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RESEARCH ARTICLE

Efficacy of Dietary *Chenopodium album* Extract on Some Health Parameters, Digestive Enzymes and Growth Performance in Juvenile *Cyprinus carpio*

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ABSTRACT

The present study was conducted to investigate the efficacy of *Chenopodium album* aqueous methanolic extract supplementation on the immunological and haematological indices, digestive enzyme activity and growth performance of the common carp (*Cyprinus carpio*). *C. album* was added to a basal diet at the rate of 0 (CA0), 0.01 (CA0.01), 0.05 (CA0.05) and 0.1 g kg^{-1} (CA0.1), and *C. carpio* was fed this diet for 45 days. Respiratory burst activity was significantly increased in all experimental groups on days 15 and 30 compared to the control ($P < 0.05$). Lysozyme activity was significantly increased over all sampling times compared to the control except in CA0.1 ($P < 0.05$). Myeloperoxidase activities were significantly increased in all experimental groups compared to

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Introduction

Farmed fish are exposed to several infectious diseases that can reduce the fish yield (Erguig et al., 2015; Syahidah et al., 2015). The use of antibiotics and chemotherapeutic agents for controlling diseases can decrease the mortality and improve the growth rates; however, they are often an expensive and unhealthy way to treat any disease (Ferguson et al., 2010; Lauzon et al., 2010). The excessive use of antibiotics as immunostimulants has led to an increase in the antibiotic

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resistance by microorganisms, which causes problems when treating the microbial infections in an aquaculture setting (Bulfon et al., 2015; Cabello et al., 2016). Moreover, the antibiotic and chemotherapeutic residues can remain in fish tissues, which may threaten the health of human consumers and cause pollution of the aquatic environment (Biswas et al., 2010; Bulfon et al., 2015; Erguig et al., 2015; Syahidah et al., 2015).

The main goals in aquaculture include better growth with less cost and maintaining a good health status of the cultured fish to achieve farmed fish sustainability. In this regard, the medicinal plants have received immense attention in the aquaculture sectors as an alternative to chemotherapeutics and antibiotics as either added to fish diets or supplied as a dietary supplement (Bulfon et al., 2015; Citarasu, 2010; Reverter et al., 2014; Syahidah et al., 2015). The use of medicinal plants significantly increased various activities in aquaculture, such as antimicrobial, antistress, immunostimulants, growth promotion, appetite stimulation, improvement of gut flora, induced secretion of digestive enzymes and increased survival rate (Citarasu, 2010; Reverter et al., 2014; Bulfon et al., 2015; Van Hai, 2015). These activities promoted by medicinal plants are due to their active properties, such as glycosides, polysaccharide, alkaloids, saponins, terpenoids, flavonoids, phenolics, steroids, pigments, vitamins, proteins, fatty acids, minerals, and essential oils (Bulfon et al., 2015; Citarasu, 2010; Govind et al., 2012; Otunola et al., 2010). These are natural substances that act as powerful antioxidants against the reactive oxygen species generation (Asimi and Sahu, 2016; Shivashri, 2013; Sönmez et al., 2015) and stimulate the immune response against the pathogens (Ahmed et al., 2011; Erguig et al., 2015; Reverter et al., 2014). They also induce digestive enzymes secretion, thereby increasing appetite and food utilisation, which in turn promotes growth and improves the overall fish health (Bhavan et al., 2013; Poongodi et al., 2012). The use of medicinal plants or their derivatives in aquaculture could also reduce treatment costs associated with the side effects of chemotherapeutic and antibiotic use. Hence, their use in aquaculture has been successful because they are available, have fewer side effects, are cheaper, safer, biodegradable, biocompatible and eco-friendly (Bulfon et al., 2015; Madhuri et al., 2012; Mohamed and Abasali, 2010; Syahidah et al., 2015).

Lambs quarters (*Chenopodium album*) is a medicinal herb belonging to the Chenopodiaceae family that is generally distributed worldwide, especially in North America, Europe, Africa and Asia (Agrawal et al., 2014). Previous studies reported that the plant is extremely nutritious and acts as a rich source of proteins, carbohydrates, fats, fibre, vitamins, minerals and microelements (Agrawal et al., 2014; Al-Snafi, 2015; Choudhary and Sharma, 2014; Gqaza et al., 2013; Kaur and Shri, 2015; Sikarwar et al., 2013). The bioactive compounds of the plant are phenols, flavonoids, alkaloids, glycosides, lignins, saponins, tannins, carotenoids, xylosides, cinnamic acid and non-polar lipids (Agrawal et al., 2014; Kaur and Shri, 2015; Sikarwar et al., 2013). It is evident from the previous studies that a *C. album* extract demonstrated several pharmacological activities, including hepatoprotective (Pal et al., 2011; Vijay and Padmaa, 2011), antibacterial (Amjad and Alizad, 2012; Korcan et al., 2013), spasmolytic and analgesic (Ahmad et al., 2012), antimicrobial and antihelmintic (Nayak et al., 2010), antipruritic and antinociceptive (Dai et al., 2002), anticancer (Ankita and Chauhan, 2012), sperm-immobilising (Kumar et al., 2007), antiulcer (Nigam and Paarakh, 2015), anti-inflammatory (Usman et al., 2010) and antioxidant (Kumar and Kumar, 2009) activities. Moreover, it has been widely utilised as a traditional medicinal herb to treat various diseases, including cough, laxative, piles, rheumatism, abdominal pain, throat trouble and diseases of the blood, heart and spleen (Arora et al., 2014; Baldi and Choudhary, 2013; Kritikar and Basu, 1975).

C. carpio is a warm freshwater fish that belongs to the Cyprinidae family. It has an omnivorous feeding habit and is one of the most widely cultured freshwater fish species due to its fast growth and good meat quality; it is economic and marketable fish species, readily accepts artificial food and is highly tolerant to environmental fluctuations (Cao et al., 2013; Jalali et al., 2013; Shirali et al., 2012; Tokur et al., 2006). It is also considered as a good model for experimental studies (Alishahi et al., 2010; Pratheepa and Sukumaran, 2014); however, *C. album* is affected by diseases caused by pathogens that have an impact on their health status, which reduces their growth rate.

A majority of studies on *C. album* focused on its pharmacological activities and there are no studies regarding its use in aquaculture. The objective of the present study was to investigate the efficacy of a *C. album* aqueous methanolic extract on the immune response, haematological parameters, digestive enzymes and growth performance of common carp (*Cyprinus carpio*).

Materials and Methods

Fish and experimental design

A total of 480 *C. carpio* with an average body weight of 2.4 ± 0.1 g were obtained from a commercial fish farm in Antalya. Fish were allowed to acclimate for 2 weeks prior to the study during which they were fed a commercial diet twice a day. *C. carpio* were randomly divided into four treatment groups: *C. album* aqueous methanolic extract was added to their diet at a rate of 0% (control), 0.01% (CA0.01), 0.05% (CA0.05) and 0.1% (CA0.1). Each treatment was conducted in triplicate (12 aquaria 110 L each, 40 fish in each aquarium). Fish were fed the experimental diets twice daily for 45 days. The study was conducted over 45 days, and on every 15th day of the study, 3 fish from each aquarium were randomly selected, anaesthetised using 0.01 mg L^{-1} of phenoxyethanol and kidney samples were collected. At the end of the study, blood samples

and the anterior intestine were also collected. Intestines were preserved at −80 ℃.

Water quality parameters during the experimental period were as follows: dissolved oxygen 6.8–7.2 mg/L; pH 7.7–7.8; and water temperature 25–26 ℃. All experimental animals were maintained according to the relevant international guidelines. The study protocol was approved in advance by the local Ethics Committee for Animal Research Studies at the Kastamonu University (KUHADYEK-17.12.2017-2017.323).

Preparation of *C. album* extract

C. album were collected from Kastamonu province, North-west Turkey. An aqueous methanolic extract of *C. album* leaves was prepared following the method of Pakravan et al. (2012) with slight modifications (Bilen et al., 2016). Briefly, leaves were ground in a mechanical grinder and 50 g samples of the ground plant were added to 1 L of 40% methanol (Sigma-Aldrich). The mixture was allowed to stand at room temperature for 5 days and was shaken every day. After 3 days, the plant extract was filtered through filter paper (Whatman filter No. 1) and the filtrate was collected and evaporated in a rotary evaporator at 55–65 ℃. The final product was dissolved in distilled water and then sprayed on the commercial diet at level 0.01%, 0.05% and 0.1%.

Non-specific Immune Parameters

Head kidney cells were isolated from euthanised *C. carpio* according to the method of Kono et al. (2012) with slight modification, as follows. Briefly, the head kidney tissue was carefully removed and gently pushed through a 100 µm nylon mesh (John Stanier & Co., Whitefield, Manchester, UK) with an RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 5% foetal bovine serum (Invitrogen) and a 1% solution of 10,000 g mL^{-1} streptomycin plus 10,000 U mL^{-1} penicillin (Invitrogen) and was then pushed again through a 40 µm nylon mesh cell strainer (Becton, Dickinson & Co., Franklin Lakes, NJ, USA). The final homogenate was placed in a 3 mL falcon tube. Head kidney cell suspensions were pelleted in a centrifuge at 1800 rpm for 3 min at 4 °C. After centrifugation, the supernatant was collected to measure myeloperoxidase (MPO) by using 3,3,5,5-teteramethyl benzidine hydrochloride (Sigma-Aldrich) as a substrate (Sahoo et al., 2005). Lysozyme was measured by using a lyophilised *Micrococcus lysodeikticus* bacterial cell solution (Sigma-Aldrich) as a substrate (Bilen et al., 2014b). The pellet was suspended with 1 mL of the same medium directly to assay nitroblue tetrazolium (NBT; Sigma-Aldrich) activity, which was determined by the reduction of NBT as a substrate, according to the method described by Biswas et al. (2013).

Haematological Parameter Analyses

White blood cells (WBC) were determined using the Neubauer counting chamber (Ivanava, 1983), and the red blood cell (RBC) count, haemoglobin (Hb) and haematocrit (Hct) were measured according to the methods described by Blaxhall and Daisley (1973). Mean cell haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were calculated according to the formulae of Lewis et al. (2001).

Digestive enzyme activity

The anterior intestine was homogenised by a Potter Elvenhjem homogeniser on ice in cold double-distilled water (0.1 g L ml^{-1}) and centrifuged at 9000 × g for 20 min at 4 ℃. The resultant supernatant was removed and stored at −80 ℃ to test the digestive enzymes activity, as follows. Trypsin activity was determined following the method of Erlanger et al. (1961) by using benzoyl-dl-arginine-p-nitroanilide (Sigma-Aldrich) as a substrate. Amylase activity was determined by using 2% starch (Sigma-Aldrich) as a substrate according to Worthington (1991). Lipase activity was determined by the hydrolysis of 4-nitrophenyl myristate (Sigma-Aldrich), according to a method described by Gawlicka et al. (2000). The protein content of the intestine supernatants was evaluated following the method of Bradford (1976).

Growth Performance Parameters

Growth performance was calculated according to the following equations: weight gain (WG, %) = 100 × (final fish weight – initial fish weight)/initial fish weight); specific growth rate (SGR %/day) = 100 × (ln final fish weight) – (ln initial fish weight)/number of experimental days; feed conversion ratio (FCR) = feed intake (g)/weight gain (g); survival rate (SR, %) = final number of fish/initial number of fish × 100.

Statistical Analysis

The result was analyzed using SPSS software. One-way ANOVA and Duncan's multiple range tests were used to determine the significant differences between the groups. All results are expressed as mean ± SE and $P < 0.05$ was considered statistically significant.

Result and Discussion

Non-specific Immune Parameters

NBT Activity

NBT activity was significantly higher in the experimental groups compared to that of the control group at 15 days ($P < 0.05$). Differences were also observed between the

experimental groups (P < 0.05), and the highest value was recorded in CA0.1 (Table 1). Similar results were observed at 30 days, except in CA0.1 that was not significantly different from the control (P < 0.05). There was no significant difference between the experimental groups and the control at 45 days (P < 0.05).

Table 1. Effect of *Chenopodium album* extract supplemented diet on NBT activity in *Cyprinus carpio*.

Groups	Experimental period		
	15th day	30 th day	45 th day
CA0	0.35 ± 0.04[a]	0.87±0.08[a]	0.99±0.37[a]
CA0.01	0.45±0.07[b]	1.26±0.13[b]	1.01±0.22[a]
CA0.05	0.52±0.05[c]	1.39±0.11[c]	0.87±0.26[a]
CA0.1	0.58±0.07[d]	0.87±0.07[a]	1.07±0.20[a]

All data are means ± SE (n = 9); different superscript letters in the same column denote statistically significant differences (P < 0.05) between groups.

Lysozyme Activity

Changes in lysozyme activity in the kidneys at different experimental times were observed in *C. carpio* when fed a *C. album* extract-supplemented diet at different concentrations (Table 2). Lysozyme activity was significantly higher in the experimental groups compared to that of the control at 15 days (P < 0.05) and there was no difference between the experimental groups at 15 days. There was no significant difference between the experimental groups and the control at 30 days, except in CA0.1 that had a significantly lower lysozyme activity compared to that of the control (P < 0.05). There was no significant difference between the experimental groups and the control at 45 days (P < 0.05).

Table 2. Effect of *Chenopodium album* extract supplemented diet on lysozyme activity in the kidneys.

Groups	Experimental period		
	15th day	30 th day	45 th day
CA0	0.36±0.03[a]	0.27 ± 0.01[a]	0.28±0.01[a]
CA0.01	0.39±0.02[b]	0.27 ± 0.01[a]	0.29±0.04[a]
CA0.05	0.39±0.03[b]	0.26 ± 0.01[a]	0.27±0.02[a]
CA0.1	0.38±0.02[b]	0.25 ± 0.03[b]	0.28±0.01[a]

All data means ± SE (n = 9 fish); different superscript letters in the same column denote statistically significant differences (P < 0.05) between groups.

MPO Activity

A *C. album* extract-supplemented diet significantly enhanced the MPO activity in the kidneys in all experimental groups and at all experimental periods (days 15, 30 and 45) compared to that of the control (P < 0.05; Table 3). There were significant differences between the experimental groups at 15 days (P < 0.05) and no significant difference between CA0.01 and CA0.05 at 30 days (P < 0.05). There was no significant difference between the experimental groups at 45 days (P < 0.05).

Table 3. Effect of *Chenopodium album* extract supplemented diet on myeloperoxidase activity in the kidneys.

Groups	Experimental period		
	15 days	30 days	45 days
CA0	116.55±9.15[a]	125.90±6.87[a]	224.15±9.89[a]
CA0.01	129.97±5.51[b]	138.351±6.01[b]	252.08±6.99[b]
CA0.05	123.51±5.95[c]	140.26±6.15[b]	247.25±21.28[b]
CA0.1	126.58±7.45[d]	132.79±11.42[c]	241.43±13.59[b]

All data means ± SE (n = 9); different superscript letters in the same column denote statistically significant differences (P < 0.05) between groups.

Haematological Parameters

The effects of a C. album extract-supplemented diet on the haematological parameters at the end of the feeding trial (45 days) are summarised in Table 4. WBC counts were significantly decreased in the experimental groups compared to that of the control (P < 0.05) and differences were also observed between the experimental groups, where the lowest WBC value was observed in CA1. There was no significant difference in the RBC count, Hb, Hct, MCH, MCV or MCHC between the experimental groups compared to those of the control (P < 0.05).

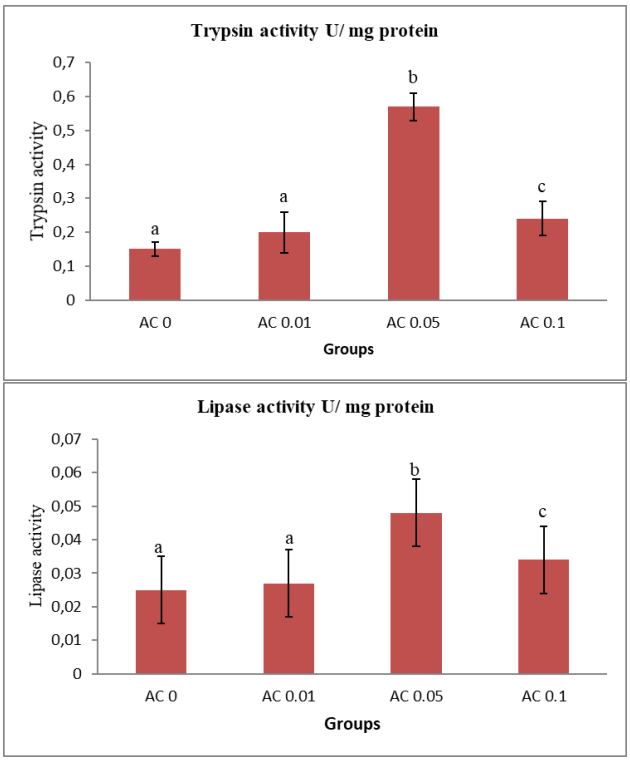
Table 4. Effect of *Chenopodium album* extract supplemented diet on haematological parameters at the end of the feeding trial (45 d).

Groups	WBC x 10^7	RBC x 10^6	HG (g/dl)	HCT (%)	MCV (pg)	MCH	MCHC (g/l)
CA0	36.17±1.17^a	1.54±0.09^a	6.58±0.75^a	24.33±1.02^a	156.82±3.40^a	41.98±4.93^a	266.16±7.22^a
CA0.01	33.17±1.47^b	1.64±0.13^a	6.37±0.82^a	24.96±2.18^a	152.7±3.61^b	39.9±4.55^a	267.17±9.02^a
CA0.05	26.83±0.75^c	1.66±0.13^a	6.67±0.76^a	26.12±1.15^a	154.28±3.73^a	40.08±6.08^a	264.66±6.31^a
CA0.1	20.33±1.86^d	1.48±0.16^a	5.97±0.77^a	22.40±2.39^a	155.88±2.71^a	40.2±1.88^a	268.5±8.87^a

All data means ± SE (n = 9); different superscript letters in the same column denotes statistically significant differences (P < 0.05) between groups.

Digestive Enzyme Activity

The effect of a *C. album* extract-supplemented diet on the digestive enzyme activity is presented in Figure 1. Trypsin activity was significantly increased in CA0.05 and CA0.1 compared to that of the control (P < 0.05), whereas it did not differ between CA0.01 and the control. Similar results were observed in lipase activity, which significantly increased in CA0.05 and CA1 compared to that of the control (P < 0.05) and CA0.01 was not different from the control. The highest trypsin and lipase activity was recorded in CA0.05. Amylase activity was significantly improved in all experimental groups compared with that of the control (P < 0.05) and the highest activity was observed in CA0.01.



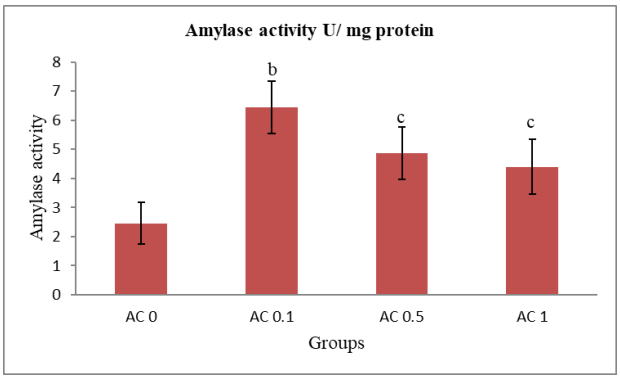


Figure 1. Digestive enzyme activity in *Cyprinus carpio* fed a diet supplemented with different concentrations of *Chenopodium album* extract. CA0, CA0.01, CA0.05 and CA0.1 indicate a *C. album* extract concentration of 0, 0.01, 0.05 and 0.1 g kg^{-1}, respectively. Different letters above the bars (mean ± SE; n = 3) denote significant differences among the trial groups (P < 0.05).

Growth Performance Parameters

Changes in the growth performance parameters at the end of the feeding trial (45 d) are summarised in Table 5. Final weight and weight gain were significantly enhanced in CA0.01 and CA0.1 compared to those of the control (P < 0.05). FCR was not significantly different between all experimental groups compared to that of the control (P < 0.05). Alternatively, SGR was significantly increased in all experimental groups compared to that of the control (P < 0.05). In addition, SR was significantly improved in all experimental groups compared to that of the control (P < 0.05).

Table 5. Effect of *Chenopodium album* extract supplemented diet on the growth *Cyprinus carpio* at the end of the feeding trial (45 d).

All data are expressed as means ± SE, different superscript letters in the same column denote statistically significant differences ($P < 0.05$) between groups.

Groups	Growth performance parameters					
	Initial weight (g)	Final weight (g)	Weight gain (%)	FCR	SGR	SR (%)
CA0	2.64±0.02[a]	3.68±0.32[a]	39.30±13.50[a]	2.14 ± 0.58[a]	0.76 ± 0.19[a]	65[a]
CA0.01	2.63±0.03[a]	5.05±0.65[b]	92.36±27.32[b]	1.42 ± 0.45[a]	1.47 ± 0.28[b]	81.25[a]
CA0.05	2.63±0.08[a]	4.75±0.39[a]	80.16±9.31[a]	1.56 ± 0.72[a]	1.34 ± 0.18[b]	85[a]
CA0.1	2.64±0.06[a]	5.44±0.23[b]	105.73±13.55[b]	1.03 ± 0.05[a]	1.65 ± 0.09[b]	85[a]

Fish have specific (adaptive immune system) and non-specific (innate immune system) defences to protect themselves against pathogens (Pratheepa and Sukumaran, 2014). Non-specific defence is the primary action when fish are infected with pathogens (Dügenci et al. 2003). The major components of non-specific defence include granulocytes, monocytes, macrophages and humoral elements such as lysozymes that complement the system (Galina et al., 2009; Magnadóttir, 2006). Immunostimulants are substances that stimulate the immune response either specifically or non-specifically, providing more resistance to various diseases (Yin et al., 2006).

In the present study, the efficacy of a C. album extract as an immunostimulant was assessed by a non-specific immune parameter index, especially NBT activity (Muñoz et al., 2000). In this study, the NBT activity was significantly increased in the experimental groups on days 15 and 30 compared to that of the control and no significant differences were observed on day 45. This increase may be due to the phytochemical constituents of the extract. Similar results were observed in Koi carp (Cyprinus carpio) (Bilen et al., 2014c), and rainbow trout (Oncorhynchus mykiss) (Bilen et al., 2011) after supplementation with a tetra (Cotinus coggygria) extract. Moreover, Park and Choi (2012) reported that Nile tilapia (Oreochromis niloticus) when fed a diet supplemented with mistletoe (Viscum album coloratum) revealed an increase in the NBT activity. Kim and Lee (2008) reported that the NBT activity in juvenile olive flounder (Paralichthys olivaceus) was

enhancement in the lysozyme activity was also recorded in juvenile P. olivaceus when fed a diet supplemented with E. cava (Kim and Lee, 2008) and in O. niloticus supplemented with V. album coloratum (Park and Choi, 2012), Sophora flavescens (Wu et al., 2013) and a Chinese herbal mixture composed of astragalus, angelica, hawthorn, liquorice root and honeysuckle (Tang et al., 2014).

MPO is an enzyme that is secreted by macrophages and neutrophils of several fish species. It utilises hydrogen peroxide to oxidise various substrates (Hampton and Kettle, 1996) and is one host defence against the invading pathogens (Rosen et al., 2002). Moreover, macrophages and neutrophils are stimulated during inflammation (Grattendick et al., 2002; increased after being fed a diet supplemented with kelp (Ecklonia cava). Haghighi and Rohani (2013) reported an elevation in superoxide anion production in O. mykiss after being fed a diet supplemented with powdered ginger (Zingiber officinale). Moreover, Devasree et al. (2012) demonstrated that a water soluble extraction of parijat (Nyctanthes arbortristis) leaves enhanced NBT in the Mozambique tilapia (Oreochromis mossambicus). Recently, Bilen et al. (2016) reported an increased NBT level in O. mykiss when fed a diet supplemented with a methanolic extract of nettle (Urtica dioica).

Lysozyme is an important humeral non-specific defence mechanism enzyme that provides defence against microbial invasion (Evelyn, 2002). The bactericidal action of this enzyme involves the hydrolyzation of the peptidoglycan layers of the bacterial cell wall, which produces cell lyses that prevent the colonisation of micro-organisms (Saurabh and Sahoo, 2008). In addition, it induces antibacterial activity in the presence of a complement (Harikrishnan et al., 2012). The present study recorded a significantly increased lysozyme activity in the experimental groups compared to that of the control on day 15 and no significant changes were observed between the experimental groups and that of the control on days 30 and 45, except in CA0.1. Similar results were observed in C. carpio when fed a diet supplemented with methanolic extracts of C. coggygria (Bilen et al., 2014a). Jian and Wu (2004) recorded an elevated lysozyme level in common carp when fed a diet supplemented with various Chinese herbal extracts. An
Lau et al., 2005). In the study, MPO activity was significantly improved in all experimental groups in all experimental periods (15, 30 and 45 days) compared to that of the control. The long-term efficiency of C. album extract was also noted in this study, which may provide better protection. In line with our results, some previous studies reported that medicinal plants improved the MPO activity in different fish species. Kim and Lee (2008) demonstrated an enhanced MPO activity in juvenile P. olivaceus when fed a diet supplemented with E. cava. Similarly, Alexander et al. (2010) observed an elevated MPO activity in O. mossambicus when fed a diet supplemented with Tinospora cordifolia leaves. Bilen et al. (2014a) found an increased MPO level in C. carpio when fed a diet supplemented with C. coggygria extract and in goldfish (Carassius auratus)

after being fed a diet supplemented with a U. dioica methanolic extract (Bilen et al., 2014c).

Haematological parameters can be useful to detect the abnormal changes in fish health. Haematological characteristics act as an effective and sensitive index to detect physiological and pathological changes in fish as a response to the stress conditions such as changes in water quality (Alwan et al., 2009; Fernandes and Mazon, 2003). In the present study, haematological indices revealed that C. carpio when fed a diet supplemented with C. album extract revealed significantly decreased WBC counts and there were no significant differences in the RBC count, Hb, Hct, MCH, MCV or MCHC between the experimental and control groups. Presumably, the supplementation of C. album extract at concentrations of 0.01, 0.05 and 0.1% does not have a negative effect on the haematological parameters of C. carpio, except for the negative effect on WBC count. This suggests that the C. album extract concentrations used in this study were at a non-toxic level. This is in accordance with the findings of Bilen et al. (2014a) who found no significant changes in Hb, Hct, MCV, MCH or MCHC in C. carpio when fed a diet supplemented with different concentrations of C. coggygria extract. Asadi et al. (2012) indicated that the oral administration of 0.01% or 0.1% of watercress (Nasturtium nasturtium) extract caused no significant difference in the RBC or WBC counts, Hct, MCV or MCH values in O. mykiss when compared with the control, whereas Hb and MCHC values were significantly increased in O. mykiss when fed diets enriched with 1% N. nasturtium extract when compared with the control. Conversely, Alishahi et al. (2010) found that C. carpio when fed a diet containing aloe (Aloe vera) revealed a significant increase in the WBC counts and no significant changes in RBC or PCV compared with the control. Babahydari et al. (2014) also found that C. carpio when fed a diet containing a 2% wood betony (Stachys lavandulifolia) extract, enhanced Hb and Hct. Labh and Shakya (2016) revealed a significant enhancement in the haematological parameters, such as WBC, RBC, Hb, HCT, MCH, MCV and MCHC in C. carpio when fed a diet supplemented with ethanolic extract of lapsi fruits. Mishra and Gupta (2017) reported that aqueous and alcoholic extracts of Eclipta alba roots, stems and leaves significantly improved RBC,WBC and Hb in the walking catfish (Clarias batrachus).

Digestive enzymes play an important role in the digestion of proteins, lipids and carbohydrates; they facilitate the absorption of digested materials through the intestinal wall for fish growth and reproduction (Furne et al., 2005). Fish digestive enzyme activities are affected by several factors including diet and feeding habits (Debnath et al., 2007; Santigosa et al., 2008), fish age, growth stage, pH and temperature (Jun-sheng et al., 2006), specific fish species and digestive system structure (Al-Saraji and Nasir, 2013). In the present study, the activity of trypsin and lipase was significantly increased in CA0.05 and CA0.1 compared with the control group and no significant changes were recorded in CA0.01. The highest trypsin and lipase activity was recorded in CA0.05. Amylase activity was significantly improved in all experimental groups when compared with the control and the highest activity was observed in CA0.01. The improvement in digestive enzyme activity may be due to the active principles of this herb, which has the ability to stimulate the endocellular digestive enzyme activity in fish and extracellular enzyme activity by modulating the intestinal microflora. Likewise, some previous studies demonstrated that medicinal herbs or their derivatives exerted enhanced digestive enzyme secretions. Sankar et al. (2011) demonstrated enhanced lipase, amylase and protease activities in black tiger shrimp (Penaeus monodon) treated with a methanolic extract of Ricinus communis at different concentrations compared to the control. Ojha et al. (2014) also reported that a dietary ethanolic extract of Mucuna pruriens seeds at different concentrations resulted in a significant increase in the intestinal amylase, protease and lipase secretions of carp (Labeo rohita). Fereidouni et al (2015) reported that a garlic extract (Allium sativum) supplement in the basal diet of Mugil cephalus larvae at different concentrations (0.5, 1 and 3%) for 30 days resulted in a significant increase in the protease, amylase and lipase activities. Alternatively, Rahimi et al. (2015) demonstrated that Mesopotamichthys sharpeyi fingerlings that were fed a normal diet mixed with Z. officinale extract at different doses did not reveal any significant change in the trypsin activity but it did improve the amylase activity in the intestine compared to the control. Djauhari et al. (2017) reported that a prebiotic from a sweet potato (Ipomoea batatas) extract supplemented in the diet of C. carpio improved the protease activity and there was no significant difference in the amylase and lipase activities compared to the control.

At the end of the study, growth performance, especially the final weight and weight gain, were significantly enhanced in CA0.01 and CA0.1 compared to the control. There was no difference in FCR or SR among all experimental and control groups. Alternatively, SGR was significantly increased in all experimental groups compared to the control. The increase in the growth performance, such as final weight, weight gain and SGR, was presumably due to the stimulation of gastrointestinal digestive enzyme secretions or through modulating the beneficial intestinal microflora that play an important role during the secretion of digestive enzymes. The increase in digestive enzymes in the present study supports the results of the increase in growth rates. This is in accordance with the previous studies who demonstrated that the active herbal properties in fish diets stimulate the secretion of digestive enzymes. This would induce appetite and eventually elevate food consumption, resulting in increased growth rates of farmed fish. Therefore, these herbs have potential as a feed additive for the sustainable development of aquaculture (Bhavan et al., 2013; Radhakrishnan, et al., 2013). In C. carpio, several investigators demonstrated that A. vera (Mahdavi et al., 2013), S. lavandulifolia (Babahydari et al., 2014), Althaea officinalis (Fallahpour et al., 2014) and Z. officinale (Ghadikolaei et al., 2017) plant extracts significantly improved

their growth performance.

Conclusion

The results obtained from the present study indicate that supplementation of a methanolic extract of *C. album* improved the non-specific immune parameters of *C. carpio.* In addition, a *C. album* extract enhanced the digestive enzyme activity and certain growth performance parameters, which might be a result of the antioxidant properties of the plant.

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