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Effects of probiotic bacteria on survival, growth and body composition of rainbow trout (*Oncorhynchus mykiss*) larvae fed diets with various fish meal

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PEER REVIEW

Peer reviewer

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Comments

The authors results on potential replacements for fishmeal in fish feed and use of probiotics in enhancing immunity of fish against disease, concur with that published by other researchers.

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ABSTRACT

Objective: To compare the the effects of the diets containing various fish viscera meal and effects of probiotic bacteria (*Lactobacillus* sp., *Bifidobacterium* sp. and *Streptococcus* sp.) on the growth, survival and carcass composition of rainbow trout larvae.

Methods: Twelve tanks consisting of triplicates for each treatment group were used. Triplicate groups of rainbow trout (176 mg bodyweight) were fed with three diets formulated with viscera meal (VM) derived from common carp (*Cyprinus carpio*), or VM derived from mullet (*Liza saliens* and *Liza auratus*) or commercial diet with in-tank probiotic respectively, and three diets without probiotic 4 times a day at 5% to 6% of body weight for 45 d. Rainbow trout larvae (average individual weight, 176 mg) were randomly distributed with density of 4 fish/L into 18 fiberglass tanks. In probiotic treatments a blend of selected bacteria at 10^4 CFU/mL were added into rearing tanks four times a day. At the end of the 45-day experiment, growth performance, survival rates and carcass composition of larvae were determined.

Results: The results indicated that change to probiotic diets significantly affect growth and survival of rainbow trout larvae ($P<0.05$). Rainbow trout larvae fed with commercial diet with in-tank probiotics showed the highest survival rates (98%), total weight (2226 mg) and specific growth rates (7.16%/d) among treatment groups.

Conclusions: VM derived from common carp can successfully replace more than half of marine fish meal in formulated diets for rainbow trout. Use of in-tank blends of *Lactobacillus* sp., *Bifidobacterium* sp. and *Streptococcus* sp., can increase survival rates and specific growth rates in rainbow trout larvae.

KEYWORDS

Rainbow trout, Fish viscera meal, Probiotic

1. Introduction

Rainbow trout culture is economically important in Iran. Bacterial infectious disease is one of the major reasons for decreasing production levels in some trout farms. The success fish culture programs are determined in part by the health conditions of early fry stages[1]. The occurrence of sub-clinical infections under aquaculture conditions can lead to reduced growth and increased mortality[2]. Several feed additives have been used for improving fry health conditions and feed

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utilization efficiency[3]. Antibiotics as feed additives were commonly used in the early 1950s[3]. Due to abuse of antibiotics as animal growth promoters, antibiotic resistance has become a common characteristic in microorganisms, and caused serious problems in treating microbial infections[4]. The aquaculture industry demands the use of alternative disease control methods to support environmentally-friendly aquaculture practices[5].

The use of beneficial microorganisms as probiotics, or their products, have been used in aquaculture to control disease, as supplements for improving growth and in some cases as alternative antimicrobial compounds[6]. Probiotics are live bacteria feed supplements, which can benefit the host animal by improving intestinal microbial balance[7]. Probiotics can improve digestive enzyme activity, digestibility of ingested nutrients and enhance growth and feeding performance in fish larvae. Microbial probiotics have been proved as a suitable prophylactic, and are now widely accepted for use in aquaculture[5,6,8-10]. This study aimed to investigate the effects of experimental diets made from viscera meal (VM) of common carp (*Cyprinus carpio*), mullet (*Liza saliens* and *Liza auratus*) and a commercial diet, and use of a blend of *Lactobacillus* sp., *Bifidobacterium* sp. and *Streptococcus* sp., added directly into the larvae cultivation system on the health of rainbow trout larvae.

2. Materials and methods

2.1. Rearing conditions and experimental design

Rainbow trout, *Oncorhynchus mykiss* (*O. mykiss*) (Walbaum) [average weight (wet weight)=176 mg] were randomly selected and stocked into 12 fiberglass tanks (10 L) at 40 fish per tank. Each tank was supplied with constant temperature (16 °C) spring water at a flow rate of 2 L/min and aeration to maintain a proper level of dissolved oxygen and a fixed photoperiod (12 h daylight:12 h dark). These groups of fish were fed four times per day to satiation, for 45 d according to normal trout culture practice. Any uneaten feed was collected 30 min after feeding and weighed, to determine actual feed intake. Fish weight was taken on a once weekly basis. Feeding was withheld 24 h prior to weighing to minimize stress to fish. Three experimental groups fed different diets were subjected to a blend of selected bacteria at 10^4 CFU/mL to evaluate the beneficial effects of probiotics use in rainbow trout larvae. The blend of above bacteria were added into the rearing tank 4 times a day.

2.2. Feeding and probiotic regime

Rainbow trout were fed one of three diets formulated with VM derived from common carp (*Cyprinus carpio*), or VM from mullet (*Liza saliens* and *Liza auratus*), or a commercial diet (Aqua™, Denmark) with in-tank probiotics (carp-p, mullet-p and commercial diet-p), respectively and three diets without in-tank probiotic (carp, mullet and commercial diet). The chemical composition of diets and fish VM are listed in Tables 1 and 2. The fish meals from two species were cooked, pressed and dried by conventional processing methods.

Table 1

Formulation of experimental diets fed to rainbow trout larvae.

Ingredients	Diets	
	Carp	Mullet
Common carp viscera meal	55	-
Mullet viscera meal	-	55
Soybean meal	5	5
Corn gluten	8	7
Sunflowermeal	8	7
Wheatmeal	16	17
Fish oil	4	5
Vitamin/mineral premix (1:1)[1]	1	1
Binder	1	1
Calsid	2	2

[1]: Per g vitamin mixture: vitamin A (342 IU), vitamin D_3 (329 IU), vitamin E (0.0274 IU), vitamin K_3 (5.48 mg), vitamin B_1 (2.05 mg), vitamin B_2 (3.42 mg), vitamin B_3 (20.5 mg), vitamin B_5 (5.48 mg), vitamin B_6 (2.05 mg), vitamin B_{12} (2.74 mg), vitamin C (24.0 mg) and per g mineral mixture: biotin (0.411 mg), folicacid (0.685 mg), Zn (12.3 mg), Mn (4.80 mg), Cu (1.64 mg), I (0.274 mg), Se (0.0274 mg), Ca (125 mg), K (189 mg).

Table 2

Proximate composition (%) of fish viscera meal and various diets tested in this study.

Composition		Protein	Lipid	Ash	Dry matter	Energy (kcal/kg)
Fish viscera meal	Common carp	52.07±1.62	15.83±0.31	11.0±1.2	93.80±2.08	5691.0±42.1
	Mullet	54.87±1.72	13.49±0.11	9.00±0.61	95.23±3.50	5469.0±35.4
Diets	Carp	51.50±1.45	16.80±0.19	9.5±1.3	–	4110.0±27.6
	Mullet	50.80±2.04	16.85±0.37	7.7±0.7		4080.0±22.5
	Commercial diet	54.00±1.39	15.00±0.51	10.8±1.5	–	–

Probiotics which contained a blend of *Lactobacillus* [*Lactobacillus dlbrueckii*, *Lactobacillus plantarum* (*L. plantarum*), *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*], *Bifidobacterium bifidum*, *Streptococcus salivarius* and *Enterococcus faecium* from the commerical product Protexin aquatic (Iran-Nikotak) were added into the rearing tank water four times daily for 45 d.

2.3. Sample collection and analyses

All fish in each tank were bulk-weighed and counted to compute the weight gain, feed conversion ratio, specific growth rate, lipid efficiency ratio (LER), protein efficiency ratio (PER) and survival rates. Fish body weight and size were measured once a week. All manipulations were performed under fish anesthesia in a solution of MS-222 (150 mg/L). At the beginning of the feeding trial, 50 fish were sampled and frozen at -25 °C for subsequent whole body proximate analysis. At the end of the feeding trial, feed was withheld for 48 h to ensure that there is no residual feed in digestive system. Five fish from each tank were euthanised and stored at -25 °C for subsequent whole-body proximate analysis. Dry matter, crude ash, crude protein and crude lipid of experimental diets were determined. Moisture content of fish and feed was determined by oven-drying to constant weight at 105 °C for diet and at 70 °C for fish[11]. Protein content of fish and diets was measured by Kjeldahl method using an auto Kjeldahl system. Lipid content of fish and diets was measured by ether extraction. Ash content of fish and diets was determined by a muffle furnace at 550 °C for 8-10 h. Gross energy content of fish and diets was measured by an oxygen calorimetric bomb[12]. For each variable, at least duplicate samples were determined and the mean of duplicate measurements was taken when the relative deviation was less than 2%.

2.4. Statistical analysis

All data are presented as means±SD. Data was transformed where necessary and statistical analysis was conducted using SPSS statistics version 21 for windows (SPSS Inc., Chicago, Illinois, USA) and accepted at the $P<0.05$ level. Data were analyzed using a One-way ANOVA. Significant differences between control and treatment groups were determined using *post-hoc* Fisher's Duncan test.

3. Results

Final weight, survival rates, and specific growth rates in rainbow trout larvae were higher when probiotics were added into culture tanks (Figures 1, 2 and Table 3). There was significant difference in the rainbow trout larval survival rates between larvae fed with and without probiotic added to culture tanks ($P<0.05$).

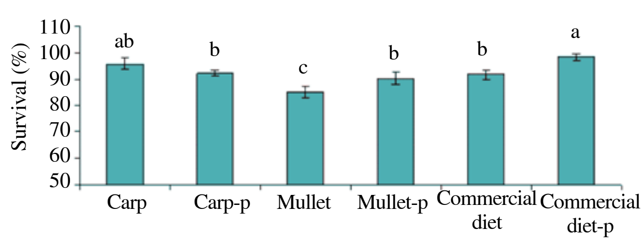

Figure 1. Survival rates in rainbow trout larvae fed with different diets, with and without probiotics added to tanks.
[a b c] showed significant differences between the treatments.

The rainbow trout larvae fed with commercial diet-p treatment (probiotic bacilli added to culture tanks) showed the highest survival (98%) among treatments (Figure 1). Lowest survival was recorded in larvae fed with diet formulated with mullet VM without probiotic added to culture tanks (85%).

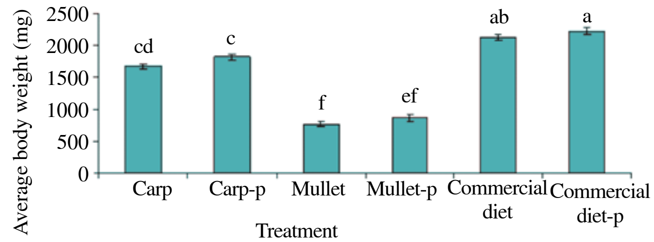

Figure 2. Average body weight of rainbow trout larvae fed with different diets.
[a b c d e f]: Significant differences between the treatments.

Table 3
Growth and indicators of feed conversion in rainbow trout larvae fed fish fed a commercial diet, or diet formulated with carp or mullet viscera meal, with and without probiotics added to culture tanks (mean±SD).

Growth indexes	Carp	Carp-p	Mullet	Mullet-p	Commercial diet	Commercial diet-p
Total length (mm)	$52.72±0.62^b$	$53.30±0.63^b$	$43.49±0.370^c$	$44.22±0.49^c$	$57.74±0.43^a$	$57.74±0.44^a$
SGR (%/d)[1]	$6.21±0.12^c$	$6.51±0.10^b$	$4.13±0.070^e$	$4.46±0.08^d$	$7.03±0.07^a$	$7.16±0.07^a$
FCR[2]	$1.33±0.06^c$	$1.16±0.04^d$	$2.10±0.060^a$	$1.89±0.06^b$	$0.83±0.02^e$	$0.80±0.02^e$
PER[3]	$1.70±0.07^c$	$1.86±0.07^b$	$0.95±0.002^e$	$1.06±0.03^d$	$2.38±0.07^a$	$2.50±0.07^a$
LER[4]	$4.97±0.23^d$	$5.44±0.20^c$	$3.32±0.090^f$	$3.74±0.14^e$	$8.58±0.24^b$	$9.02±0.28^a$

[1]: SGR%=$[LnWt_2-LnWt_1/t_2-t_1]×100$[13]; [2]: FCR=g dry feed eaten/g live weight gain[13]; [3]: PER=g live weight gain/g protein intake[14]; [4]: LER=g live weight gain/g lipid intake[13]. [a b c d e]: significant differences between the treatments. Carp-p: Carp and probiotic; Mullet-p: Mullet and probiotic; Commercial diet-p: Commercial diet with probiotic.

The recorded total length and weight of rainbow trout larvae fed with different diets was highest in commercial diet-p (57.74 mm and 2 226 mg). There was significant difference in the rainbow trout larval total length and weight recorded between larvae with probiotic diets and without probiotic ($P<0.05$). Lowest total length (43.49 mm) and weight (775 mg) were recorded in larvae fed with diet formulated with mullet VM. Food conversion ratio (FCR), PER and LER were significantly higher when probiotic were not added to culture tanks (Table 3).

In order to determine the nutritional effects of probiotics on rainbow trout larvae, the biochemical composition of carcass was analyzed. The results are represented in Table 4. Protein values of carcass in all probiotic treatment groups were significantly higher than other treatment groups. The best result was obtained in commercial diet-p group (67.14%). Significantly different crude lipid, ash and gross energy of carcass were observed in probiotic treatment groups, compared to the other treatment groups.

Table 4
Chemical composition of carcass in fish fed a commercial diet, or diet formulated with carp or mullet viscera meal, with and without probiotics added to culture tanks (mean±SD).

Treatment	Dry matter	Crude protein	Crude lipid	Ash	Gross energy
Initial	15.04	62.25	17.98	7.48	4580.00
Commercial diet	$23.11±2.19^a$	$66.41±1.40^b$	$18.07±0.20^e$	$6.16±0.40^c$	$4656.70±26.12^d$
Commercial diet-p	$24.19±3.15^a$	$67.14±0.40^a$	$17.68±0.13^f$	$7.09±0.21^a$	$4549.47±26.47^f$
Carp	$22.84±1.75^a$	$64.42±1.20^d$	$21.06±0.31^c$	$5.89±0.14^e$	$4762.63±31.24^b$
Carp-p	$23.02±3.23^a$	$66.07±0.53^c$	$19.78±0.23^d$	$6.05±0.31^d$	$4604.92±45.33^e$
Mullet	$16.26±2.05^c$	$62.47±0.52^f$	$24.62±0.43^a$	$6.39±0.23^b$	$4795.10±30.13^a$
Mullet-p	$20.75±1.57^b$	$63.78±0.72^e$	$21.64±0.40^b$	$7.12±0.37^a$	$4752.92±35.05^c$

[a b c d e f]: Significant differences between the treatments. Carp-p: Carp and probiotic; Mullet-p: Mullet and probiotic; Commercial diet-p: Commercial diet with probiotic.

4. Discussion

Fishery products, either in the form of low-value trash fish or rendered as fish meal, are presently the major source of protein for the grow-out in aquaculture, and constitutes up to 70% by weight of cultured fish diet[15]. As the demand for fish meal and marine fishery products in aquaculture increases, their availability decreases, and the cost is expected to rise. A dependable supply of cost-effective, alternative sources of protein must be provided for fish aquaculture to be profitable. Feeding less fish meal in diets will help make commercial fish farming more sustainable[16]. Our results showed that carp or mullet VM are a good alternative source of protein or energy for rainbow trout even at high inclusion levels, at least within the conditions of this experiment. In view of the high protein requirement of rainbow trout, the findings of the present study are considered significant with respect to an alternative protein source. VM is a cheaper source of protein than fish meal, and is available in large quantities, especially in wild fishery producing regions. Feed costs can be substantially reduced with the inclusion of greater quantities of VM in the diets for rainbow trout, and possibly in the diets of other cultured carnivorous fish species. In this study, diets formulated with VM were found to contain high levels of proteins and lipids, similar to that reported by several scientists[17,18]. Good quality viscera can be used to replace 50% of the fish meal without amino acid supplementation, in diets for rainbow trout without a significant reduction in growth performance. The fact that viscera protein can provide 50% of the total dietary protein means that feed costs in aquaculture production can be significantly reduced. Kechaou *et al.* suggested that sardines and cuttlefish viscera hydrolysates can be a good source of high-nutritional quality products for formulating aquaculture feeds[19].

In the present study, use of VM in rainbow trout diets showed acceptable result in growth performance and feed conversion efficacy. PER and FCR of fish fed diet with carp VM were significantly lower than fish fed commercial diet. Giri *et al.* reported that protease activities in catfish fed fish viscera and chicken viscera were very high[17]. However, the enzyme activity was significantly lower in fish fed only on plant protein, as reported by Venkatesh *et al.*[20]. Dried fish and chicken viscera can be used as alternate animal protein sources for *Clarias batrachus* juveniles, without affecting nutrient digestibility and hence can be used as a replacement for expensive fish meal in diet[17]. Habib *et al.* observed that replacement of fish meal by other animal protein sources did not reduce the apparent protein digestibility[21]. Possible reasons for the reduced growth of rainbow trout at total replacement of fish meal may be due to deficiencies in essential nutrients such as essential amino acids[22].

Our results showed that probiotics added into culture tanks enhanced growth in rainbow trout larvae. The main beneficial effects of probiotic use in cultured fish are improvements in growth performance[23,24], and disease control through enhancement of immune system[24-26]. These probiotic bacteria can also improve digestive activity via synthesis of vitamins and cofactors or via enzymatic enhancement[8]. This stimulation in growth reinforces the idea on the capacity of probiotics for growth improvement in important cultured species of teleosts (*Pinctada maxima*: Gatesoupe, 1991; *Oreochromis niloticus*: El-Haroun *et al.,* 2006; *Dicentrarchus labrax*: Carnevali *et al.*, 2006; *Paralichthys olivaceus*: Taoka *et al.*, 2006)[27-30].

Other authors have reported that *Lactobacillus bulgaricus*, *Lactobacillus acidophillus*, *Lactobacillus sporogenes*, *Lactobacillus casei*, *L. plantarum* and *Streptococcus thermophillus* are effective as probiotics in animal nutrition[31-33]. The beneficial effects of *Lactobacillus* sp. on growth response have been observed in Nile tilapia by Lara-Florest *et al.*[34], sea bream, *Sparus aurata* (Suzer *et al.*)[35], and European sea bass *Dicentrarchus labrax* (Carnevali *et al.*)[29]. The improvement of feed utilization in fish fed diet supplemented with *L. plantarum* NIOFSD018 could be due to improvement in intestinal microbial flora balance which in turn will lead to better nutrient digestibility, improved absorption, increased enzyme activities[9,34,36-38], and greater degradation of higher molecular weight proteins to lower molecular weight peptides and amino acids[39].

In this study, rainbow trout larvae in commercial diet-p treatment group (larvae fed with commercial diet with probiotic bacilli) showed the highest survival (98%) among treatments. Dietary administration of lactic acid bacteria (*Lactococcus lactis* CLFP 100, *Leuconostoc mesenteroides* CLFP 196, and *Lactobacillus sakei* CLFP 202) at 10^6 CFU/g for 2 weeks significantly increased the survival rate by 32.2%-34.2% in rainbow trout, *O. mykiss*, challenged with *Aeromonas salmonicida*[40]. *O. mykiss* fed Lactobacillus rhamnosus-supplemented diets at 10^9 and 10^{12} CFU/g for 51 d showed increased survival rates of 33.7% and 6.3%, respectively, following challenge with *Aeromonas salmonicida*[41]. Kuruma shrimp (*Penaeus japonicus*) fed with Gram-negative probiotics from *Bifidobacterium thermophilum* significantly increased shrimp body weight and survival. *Lactobacillus acidophilus* and *Lactobacillus sporogenes* significantly improved growth of *Macrobrachium rosenbergii* postlarvae, but not survival rate[33]. Similarly, *Penaeus indicus* larvae fed with *L. plantarum* improved FCR compared to the control fed with non-LAB diet[42]. Treatment of *Litopenaeus stylirostris* infected by *Vibrio nigripulchritudo* with LAB probiotic, *Pediococcus acidilactici*, significantly improved the survival of the shrimp in pond A (+7%) and pond B (+15%) with lower FCR correlated to the increase of hepatopancreas (storage organ) dry weight as well as specific activities of a-amylase and trypsin in the digestive gland by 35% and 55%, respectively in probiotic fed shrimp. The rise in total trypsin activity following morning feeding was also enhanced by the probiotic treatment[43].

Olafsen stated that the use of probiotics which has proven advantageous in domestic animal or poultry production and microbial management may also have a potential in aquaculture[44]. The gastrointestinal microbiota of fish are peculiarly dependent on the external environment. Most bacterial cells are transient in the gut, with continuous introduction of microbes coming in with food and water[8]. In addition, by feeding fish with probiotics bacteria, these bacteria present in surrounding water will colonise fish skin and other parts of body[41]. The most likely explanation of the effective role of probiotics is their effect on suppressing pathogenic coli forms in the stomach and intestine and improving the absorption of nutrients by reducing the thickness of intestinal epithelium[33]. The improvement in live body weight in probiotic treated groups of fish may mainly be due to an increase in beneficial bacteria such as *Lactobacillus* in the intestinal tract, which can competes with undesirable organisms for space and nutrients, as reported by Jena *et al*.[45]. Such useful bacterial growth facilitates the fermentation process, which is of nutritional significant in producing various types of vitamins[46], and organic acids, and providing energy to the host and stimulating growth.

There were significant (P<0.05) differences in dry matter, crude protein and crude lipid content in *O. mykiss* carcass subjected to probiotic treatments versus other treatments. Crude lipid and gross energy were decreased by probiotic treatments while crude protein was increased. Results showed that crude lipid and moisture content in rainbow trout carcass decreased after feeding with probiotics while crude protein levels were increased[47,48]. El-dakar reported that with use of a dietary probiotic/prebiotic in spinefoot rabbitfish (*Siganus rivulatus*) though significant differences in moisture level and crude protein proportion are apparent among treatments[49]; these do not follow a trend and thus suggest that variations among results due to inherent variation associated with using wild undomesticated fish stock in research.

Our results showed that fish VM especially common carp can successfully replace more than half of the protein from marine fish meal in formulated diets for rainbow trout, a carnivorous fish. However, the use of viscera as the sole protein source in the diets of rainbow trout might be constrained by lowered nutrient digestibility and limiting essential amino acids. Further research with longer-term feeding trials is currently being carried out to evaluate the nutritive value of viscera meal in diet for other fish species. The results of these studies showed that the blend of Lactobacillus sp., Bifidobacterium sp. and Streptococcus sp., can increase the growth and feeding efficiency in rainbow trout larvae.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Due to depleting wild fisheries, fish meal as a source of protein for formulating aquaculture fish feed is unsustainable. Intensification of aquaculture practices has led to conditions favoring the increased frequency and severity of disease outbreaks, often resulting in severe economic losses.

Research frontiers

Research on sustainable alternative sources of protein for formulating aquaculture feeds, to replace fish meal derived from depleted wild fisheries, and the beneficial effects of probiotics usage for enhancing fish health under intensive aquaculture conditions, have received much interests in recent decades.

Related reports

This article refers to the many beneficial effects of probiotic usage as an in-feed supplement, such as better growth and survival rates post-challenge with pathogenic bacteria.

Innovations and breakthroughs

The authors showed that probiotics added directly into culture water produced similar beneficial effects to probiotics added into feed.

Applications

The potential for application of a fishmeal replacement for use in formulating fish feeds, and probiotics as an alternative means of control for fish diseases in intensive aquaculture, is immense.

Peer review

The authors results on potential replacements for fishmeal in fish feed and use of probiotics in enhancing immunity of fish against disease, concur with that published by other researchers.

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