Aquaculture 496 (2018) 211–220

Contents lists available at ScienceDirect

Aquaculture

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

Effects of dietary FARMARIN® XP supplement on immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*)

Sevdan Yilmaz[a,*], Sebahattin Ergün[a], Murat Yıgıt[b]

[a] Department of Aquaculture, Faculty of Marine Sciences and Technology, Canakkale Onsekiz Mart University, Canakkale 17100, Turkey
[b] Department of Marine Technology, Faculty of Marine Sciences and Technology, Canakkale Onsekiz Mart University, Canakkale 17100, Turkey

ARTICLE INFO

Keywords:
Rainbow trout
Humic substances
Sulphadiazine/trimethoprim
Digestive enzyme
Yersiniosis

ABSTRACT

The study was performed to determine the effects of FARMARIN® XP and INFISH-AQUA® on growth performance, proximate composition, biometric indices, serum biochemical variables, hematological parameters, non-specific immune responses, digestive enzyme activities and disease resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles against *Yersinia ruckeri*. Four experimental groups of fish were fed an additive free basal diet (control) and FARMARIN® XP incorporated test diets at increasing levels (0.1%-F1, 0.2%-F2, 0.4%-F4) for 60 days. Additionally, a fifth group of test diet was antibiotic medicated (0.1%), prepared with the commercial product INFISH-AQUA® (sulphadiazine 20% and trimethoprim 4%). When fish were challenged with *Yersinia ruckeri* after the 60-days feeding trial, and mortality was recorded over an additional 20-days period, no influence of FARMARIN® XP and antibiotic supplemented diets were observed on growth performance and hematological parameters of rainbow trout. However, the intestinal lipase activities in F1, F2 and AMF groups were significantly higher than the other treatments. Serum glucose level was significantly lower in the F4 group, and triglyceride levels decreased significantly when fish were fed with FARMARIN® XP or antibiotic supplemented diets. The dietary FARMARIN® XP especially at 0.1% and 0.2% significantly increased the respiratory burst activity. A decreasing potential killing activity and phagocytic index were found in the F4 and AMF groups. At the end of the 20-day challenge period the survival rates were significantly higher in the F2 and AMF groups compared to all other treatment groups. Thus FARMARIN® XP can be used as a replacement for antibiotic in rainbow trout diets for the control of yersiniosis.

1. Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most cultured finfish species in the world (Cowx, 2006), reaching 814.091 tons of production with a commercial value of around 3.4 billion USD in 2016 (FAO, 2018). The increased production of rainbow trout in intensive conditions may affect fish health due to decreased fish welfare under stressful culture environments. *Yersinia ruckeri* is widely seen in *O. mykiss* causing Enteric Redmouth (ERM) disease (Austin et al., 2003; Austin and Austin, 2016), which can be controlled by vaccination (Ispir and Dorucu, 2010; Chettri et al., 2015) and antimicrobial drugs, particularly sulphamethazine, chloramphenicol or oxytetracycline (Tobback et al., 2007). However, vaccination requires labor efforts and it may cause handling stress. On the other hand, when in excessive, the use of antibiotics may increase the resistance of fish pathogens against antibiotics, since *Y. ruckeri* strains are capable to developed resistance to a variety of antimicrobial agents in aquaculture facilities (Shah et al., 2012; Huang et al., 2014).

In order to fight fish diseases, many investigations have been focused on a variety of antibiotics or chemotherapeutics in aquaculture. However, antibiotics or chemotherapeutics are not environmentally sustainable and may have side effects or residues undesired for farmed terrestrial animals and human beings.

Humic substances (HUMS), produced by organic materials of rotted or dead plants and animal tissues through microbial activity (Herzig et al., 2001), are important antioxidants with their significant free radical scavenger properties (Ozkan et al., 2015), and also known to have antimicrobial (Van Rensburg et al., 2000), anti-inflammatory (Van Rensburg et al., 2001) and immunostimulatory (Vucskits et al., 2010) effects, through which they can be considered as useful dietary additives in fish feed.

Few reports are available on the use of HUMS in poultry (Eren et al., 2000; Kocabağli et al., 2002), however information regarding the effects of dietary HUMS in fish species is scarce and limited to

* Corresponding author.
E-mail address: sevdanyilmaz@comu.edu.tr (S. Yilmaz).

https://doi.org/10.1016/j.aquaculture.2018.07.024
Received 27 April 2018; Received in revised form 16 July 2018; Accepted 17 July 2018
Available online 19 July 2018
0044-8486/

Dicentrarchus labrax (Soytaş, 2015) and *Cyprinus carpio* (Sharaf and Tag, 2011; Rousdy and Wijayanti, 2015). Improved fish growth was reported in *C. carpio* by dietary inclusion of HUMS (Sharaf and Tag, 2011), whereas no growth-promoting effects of dietary HUMS were noted in *D. labrax* (Soytaş, 2015) or *C. carpio* (Rousdy and Wijayanti, 2015). There are discrepancies between earlier studies regarding the effects of HUMS in fish diets, therefore it seems necessary to further clarify the effect of dietary HUMS in fish feed.

FARMARIN® XP is a commercial product specially developed as a biostimulator, bioregulator and high activity organic performance improver for finfish species by the Scientific Work Group of Farmavet. The content of the product includes mainly humic acids along with fulvic, ulmic and fulfonic acids. However, there is no scientific information so far regarding the effects of commercial products of humus sources produced for finfish species and this study is the first attempt in this regards. Therefore, in the present study the effects of different inclusion levels of dietary FARMARIN® XP on growth performance, chemical composition, serum biochemical variables, haemato-immunologic parameters and survival rate against *Y. ruckeri* have been investigated in rainbow trout for the first time.

2. Materials and methods

2.1. Experimental diet

FARMARIN® XP was obtained from FARMAVET ILAC San Tic AS Co., and incorporated to a laboratory-manufactured feed at levels of 0% (control group), 0.1%, 0.2%, and 0.4% for diets designated as C0, F1, F2, and F4, respectively. These dietary incorporation levels of FARMARIN® XP were followed by the recommended dose inclusion levels of the Farmavet company. In addition, an antibiotic medicated diet (AMD) was prepared with a commercial product of INFISH-AQUA® (sulphadiazine 20% and trimethoprim 4%; Indukern Istanbul Kimya San Tic Ltd. Sti Co.) and 0.1% (corresponding to 50 mg/100 g antibiotic in fish feed) added to the experimental feed. Fish fed on a diet without supplementations of both FARMARIN® XP and antibiotic served as a control group. All ingredients (Table 1) were mixed in a laboratory blender and a pelleting machine (La Monferrina P3, Italy) with a 2-mm die was used to produce the pellets, which were then dried in a drying cabinet at 40 °C until the moisture content of pellets declined to 10%. Thereafter, the pellets were stored in plastic bags and kept in a deep freezer at −20 °C until used.

Table 1
Percentage and proximate composition of the experimental diets.

	C0	F1	F2	F4	AMF
Ingredients (% dry matter)					
Fish meal (anchovy meal)	58	58	58	58	58
Fish oil (anchovy oil)	13	13	13	13	13
Soybean meal	12	12	12	12	12
Wheat flour	10	10	10	10	10
Wheat starch	3.99	3.89	3.79	3.59	2.89
Carboxymethyl cellulose	1	1	1	1	1
FARMARIN®	0	0.1	0.2	0.4	0
Antibiotic	0	0	0	0	0.1
Vitamin mix	1	1	1	1	1
Mineral mix	2	2	2	2	2
BHT	0.001	0.001	0.001	0.001	0.001
Total	100	100	100	100	100
Chemical analyses (% DM)					
Protein	44.63	44.63	44.63	44.64	44.63
Fat	18.27	18.27	18.28	18.28	18.27
Ash	9.26	9.35	9.45	9.64	9.35
NFE[a]	16.15	16.07	15.99	15.82	16.07
Energy (kj/g)[b]	20.50	20.48	20.47	20.44	20.48

[a] Nitrogen-free extracts (NFE) = dry matter − (crude lipid + crude ash + crude protein).
[b] Energy was calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid, and 17.0 kJ/g NFE.

2.2. Fish and experimental design

O. mykiss juveniles, obtained from a trout farm (Keskin Alabalik Co., Canakkale – Turkey) were transported to the research facilities of the Canakkale Onsekiz Mart University. For the visual and external inspection of each experimental fish, the US-EPA (United States Environmental Protection Agency) guidelines for qualitative assessment of fish health were followed (Klemm et al., 1993). Fish were acclimatized to the experimental conditions for a period of two weeks before the start of the experiment, and fed a commercial trout feed (Pınar Camli Co. Turkey, 49% protein/19% lipid, 2 mm) until satiation. A total of 15 experimental fiberglass tanks were stocked with 25 fish per tank (21.73 ± 0.43 g mean ± SD, $n = 375$), in a triplicate design. Each of the experimental tanks (140 L water volume) was supplied with recirculated freshwater at a flow rate of 145 L/h and aerated with air stones. All experimental groups were fed until satiation two times a day at 08.00 and 17.00 h over a period of 60 days. Water temperature was controlled by a heater/chiller (Tuna Mac®, Çanakkale Turkey) and photoperiod regime followed a 12:12 light:dark cycle throughout the study. Water quality parameters such as temperature, oxygen, conductivity and pH were measured daily, while ammonia, nitrite and nitrate were analyzed weekly. Temperature, pH, dissolved oxygen, conductivity, total ammonia, nitrite, and nitrate were recorded as: 17.5 ± 0.2 °C, 7.8 ± 0.2, 7.7 ± 0.10 mg/L, 450 ± 10.2 μS, 0.015 ± 0.0012 mg/L, 0.05 ± 0.001 mg/L, and 0.5 ± 0.11 mg/L, respectively during the course of the study.

2.3. Calculation of growth performance and biometric indices

Calculations for the relative growth rate (RGR, %), specific growth rate (SGR, % per day) and feed conversion ratio (FCR) were performed using following equations:

$$\text{RGR (\%)} = 100\,(\text{final fish weight} - \text{initial fish weight})/\text{initial fish weight}$$

$$\text{SGR (\%/day)} = 100\,(\ln\ \text{final fish weight}) - (\ln\ \text{initial fish weight})/\text{experimental days}$$

$$\text{FCR} = \text{feed intake/weight gain}$$

Right after dissection, the liver, viscera and visceral fat were removed from fish and weighed for the calculated of biometric indices using the formulae given below:

$$\text{Hepatosomatic index (HSI)} = \{\text{wet weight of liver (g)}/[\text{wet body weight (g)} - \text{wet weight of liver (g)}] \times 100\}$$

$$\text{Viscerosomatic index (VSI)} = \{\text{wet weight of viscera and associated fat (g)}/[\text{wet body weight (g)} - \text{wet weight of viscera and associated fat (g)}] \times 100\}$$

$$\text{Visceral fat index (VFI)} = \{\text{wet weight of visceral fat (g)}/[\text{wet body weight (g)–wet weight of visceral fat (g)}] \times 100\}$$

$$\text{Spleen–somatic index (SSI)} = \{\text{wet weight of spleen (g)}/[\text{wet body weight (g)–wet weight of spleen (g)}] \times 100\}$$