Fish & Shellfish Immunology 57 (2016) 170–178

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Probiotics as an environment-friendly approach to enhance red sea bream, *Pagrus major* growth, immune response and oxidative status

Mahmoud A.O. Dawood [a, c, *], Shunsuke Koshio [b], Manabu Ishikawa [b], Mabrouk El-Sabagh [d], M. Angeles Esteban [e], Amr I. Zaineldin [f]

[a] The United Graduate School of Agriculture Sciences, Kagoshima University, 1-21-24 Korimoto, Kagoshima, 890-0056, Japan
[b] Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries, Kagoshima University, 4-50-20, Kagoshima, 890-0056, Japan
[c] Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt
[d] Department of Nutrition and clinical nutrition, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt
[e] Fish Innate Immune System Group, Department of Cell Biology and Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100, Murcia, Spain
[f] Animal Health Research Institute(AHRI-DOKI), Egypt

ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form
11 August 2016
Accepted 14 August 2016
Available online 16 August 2016

Keywords:
Red sea bream
Probiotics
Environmental approach
Growth
Gut microbiota
Oxidative status
Humoral immunity
Mucosal immunity

ABSTRACTS

A usual strategy in modern aquaculture to combat production bottlenecks associated with intensification is preventive health care through the use of consumer and environment-friendly alternatives including probiotics. The current study evaluates the influence of *Lactobacillus rhamnosus* (LR), a lyophilized probiotic bacterium, on health status and performance of red sea bream (*Pagrus major*). Probiotics were incorporated in the diets at four different concentrations: 0 (control diet, LR0), 10^2 (LR1), 10^4 (LR2) and 10^6 (LR3) cells g^{-1} and diets were administered to the fish for a period of 8 weeks. After the feeding trial, final body weight, body weight gain, specific growth rate, protease activity, protein digestibility, *Lactobacillus* sp. intestinal count, and superoxide dismutase were significantly higher in all probiotic-fed groups ($P < 0.05$). In addition, lipid and dry matter digestibility, reactive oxygen metabolites, biological antioxidant potential, and humoral and mucosal immune parameters including (total serum protein, alternative complement pathway, bactericidal and peroxidase activities) were also significantly elevated in fish fed probiotic supplementations being the effects dose-dependent. All growth, feed utilization, immune and oxidative parameters were significantly improved following probiotic administration. Present results revealed that *L. rhamnosus* is a promising probiotic candidate employed to help red sea bream protect themselves, thus promoting safe farming that would be less dependent on chemotherapy against infectious diseases.

1. Introduction

Great and fast development of aquaculture in recent decades has increased the interest in studies focus on the physiological aspects of the stress on fish mainly due to the fact that these situations could increase the risk of fish disease outbreaks [1]. Although application of antibiotics and chemotherapeutics is considered quite effective, drug resistance and serious environmental hazards are considered as negative impacts of their use. Furthermore, other undesirable effects such as tissue accumulation, immunosuppression, development of antibiotic resistant bacteria and/or destruction of environmental microbiota have also been recorded [2]. For these reasons it is assume that a good way to avoid such problems, and concomitantly to enhance the fish survival rate on farms, would be to use natural preventive approaches [3].

Probiotic supplements have recently received extensive attention as an alternative method to antibiotic treatment [4–10]. Probiotics have been described as efficient tools to control pathogens and improve aquaculture production through different mechanisms [11–15]. In fact, probiotics may boost the quality of both the fish (e.g. growth, well-being and health status) and the fish environment [16]. Abundant efforts have been placed on probiotics' capability to modulate fish humoral immunity as they have been shown potent modulators of mucosal immunity even after

* Corresponding author. The United Graduate School of Agriculture Sciences, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0056, Japan.
E-mail address: omdawood_eg@yahoo.com (M.A.O. Dawood).

http://dx.doi.org/10.1016/j.fsi.2016.08.038
1050-4648/

compromised situations such as microbial infections or stress [15,16]. However, at present, much more efforts are still needed in this important research area.

There are many options for choosing a probiotic being the genus *Lactobacillus* the main strain used in aquaculture feed additives [9,11,17–19]. In previous studies, different beneficial effects especially on growth performance, hematological and oxidative status, immunoregulatory stimulation, modulation and expression of cytokine genes and disease resistance by dietary supplementation of *Lactobacillus* has been demonstrated in different fish species like rainbow trout, *Oncorhynchus mykiss* [18,20–25]; Nile tilapia, *Oreochromis niloticus* [26,27] and zebrafish, *Danio rerio* [28]. However, to the best of our knowledge, the information regarding the effects of *Lactobacillus rhamnosus* (LR) for red sea bream (*Pagrus major*) is scarce. This fish species was selected for the present work due to its good taste, high market value, rapid growth and strong resistance to stress. These properties make red sea bream a great fish candidate for intensive aquaculture in many countries and it is one of the most commercially important species in Japan [5,13]. Despite this, there have been some issues facing this fish species at the fry stage, such as growth retardation, high mortality and low feed utilization which have provoked important economic losses.

Taken into account all these considerations, the aim of the present study was to investigate the effects of dietary administration of LR on different parameters of red sea bream including growth performance, body composition, nutrient digestibility, gut microbiota, immune response and oxidative status. These data could be the base for future incorporations of probiotics not only to this important fish species, but also to other species sharing the same feeding habits.

2. Materials and methods

2.1. Probiotic preparation

Lactobacillus rhamnosus (LR) lyophilized bacteria was obtained from Morinaga Milk Industry CO., LTD. (Kanagawa, Japan), and the concentration of LR in the dry product is 1×10^9 cells g^{-1}. α-Cellulose powder used to adjust to the required concentrations of LR. The confirmation of the bacterial strain was based on colony and cell morphology and Gram staining. Briefly, the bacterium was cultured on to plates of DeMan, Rogosa and Sharpe agar (MRS, Merck, Darmstadt, Germany) by cultivating it for 48 h at 37 °C. The viability of bacteria was determined by plate counting on MRS agar. Colony forming units (CFU g^{-1}) were determined for viable bacterial populations. The bacteria cells were stored at −20 °C until use [13].

2.2. Experimental diets

Detailed composition and proximate analysis of the basal diet used in the present study described previously by Dawood et al. [5]. Experimental diets were prepared by supplementing a basal formulated diet with LR at four different levels (0 as control, 10^2, 10^4 and 10^6 cells g^{-1}). Accordingly, the experimental diets were named as LR0, LR1, LR2, and LR3, respectively. Graded doses of LR were added, in 50 ml of soybean lecithin oil, to the basal diet at the expense of α-Cellulose to obtain the required levels followed by mixing with a blender. All the dietary ingredients of the experimental diets were mixed with water and cold press and extruded in order to produce 1.6–2.1-mm pellets, which were dried at room temperature under sterile conditions and stored at 4 °C. To keep up LR viability, new batches of feed were produced every two weeks. The total viable LR counts present in the diet were determined by spread plating on MRS agar (de Man, Rogosa and Sharpe; MRS, Merck, Darmstadt, Germany) and TSA (Trypto-Soya agar, Nissui Pharmaceutical Co. Ltd., Japan) [18]. To do this, diet samples were first completely powdered and serially diluted with sterile saline [phosphate-buffered saline (PBS, pH = 7.4)]. The agar plates were inoculated with each dilution and they were incubated anaerobically at 37 °C for two days. Colony forming units (CFU g^{-1}) were determined for viable bacterial populations. The control feed wasn't supplemented with LR probiotic.

2.3. Fish culture and feeding trial

Red sea bream fry (mean weight 3.31 ± 0.01 g) were obtained from Akahoshi farm (Kumamoto Prefecture, Japan) were acclimatized to the experimental conditions for 1 week. During this period, a commercial diet (50% crude protein; Higashimaru, Japan) was supplied to the fish. Before the feeding trial, health condition of fishes was checked visually through their movements, infectious diseases symptoms and physical appearance all over the body and fins of the fish. Thereafter, fish were randomly allocated into twelve 100-L tanks (twenty fish per tank and triplicate tanks per treatment) in a flow-through seawater system where each tank was equipped with an inlet, outlet, and continuous aeration. All the tanks were maintained under natural light/dark photoperiod. Water temperature, dissolved oxygen content and pH were monitored daily and maintained at 22.1 ± 1.8 °C, 6.2 ± 0.5 mg L^{-1} and 8 ± 0.5, respectively [13]. During the rearing experiment (8 weeks), fish were hand fed to apparent satiation twice a day (at 09:00 and 16:00 hours). Any uneaten feed left was removed after feeding and dried using a freeze drier. Afterwards, the uneaten feed was weighted and subtracted from the total feed intake. All fish were weighed in bulk at 2 weeks' interval to determine growth, check their health condition and ration was adjusted according to mean fish weight.

2.4. Determination of growth performance, feed utilization and survival rate

The growth performance parameters including weight gain (WG), specific growth rate (SGR), condition factor (CF), feed intake (FI), feed and protein efficiency ratio (FER and PER), protein gain (PG) and survival rate (SR) were calculated according to the following formulae:

$$\mathrm{WG}(\%) = W_2 - W_1,$$

$$\mathrm{SGR} = 100(\ln W_2 - \ln W_1)/T,$$

$$\mathrm{CF} = \text{weigh of fish (g)} \Big/ (\text{length of fish})^3 (\text{cm})^3 \times 100$$

$$\mathrm{FI}\,(\text{g/fish/8 weeks}) = (\text{dry diet given} - \text{dry remaining diet recovered})/\text{no. of fish}$$

$$\mathrm{FER} = \mathrm{WG}/\mathrm{FI},$$

$$\mathrm{PER} = \mathrm{WG}/\text{dry protein intake (g)}$$

$$\mathrm{PG}\,(\text{g kg}^{-1}) = \{(W_2 \times \text{final whole body protein content}\,(\%)/100) - (W_1 \times \text{initial whole body protein content}\,(\%)/100)\}/\mathrm{WG} \times 1000$$

where W_1 is the initial weight (g), W_2 is the final weight (g), T is time (d) and WG is the weight gain (g).

$$\mathrm{SR} = \left(N_f \Big/ N_0\right) \times 100$$

where N_0 is the initial number of fish and N_f is the final number of fish.