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# Enhanced growth performance, oxidative capacity and immune responses of common carp, *Cyprinus carpio* fed with *Artemisia absinthium* extract-supplemented diet

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ABSTRACT

In the present study, the effects of Afsanteen (*Artemisia absinthium*) aqueous extract (AE) were investigated on growth performance, innate immunity, and oxidative status of common carp, *Cyprinus carpio*. Common carp juveniles were fed experimental diets that contained 0, 0.5, 1 or 1.5% of AE for 60 days (75 fish/treatment). Thereafter, the fish growth performance, feed efficiency, serum immune-related, biochemical, and antioxidant parameters were determined. The results showed that diets containing 0.5 and 1% of AE enhanced the growth performance and feed efficiency of the fish. Fish fed with AE supplemented diets (at all concentrations) also displayed higher levels of serum lysozyme, alternative complement, total immunoglobulin, superoxide dismutase, and total protein levels compared to the control diet; the highest levels were observed at 1% AE level. Serum albumin levels of all AE-supplemented treatments were significantly higher than the control treatment. Dietary AE supplementation significantly increased plasma glutathione peroxidase activities; the highest value was related to the fish fed 0.5% AE. All AE treatments showed significantly lower serum catalase activities compared to the control; the lowest value was related to 1.5% AE. 0.5 and 1% AE significantly decreased serum malondialdehyde levels. All AE- treated fish exhibited significantly lower serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities; the lowest values were observed in 0.5% or 1% AE. In conclusion, dietary inclusions of AE in juvenile common carp increased growth performance and feed efficiency, improved the innate immunity and antioxidant response, and improved hepatic health. AE at 0.5–1% levels is recommended for common carp diet formulation.

## 1. Introduction

Aquaculture growth is vital to provide a stable protein source for the increasing population (Béné et al., 2016). However, the intensification of the systems to achieve higher productions entails important secondary challenges (Segner et al., 2012). Rearing the animals at high densities not only compromises animal welfare by overcrowding but also by poor water quality, which might lead to internal homeostasis disruption, immunosuppression, and oxidative stress (Barton and Iwama, 1991; Yang et al., 2017). High fish densities also facilitate the spread and evolution of pathogen virulence, which combined with environmental stress can result in high mortalities (Pulkkinen et al., 2010; Segner et al., 2012). Antibiotics and other veterinary drugs (e.g. disinfectants) have been widely used in aquaculture to avoid disease outbreaks and stress-

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related mortality (Carmichael et al., 1984; Lulijwa et al., 2020). However, antibiotic use has numerous environmental and health side effects, including antibiotic-resistant bacteria emergence (Lulijwa et al., 2020). The need for sustainable alternatives that increase the system resilience while respecting the environment and especially that do not contribute to the antimicrobial resistance pool, has therefore become an international priority (Lieke et al., 2019; Reverter et al., 2020a).

The use of functional feed supplements (e.g. plants, prebiotics, probiotics) with beneficial effects on fish fitness and their immune system, has increased its popularity over the last couple decades (Reverter et al., 2014; Guerreiro et al., 2018; Ringø et al., 2020; Yousefi et al., 2020a; Abdel-Tawwab et al., 2021; Adeshina et al., 2021). Herbal supplementation is especially attractive because it is a relatively inexpensive alternative available to both small and large-scale fish farmers that, besides, effectively reducing disease-associated mortality can also improve fish growth and feeding efficiency (Awad and Awaad, 2017; Abdel-Tawwab et al., 2018; Adeshina et al., 2019; Reverter et al., 2020b; Abdel-Tawwab and El-Araby, 2021; Harikrishnan et al. 2021 a, b). Furthermore, the traditional application of therapeutic herbs in human and animal medicine has a long history in Asia and the Middle East, where most of the aquaculture production takes place, facilitating their implementation by fish farmers (Caruso et al., 2013).

Afstanteen (*Artemisia absinthium* L.), also commonly known as wormwood, is commonly found in Asia, Middle East, Europe, and North Africa (Grieve, 1971; Leporatti and Ghedira, 2009). Afsanteen has traditionally been used in human and animal medicine (Leporatti and Ghedira, 2009; Habibi et al., 2016; Bhat et al., 2019), and possesses a broad range of bioactivities including antimicrobial, antiviral, antistress, hepatoprotective, antioxidant and anticancer (Amat et al., 2010; Batiha et al., 2020). Afsanteen contains different types of chemical compounds such as lactones, terpenoids (e.g., myrcene, germacrene D, camphor, chamazulene), flavonoids and flavonoids glycosides (Bhat et al., 2019; Batiha et al., 2020), some of which (i.e., myrcene) enhanced fish antioxidant enzymes (Ahmadifar et al., 2020; Hoseini et al., 2020; Khalili et al., 2020). Some studies have suggested that the use of complex extracts (i.e., that contain a mixture of compounds) can display higher activities than isolated pure compounds, most likely due to synergetic effects between compounds (Lee et al., 2009; Rattanachaikunsopon and Phumkhachorn, 2010). Despite the rich chemical composition of afstanteen and its multiple bioactivities, the effects of afstanteen extract on fish health status are still unknown. The common carp is one of the most popular freshwater fish species cultured throughout the world. It is the fourth most cultured fish with production of more than 4.1 million tons in 2018 contributing 7.7% of the world's total fish aquaculture production (FAO, 2020).

In this study, the effects of dietary supplementation with afsanteen aqueous extract have investigated on growth performance, innate immunity, biochemical, and antioxidant parameters of common carp, *Cyprinus carpio*, juveniles.

## 2. Materials and methods

### 2.1. AE preparation

We purchased the afsanteen (*A. abstinthium*) fresh leaves from a local grocery in Iran. The plant species and health of purchased leaves was confirmed by the Gonbad-Kavous University botanical laboratory (Golestan province, Iran). We extracted the afstanteen leaves using hot water as described by Wu et al. (2010). Briefly, afsanteen leaves were rinsed with sterile water and dried under a constant air flow. Then, 100 g of afsanteen dried powder was mixed with 1000 mL of water, placed on a flame, and boiled for 2 h. After that time, the solid material was removed using a nylon filter and a vacuum pump.

### 2.2. Diet preparation

The diet ingredients (Table 1) were purchased, ground into powder separately, sieved, weighted near 0.01 g and mixed thoroughly by using a mixer. We prepared four experimental diets (control, 0.5, 1 and 1.5% of AE) by combining one kg of the powdered dietary mixture (i.e. basal diet, Table 1) with 300 mL of water solution, which contained four different levels of AE (0, 5, 10 and 15 mL). The resultant mixtures were then passed through a meat grinder, after which the fish pellets were obtained. The food was kept at 4 °C until use.

### 2.3. Experimental protocol

We conducted the experiment in accordance with the Animal Ethics directions of the Gonbad-Kavous University (Iran). Four hundred common carp juveniles were purchased from a local farm (Guilan province) and transferred to our laboratory in Gonbad-Kavous University (Gonbad-Kavous, Golestan province, Iran). For acclimation, the fish were distributed in two 400 L concrete tanks for 14 days. After the acclimation period, 300 healthy juveniles (initial weight 22.73 ± 0.89 g; body length: 10.51 ± 0.25 cm) were randomly distributed into 12 tanks (70 L, 3 tank replicates per treatment), and fed for 60 days with the corresponding diets (control, 0.5, 1, 1.5% AE).

The juveniles were hand-fed three-times a day at 2% of biomass. The feed amounts were adjusted biweekly. Unconsumed feed and fish feces were siphoned daily. Tanks had constant aeration and a daily water turnover of 50%. The average temperature, dissolved oxygen, pH, total suspended solids, and electrical conductivity (EC) were measured using portable apparatus (Hach HQ40d portable apparatus, Loveland, Colorado, USA). The total ammonia nitrogen was determined by digital photometer (Wagtech 7100, Berkshire, UK).

### 2.4. Growth and feeding parameters

After the 60-day trials, all juveniles were weighted. We measured the following parameters: mortality rate (MR%) = (final number of fish)/

**Table 1**
Dietary formulation and proximate composition analysis of experimental diets (% on dry matter basis) containing different levels of Afsanteen extract.

| Feedstuffs | Control | 0.5% AE | 1% AE | 1.5% AE |
|---|---|---|---|---|
| Fishmeal[1] | 15 | 15 | 15 | 15 |
| Poultry meal[2] | 15 | 15 | 15 | 15 |
| Soybean meal | 20 | 20 | 20 | 20 |
| Wheat flour | 34 | 33.5 | 33 | 32.5 |
| Fish oil | 1.5 | 1.5 | 1.5 | 1.5 |
| Soybean oil | 1.5 | 1.5 | 1.5 | 1.5 |
| Corn flour | 10 | 10 | 10 | 10 |
| L-Lysine[3] | 0.4 | 0.4 | 0.4 | 0.4 |
| L-Methionine 100[3] | 0.6 | 0.6 | 0.6 | 0.6 |
| Vitamin premix[a] | 1 | 1 | 1 | 1 |
| Mineral premix[b] | 1 | 1 | 1 | 1 |
| Afsanteen extract | 0 | 0.5 | 1 | 1.5 |
| Dry matter | 91.4 | 91.3 | 90.5 | 91.0 |
| Crude protein (%) | 38.3 | 38.2 | 37.8 | 38 |
| Crude fat (%) | 8.52 | 8.44 | 8.59 | 8.67 |
| Crude ash (%) | 8.90 | 8.83 | 8.64 | 9.11 |

1- Pars kilka Co., Mazandaran, Iran (Kilka powder analysis; Protein: 70–72%, Fat: 8–11%, Ash: 11.6%, Moisture: 7–9%). 2- Makianmehr Co., Golestan, Iran. 3- Morghenojan.Co., Tehran, Iran.

[a] Vitamin premix (per kg of diet): vitamin A, 2000 IU; vitamin $B_1$ (thiamin), 5 mg; vitamin $B_2$ (riboflavin), 5 mg; vitamin $B_6$, 5 mg; vitamin $B_{12}$, 0.025 mg; vitamin $D_3$, 1200 IU; vitamin E, 63 mg; vitamin $K_3$, 2.5 mg; folic acid, 1.3 mg; biotin, 0.05 mg; pantothenic acid calcium, 20 mg; inositol, 60 mg; ascorbic acid (35%), 110 mg; niacinamide, 25 mg.

[b] Mineral premix (per kg of diet): $MnSO_4$, 10 mg; $MgSO_4$, 10 mg; KCl, 95 mg; NaCl, 165 mg; $ZnSO_4$, 20 mg; KI, 1 mg; $CuSO_4$, 12.5 mg; $FeSO_4$, 105 mg; Co, 1.5 mg.

(initial number of fish) × 100, weight gain (WG) = final weight (g) – initial weight (g), specific growth rate (SGR; $day^{-1}$%) = (ln final weight – ln initial weight)/days×100, and feed conversion ratio (FCR) = consumed feed/gained biomass.

### 2.5. Blood sampling

After the 60-day feeding trials, the fish were fasted for 1 day. Then, 3 fish from each tank (9 fish per treatment) were captured, anaesthetized in a bath of clove oil (100 mg/l), and blood sampled. Blood samples were immediately collected from the caudal vein using syringes (without anticoagulant) and transferred into a sterile tube. After coagulation, the samples were centrifuged (7000 rpm) for 10 min at 4 °C, and the sera were stored at −80 °C until the biochemical measurements.

### 2.6. Blood biochemical, immunological, and antioxidant parameters

We measured the alternative complement (ACH50) activity using the method described by Sunyer and Tort (1995) with some modifications (Yeh et al., 2008). The lysozyme-sensitive bacterium, *Micrococcus luteus* was used as previously defined by Demers and Bayne (1997) to measure the serum lysozyme activities. Total immunoglobulin levels were estimated according to the method presented by Siwicki (1993). Total protein concentrations were measured with a chemical colorimetric assay kit (Pars azmoon Company, Karaj, Iran) following the advised protocol. We used the bromocresol green binding protocol to measure the serum albumin levels (Doumas et al., 1971).

We determined the serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities using commercial kits (Pars Azmun, Karaj, Iran) and an automated biochemical analyzer (Beckman Coulter, USA). Also, the estimation of MDA levels was done colorimetrically as suggested by Draper and Hadley (1990). Serum superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were assayed following the methods of McCord and Fridovich (1969), Aebi (1984) and Noguchi et al. (1973), respectively.

Total antioxidant capacity (TAC) was determined spectrophotometrically (oxidation-reduction method) using commercial kit (ZellBio, GmbH, Veltinerweg, Germany) based on oxidation-reduction method according to the manufacturer recommendation.

For assessment of bactericidal activity of AE, *Aeromonas hydrophila* was cultured on nutrient agar plate, suspended in phosphate-buffered saline (pH 7) to reach an optical density of 0.5 at 546 nm, and diluted with the same buffer eight times. The extract was then mixed 1:1 with phosphate buffer. 200 µl of AE were mixed with 2 ml of the diluted bacterial suspension and incubated for 24 h. Then, the suspension was cultured on nutrient agar and the colonies were counted after 24 h. Finally, the number of colonies formed in the control group medium ($5 \times 10^5$ cfu/ml buffer equivalent to half McFarland concentration containing *Aeromonas hydrophila*) was divided by the number of colonies formed in the medium containing the AE, and the antibacterial activity of the extract was expressed as a fold decrease in cfu/ml.

Total polyphenol content of AE was determined according to Agbor et al. (2014) using Folin-Ciocalteu reagent and after extraction by warm methanol;water solvent.

### 2.7. Statistical analysis

All statistical analyses of data were performed using the SPSS software version 22. Prior to the use of parametric tests, we checked the data normality (Shapiro-Wilk test) and heteroscedasticity (Levene's test). Then, we used a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to detect significant differences between the group means. All the data presented in the manuscript is expressed as mean ± SE.

## 3. Results

Total polyphenol content (11.08 ± 1.91 g gallic acid/100 g dry mater), TAC (1528.67 ± 26.40 µmol/l), and bactericidal activity against *A. hydrophila* (120,160 ± 4640 $1^{-8}$ cfu/ml) of AE were shown in Table 2.

During the 60-day feeding trial, no mortality was observed in any treatments. The water quality parameters were also periodically controlled, and they were stable (i.e. no significant difference) throughout the experiment and between all treatments ($P > 0.05$). The average temperature, dissolved oxygen, pH, total suspended solids, and electrical conductivity (EC) were 24.51 ± 0.46 °C, 6.9 ± 0.32 mg/l, 7.78 ± 0.13, 402.72 ± 9.1 mg/l and 820.22 ± 12/08 µmos/cm, respectively. The total ammonia nitrogen was 0.06 ± 0.02 mg/l during the experimental period.

The fish growth parameters as well as feed efficiency indices are shown in Table 3. The highest WG percentage, SGR, and FCR were observed in the fish fed with 0.5% and 1% AE. However, there were no significant differences in growth performance between the control and 1.5% AE treatments.

Dietary AE supplementation significantly enhanced serum total protein levels and 1% AE exhibited the highest value (Table 4). All AE treatments exhibited similar serum albumin levels, which were significantly higher than that of the control treatment (Table 4). All AE treatments exhibited significantly lower serum AST, ALT, and ALP activities. The lowest AST and ALT activities were observed in 0.5% AE treatment; whereas, the lowest ALP activity was observed in 0.5% and 1% AE treatments (Table 4).

AE inclusion significantly increased serum ACH50, lysozyme activity, and total immunoglobulin; the highest values were related to 1% AE treatment (Fig. 1).

Serum SOD activity significantly increased in the AE-supplemented treatments and the highest value was related to 1% AE (Fig. 2). Moreover, dietary AE supplementation significantly increased serum GPx activity, but the highest activity was observed in 0.5% AE treatment (Fig. 2). All the AE-treated fish exhibited significantly lower serum CAT activities, which the lowest activity was observed in 1.5% AE treatment (Fig. 2). The fish fed diets supplemented with 0.5% and 1% presented significantly lower serum MDA levels compared to the control treatment. However, there was no significant difference in serum MDA between the control and 1.5% AE treatments (Fig. 2).

## 4. Discussion

Multiple studies have exposed the beneficial effects of using medicinal plant-based supplements in aquaculture, such as better growth, enhanced immune defenses and decreased pathogen and stress susceptibility (Hoseinifar et al., 2020a; Reverter et al., 2020b; Yousefi et al., 2020a; Yousefi et al., 2021). Here, we show the potential of afsanteen (*A. absinthium*) aqueous extracts (at 0.5 and 1% of AE) to improve growth performance, feeding efficiency, antioxidant (SOD, GPx) and immune defenses (lysozyme, ACH50, immunoglobulin, total protein) of common carp juveniles.

Many medicinal plants have shown to enhance the activity of digestive enzymes, leading to increased growth rates and better feeding efficiencies (Awad and Awaad, 2017). Here, we observed that oral

**Table 2**
Total polyphenol content, total antioxidant capacity (TAC), and bactericidal activity against *A. hydrophila* of *Artemisia absinthium* extract ($N = 3$).

| *Artemisia absinthium* extract | Test result | Unit |
|---|---|---|
| Total polyphenol content | 11.08 ± 1.91 | g gallic acid/100 g dry mater |
| Total antioxidant capacity (TAC) | 1528.67 ± 26.40 | µmol/l |
| Bactericidal activity against *A. hydrophila* | 50.82 ± 11.96 | Fold decrease in cfu/ml |

**Table 3**
Growth performance and feed efficiency of Common carp fed with *Artemisia absinthium* extract after 60 days feeding trial.

| | Control | 0.5% AE | 1% AE | 1.5% AE |
|---|---|---|---|---|
| IW (g) | 22.84 ± 0.7 | 22.45 ± 1.12 | 22.62 ± 0.88 | 23.04 ± 0.89 |
| FW (g) | 48.39 ± 1.65$^{c}$ | 51.11 ± 2.18$^{ab}$ | 52.95 ± 1.59$^{a}$ | 49.47 ± 1.75$^{bc}$ |
| WG (g) | 25.55 ± 1.71$^{b}$ | 28.66 ± 2.18$^{a}$ | 30.33 ± 2.03$^{a}$ | 26.43 ± 1.35$^{b}$ |
| WG (%) | 112.03 ± 9.28$^{b}$ | 128.06 ± 12.67$^{a}$ | 134.44 ± 12.82$^{a}$ | 114.84 ± 6.86$^{b}$ |
| FI (g) | 46.50 ± 1.45 | 46.42 ± 1.14 | 46.40 ± 1.77 | 46.25 ± 1.48 |
| SGR (%/d) | 1.25 ± 0.07$^{b}$ | 1.37 ± 0.09$^{a}$ | 1.41 ± 0.09$^{a}$ | 1.27 ± 0.05$^{b}$ |
| FCR | 1.82 ± 0.12$^{a}$ | 1.62 ± 0.11$^{b}$ | 1.53 ± 0.10$^{b}$ | 1.75 ± 0.09$^{a}$ |
| SR (%) | 100 | 100 | 100 | 100 |

Data are presented as mean ± SEM. Data was analyzed through one-way ANOVA besides Duncan comparisons. IW, initial weight; FW, final weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; SR, Survival rate.
different letter in a row denote significant difference (P<0.05)

**Table 4**
Innate immune and biochemical responses of common carp fed with *Artemisia absinthium* extract after 60 days.

| | Control | 0.5% AE | 1% AE | 1.5% AE |
|---|---|---|---|---|
| Total protein (g/dl) | 2.74 ± 0.015$^{d}$ | 2.85 ± 0.036$^{c}$ | 2.99 ± 0.015$^{a}$ | 2.89 ± 0.015$^{b}$ |
| Albumin (g/dl) | 1.32 ± 0.030$^{b}$ | 1.43 ± 0.017$^{a}$ | 1.47 ± 0.034$^{a}$ | 1.45 ± 0.026$^{a}$ |
| AST (U/l) | 55.91 ± 1.12$^{a}$ | 20.63 ± 0.41$^{d}$ | 25.16 ± 0.42$^{c}$ | 29.00 ± 0.37$^{b}$ |
| ALT (U/l) | 29.52 ± 0.50$^{a}$ | 12.16 ± 0.76$^{c}$ | 15.47 ± 0.51$^{b}$ | 15.70 ± 1.25$^{b}$ |
| ALP (U/l) | 281.43 ± 1.81$^{a}$ | 165.83 ± 3.95$^{c}$ | 165.39 ± 0.76$^{c}$ | 214.40 ± 2.44$^{b}$ |

Data are presented as mean ± SEM. Data was analyzed through one-way ANOVA besides Duncan comparisons. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.
different letter in a row denote significant difference (P<0.05)

supplementation with 0.5 and 1% afstanteen aqueous extract improved the weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and lipid efficiency ratio. However, supplementation with 1.5% of AE did not improve any of the growth parameters, suggesting as previously observed in a recent meta-analysis (Reverter et al., 2020b) that the beneficial effects of plant supplementation on fish growth are dose-dependent and if optimal doses are exceeded the benefits might be lost. A recent study that evaluated the effect of dietary supplements with a related plant (*Artemisia dracunculus*) also observed the highest growth in rainbow trout that were fed the intermediate plant dose, although the highest inclusion level still enhanced fish growth (Gholamhosseini et al., 2021). In another study, Mbokane and Moyo (2018) also showed higher growths in lower inclusion doses of *Artemisia afra* in the diet of tilapia *Oreochromis mossambicus*, although their results were not statistically different.

Besides growth promoting activities, many bioactive plants also present immunostimulant activities, which can increase the animals' resistance to disease and stress factors (Awad and Awaad, 2017; Hoseinifar et al., 2020a). Here we show that the use of afsanteen aqueous extract as a feed supplement increased the alternative complement (ACH50) and lysozyme activities and the total immunoglobulin and total protein levels in common carp juveniles. Both lysozyme and alternative complement are important components of the innate immune system, by contributing to the lysis of pathogen cells, whilst immunoglobulin are responsible for the production of specific antibody responses against antigens (Gomez et al., 2013; Rombout et al., 2014). Therefore, higher levels of ACH50, lysozyme and immunoglobulins might contribute to increase the fish resistance to pathogens. Similar observations have been previously reported in fish fed with plant-supplemented diets. For example, dietary supplementation with lemon verbena (*Aloysia citrodora*) increased serum and skin mucus lysozyme and immunoglobulin levels in rainbow trout (Hoseinifar et al., 2020b). Mbokane and Moyo (2018) also found that *A. afra* supplementation increased lysozyme activity *in O. mossambicus*, while Mehrabi et al. (2019) observed increased lysozyme and alternative complement activities in rainbow trout fed with a diet enriched in *Aloe vera*.

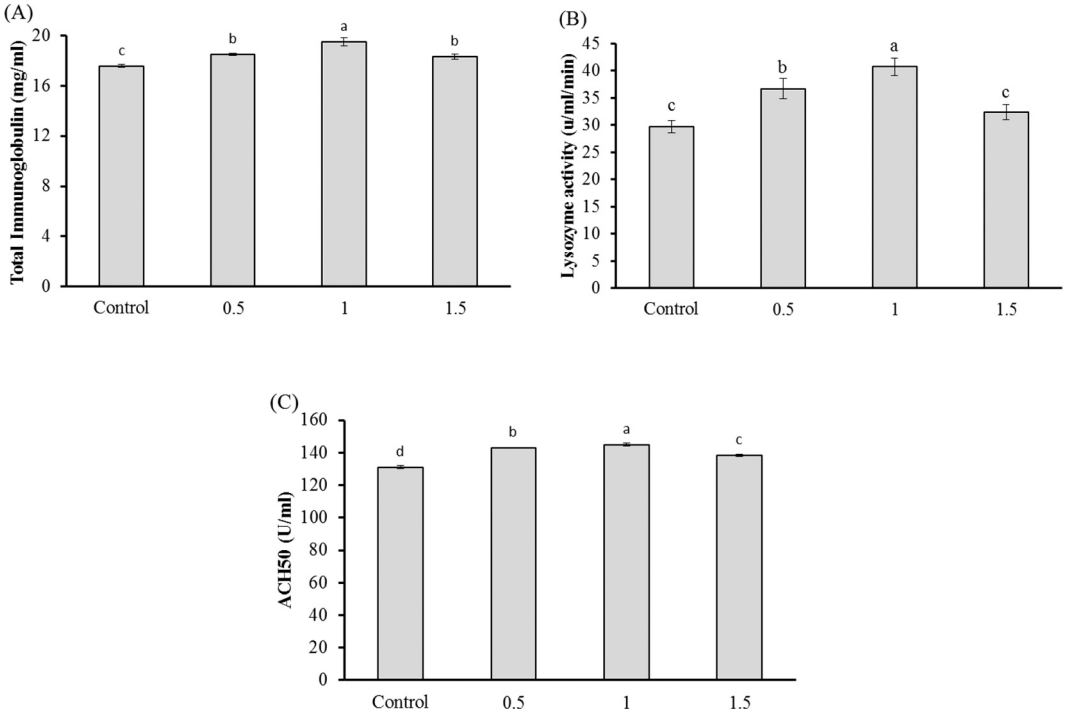


**Fig. 1.** Serum immune parameters in common carp fed with *Artemisia absinthium* extract after 60 days. Bars assigned with different letters denote significant difference ($P < 0.05$).

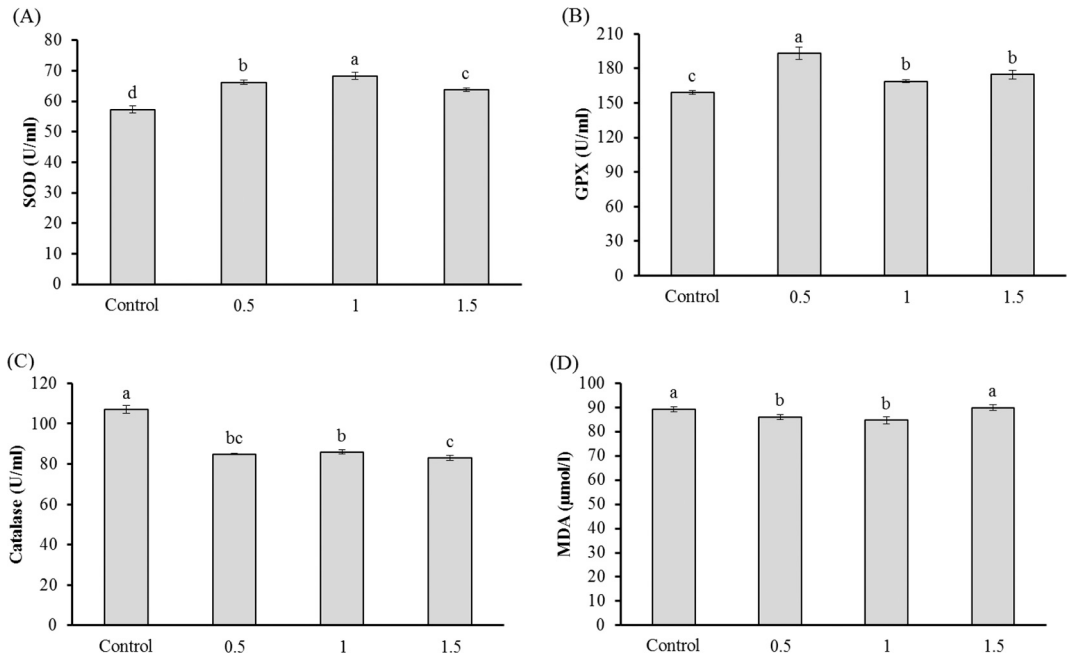


**Fig. 2.** Serum antioxidant enzymes activity in common carp fed with *Artemisia absinthium* extract after 60 days. Bars assigned with different letters denote significant difference ($P < 0.05$).

Changes in some biochemical parameters such as metabolic enzymes have been proposed as a diagnostic tool to assess the fish health status and identify metabolic dysfunctions or target organ injuries (Coz-Rakovac et al., 2005; Ramaiah, 2007). Higher levels of aminotransferases (ALT, AST, amino acid metabolism) and ALP (involved in membrane transport activities) in blood are often linked to liver damage (Ramaiah, 2007). Therefore, lower serum ALT, AST and ALP levels observed in fish fed with 0.5 and 1% of afsanteen could be interpreted as positive effects of plant supplementation on common carp fingerling metabolism, which could be related to significantly lower stress levels. The reduction of hepatic enzymes was also detected in rainbow trout fed with a trans-cinnamic acid-enriched diet (Yılmaz and Ergün, 2018) and with acorn (*Quercus brantii*) alcoholic extract (Rashidian et al., 2018).

Antioxidant system is pivotal in fish well-being and responsible for protection of the fish against oxidative stress (Ahmadifar et al., 2019). The present study suggests that AE at 0.5% and 1% decreased oxidative stress (lower MDA). Our results are in line with previous works on common carp. Abdel-Latif et al. (2020) found modulation of antioxidant responses in common carp by dietary supplementation with oregano extract. Hoseinifar et al. (2019) observed that dietary white-bottom mushroom supplementation significantly increased antioxidant parameters of common carp. Such antioxidant effects of AE might be either due to the radical scavenging nature of its compounds such as flavonoids, or modulation of antioxidant enzymes (Edenharder and Grünhage, 2003). Here we observed that AE-supplementation increased SOD and GPx, but decreased CAT activities. Although such a controversy needs more specific research, the difference in AE effects on the antioxidant enzymes might be due to their specific functions. For example, beside their presence in the cytosol, GPx and SOD are present in mitochondria and protect the organelle against reactive oxygen species during cell respiration and ATP production. However, CAT has no role in ATP production as is only present in the cell cytosol (Ighodaro and Akinloye, 2018). Therefore, higher SOD and GPx activities suggest higher aerobic ATP production in AE-treated fish that might explain higher fish growth performance. On the other hand, lower CAT activity and MDA levels could suggest lower oxidative conditions in these treatments. This is partly supported by lower serum ALT, AST, and ALP activities, as oxidative conditions damage hepatocyte and increase these enzymes in blood stream (Yousefi et al., 2020b). However, assessment of further oxidative stress biomarkers such as other products of lipid peroxidation (e.g. nitric oxide, hydrogen peroxide, protein carboxylase) are needed to confirm cell damage.

## 5. Conclusion

In conclusion, dietary 0.5% and 1% AE are suggested for common carp feed formulation, as they stimulate the fish growth performance, immune system, and antioxidant status.

## Author contributions

Morteza Yousefi - Supervision, analysis of data; Saeed Zahedi - Drafting the manuscript; Miriam Reverter - Drafting the manuscript; Hossein Adineh - performed the experiments; Seyyed Morteza Hoseini - performed the experiments; Seyed Hossein Hoseinifar - Conceptualization; performed the experiments; Hien Van Doan - Funding acquisition, Conception and design of study; Ehab R. El-Haroun - revising the manuscript critically for important intellectual content.

## Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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