Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

The use of *Avena sativa* extract against *Aeromonas hydrophila* and its effect on growth performance, hematological and immunological parameters in common carp (*Cyprinus carpio*)

Esin Baba, Ümit Acar, Canan Öntaş, Osman Sabri Kesbiç & Sevdan Yilmaz

To cite this article: Esin Baba, Ümit Acar, Canan Öntaş, Osman Sabri Kesbiç & Sevdan Yilmaz (2016) The use of *Avena sativa* extract against *Aeromonas hydrophila* and its effect on growth performance, hematological and immunological parameters in common carp (*Cyprinus carpio*), Italian Journal of Animal Science, 15:2, 325-333, DOI: 10.1080/1828051X.2016.1185977

To link to this article: https://doi.org/10.1080/1828051X.2016.1185977

ITALIAN JOURNAL OF ANIMAL SCIENCE, 2016
VOL. 15, NO. 2, 325–333
http://dx.doi.org/10.1080/1828051X.2016.1185977

Taylor & Francis
Taylor & Francis Group

PAPER

OPEN ACCESS

The use of *Avena sativa* extract against *Aeromonas hydrophila* and its effect on growth performance, hematological and immunological parameters in common carp (*Cyprinus carpio*)

Esin Baba[a], Ümit Acar[a], Canan Öntaş[a], Osman Sabri Kesbiç[b] and Sevdan Yilmaz[c]

[a]Department of Aquaculture, Mugla Sitki Kocman University, Mugla, Turkey; [b]Inebolu Vocational School, Kastamonu University, Inebolu, Turkey; [c]Department of Aquaculture, Canakkale Onsekiz Mart University, Canakkale, Turkey

ABSTRACT

In this research the effects of oat *Avena sativa* extract on the non-specific immune system of common carp (*Cyprinus carpio*) was examined. For this purpose, the fishes (average weight 9.91 ± 1.52 g) were fed with 5 g kg^{-1}, 10 g kg^{-1} and 20 g kg^{-1} oat extract supplemented diets for 60 days. Following 60 days of feeding, the fishes were injected with *Aeromonas hydrophila* and mortalities were recorded. Lysozyme and myeloperoxidase activity, improved in all groups that received feed supplemented with oat extract. Serum glucose and cholesterol decreased while total protein and albumin in fish increased with the use of the diet supplement with oat extract. Haemoglobin (Hb), mean cell hemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) increased with diet supplemented with oat extract. Oat extract at the concentration of 10 g kg^{-1} showed significantly higher relative percentage survival (67%) when compared with the control against *A. hydrophila* injection. Also the dietary supplementation with oat extract caused a significant increase in growth parameters (final weight (FW), weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR), when compared to non-supplemented control basal diet. The results suggest that *A. sativa* extract can be used as a feed supplement to enhance fish immune response and disease resistance against *A. hydrophila*.

ARTICLE HISTORY
Received 19 February 2016
Accepted 2 May 2016

KEYWORDS
Aeromonas hydrophila; *Avena sativa*; *Cyprinus carpio*; immune system; oat extract

Introduction

Common carp (*Cyprinus carpio*), is a freshwater fish inhabiting several freshwater ecosystems such as lakes, ponds and dams and it is widely distributed thoughout the world especially in Asia, Europe and the Middle East (Eddy & Underhill 1974). Moreover, common carp is the third most frequently introduced species worldwide (Saikia & Das 2009). However, the diseases caused by pathogens bacterial in common carp culture are becoming severe resulting in significant morbidity and mortality. The culture of *C. carpio* in fresh water aquaculture has suffered due to bacterial infections particularly by the occurrence of "motile aeromonas septicemia" caused by *Aeromonas hydrophila*, which results in heavy losses and causes economic loss to fish farmers (Austin & Adams 1996). In several fish species, including carps, *A. hydrophila* is regarded as an opportunistic pathogen both in the farm and field. The disease is frequently associated with haemorrhagic septicemia (Kuge et al. 1992; Roberts et al. 1992; Zhang et al. 2014). In order to control the proliferation of these bacteria, antibiotics are widely used in intensive aquaculture. Nevertheless prolonged use of antibiotics could lead to many negative effects such as antibiotic resistance in bacteria or antibiotics residues in environment and fish products (Cabello 2006). Therefore, the research for new methods to prevent infectious diseases has become crucial in common carp culture. Immunostimulants increase resistance to infectious diseases, not only stimulating the acquired immune response, but also enhancing innate immune mechanisms (Galindo-Villegas & Hosokawa 2004). The immunostimulants also have additional advantages, such as growth enhancement and increase in the survival rates of the fish under stress (Heo et al. 2004). Many immunostimulants have been found to be effective in common carp (Harikrishnan et al. 2003; Yin et al. 2009; Maqsood et al. 2010; Anbazahan et al. 2014; Jagruthi et al. 2014; Wang et al. 2015).

Avena sativa L. (Gramineae) commonly known as oat, groats, haber, hafer, avena, straw, oatmeal is an edible plant and it is a species of grain cultivated for

CONTACT Dr. Ümit Acar umitacar@mu.edu.tr Department of Aquaculture, Faculty of Fisheries, Muğla Sïtkï Koçman University, Muğla, 48000, Turkey

its seed (Coffman 1977). The bran of this plant has also been used as a traditional folk medicine for the treatment of rheumatism, gout, and liver and skin diseases on the benefit of its diuretic and sedative effects (Wenzig et al. 2005). Up till now, saponins (Waldemar 1982), flavonoids (Popovici et al. 1977; Peterson 2001), and beta-D-glucan (Ahmad et al. 2010) have been found in the plant. Also the oat is rich in protein, has lots of beneficial minerals such as iron, calcium, potassium, magnesium, copper, zinc, silicon, selenium and contains several vitamins like Vitamin B_1, B_2, B_6, B_{12}, Niacin, Vitamin C, Vitamin A, Vitamin E. Different chemical constituents like carbohydrates, proteins, avenanthramides, lipids (9 glycolipids and 11 phospholipids), an indole alkaloid-gramine, number of flavonoids, 3 flavonolignans, saponins and sterols have been reported from *A. sativa*. Oats and its constituents are reported to possess varied pharmacological activities like lowering blood cholesterol and blood sugar, as well as being immunomodulatory, anticancer, antioxidant, antiatherogenic, and topical anti-inflammatory (Singh et al. 2013).

The aim of the study was to assess oral administration of three different concentrations of oat extract (OE) derived from *Avena sativa* on biometrical, haematological, biochemical and immunological indices of common carp (*Cyprinus carpio*) in experimental conditions. A further aim was to investigate the effects of supplemental dietary oat extract on disease resistance of *C. carpio* against *A. hydrophila*.

Materials and methods

Preparation of oat extract

Samples of *A. sativa* were attained from their natural environment from Balïkesir region of Turkey. One hundred grams of dried ground of oat were extracted with 100 mL water in 1000 mL conical flasks at 60 °C in a water bath incubated for 24 h in a water bath and then filtered (0.45 µ Whatman filter). The same process was repeated three times for the complete extraction. Water was evaporated using a lyophilizator for the complete extraction. The extract were stored in a refrigerator at 4 °C for future use (Lee et al. 2000; Tanker & Tanker 2003).

Fish and experimental design

Healty common carp, *Cyprinus carpio* (9.91 ± 1.52 g) were obtained from Antalya-Kepez (Antalya, Turkey). The study was carried out in triplicate (three aquarium per experimental groups) with 216 fish allocated into 50 L aquariums (18 fish/aquarium). The oat extract was added to the feed at 0 g kg^{-1} (OE0) as control, 5 g kg^{-1} (OE5), 10 g kg^{-1} (OE10) and 20 g kg^{-1} (OE20). The control diet contained no supplementation (OE0) (Table 1). The fishes were fed with 2% of body weight during the trial. Water was changed daily at a rate of ~10% of the total volume. After 60 days of feeding, nine fishes from each group were randomly chosen and their blood samples were collected. Moreover, at the end of the feeding period, all the groups were injected intraperitoneally (i.p.) with 100 µl PBS containing *A. hydrophila* at 1.5×10^6 CFU mL^{-1}. During the experimental period the main parameters of water were measured as: temperature 25.2 ± 0.6 °C, pH 7.6 ± 0.6 and dissolved oxygen 5.63 ± 0.68 mg L^{-1}.

Table 1. Percentage and proximate composition of the experimental diets containing supplement of different oat extract (OE) rate.

	Experimental diets			
	OE0	OE5	OE10	OE20
Ingredients, %				
Fish meal	23.00	23.00	23.00	23.00
Soybean meal	37.00	37.00	37.00	37.00
Wheat flour	12.00	12.00	12.00	12.00
Fish oil	5.00	5.00	5.00	5.00
Vitamin–mineral mix[a,b]	4.00	4.00	4.00	4.00
Starch	19.00	18.50	18.00	17.00
Oat extract	0	0.50	1.00	2.00
Total	100	100	100	100
Chemical analyses				
Protein, %DM	35.10	35.54	35.11	35.42
Fat, %DM	7.72	7.53	7.75	7.62
Ash, %DM	5.94	5.32	5.45	5.92
NFE[c], %DM	47.16	47.17	47.18	47.17
Energy[d], kj/g	19.35	19.38	19.36	19.39

[a]Vitamin Mix: Vit. A, 18,000 IU; Vit. D3, 2500 U; Vit. E, 250 mg/kg; Vit. K3, 12 mg/kg; Vit. B1, 25 mg; Vit. B2, 50 mg; Vit. B3, 270 mg; Vit. B6, 20 mg; Vit. B12, 0.06 mg; Vit. C, 200 mg; Folic acid, 10 mg; Calcium d–pantothenate, 50 mg; Biotin, 1 mg; Inositol, 120 mg; Choline chloride, 2000 mg.

[b]Mineral Mix: Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg.

[c]Nitrogen–free extracts (NFE) = dry matter – (crude lipid + crude ash + crude protein + crude fiber).

[d]Energy calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, and 17.0 kJ g^{-1} NFE.

Blood samples and analyses

Nine fishes from each groups (three fishes from per aquarium) were selected and anesthetized by 0.01 mg L^{-1} of phenoxyethanol. After that blood samples were obtained from caudal vein using syringe at the end of the 60 days feding trial. Newly collected blood samples were used to determine the hematological parameters. Blood was centrifuged for 15 min. at 3500 *g*. After centrifugation, serums were stored at −20 °C for future analysis.

Hematological assay

The hematological indices Red blood cell (RBC) count ($\times 10^6$ per mm3), hematocrit (Hct; %), and hemoglobin (Hb) concentrations (g/dL) were designated Blaxhall and Daisley (1973) method. Mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and mean corpuscular Hb concentrations (MCHCs) were calculated according to Bain et al. (2006).

Biochemical assay

The determination of plasma glucose (GLU), total protein (TPROT), albumin (ALB), globulin (GLO), triglyceride (TRIG), and cholesterol (CHO) were determined using commercially diagnostic kit (Bioanalytic Diagnostic Industry, Co.).

Lysozyme activity

Serum lysozyme (Lyso) was assessed using the turbidometric assay. A *Micrococcus lysodeikticus* suspension of 875 µl (Sigma, ATCC 4698) at a concentration of 0.2 mg/ml (in PBS) was added to 25 µl of serum samples and were measured spectrophotometrically at 530 nm after 0.5 and 4.5 minutes at 25 °C, with a spectrophotometer. A unit of lysozyme activity was defined as the amount of serum caused reduction in absorbance of 0.001 min^{-1}.

Myeloperoxidase activity

Total myeloperoxidase (MPO) content in blood serum was measured according to Quade and Roth (1997) with minor modifications. Thirty microliters serum was diluted with 370 ml of HBSS without Ca2 + or Mg2 + in eppendorf tubes. Hundred microliters of 0.1 mg/ml (w/v) 3,3′,5,5′-tetramethylbenzidine dihydrochloride and 0.06% fresh hydrogen peroxide were added. The reaction was followed kinetically by measuring the increase of absorbance. Reaction velocities were determined as IU, defined as the amount of enzyme required to produce an 0.001 increase in absorbance per minute 0.5 ml of reaction mixture (ΔA 450/min/ml).

Challenge study

To study the resistance of the common carp to *A. hydrophila*, 45 fish from each experimental and control groups were used. After 60 days of feeding and blood samplings, the fish were injected intraperitoneally with 0.1 mL of a 1.5×10^6 CFU mL^{-1} *A. hydrophila* was suspended in phosphate buffered saline. The fish were checked regularly with eyes for any overt signs of disease including behavioural abnormalities and dead fish taken slowly from aquariums without creating stress factors. Mortality was noted in all the groups for 6 days of post infection. The confirmation of the infection was accomplished after re-isolating the bacteria from the dead fishes. Reisolated bacteria identification was obtained by using classical biochemical (Austin & Austin, 2007) and API 20 Strep kit (Biomerieux, France).

Relative percentage survival

Recorded mortality data was used for calculating Relative Percentage Survival (RPS) following Amend (1981).

$$\text{RPS} = 1 - [(\text{Mortality } (\%) \text{ in treated group})/ (\text{Mortality } (\%) \text{ in control group})] \times 100$$

Growth performance

At the end of 60 days, fishes in each aquarium were individually weighed. Growth performance was calculated as following formulae:

$$\text{WG (weight gain) } (\%) = 100 \times \frac{(\text{final body weight} - \text{initial body weight})}{\text{initial body weight}}$$

$$\text{SGR (specific growth ratio)} = 100 \times \frac{\ln[\text{final eight/initial weight}]}{\text{days of experiment}}$$

$$\text{FCR (feed conversion ratio)} = \frac{\text{dry feed fed (g)}}{\text{weight gain (g)}}$$

Crude protein, crude lipid, moisture and ash in feed ingredients and diets were determined following standard methods (AOAC 1998).

Statistical analysis

Statistical analysis of the data involved one-way analysis of variance (ANOVA) followed by Tukey's pairwise multiple comparison test. The data were expressed as arithmetic means and standard error (SE). Differences were considered significant at $p < 0.05$.

Results

There was no mortality at the end of the study in all the groups. Growth performance parameters of common carp feed with oat extracted suplemented diets shown in Table 2. WG of carps fed with the OE10 diet was tended to increase than fish fed with other diets ($p < 0.05$). The oat extract influenced FCR. The FCR value was significantly different other groups were vary depending on the OE10 diet. Best specific growth ratio (SGR) was obtained for common carp fed with the OE10 diet ($p < 0.05$).

Hematological variables

The effects of oat extract on common carp hematological variables are presented in Table 3. There were no significant differences among the Hct, RBC and MCV levels in any of the experimental groups or the control group ($p > 0.05$). Besides the Hb, MCH and MCHC were also found to be significantly higher in the experimental groups ($p < 0.05$). Among the groups, the OE10 showed the highest level of Hb.

Biochemical variables

The effects of different concentrations of oat extract on serum biochemical parameters of common carp are summarized in Table 3. Serum GLU value was tended to increase in carp fed with OE20 diet ($p < 0.05$). Other serum parameters, which are TPROT, ALB, GLO values were fed with OE10 diet were significantly higher than the control values ($p < 0.05$). The CHOL level was significantly higher in control group ($p < 0.05$). The addition of OE reduced the CHOL levels. There were no significant differences in TRIG levels among the groups ($p > 0.05$).

Table 2. Weight gain, specific growth rate (SGR) and feed conversion rate (FCR) in carp fed the experimental diets.

Parameters	OE0	OE5	OE10	OE20
WG, %	71.91 ± 9.04^{b}	77.42 ± 7.50^{ab}	93.09 ± 5.51^{a}	75.81 ± 0.69^{b}
SGR	0.90 ± 0.09^{b}	0.95 ± 0.07^{ab}	1.10 ± 0.10^{a}	0.94 ± 0.01^{ab}
FCR	1.92 ± 0.15^{a}	1.78 ± 0.08^{a}	1.47 ± 0.10^{b}	1.70 ± 0.04^{ab}

Data were presented as mean ± SE ($n = 3$/group). Values within the same row having different superscripts are significantly different ($p < 0.05$).

Lysozyme activity

As it is shown in Table 3, serum lysozyme activity, significantly increased in the oat extract supplemented diet fed groups at all concentrations in *C. carpio*. The differences were significant ($p < 0.05$) throughout the trial period. In all concentrations of oat extract-added diet fed fish serum showed higher lysozyme activity compared to the control. Among the concentrations, the OE20 group showed the highest lysozyme activity.

Myeloperoxidase activity (MPO)

The effect of oat extract supplemented diet fed on fish on the leukocyte myeloperoxidase activity in serum is depicted in Table 3. The highest significant myeloperoxidase values ($p < 0.05$) were recorded in fish fed with the OE10 and OE20 concentration of oat extract. All concentrations of oat extract led to significantly higher myeloperoxidase than the control group.

Challenge test with A. hydrophila

After 60 days of feeding, fishes were challenged with *A. hydrophila* and cumulative mortality was recorded for 6 days (Figure 1). The OE10 and OE20 groups

Table 3. Serum biochemical and hemato-immunological parameters of *C. carpio* juveniles fed diets containing oat extract for 60 days.

Paremeters	Control	OE5	OE10	OE20
Myeloperoxidase, U/L	33.65 ± 0.75^{c}	38.97 ± 0.52^{b}	48.40 ± 0.76^{a}	49.78 ± 0.29^{a}
Lysozyme activity, U/L	533.3 ± 6.66^{d}	933.3 ± 6.66^{c}	1200.00 ± 8.64^{b}	1770.77 ± 9.68^{a}
GLU, mg/dL	54.25 ± 6.42^{b}	53.14 ± 3.15^{b}	50.84 ± 9.65^{a}	65.90 ± 12.19^{a}
TRİG, mg/dL	56.41 ± 8.03^{a}	64.10 ± 13.54^{a}	66.41 ± 13.09^{a}	68.72 ± 10.75^{a}
CHOL, mg/dL	65.64 ± 13.33^{a}	62.93 ± 18.01^{a}	64.48 ± 9.73^{a}	48.39 ± 14.15^{b}
TPROT, g/dL	1.96 ± 0.18^{b}	2.07 ± 0.26^{b}	2.96 ± 0.39^{a}	2.24 ± 0.29^{b}
ALB, g/dL	1.36 ± 0.15^{b}	1.43 ± 0.33^{b}	1.99 ± 0.35^{a}	1.40 ± 0.22^{b}
GLO, d/dL	0.60 ± 0.27^{a}	0.64 ± 0.39^{a}	0.97 ± 0.24^{a}	0.85 ± 0.46^{a}
Hct, %	24.11 ± 0.48^{a}	25.33 ± 0.52^{a}	24.11 ± 0.42^{a}	24.00 ± 0.28^{a}
Hb, g/dL	8.88 ± 0.21^{c}	13.20 ± 0.72^{b}	15.90 ± 0.59^{a}	14.49 ± 0.79^{ab}
RBC, $\times 10^{6}$ mm^{3}	4.66 ± 0.24^{a}	5.27 ± 0.15^{a}	4.97 ± 0.21^{a}	5.17 ± 0.35^{a}
MCV, µm^{3}	53.33 ± 4.08^{a}	48.44 ± 1.89^{a}	49.46 ± 2.92^{a}	48.33 ± 3.57^{a}
MCH, pg cell^{-1}	19.58 ± 1.36^{c}	25.23 ± 1.61^{b}	32.57 ± 2.29^{a}	28.52 ± 1.55^{ab}
MCHC, %	37.02 ± 1.28^{c}	52.02 ± 2.34^{b}	66.00 ± 2.34^{a}	60.42 ± 3.37^{a}

Values are mean ($n = 9$). Mean ± SE with common superscripts in the same line are not significantly different ($p > 0.05$).
GLU: glucose; Trïg: triglyceride; CHOL: cholesterol; TPROT: total protein; ALB: albumin; Hct: hematocrit; Hb: hemoglobin; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration.

showed reduced mortality compared to the control group ($p < 0.05$). The relative percentage survival (RPS) and survival rate of groups challenged with *A. hydrophila* are presented in Table 4. A significantly higher survival rate was determined in fish fed with diets supplemented with both 10 g kg^{-1} and 20 g kg^{-1} concentrations of oat extract following exposure to *A. hydrophila*. More specifically, the highest protection was recorded in fishes of OE10 group followed by the OE20group. The mortality percentage was highest (80%) in the control group and lowest (26.66%) in OE10 group. The relative percentage survival was highest (67%) in OE10 group and lowest in OE5 (5%) group.

Discussion

The use of chemicals as growth promoter and resistance of bacterial disease in fish culture reason to many problems like resistance to antibiotics while the utilization of chemicals can be harmful to fish health, consumers and environment (Alderman & Hastings 1998). There from, most of the attention has been paid to natural products in order to substitute antibiotics in aquaculture. The most important of the possibilities of natural products is the use of herb extracts. They are obtained from many plant materials such as flowers, buds, seeds, leaves, fruits (Rattanachaikunsopon & Phumkhachorn 2009). This study evaluated the effect of oat extract on growth performance, non-specific immune response and disease resistance of common carp against *Aeromonas hydrophila*.

In fishes, blood is a patho-physiological reflector of the entire body and the counts of hematological parameters in blood give an indication of the health statue by determining any abnormality occurring owing to the use of immunostimulants (Tewary & Patra 2011).

Table 4. Relative Percentage Survival (RPS) (%) of challenged *Cyprinus carpio* fed oat extract supplemented diet and the control diet.

Groups	Survival (%)	Mortality	RPS (%)
Control	20^{c}	80	–
OE5	23.33^{c}	76.66	5
OE10	73.33^{a}	26.66	67
OE20	66.66^{b}	33.33	59

Data are represented as mean ± SE ($n = 30$). Different letters represent the significantly different ($p < 0.05$).

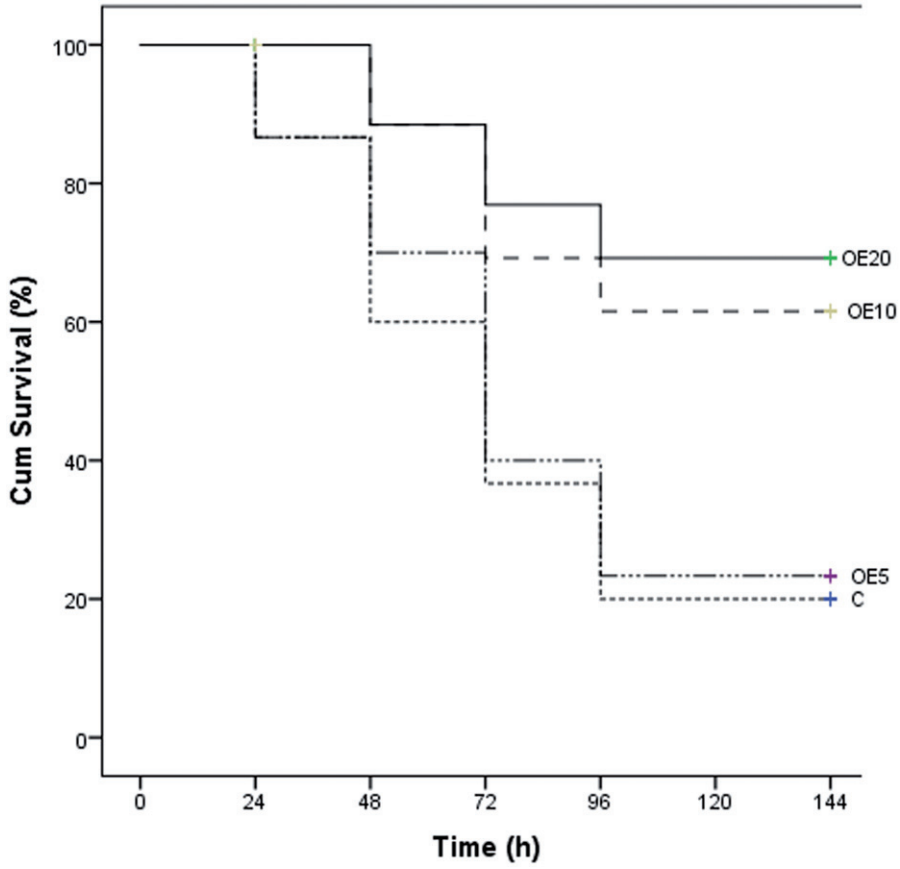

Figure 1. Kaplan–Meier survivorship curves (cumulative survival [%] over time [h]) for common carp after challenge with *Aeromonas hydrophila*; the fish were fed with oat extract supplemented diets (0, 5, 10 or 20 g of OE/kg of feed; control diets, OE5, OE10, and OE20, respectively) prior to bacterial challenge.

The hematocrit value is an important tool of health status of fish in aquaculture (Mulero et al. 1998). In the present study no significant changes were observed in hematocrit level. Many authors reported that there was no enhancement of hematocrit level after using immunostimulant compounds in fishes (Eslamloo et al. 2012; Binaii et al. 2014). In our study the Hb, RBC, MCH and MCHC levels significantly increased in the group fed with especially 10 g kg^{-1} oat extract supplemented diet. Also parallel works have been documented in different fish species such as common carp, *C. carpio*, (Harikrishnan et al. 2005), juvenile beluga, *Huso huso*, (Binaii et al. 2014). The increase in the levels of serum protein, albumin and globulins in fish is thought to be associated with a stronger innate immunity response (Wiegertjes et al. 1996). The present study showed an enhancement of total protein in group fed with 10 g kg^{-1} oat extract supplemented diet that recorded the highest values compared to the other groups. This is in agreement with previous studies conducted using *Astragalus membranaceus*, *Polygonum multiflorum*, *Isatis tinctoria* and *Glycyrrhiza glabra* (Yuan et al. 2007), garlic (Nya & Austin 2011), *Nigella sativa* and quercetin (Awad et al. 2013) in the sense that they have all enhanced serum total protein level in different fishes. Also Binaii et al. (2014) recorded increases in total protein level in juvenile beluga fed with nettle. These studies suggested that high concentration of total protein in fish serum was likely to be a result of the enhancement of non-specific immune response. Albumin and globulin are the main plasma proteins in fishes (Gunter et al. 1961). The present results indicate that the albumin and globulin values increased along with the use of oat extract enriched diets. Similar results in globulin were reported in rainbow trout fed with garlic enriched diets (Nya & Austin 2009). Increasing albumin level was reported by Jagruthi et al. (2014) in carp fed with astaxanthin supplemented diet for 4 weeks. An increase in glucose level was one of the stress indicators in fishes (Morgan & Iwama 1997). In this study oat extract supplemented diet decreased glucose values in common carp compared to the control group. As the value of oat extract increased in diet, the level of glucose decreased. This might be due to the capability of the plant extract to reduce the effects of stressors. This is in agreement with the reports of Citarasu et al. (2006), Sahu et al. (2007) and Abasali & Mohamad (2010) that glucose levels were reduced in different fish fed with herbal immunostimulant diets. In the present study, cholesterol level had significantly decreased in the 20 g kg^{-1} group compared to the control group. The triglyceride levels were slightly higher in oat extract groups but there was no significant difference. Similar results were obtained for various fish species fed with herbal enhanced diets (Immanuel et al. 2009; Metwally 2009).

Lysozyme activity is another ingredient in the first line of barrier in innate immune system (Magnadóttir 2006). Biological and syntetic immunostimulant products are considered to increase serum lysozyme activity (Engstad et al. 1992). In the present study, fish fed diets supplemented with different levels of oat extract showed significantly higher lysozyme activities when compared to the control group. Similar results have also been reported in common carp fed with herbal immunostimulant diets (Abasali & Mohamad 2010; Anbazahan et al. 2014; Jagruthi et al. 2014; Wang et al. 2015). The increased lysozyme activity observed in this study supported a higher non-specific immune response in the common carp fed with oat extract supplemented diets. Myeloperoxidase (MPO) is another important enzyme which plays a role in the killing of microorganisms (Johnston 1978). In this study, MPO activity of serum in the experimental groups showed an increase compared to the control, especially after feeding with 2% oat extract supplemented diet. Similarly, MPO activity increased significantly in common carp fed with diets supplemented with different levels of extract of caretenoids (Sowmya & Sachindra 2015). Many authors reported an enhancement of MPO activity after using immunostimulant compounds in fishes (Awad et al. 2013; Kumar et al. 2013; Wu et al. 2013).

This study demonstrated that fishes fed with oat extract supplemented diet remarkably increased the survival rate of *C. carpio* against *A. hydrophila* pathogen. This result indicated that oat, *A. sativa*, extract had a positive effect on the survival rate of common carp and this could be due to the cooperative effects of the active compounds in the extract. According to several studies (Nya & Austin 2009; Awad & Austin 2010; Nya & Austin 2011), immunostimulants can enhance resistance of fish to several bacterial pathogens including *A. hydrophila*. In other studies, a lower mortality on *A. hydrophila* challenge was reported in *C. carpio* fed *Azadiracta indica* (Harikrishnan et al. 2003), astaxanthin (Jagruthi et al. 2014), carotenoids (Anbazahan et al. 2014; Wang et al. 2015). The results showed that 10 g kg^{-1} oat extract supplemented diets increased the weight gain.

Several herbs were tested for their growth promoting activity in aquatic animals (Citarasu et al. 2002). Wang et al. (2015) observed that dietary supplementation of *Rehmannia glutinosa* increased the growth rate

C. carpio. Positive effects of herbal extracts on growth performance of different fish have been reported by other authors (MacLennan et al. 2002; Immanuel et al. 2009; Talpur & Ikhwanuddin 2013; Kanani et al. 2014). The results shown in the present study also indicate that oat extract included in the diet is useful for improving the growth performance of common carp. Especially the feed conversion ratio and specific growth rate of the study showed that fishes fed with OE10 was significantly superior when compared to other the groups.

The present results showed that the oat extract has a potential value for aquaculture both in terms of increased growth, immune response and resistance to *A. hydrophila* when diet is supplemented with 10 g kg^{-1} oat extract. Further studies focusing on the potential application of oat extract in other fishes and pathogens as an immunostimulant for the use in aquaculture are strongly recommended.

The authors wish to thank Süleyman BABA, Dr. Murat YABANLI and Aykut YOZUKMAZ for their help.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding information

This study was approved by the local ethics committee for animal experiments of Adnan Menderes University, Aydïn, Turkey (Approval Number: 64583101/2015/141).

References

Abasali H, Mohamad M. 2010. Immune response of common carp (*Cyprinus carpio*) fed with herbal immunostimulants dies. Agr J. 5:163–172.

Ahmad A, Anjum FM, Zahoor T. 2010. Extraction and characterization of beta-D-glucan from oat for industrial utilization. Int J Biol Macromol. 46:304–309.

Alderman DJ, Hastings TS. 1998. Antibiotic use in aquaculture: development of antibiotic resistance-potential for consumer health risk. Int J Food Sci Tech. 33:139–155.

Amend DF. 1981. Potency testing of fish vaccines. In: Anderson DP, Hennessen H, editors. Fish biologies: serodiagnostics and vaccines. Development in biological standardization 49. Basel, Switzerland: Karger Publishers, p. 447–454.

Anbazahan SM, Mari LSS, Yogeshwari G, Jagruthi C, Thirumurugan R, Arockiaraj J, Velanganni AAJ, Krishnamoorthy P, Balasundaram C, Harikrishnan R. 2014. Immune response and disease resistance of carotenoids supplementation diet in *Cyprinus carpio* against *Aeromonas hydrophila*. Fish Shellfish Immunol. 40:9–13.

AOAC. 1998. Official methods of analysis of AOAC International. Gaithersburg, MD: AOAC International.

Austin B, Adams C. 1996. Fish pathogens. In: Austin B, Altwegg M, Gosling PJ, Joseph S, editors. The genus aeromonas. Chichester, UK: John Wiley and Sons. p. 197–243.

Austin B, Austin DA. 2007. Bacterial fish pathogens diseases of farmed and wild fish. 4th ed. London, UK: Springer-Verlag, Praxis Publishing. p. 552.

Awad E, Austin B. 2010. Use of lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, as feed additives to prevent *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis. 33:413–420.

Awad E, Austin D, Lyndon AR. 2013. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture. 388-391:193–197.

Bain BJ, Lewis SM, Bates I. 2006. Basic haematological techniques. In: Lewis SM, Bain BJ, Bates I, editors. Dacie and Lewis practical haematology. 10th ed. Philadelphia (PA): Churchill Livingstone Elsevier. p. 26–54.

Binaii M, Ghiasi M, Farabi SMV, Ourgholam R, Fazli H, Safari R, Alavi SE, Taghavi MJ, Bankehsaz Z. 2014. Biochemical and hemato-immunological parameters in juvenile beluga (*Huso huso*) following the diet supplemented with nettle (*Urtica dioica*). Fish Shellfish Immunol. 36:46–51.

Blaxhall PC, Daisley KW. 1973. Routine hematological methods for use with fish blood. J Fish Biol. 5:771–781.

Cabello FC. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol. 8:1137–1144.

Citarasu T, Babu MM, Sekar RRJ, Marian MP. 2002. Developing Artemia enriched Herbal diet for producing quality larvae in *Penaeus monodon*, Fabricius. Asian Fisheries Sci. 15:21–32.

Citarasu T, Sivaram V, Immanuel G, Rout N, Murugan V. 2006. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp, Penaeus monodon with reference to haematological, biochemical and immunological changes. Fish Shellfish Immunol. 21:372–384.

Coffman FA. 1977. Oat history, identification and classification. Technical Bulletin No 1516. Washington D.C., United States: United States Department of Agriculture, Agricultural Research Service. p. 356.

Eddy S, Underhill JC. 1974. Northern fishes with special reference to the upper Mississippi Valley. 3rd ed. Minneapolis (MN): University of Minnesota Press.

Engstad RE, Robertsen B, Frivold E. 1992. Yeast glucan induces increase in activity of lysozyme activity in Atlantic salmon blood. Fish Shellfish Immunol. 2:287–297.

Eslamloo K, Falahatkar B, Yokoyama S. 2012. Effects of dietary bovine lactoferrin on growth, physiological performance, iron metabolism and non-specific immune responses of Siberian sturgeon Acipenser baeri. Fish Shellfish Immunol. 32:976–985.

Galindo-Villegas J, Hosokawa H. 2004. Immunostimulants: towards temporary prevention of diseases in marine fish. In: Cruz Suárez LE, Ricque Marie D, Nieto López MG, Villarreal D, Scholz U, y González M, editors. Avances en Nutrición Acuícola VII. Memorias del VII Simposium Internacional de Nutricioen AcuÚcola, Noviembre, 2004. Hermosillo, Sonora, México.

Gunter G, Sulya LL, Box BE. 1961. Some evolutionary patterns in fishes blood. Biol Bull. 121:302–306.

Harikrishnan R, Rani NM, Balasundaram C. 2003. Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. Aquaculture. 221:41–50.

Harikrishnan R, Balasundaram C, Bhuvaneswari R. 2005. Restorative effect of *Azadirachta indica* aqueous leaf extract dip treatment on haematological parameter changes in *Cyprinus carpio* (L) experimentally infected with *Aphanomyces invadans* fungus. J Appl Ichthyol. 21:410–414.

Heo GJ, Kim JH, Jeon BG, Park KY, Ra JC. 2004. Effects of FST-Chitosan mixture on cultured rockfish (*Sebastes schlegeli*) and olive flounder (*Paralichthys olivaceus*). Kor J Vet Pub Health. 25:141–149.

Immanuel G, Uma RP, Iyapparaj P, Citarasu T, Punitra Peter SM, Babu MM, Palavesam A. 2009. Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*. J Fish Biol. 74:1462–1475.

Jagruthi C, Yogeshwari G, Anbazahan SM, Mari LSS, Arockiaraj J, Mariappan P, Sudhakar GRL, Balasundaram C, Harikrishnan R. 2014. Effect of dietary astaxanthin against *Aeromonas hydrophila* infection in common carp, *Cyprinus carpio*. Fish Shellfish Immunol. 41:674–680.

Johnston RB. 1978. Oxygen metabolism and the microbicidal activity of macrophages. Fed Proc. 37:2759–2764.

Kanani HG, Nobahar Z, Kakoolaki S, Jafarian H. 2014. Effect of ginger- and garlic-supplemented diet on growth performance, some hematological parameters and immune responses in juvenile Huso huso. Fish Physiol Biochem. 40:481–490.

Kuge T, Takahashi K, Barcs I, Hayashi F. 1992. *Aeromonas hydrophila*, a causative agent of mass mortality in cultured Japanese catfish larvae (*Silurus asotus*). Fish Pathol. 27:57–62.

Kumar S, Raman RP, Pandey PK, Mohanty S, Kumar A, Kumar K. 2013. Effect of orally administered azadirachtin on non-specific immune parameters of goldfish *Carassius auratus* (Linn. 1758) and resistance against *Aeromonas hydrophila*. Fish Shellfish Immunol. 34:564–573.

Lee TH, Qiu F, Waller GR, Chou CH. 2000. Three new flavonol galloylglycosides from leaves of *Acacia confusa*. J Nat Prod. 63:710–712.

MacLennan AH, Wilson DH, Taylor AW. 2002. The escalating cost and prevalence of alternative medicine. Prev Med. 35:166–173.

Magnadóttir B. 2006. Innate immunity of fish (overwiew). Fish Shellfish Immunol. 20:137–151.

Maqsood S, Singh P, Samoon MH, Balange AK. 2010. Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio* challenged with *Aeromonas hydrophila*. Int Aquat Res. 2:77–85.

Metwally MAA. 2009. Effects of Garlic (*Allium sativum*) on Some Antioxidant Activities in Tilapia Nilotica (*Oreochromis niloticus*). World J Fish Mar Sci. 1:56–64.

Mohomad S, Abasali H. 2010. Effect of plant extracts supplemented diets on immunity and resistance to Aeomonas hydrophila in common carp (*Cyprinus carpio*). Agri J. 5:119–127.

Morgan JD, Iwama GK. 1997. Measurements of stressed states in the field. In: Iwama GK, Pickering AD, Sumpter JP, Schreck CB, editors. Fish stress and health in aquaculture. Cambridge, UK: Cambridge University Press. p. 247–270.

Mulero V, Esteban A, Munoz J, Meseguer J. 1998. Dietary intake of levamisole enhances the immune response and disease resistance of the marine teleost gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol. 8:49–62.

Nya E, Austin B. 2009. Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, (*Oncorhynchus mykiss*). J Fish Dis. 32:971–977.

Nya EJ, Austin B. 2011. Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. Fish Shellfish Immunol. 30:845–850.

Peterson DM. 2001. Oat antioxidants [J]. J Cereal Sci. 33:115–129.

Popovici G, Weissenböck G, Bouillant ML. 1977. Isolation and characterization of flavonoids from *Avena sativa* Linn. [J]. Z Pflanzenphysiol. 85:103–115.

Quade JM, Roth JA. 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet Immunol Immunopathol. 58:239–248.

Rattanachaikunsopon P, Phumkhachorn P. 2009. Protective effect of clove oil-supplemented fish diets on experimental Lactococcus garvieae infection in tilapia. Biosci Biotechnol Biochem. 73:2085–2089.

Roberts RJ, Frerichs GN, Miller SD. 1992. Epizootic ulcerative syndrome the current position. In: Shariff, M, Subasinghe RP, Arthur JR, editors. Diseases in Asian aquaculture I. Fish health section. Manila, Philippines: Asian Fisheries Society. p. 431–436.

Saikia SK, Das DN. 2009. Feeding ecology of common carp (*Cyprinus carpio* L.) in arice-fish culture system of the *Apatani plateau* (Arunachal Pradesh, India). Aquat Ecol. 43:559–568.

Sahu S, Das BK, Pradhan J, Mohapatra BC, Mishra BK, Sarangi NN. 2007. Effect of *Mangifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. Fish Shellfish Immunol. 23:109–118.

Singh R, De S, Belkheir A. 2013. *Avena sativa* (Oat), A potential neutraceutical and therapeutic agent: an overview. Crit Rev Food Sci Nutr. 53:126–144.

Sowmya R, Sachindra NM. 2015. Enhancement of non-specific immune responses in common carp, *Cyprinus carpio*, by dietary carotenoids obtained from shrimp exoskeleton. Aquacult Res. 46:1562–1572.

Talpur AD, Ikhwanuddin M. 2013. *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish Shellfish Immunol. 34:254–264.

Tanker M, Tanker N. 2003. Pharmacognosy. Ankara, Turkey: Ankara University Pharmacy Press.

Tewary A, Patra BC. 2011. Oral administration of baker's yeast (*Saccharomyces cerevisiae*) acts as a growth promoter and immunomodulator in *Labeo rohita* (Ham.). J Aquacult Res Dev. 2:1–7.

Waldemar E. 1982. Incorporation of [4-14C] cholesterol into steryl derivatives and saponins of oat (*Avena sativa* L.) plants [J]. Plant Cell Rep. 1:253–256.

Wang JL, Meng X, Lub R, Wu C, Luo YT, Yan X, Li XJ, Kong XH, Nie GX. 2015. Effects of *Rehmannia glutinosa* on

growth performance, immunological parameters and disease resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio* L.). Aquaculture. 435:293–300.

Wenzig E, Kunert O, Ferreira D. 2005. Flavonolignans from *Avena sativa* [J]. J Nat Prod. 68:289–292.

Wiegertjes GF, Stet RJ, Parmentier HK, Van Muiswinkel WB. 1996. Immunogenetics of disease resistance in fish: a comparative approach. Dev Comp Immunol. 20: 365–381.

Wu Y, Gong O, Fang H, Liang W, Chen M, He R. 2013. Effect of *Sophora flavescens* on non-specific immune response of tilapia (GIFT *Oreochromis niloticus*) and disease resistance against *Streptococcus agalactiae*. Fish Shellfish Immunol. 34:220–227.

Yin G, Ardo L, Thompson K, Adams A, Jeney Z, Jeney G. 2009. Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. Fish Shellfish Immunol. 26:140–145.

Yuan C, Li D, Chen W, Sun F, Wu G, Gong Y, Tang J, Shen M, Han X. 2007. Administration of a herbal immunoregulation mixture enhances some immune parameters in carp (*Cyprinus carpio*). Fish Physiol Biochem. 33:93–101.

Zhang X, Yang W, Wu H, Gong X, Li A. 2014. Multilocus sequence typing revealed a clonal lineage of *Aeromonas hydrophila* caused motile *Aeromonas* septicemia outbreaks in pond-cultured cyprinid fish in an epidemic area in central China. Aquaculture. 432:1–6.