conclusion was to infer a relationship between gut microbiota and the reproductive performance of fish (Gioacchini et al., 2014).

The most widely used and commercially available probiotic is *L. rhamnosus*. However, the effect of a mixture of four indigenous bacterial genera composed of *Bacillus, Pseudomonas, Acinetobacter* and *Flavobacterium* on egg incubation and larval viability of *Clarias gariepinus* was investigated by Ariole et al. (2012) .Up to date, different probiotic strain applied in fish for improvement of reproductive performance and gamete quality are given in Table 1 (Table 1). Based on their results, they implied that the incubation time, hatching rate and larval survival of *C. gariepinus* increased with increase in bacterial load of water up to 10^8 cells/ml, which was the highest dose they had applied. They were suggested that further investigations could be performing in order to establish the optimal and threshold doses (Ariole et al., 2012).

Table 1. Different Probiotic Strain Applied in Fish for Improvement of Reproductive Performance and Gamete Quality.

Probiotic	Application	Species	Benefites mentioned	References
Bacillus subtilis	Feed additive	*Poecilia reticulata* *Poecilia sphenops Xiphophorus helleri and maculatus*	Fecundity ↑ Viability of eggs ↑ Larval quality	Ghosh et al., (2007)
Bacillus, Pseudomonas, Acinetobacter	Immersion	*Clarias gariepinus*	Reproductive performance ↑ Gamete quality ↑	Ariole et al., (2012)
Lactobacillus acidophilus ***Lactobacillus casei*** ***Enterococcus faecium*** ***Bifidobacterium thermophilum***	Feed additive	*Xiphophorus helleri*	Gonadosomatic indices Fecundity ↑ Larval quality ↑	Abasali & Mohamad, (2010)
Lactobacillus rhamnosus **IMC 501 (Synbiotec)**	Feed additive	*Danio rerio*	Oocyte maturation Fecundity ↑ Reproduction improvement Numbers of ovulated eggs	Gioacchini et al., (2010a,b,c; 2011)
Lactobacillus rhamnosus **IMC 501 (Synbiotec)**	Feed additive	*Danio rerio*	Follicle growth phase ↑ Gonadosomatic index (GSI) Oocyte development	Gioacchini et al., (2011)
Lactobacillus rhamnosus **IMC 501 (Synbiotec)**	Feed additive	*Danio rerio*	Follicle development ↑ Ovary →	Gioacchini et al.,(2012)
Lactobacillus rhamnosus **IMC 501 (Synbiotec)**	Feed additive	*Danio rerio*	Follicular apoptosis ↓ Follicular survival ↑	Gioacchini et al.,(2013)
Lactobacillus rhamnosus **IMC 501 (Synbiotec)**	Immersion	*Anguilla anguilla*	Spermatogenesis process ↑ Sperm volume Sperm motility ↑	Vílchez et al., (2015)
Pediococcus acidilactici **(Bactocell®)** **(probiotic along with nucleotide)**	Feed additive	*Carassius auratus*	Reproductive performance Percentage of motile cells ↑ Duration of spermmotility Absolute fecundity Spermatocrit ↑ Fertilization	Mehdinejad et al., (2018)
Pediococcus acidilactici **(Bactocell®)**	Feed additive	*Danio rerio*	Testicular cells Male reproductive performance ↑	Valcarce et al., (2015)

↑-Increase, →-No Change, ↓-Decrease.

Effects of probiotics on the gamete and larval quality of fish

Fish reproductive health is crucial for their well-being as well as reproduction and health of gametes and embryos. Fish gamete quality is also a very important issue for the fish farming industry, especially for intensively propagated species; one of the most serious impediment in the development of commercial fish farming is the control of gamete quality in captivated fish. Since most of them exhibit poor quality of gametes

Certain probiotics not only promote ovulation and fecundity but can also effect gamete quality and may support the hatching and survival of embryo and larvae. Among the probiotics the *L. rhamnosus* has emerged as the most widely used potent probiotic for gamete and larval quality in fish (Gioacchini et al., 2011; 2012; 2013; Carnevali et al., 2013; Vílchez et al., 2015; Ahmadnia-Motlang et al., 2017). This probiotic *L. rhamnosus* administration resulted, at the physiological level, in a significant increase of fecundity and egg quality.

Investigations in female ornamental fish shown that the use of *Bacillus subtilis* can increase fecundity and viability of eggs and larval quality (Ghosh et al., 2007).

A recent remarkable study by Mehdinejad et al. (2018), shown the effect of dietary supplementation of probiotic, *Pediococcus acidilactici* and nucleotide on reproductive performance including semen quality (motility and density) and egg indices (egg diameter, ovum diameter, absolute fecundity, relative fecundity gonadosomatic and hepatosomatic index, fertilization and hatching rate) in goldfish, *Carassius auratus*. Combined or individual effects of dietary probiotic *P. acidilactici* and nucleotide on gamete quality was a novel investigation. The effects of nucleotide were not previously studied. This study revealed that, dietary probiotic combined with nucleotide or applied solely have a positive role on sperm and egg quality (Mehdinejad et al., 2018).

The effects of a commercial probiotic diet supplement (Bactocell®)containing lactic acid bacteria *Pediococcus acidilactici* were evaluated by identifying five zebrafish sperm quality markers (Valcarce et al., 2015). *P. acidilactici* was applied to zebrafish males as feed probiotic supplement during a 10 days test period. The transcripts were previously described as male zebrafish quality markers; brain-derived neurotrophic factor (bdnf), BCL2-interacting killer (bik), double-sex and mab-3 related transcription factor 1 (dmrt1), follicle-stimulating hormone beta subunit (fshb) and leptin a (lepa). After exposition, males fed on with the supplemented diet with a commercial probiotic containing *P. acidilactici* presented an over-expression on three fertility markers comparing to the control group: lepa, dmrt and bdnf (they are fertility markers). Their results suggested that, *P. acidilactici* had a potential use as probiotic supplement in male zebrafish diet for the improvement of molecular parameters in testicular cells, indicating that probiotic supplementation could affect male zebrafish reproductive performance, sperm and semen quality (Valcarce et al., 2015).

Male European eels (*Anguilla anguilla*) were weekly treated with three different concentrations (10^3, 10^5, 10^6 CFU/Ml) of probiotic *L. rhamnosus* which were daily added to the water (Vílchez et al., 2015). After completion of the experiment, males from the treated and control, groups were sacrificed. Sperm and testis samples were analyzed. The authors concluded that treatment with probiotic *L. rhamnosus* at 10^5 CFU/mL could improve sperm quality parameters and spermatogenesis process in *A. anguilla*.

Conclusion

The stimulatory effects of probiotic *L. rhamnosus* supplementation as a feed additive in zebrafish follicle maturation, fecundity, egg quality was comprehensively investigated by Gioacchini et al. (2012, 2013). They found out that the probiotic *L. rhamnosus* has a significant potential for ovarian physiology and for embryo development. Her team also claims that zebrafish has been used as a vertebrate model system for biomedical research, and the results obtained by them, may have a potential application in reproduction technology for all vertebrates, including mammals and even humans.

As it is clear from our review, numerous studies have addressed the efficacy of probiotics in female fish species but very few studies have focused on sperm and semen quality (motility, concentration, quality of seminal plasma, sperm morphology and volume). Thus, it is indispensable to take action regarding to the effects of probiotics on male reproductive performance.

In the near future, it is necessary to conduct more research relating to the effects of some other probiotics other than *L. rhamnosus* and some other commercially important fish species other than zebrafish. In order to make a conclusion, the effects of probiotics on fish reproduction, male and female gamete quality should be expand to other marine and freshwater fish species. Thus, this eco-friendly method can be use in fish culture and reproduction.

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