Aquaculture 495 (2018) 611–616

Contents lists available at ScienceDirect

Aquaculture

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

Effect Of dill (*Anethum graveolens*) and garden cress (*Lepidium sativum*) dietary supplementation on growth performance, digestive enzyme activities and immune responses of juvenile common carp (*Cyprinus carpio*)

Soner Bilen[a], Oğuz Özkan[b], Kerem Alagöz[b], Keriman Yürüten Özdemir[b,*]

[a] Kastamonu University, Faculty of Fisheries, Department of Aquaculture, Kastamonu, Turkey
[b] Kastamonu University, Institute of Science, Department of Aquaculture, Kastamonu, Turkey

ARTICLE INFO

Keywords:
Cyprinus carpio
Growth performance
Digestive enzyme activity
Immunostimulant
Anethum graveolens
Lepidium sativum

ABSTRACT

In this study, Bilen et all, studied that the effect of dietary supplementation of dill (*Anethum graveolens*) and garden cress (*Lepidium sativum*) on the growth performance, digestive enzyme activities and immune responses of juvenile common carp (*Cyprinus carpio*) in addition to their disease resistance to *Aeromonas hydrophila* and *Edwardsiella tarda*. Common carp were fed for 45 days with two different doses of extracts of plant (1 and 2 g kg^{-1}) in four treatment groups: dill 1 g kg^{-1} (D1) and 2 g kg^{-1} (D2) and garden cress 1 g kg^{-1} (G1) and 2 g kg^{-1} (G2). At the end of the study, the fish were challenged with *A. hydrophila* and *E. tarda*. Results showed that the final fish weight and specific growth rates were higher in the G2 group than in the control group (C) and other experimental groups (D1, D2 and G1) ($P < 0.05$). Compared with the control group, there were no differences in the feed conversion ratio (FCR) in the experimental groups, except the D1 group, ($P > 0.05$). In the D1 group, FCR was significantly increased. Trypsin activity was significantly decreased in the G2 group. Compared with the control group, amylase activity was the highest in the D2 group ($P < 0.05$). There were no differences in lipase activities among the groups. Lysozyme activity was significantly increased on the 15th day of the study in D1 and G2 groups compared with that in the control group ($P < 0.05$). On the 30th day, lysozyme activity was increased in the D2 group compared with that in the control group ($P < 0.05$). No differences were observed in lysozyme activity among the groups on the 45th day. Myeloperoxidase (MPO) activity was elevated in the G2 group on the 15th day ($P < 0.05$). The highest MPO activity was observed in the D1 group ($P < 0.05$) on the 30th day of the study. On the 45th day, the MPO activity in all experimental group's ($P < 0.05$) was higher than that in the control group. At any sampling time of the study, superoxide anion production was elevated in all experimental groups compared with that in the control group. Moreover, when challenged with *A. hydrophila*, survival rates of common carp in G1, G2 and D1 groups were significantly increased, and when challenged with *E. tarda*, those of common carp in G2 and D1 groups were also elevated. The results of the study showed that cress had immunostimulatory effects in common carp and increased the fishes' growth rate.

1. Introduction

Fisheries and aquaculture are increasingly important as sources of food, nutrition, income and livelihoods for hundreds of millions of people worldwide; the world per capita fish supply reached a new record high of 20 kg in 2014 (FAO, 2016). The aquaculture sector has shown rapid growth over the last 30 years and remains the main source for increasing fish supplies. However, rapid development of the aquaculture industry has resulted in increasing disease problems arising from the magnification of stressors for fish, such as high stocking densities, handling and transportation. As a result of these activations, aquaculturists have been faced with either partial or total loss of production (Ingram et al., 2005).

To avoid disease-related economic losses, antibiotics and other veterinary drugs are regularly administered as additives in fish feed and sometimes in baths and injections for both prophylactic and therapeutic purposes (Rico et al., 2013). However, side effects of veterinary drugs, such as antibiotic resistance in bacterial strains and potential harm to both the environment and human health, has forced fish farmers to find a much more environmentally safe way to avoid disease-related losses.

The common carp (*Cyprinus carpio*) is one of the most economically important species in the world's aquaculture (FAO, 2016). Although the

* Corresponding author at: Kastamonu University, Institute of Science, Department of Aquaculture, Kuzeykent 37150, Kastamonu, Turkey.
E-mail address: k.yuruten@gmail.com (K.Y. Özdemir).

https://doi.org/10.1016/j.aquaculture.2018.06.037
Received 21 February 2018; Received in revised form 11 June 2018; Accepted 14 June 2018
Available online 18 June 2018

common carp can be cultured easily, problems that impede carp culturing can arise from causative agents, such as *Aeromonas hydrophila* and *Edwardsiella tarda*. *A. hydrophila*, a ubiquitous bacterium responsible for primary or stress-associated pathogenicity in both warm- and cold-water fish (Harikrishnan and Balasundaram, 2005), is associated with small surface lesions, local haemorrhaging and septicaemia (Yin et al., 1997), dropsy, exophthalmia and fin and tail rot (Austin and Austin, 1993). *E. tarda*, which is a gram-negative bacterium belonging to the family Enterobacteriaceae, can cause excessive mortality in tilapia, catfish and trout. It also causes systematic infection in both common carp (Sae-Qui et al., 1984) and koi carp (*Anabas testudineus*) (Ahmed et al., 2007).

Dill is an important medicinal plant that is cultured intensively and used for human consumption. It contains essential oil, vitamin C, carotenoids and polyphenols (Zofia et al., 2006). Garden cress, similar to dill, is a commercially cultured plant species that has many medicinal components. Cress is a good source of antioxidants and vitamins, including vitamins A, C and K, in addition to the B-group vitamins riboflavin and folate (Günay, 1984).

Many medicinal plants have been used as immunostimulants for fish. Although many medicinal plants show an immunomodulatory effect and protection against pathogens, lack of cost-effectiveness and easy methods to obtain these plants seem to be the main impediments to the use of medicinal plants. The aim of this article was to determine the potential usage of extracts of dill and garden cress as sustainable and effective immunostimulants with mass culture production. This is also the first report related to the effects of dill and garden cress on immune response, growth performance and disease resistance against *A. hydrophila* and *E. tarda* in carp.

2. Material and methods

2.1. Experimental design and fish

This study was conducted in triplicate over 45 days in 30 aquariums, separetly for both diseases, each with a 90 L capacity, in the Aquarium Units at Kastamonu University Faculty of Fisheries. Experimental common carp, with an average body weight of 3.46 ± 0.1 g, were obtained from the Mediterranean Fisheries Research, Production And Training Institute. A total of 46 fish were stocked in aquariums in 3 replicates. During the study, the water temperature was kept at 20 °C, and 20% of the water was changed daily. Aqueous methanolic extracts of dill (D) and garden cress (G) were added to the fishes' basal diet at the rate of 0 (Control), 1 (D1 and G1) and 2 (D2 and G2) g kg^{-1} by spraying. All groups were fed with the diets twice a day ad-libitum.

All experimental animals were directed according to the relevant international guideines. Study protocol was approved in advance by the local Ethics Committee for Animal Research Studies at the Kastamonu University (KUHADYEK- 03.04.2017-2017.06).

2.2. Aqueous methanolic extraction of dill (Anethum graveolens) and garden cress (Lepidium sativum)

Dill and garden cress were purchased from wholesalers and dried under shade in natural conditions; extracts of the plants were prepared according the procedure described by Bilen et al. (2016).

2.3. Growth parameters

Growth performance parameters of weight gain (WG; %), specific growth rate (SGR) and feed conversion ratio (FCR) were included according to the method described by Rıcker (1979).

$$\text{Weight gain (\%)} = [(\text{final weight} - \text{initial weight})/\text{initial weight}] \times 100$$

$$\text{Specific growth rate (SGR)} = [(\ln \text{ final weight} - \ln \text{ initial weight})/\text{days}] \times 100$$

$$\text{Feed conversion Ratio (FCR)} = \text{Feed Offered/Weight Gain (g)}$$

2.4. Non-specific immune parameters

In this study, superoxide anion production in head kidney-derived macrophages (200 mg) was determined using the reduction of nitroblue tetrazolium [(NBT) Sigma–Aldrich, St. Louis, MO, USA] assay on the 15th, 30th and 45th days, as per a previously described method (Biswas et al., 2013). Lysozyme activity (LA) was determined according to method used by Bilen et al. (2016). Total myeloperoxidase (MPO) activity present in the serum (total of 2 ml) was measured according to method used by Sahoo et al. (2005), with slight modifications according to method used by Bilen et al. (2016).

2.5. Challenge test

A. hydrophila (ATCC 20662) with 1×10^8 CFUs and *E. tarda* (isolated from carp) with 1×10^9 CFUs, mixed in 100 µL PBS separately, were intraperitoneally injected in all fish following dietary administration of extracts of dill and cress. The survival rates of each experimental group were recorded over 14 days.

2.6. Digestive enzyme activity

Digestive enzyme (amylase, lipase and trypsin) activities were assayed at the end of the study. The whole digestive tract and hepatopancreas were thoroughly homogenized in ice cold distilled water and centrifuged at 15000*g* for 45 min at 4 °C. The supernatant was used as a crude enzyme source. Amylase activity was analysed by the starch-hydrolysis method used by Bernfeld (1995). Lipase activity was determined using the method described by Furne et al. (2005). Trypsin was determined using N-a-benzoyl-DL-arginine 4-nitroanilide hydrochloride as a substrate (Erlanger et al., 1961). Specific activities of all digestive enzymes were calculated as milligrams of protein. All enzyme activity units were calculated using the following equation:

$$\text{Amylase} = [(\text{Sample} - \text{Blank})^2 \times 7,712] - [1,082 \times (\text{Sample} - \text{Blank})] + 0,082 = \text{Result/mgprotein}$$

$$\text{Lipase} = [(\text{Sample} - \text{Blank}) \times (0,2359 + 0,0153)^2]/\text{mg protein}$$

$$\text{Trypsin} = [(\text{Last Result} - \text{First Result})/10 \text{ min}] = \text{Absorption Result } [(\text{Absorption Result} \times 1 \text{ million})/8.800]/2 = \text{Result/mg protein}$$

2.7. Statistical analyses

All statistical analyses were performed using statistical software package SPSS 22 (SPSS Inc., Chicago, IL, USA). Firstly, the variance of data was analysed using ANOVA, and Duncan's multiple range tests were then performed to determine significant differences among groups. The accepted level of significance was $P < 0.05$.

3. Results

In this study, effects of methanolic extracts of dill and garden cress on growth are summarized in Table 1. Final fish weight in the G2 group was significantly higher than was that in the control and other experimental groups ($P < 0.05$). A significant increase in WG and SGR was observed in both experimental groups compared with that in the control group and higher levels were recorded in the W2 group. FCR was significantly increased in all treated groups compared with that in