Iran. J. Ichthyol. (June 2017), 4(2): 150-161
© 2017 Iranian Society of Ichthyology
P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org
Received: September 23, 2016
Accepted: April 29, 2017
doi: 10.7508/iji.2017

Research Article

Reproductive performance and intestinal bacterial changes of *Carassius auratus* fed supplemented lactoferrin and *Lactobacillus rhamnosus* PTCC 1637 diet

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Abstract: This study broadens research in to improving the reproduction and intestinal microflora of *Carassius auratus* broods stocks via lactoferrin and *Lactobacillus rhamnosus* PTCC 1637 in 120 days. The experimental treatments included: *L. rhamnosus* PTCC 1637 (10^6 CFU g diet^{-1}), lactoferrin (200mg kg diet^{-1}) a combined treatment (*L. rhamnosus* PTCC 1637 with lactoferrin) and a control group in three replications. On the basis of results, the highest ($p<0.05$) working fecundity, absolute fecundity, gonadosomatic index (GSI) were observed in *L. rhamnosus* PTCC 1637 treatment. Egg and one-day-old larvae characteristics and fertilization rate were not affected significantly. As the results showed, the highest survival rate of larvae was found in lactoferrin treatment but not significant. While the counts of viable lactic acid bacteria in the probiotic and in the combined treatment were higher than those of lactoferrin treatment and control group ($p<0.05$), the total count of aerobic bacteria was not affected by the probiotic and lactoferrin. According to our findings, it is recommended to use *L. rhamnosus* PTCC 1637 (10^6 CFU g diet^{-1}) to increase reproduction and the combination of *L. rhamnosus* PTCC 1637 and lactoferrin to control the reproduction efficiency of the *C. auratus*.

Keywords: Reproduction, Bovine lactoferrin, Probiotic, Gold crucian carp.

Citation: Ahmadnia-Motlagh, H.; Hajimoradlo, A.; Gorbani, R.; Agh, N.; Safari, O. & Lashkarizadeh-Bami, M. 2017. Reproductive performance and intestinal bacterial changes of *Carassius auratus* (L., 1758) fed supplemented lactoferrin and *Lactobacillus rhamnosus* PTCC 1637 diet. Iranian Journal of Ichthyology 4(2): 150-161.

Introduction

One of the most important ways to increase the production of fish stocks and preserve them is to increase their reproduction and improve the quality of larvae and fry through improved nutrition of brood stocks. In this respect, various factors like race and quality of brood stocks, their rearing and breeding conditions, food type and quality, water quality and fish health status may affect the hatchery production (Ahmadian et al. 2012). However, the nutrition of brood stocks is particularly important, since it affects all aspects of fertility from maturation to gametogenesis. Therefore, researchers have drawn special attention to the constituent of brood stocks diets over the past two decades

(Gioacchini et al. 2010). Hormones, antibiotics, nutrient combinations and herbal products are used as nutritional supplements for brood stocks, but applying some of these growth stimulants can distract advantageous microbial activities of the digestive tract, which may easily expose brood stocks to diseases caused by opportunistic pathogens. In such circumstances, probiotic supplements can cover such deficits (Ghosh et al. 2007).

Probiotics—as live microorganisms—improve the digestion performance of dietary proteins and lipids by producing essential nutrients like essential fatty acids and digestive enzymes. Therefore, they cause an improvement in the general and nutritional status of the host and a probable increment in its reproductive efficiency. The use of microbial supplements, especially probiotics, has attracted lots of attention in recent decades; extensive research has been done in this respect (Carnevali et al. 2016; Hamdan et al., 2016; Jafariyan et al. 2015; Hoseinifar et al. 2015). Such research has usually concentrated on the determination of the effects and the mechanisms of the effects on growth indices (Ahmadnia Motlagh et al. 2012), immune response (Batista et al. 2015), and disease resistance (Park et al. 2016). However, despite much interest in the use of probiotics, few studies on the effects of probiotics on fish reproduction have been carried out (Carnevali et al. 2013; Gioacchini et al. 2010). The lactobacillus sp., which includes more than 50 species, is among the most important, practical and successful probiotics in the aquaculture industry (Tannock 2004). Some researchers have investigated the effects of *L. rhamnosus* on the reproduction of zebra fish (*Danio rerio*) (Gioacchini et al. 2010; Carnevali et al. 2013; Standen et al. 2013). After 10 days of treatment with the bacteria at 106 CFU g diet^{-1}, daily spawning, ovulation and hatching rate increased significantly (Carnevali et al. 2013). In the case of gonadal histological studies, improvements in follicular growth and gonadal index were reported, indicative of the increment in yolk follicles in partnership with probiotics. In addition to probiotics, other nutritional supplements of natural origin—such as lactoferrin—have also attracted researchers' attention.

Lactoferrin is a glycoprotein that is extracted from cow milk on a commercial scale (Levay & Viljoen 1995) and has been considered over the past decade because of its antibacterial, antiviral, anti-fungal and anti-cancer characteristics (Wang et al. 2013). Recent studies have reported the production of lactoferrin in fish (González-Chávez et al. 2009). It is among those important components of the non-specific immune system. Several studies have investigated the effects of lactoferrin on fish immune system and growth (Wang et al. 2008; Rahimnejad et al. 2012), but few studies have focused on the effects of lactoferrin on the reproduction of fishes.

Therefore, the current study was carried out to investigate the effect of using lactoferrin and *L. rhamnosus* bacteria on reproductive indices GSI, oocyte and egg characteristics (weight and diameter), larva characteristics (total length, weight and survival), and intestinal microbiota (total counts of aerobic bacteria and lactic acid bacteria) of gold fish (*Carassius auratus gibelio*).

Materials and Methods

Experimental design. The experiment was carried out in the form of a completely randomized design (Three treatments and a control group, triplicated). The experimental treatments included a probiotic treatment (10^6 *L. rhamnosus* PTCC 1637 CFU g diet^{-1}), a lactoferrin treatment (200mg kg diet^{-1}), a combined treatment (*L. rhamnosus* at 10^6 CFU g diet^{-1} combined with lactoferrin at 200mg kg diet^{-1}) and a control group.

The bacteria for the current study was purchased from Scientific and Industrial Research Organization of Iran and was then cultured for 24h in MRS Broth medium (Merck®, Germany) at 37˚C. Then, the cultured bacteria were separated from the medium

precipitating by centrifuges at 3000g for 10 minutes. The extracted bacteria were rinsed out using a sterile serum physiology. For determination the concentration of bacteria, the optical densitometry at a wavelength of 620nm was applied using a spectrophotometer (BK-F96RO, BIOBASE®, China). The purchased Lactoferrin (Biopole SA®, Belgium) was stored at 4˚C until use.

Running the experiment. The probiotic and lactoferrin diets were prepared by supplementing the ornamental fish diet (Energy® Dry matter 69.74±2.25, Crude protein 40.92±1.37, Crude Lipid 37.15±0.70, Ash 2.72±0.59) with *L. rhamnosus* and lactoferrin. Gelatin was splashed on the experimental diets at a concentration of 4g kg diet^{-1}, too, as a protective agent. The control diet was prepared using only gelatin, without adding lactoferrin or bacteria. The feed was air-dried at 20°C for 2h and kept at 4°C until use. The quality of the probiotic added diets was monitored by MRS plate culture. The experimental diets were prepared on a weekly basis.

A total of 225 healthy *C. auratus* fry (12.25±0.50gr) was maintained from a commercial ornamental fish supplier. The fish were acclimatized to the laboratory conditions and observed for clinical health for 14 days prior to starting the experiment. They were distributed randomly in the 12 experimental tanks (300l). The fish were fed three times a day with the given amount of diets in accordance with the standard of 2.5% of total body weight of biomass in each tank. The experiment lasted for 120 days until sexual maturity. Physicochemical parameters of the water, such as temperature (19.2±3˚C), DO (6.0±0.5ppm) and pH (7.1±0.5), were maintained in accordance with the standard culture conditions.

***Sampling and studied indices*.** At the end of the experiment, the fish were anaesthetized using ground cloves extract (5ppt) and ovaries were removed. A digital balance, with the accuracy of0.001, was used to weigh ovaries and ovules. 1 gram of ovules obtained from each female brood stock was fixed in 10% formalin to determine the weights and diameters of ovules. Diameters of 30 ovules from each fish were measured using a loop (ZEISS, KF2, Germany) equipped with the eye micrometer (100µm accuracy). The following equations were used to calculate the amounts of absolute fecundity and gonadal index:

Absolute fecundity= (Ovary weight (g)×ovule count in the subsample)/subsample weight

GSI=(gonad weight (g)/body weight (g))×100

Fertilization rate (%)= (No. of fertilized eggs/Total no. of eggs)×100

After hand-breeding, the total weight of ovules obtained from each brood was assigned instantly to calculate the amount of working fecundity. The egg samples were fixed in 4% formalin for measuring their diameters and average weights. Also, after hatching, a sampling of one-day-old and four-day-old larvae was done to measure their total lengths and weights. The measurement of larvae lengths was performed using a loop, equipped with an eye micrometer (100µm accuracy) (Lorenzoni et al. 2007). Thirty days post-hatching, the number of surviving larvae was counted, and the survival rate was calculated using the following formula:

Survival rate (%)= (No. of survived larvae/Total no. of larvae)×100

At the end of the feeding trial, the fish were anesthetized, disinfected by 70% ethanol and the whole gut was removed, to obtain total aerobic bacteria and total lactobacillus count in accordance with Mahious et al. (2005). The cultures Plate Count Agar and MRS Agar were used for counting the total countable aerobic bacteria and total countable lactic acid bacteria (LAB). The culture plates were then incubated at 37˚C for 48h, and the number of colonies was accurately counted.

Statistical analyses. All percentage data were transformed using the arcsine method. After confirming the homogeneity of variance and

normality of the data using Leaven and Kolmogorov–Smirnov tests, respectively then data were subjected to a one-way ANOVA to test the effects of dietary lactoferrin and *L. rhamnosus* supplementation on the reproductive indices and gut microbiota of *Carassius auratus*. Duncan's multiple post hoc was applied in order to compare the significant differences among the treatment means (p<0.05). All analysis was performed using SPSS 17 (IBM SPSS Software, USA).

Results

Gonadosomatic index, fecundity and fertilization rate. According to the results (Fig. 1), the highest (19.58±1.17) and lowest amounts of female GSI were observed in *L. rhamnosus* and combined treatments (p<0.05).

As the results demonstrated (Fig. 2), the highest amount of absolute fecundity (7624 pieces) was observed in *L. rhamnosus* treatment and the lowest amount (3845 pieces) was observed in combined treatment (p<0.05). Moreover, the results showed that no significant differences were found (p>0.05) in the amounts of these two indices between the control group, probiotic and lactoferrin treatments.

Figure 3 shows the averages of working fecundity (ovulated oocytes). Based on the obtained results, the highest amount of ovulated oocytes (5.15g), were found in probiotic treatment and the lowest amount (2.07g) per each spawning brood stock was found in combined treatment, which showed a significant difference (p<0.05) between these two treatments.

Comparison of the averages of the fertilization rate (Fig. 4) in experimental treatments showed no significant difference between control and other treatments.

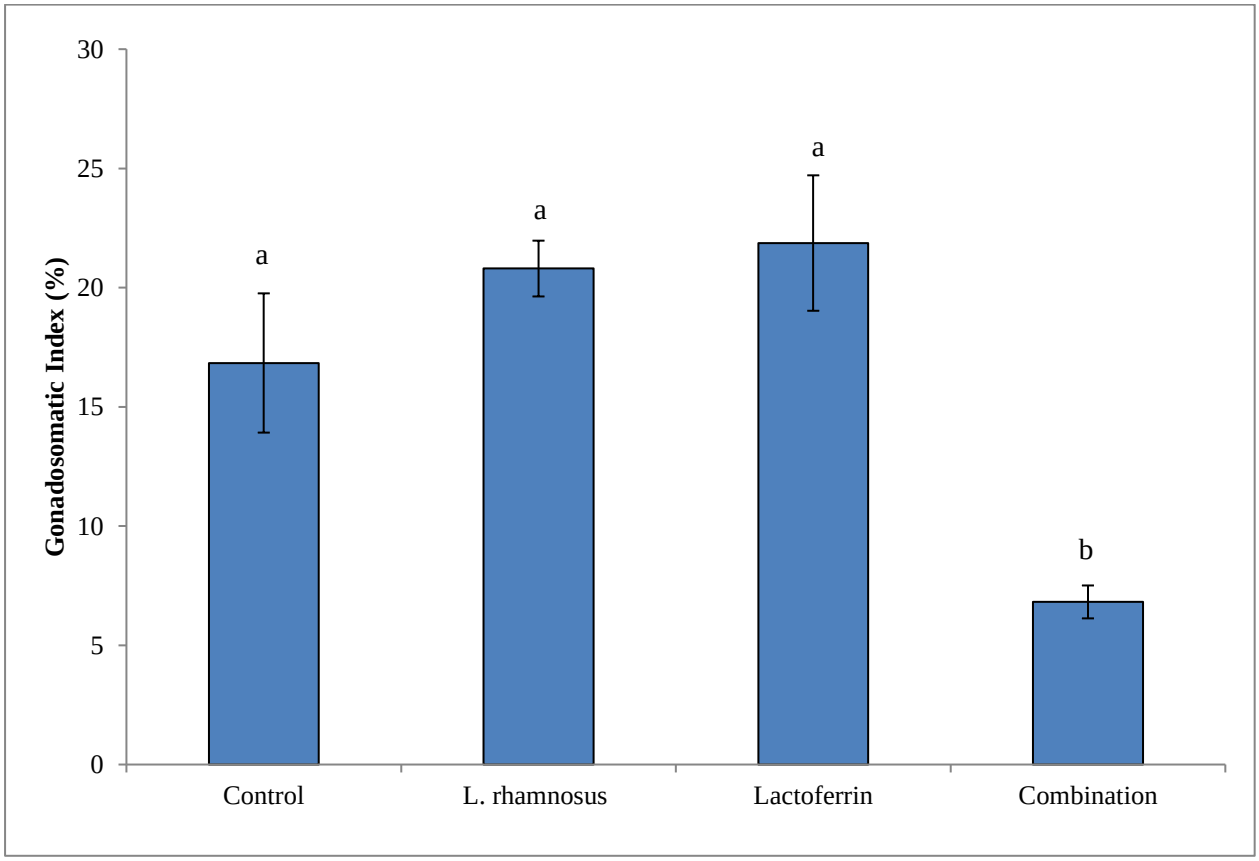

Fig. 1. Mean (±SEM) Gonadosomatic Index (GSI) of female *C. auratus* fed lactoferrin and *L. rhamnosus* in different treatments (n=3).

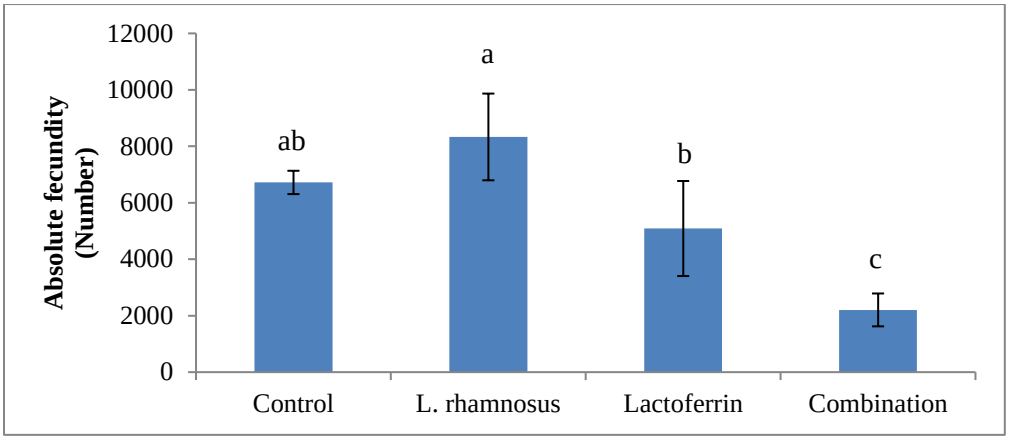

Fig. 2. Mean (±SEM) weight of absolute fecundity (number) of *C. auratus* fed lactoferrin and *L. rhamnosus* in different treatments (n=3).

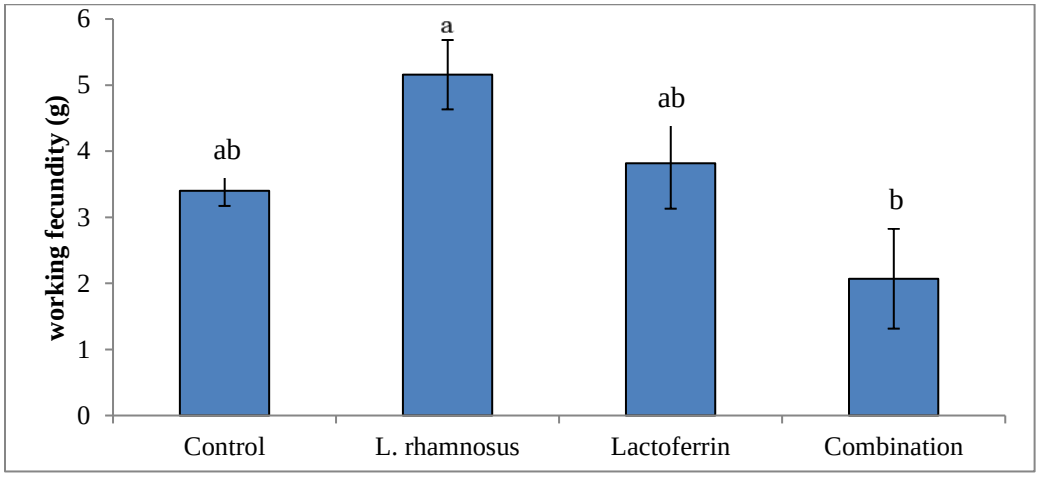

Fig. 3. Mean (±SEM) working fecundity (ovulated oocytes (g)) from *C. auratus* fed lactoferrin and *L. rhamnosus* in different treatments (n=3).

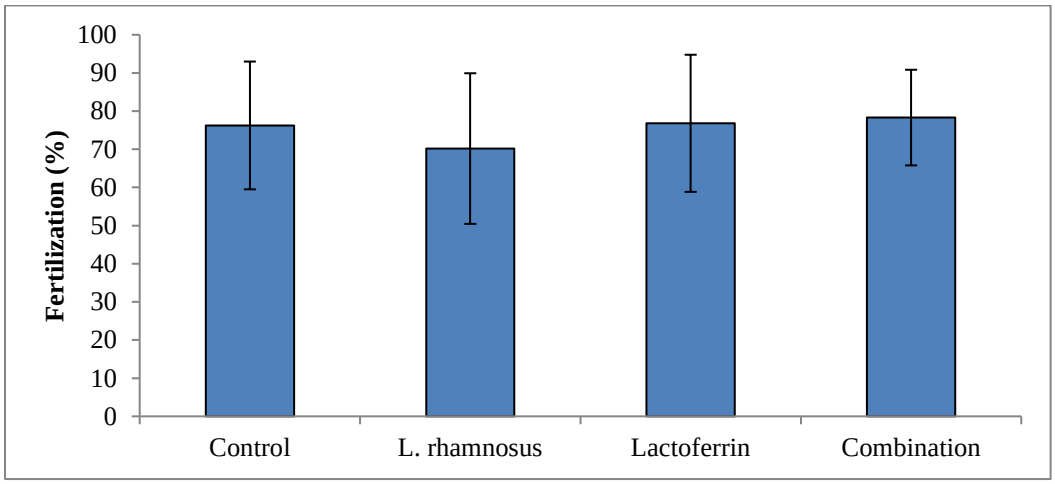

Fig. 4. Mean (±SEM) fertilization rate of *C. auratus* eggs fed lactoferrin and *L. rhamnosus* in different treatments (n=3).

Oocyte, egg and larvae characteristics. *Lactobacillus rhamnosus* treatment had a significantly (p<0.05) higher oocyte diameter increment than the control group and combined treatment (Table 1). Also, *L. rhamnosus* treatment showed a significantly (p<0.05) higher oocyte weight (0.71±0.10g) compared to control group (0.47±0.19g) and combined treatment (0.26±0.25g). The highest egg diameter (1.29±0.56mm) was observed in lactoferrin treatment and the lowest egg diameter (1.14±0.12mm) was found in combined treatment (p<0.05). No significant differences were observed (p>0.05) in the egg weight amounts between the control group and other treatments.

As shown in Table 2, no significant differences were found (p>0.05) in the lengths of one-day-old larvae between the control group and other treatments. Also, no significant differences were observed (P>0.05) in the weights of one-day-old larvae between the control group and other treatments. Probiotic treatment showed a significantly (p<0.05) higher four-day-old larvae weight (5.79±0.25mm) compared to other treatments. Moreover, four-day-old larvae had significantly (p<0.05) higher (1.40±0.17g) and lower (0.50±0.06g) weights in *L. rhamnosus* and combined treatments compared to other treatments.

Effects on brood stocks intestinal bacteria population. It was found out by investigating total intestinal bacteria counts at the end of the experiment (Table 3) that lactoferrin and probiotic bacteria did not raise the total aerobic bacteria counts in the digestive tract of gold crucian carp significantly. *Lactobacillus rhamnosus* clearly enhanced lactic acid bacteria counts of the probiotic treatment (p>0.05). Lactic acid bacteria levels were too few to enumerate in the intestine of control and lactoferrin treatment.

Table 1. Mean (±SEM) Diameter and weight of the oocytes and the eggs from *C. auratus* brood stocks fed lactoferrin and *L. rhamnosus* in different treatments (r=3).

	Oocyte		Egg	
	Diameter (mm)	Weight (g)	Diameter (mm)	Wight (g)
Control	0.69±0.28 ^{b}	0.47±0.19 ^{c}	1.19±0.10 ^{bc}	0.95±0.11
L. rhamnosus	0.85±0.29 ^{a}	0.71±0.10 ^{bc}	1.18±0.10 ^{bc}	1.22±0.15
Lactoferrin	0.79±0.32 ^{ab}	0.79±0.11 ^{ab}	1.27±0.08 ^{ab}	1.00±0.12
Combination	0.54±0.23 ^{c}	0.26±0.25 ^{c}	1.14±0.12 ^{c}	1.04±0.20

Data with different letter in each column are significantly different (p<0.05).

Table 2. Mean (±SEM) Length and Wight of the larvae from *C. auratus* brood stocks fed lactoferrin and *L. rhamnosus* in different treatments (r=3).

	One-day-old larvae		four-day-old larvae	
	Length (mm)	Wight (g)	Diameter (mm)	Wight (g)
Control	3.98±0.11	0.85±0.10	5.22±0.11 ^{bc}	0.71±0.08 ^{bc}
L. rhamnosus	4.22±0.20	1.00±0.12	5.79±0.25 ^{a}	1.40±0.17 ^{a}
Lactoferrin	4.02±0.25	0.84±0.10	5.17±0.20 ^{c}	1.16±0.14 ^{ab}
Combination	4.12±0.41	0.87±0.12	5.61±0.41 ^{ab}	0.50±0.06 ^{c}

Data presented in each column with non-common characters were significantly different (p<0.05)

Table 3. The mean (±SEM) total number of bacteria and lactic acid bacteria count per gram of intestinal *C. auratus* brood stock fed lactoferrin and *L. rhamnosus* in different treatments (r=3).

	Total count	LAB count
Control	$1.95\pm0.43\times10^{6}$	$0.00\pm0.00\times10^{3}$ b
L. rhamnosus	$2.34\pm0.95\times10^{6}$	$8.77\pm2.65\times10^{3}$ a
Lactoferrin	$1.10\pm0.58\times10^{6}$	$0.00\pm0.00\times10^{3}$ b
Combination	$1.64\pm0.80\times10^{6}$	$5.44\pm1.10\times10^{3}$ a

Data presented in each column with non-common characters were significantly different (p<0.05)

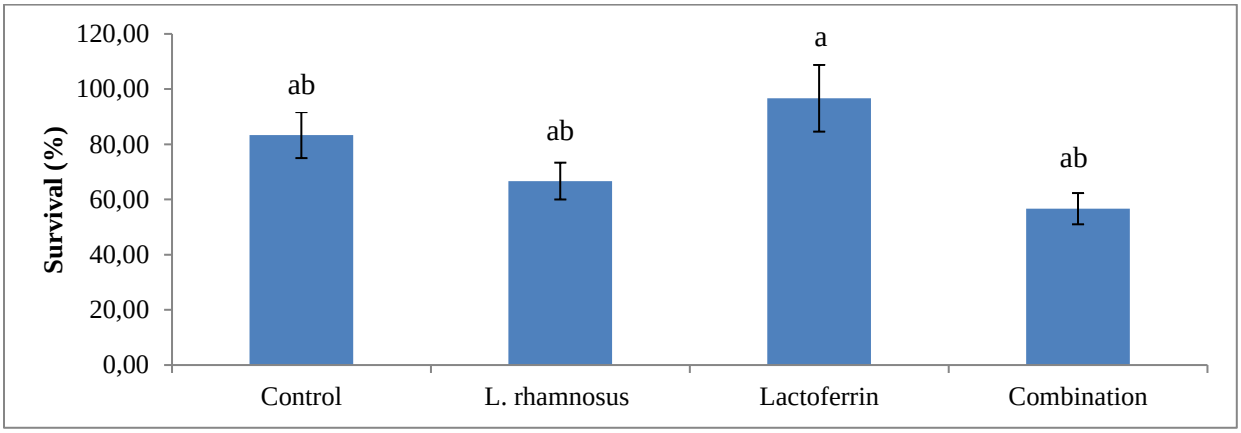

Fig. 5. The mean (±SEM) survival rate of thirty-day old *C. auratus* larvae from brood stock fed lactoferrin and *L. rhamnosus* in different treatments (n=3).

Larvae survival. The results of thirty-day old larvae survival (Fig. 5), showed that the highest survival (96.67%) was related to lactoferrin treatment while no significant differences were found in the survival amounts between the control group and other treatments.

Discussions

Nutrition is among those important factors that determine the fish growth rate (Li et al. 2004) and affect the capability of fish to present its genetic potential for growth (Ownagh et al. 2013). Therefore, preparing an ideal diet that results in the growth increment is essential to achieve the maximum efficiency in the aquaculture industry. Nowadays, the use of dietary growth stimulants in addition to essential nutrients is highly regarded in the aquaculture industry.

The first and the most important purpose of constructing fish hatcheries is the production of the maximum number of high-quality eggs and fries. It is well known that fecundity and quality of eggs are related not only to the genetics of broods, but also to their rearing condition, such as feeding, water quality and fish health (Okumuş 2002).

Reproduction is gated by the state of body energy reserves and is sensitive to different metabolic cues (Zohar et al. 2010). Gonadosomatic Index (GSI), which is the ratio of gonad weight to body weight, is one of the most important indices to assess reproduction. This index had increased in *L. rhamnosus* and lactoferrin treatments compared to combined treatment. The GSI increment has also been reported in four live-bearing fish species treated with *Bacillus subtilis* bacteria, including guppy (*Poecilia reticulate*), molley (*Poecilia sphenops*), green swordtail (*Xiphophorus helleri*) and platy (*Xiphophorus maculatus*) (Ghosh et al. 2007). Moreover, GSI has been

reported to increase in mummichog (*Fundulus heteroclitus*) treated with *L. rhamnosus* bacteria (Gioacchini et al. 2010).

L. rhamnosus PTCC 1637 treatment showed the highest fecundity, which was significantly higher than that of the combined treatment. This could be as the result of higher oocyte weight and, therefore, higher ovary weight in fish that received *L. rhamnosus* PTCC 1637 compared to other treatments. Previous studies have shown that the incorporation of fatty acids into the brood stock diets would improve reproductive performance (Bruce et al. 1999; Izquierdo et al. 2001; Liang et al. 2014). The production of fatty acids by lactic acid bacteria has also been demonstrated (Ratledge 2004). Therefore, it seems that the increased number of lactic acid bacteria in the intestines of probiotic-receiving treatments led to an increase in the production of fatty acids. According to the results, the maximum and minimum amounts of working fecundity (ovulated oocytes) per spawning brood stock were observed in *L. rhamnosus* PTCC 1637 and combined treatments. Similar results have been found in zebrafish (*Danio rerio*) treated with *L. rhamnosus* IMC 501 (Carnevali et al. 2013). It seems that probiotics are capable of increasing ovulation in zebrafish by preparing essential nutrients and affecting the expression of cox2a gene and, therefore, activating this gene (Wang et al. 2008). The highest amounts of absolute and relative fecundities were observed in *L. rhamnosus* PTCC 1637 treatment, while the lowest amounts were found in combined treatment.

Producing big-yolk eggs is the common principle among all fish species in evolution (Izquierdo et al. 2001). As the results demonstrated, the highest diameter and weight of non-ovulated oocytes were found in probiotic treatments and the lowest amounts were found in the control group and combined treatment. According to the similar results in zebrafish, producing bigger and heavier oocytes could be the result of higher absorption of yolk by oocytes, and could be regarded as the better intestinal provision of essential fatty acids (Gioacchini et al. 2010). The weight and diameter of eggs did not show any significant difference between different treatments and control group. Fish oocytes absorb water during fertilization time, and different factors affect water absorption ability of eggs (Gioacchini et al. 2010). As the amount of water absorbed by the egg can affect the egg weight, so it seems that the diameter and weight of oocyte can be more precise criteria than the diameter and weight of egg to judge the effects of different factors on reproduction.

Four-day-old larvae showed higher weights and lengths in *L. rhamnosus* treatment compared to other treatments. Gold crucian carp larvae fed actively after four days at 10–20˚C, though this happened approximately one day earlier in the case of lactoferrin and *L. rhamnosus* treatments. The rapid embryonic development of zebrafish that received *L. rhamnosus* bacteria has been reported by other researchers (Gioacchini et al., 2010). In addition, the production of large-size larvae in the case of treatments that received probiotic could be the result of a balance in producing essential fatty acids by probiotic bacteria in the intestines, which promotes the production of large eggs and larvae (Irianto & Austin 2002).

A lot of research has been done to investigate the immunogenicity properties of lactoferrin and its effects on survival and disease resistance of fish, but no studies are found that have investigated the effects of lactoferrin on survival or immunity status of larvae produced of treated broods. Prior to complete maturation of their own immune systems, developing embryos and larvae rely on maternally inherited immune-relevant molecules for protection against invading pathogens (Qin et al. 2014). Previous studies on several fish species have investigated transmission to offspring of maternal immunization in zebrafish (*Danio rerio*) (Qin et al. 2014), sea bream (*Sparus aurata*) (Hanif et al. 2004, 2005) and *Labeo rohita* (Swain et al. 2006). The survival increment of larvae produced by lactoferrin

treatment might be related to the vertical transmission of immunity from the mother to the larvae.

Probiotic supplements can cover the shortages resulting from suppressing beneficial activities of digestive tract bacteria in brood stock fed on hormones, antibiotics, and herbal products as food supplements (Ghosh et al. 2007). Investigating the total count of intestinal bacteria in female gold crucian carp demonstrated that lactoferrin and probiotic bacteria used had no significant effect on the total count of aerobic bacteria in the digestive tract of this fish. Researchers, however, have observed an increase in total aerobic bacteria count in the digestive tract of green swordtail (*X. helleri*) when treated with lactic acid bacteria (*Lactobacillus acidophilus*) (Hoseinifar et al. 2015). Other researchers have reported that using *Bacillus sp.* bacteria in Indian white shrimp (*Fenneropenaeus indicus*) has no effect on total bacteria count of the digestive tract (Ziaei-Nejad et al. 2006), which is in agreement with findings of the current research.

According to the results, *L. rhamnosus* PTCC 1637 had clear modulating effects on the gut microbiota of *C. auratus* adults that were fed the probiotic diet. Lactic acid bacterial levels were 8.77×103± 2.65x103 $CFUg^{-1}$ of *C. auratus* gut in the probiotic-fed fish, whereas acid lactic bacteria levels were too low to enumerate in the control and lactoferrin groups. This evidence could serve as a demonstration of the high competitiveness of probiotics used, which might result from the secretion of products inhibiting the growth of other intestinal bacteria or possession of bacteria binding sites in the intestine. Similar reports are found on Nile tilapia (*Oreochromis niloticus*) treated with *P. acidilactici* bacteria (Standen et al. 2013) and green swordtail (*X. helleri*) treated with *L. acidophilus* bacteria (Ghosh et al. 2007).

As regards lactoferrin's antibacterial properties against non-probiotic and pathogenic bacteria, it was expected that a significant increase would be observed in lactic acid bacteria population in combined treatment with growth inhibition of other bacteria by lactoferrin. However, the results demonstrated that no significant difference was found in the counts of lactic acid bacteria between combined and *L. rhamnosus* treatments. It could be the result of the production of secondary metabolites by lactoferrin—such as lactoferricin—which is said to be several times greater than that of lactoferrin in antibacterial properties (González et al. 2009). The final judgment in this case requires more extensive studies. Morshedi et al. (2015) have reported that 400 and 800 mg lactoferrin used per kg diet of sobaity sea bream fries (*Sparidentex hasta*) had no effects on intestinal bacteria flora, concluding that lactoferrin has been probably unsuccessful to establish a new bacteria balance in the intestine of *S. hasta* fries.

The output of this survey revealed that lactoferrin and *L. rhamnosus* PTCC 1637 enhanced the reproduction efficiency to some extent, but their combination sharply suppressed the reproduction. It could be dependent on digestive tract properties, breeding conditions, the existence of some unidentified materials in the diet, and their interaction with lactoferrin and bacterial metabolites (Eslamloo et al. 2012). As the combination of *L. rhamnosus* bacteria and lactoferrin somehow resulted in the growth inhibition of sexual gonads, it is suggested using this combination to control and postpone reproduction.

Acknowledgements

The authors are grateful to Seyed Hosein Hoseinifar and Ali Jafar for their kind help and guidance. They also thank Mr. Morteza Ghasemi for his support during the study.

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Iran. J. Ichthyol. (June 2017), 4(2): 150-161
© 2017 Iranian Society of Ichthyology
P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org
Received: September 23, 2016
Accepted: April 29, 2017
doi: 10.7508/iji.2017

مقاله پژوهشی

تأثیر لاکتوفرین و *Lactobacillus rhamnosus* PTCC 1637 جیره غذایی بر عملکرد تولیدمثلی و تغییرات میکروبی روده ماهی قرمز

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چکیده: این مطالعه به‌منظور بررسی اثر استفاده از لاکتوفرین و باکتری *Lactobacillus rhamnosus* به مدت ۱۲۰ روز بر شاخص‌های تولیدمثلی و فلور باکتریایی روده مولدین کاراس طلایی (*Carassius auratus*) (میانگین وزن ۰/۵۰±۱۲/۲۱ گرم) طراحی شد. تیمارهای آزمایشی شامل تیمار پروبیوتیکی: باکتری*L. rhamnosus* PTCC 1637 (۱۰۶ باکتری در گرم جیره) ، تیمار لاکتوفرین: ۲۰۰ میلی‌گرم لاکتوفرین در کیلوگرم جیره، تیمار ترکیبی: *L. rhamnosus* PTCC 1637 (۱۰۶ باکتری در گرم جیره) به همراه لاکتوفرین (۲۰۰ میلی‌گرم در کیلوگرم جیره) و شاهد (جیره غذایی بدون افزودنی) در سه تکرار بود. بر اساس نتایج، بالاترین میزان هم‌آوری کاری و مطلق و شاخص گنادی در تیمار *L. rhamnosus* مشاهده شد. خصوصیات تخم (قطر و وزن) و لارو یک‌روزه (طول و وزن) و همچنین میزان لقاح به‌صورت معنی‌داری تغییر نکردند. نتایج نشان داد که بیشترین میزان بقای لارو مربوط به تیمار لاکتوفرین بود. هرچند که تعداد کل باکتری‌های هوازی موجود در روده اختلاف معنی‌داری بین تیمارهای آزمایشی نشان نداد، تعداد کل باکتری‌های اسیدلاکتیک در روده ماهیان تیمارهای پروبیوتیک و ترکیبی بیشتر از شاهد و لاکتوفرین بود. با توجه به نتایج به دست آمده، از باکتری *L. rhamnosus* (۱۰۶ باکتری در گرم جیره غذایی) جهت افزایش کارایی تولیدمثل و تیمار ترکیبی برای کنترل تولیدمثل در ماهی کاراس طلایی پیشنهاد می‌شود.

کلیدواژه‌ها: تولیدمثل، لاکتوفرین گاوی، پروبیوتیک، کاراس طلایی .