

Original Article

Effects of different levels of chamomile (*Matricaria recutita*) extract on growth and antioxidant parameters of zebra fish (*Danio rerio*)

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Abstract: An eight-week feeding trial was conducted to investigate the effects of different levels of dietary chamomile (*Matricaria recutita*) aqueous extract (CAE) on growth performance and antioxidant status of zebra fish, *Danio rerio*. Fish (initial weight of 0.20±0.02 g) were stocked at 12 tanks (50 fish per tank) and fed with a basal diet (32% protein and 8% lipid) supplemented with 0 (control), 0.5, 1, and 2% CAE in triplicate groups for 8 weeks. The results showed that fish fed 2% CAE had a significantly higher growth and biomass gain compared to the control group ($P<0.05$). Condition factor had a significant increase in 2% CAE treatment compared with other treatments ($P<0.05$). There were no significant differences in catalase, glutathione peroxidase, and superoxide dismutase activity amongst treatments ($P<0.05$). Finally, it seems the use of a 0.5-2.0% CAE can be effective in improving the growth and antioxidant system of zebra fish, *D. rerio*.

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Introduction

Under intensive and super-intensive fish culture systems, fish are exposed to a variety of stresses such as poor water quality, high density, and bad handling, which play a main role in fish disease. Hence, preventing disease by chemical drugs is crucial to deal with the high cost of treatments (Goda et al. 2008; Volpatti et al., 2013). Indeed, in intensive systems, improved nutritional condition plays an important role in promoting growth and maintaining fish health and welfare (Lee et al., 2015; Abdel-Latif et al., 2020a, b; Abdel-Tawwab et al., 2020a, b). Therefore, the most important challenge of the aquaculture industry to date is improving the diets formulated for optimal growth and promotion of aquatics health (Akrami et al., 2010). Nutritional manipulations and the addition of various supplements can increase fish health and safety (Lee et al., 2015; Abdel-Latif et al., 2020a, b; Abdel-Tawwab et al., 2020a, b). So far, many studies have been done on food additives such as plant extracts, immune stimulants, enzymes, prebiotics, probiotics, and organic acids as alternatives to

antibiotics (Lee et al., 2015).

Valuable herbal medicines have a special place in the treatment because of some features as economic value, low-cost production, non-destructive effects on the environment, few side effects compared to chemical drugs, lack of relative resistance to pathogens, uniqueness in the treatment of some diseases, and the existence of various clinical experiences in this field (Ghasemi Pirbaluti et al., 2011). Herbal chemicals, including alkaloids, flavonoids, terpenes, phenolic compounds, pigments, and steroids have positive effects comprising increased growth, reduced stress, stimulating the immune system, and antimicrobial and antioxidant properties in fish (Abdel-Razek et al., 2019). These medicinal resources not only can be used as new drugs and bioactive compounds in increasing fish production, product quality, and fish health, but also they are eco-friendly due to their plant origin (Chakraborty et al., 2014).

Chamomile recutita is known as German chamomile (McKee and Bloomberg, 2006; Harbren et

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al., 2009). Medicinal and health-promoting properties of chamomile are mainly attributed to two groups of bioactive compounds present in chamomile flowers, which include essential oil (essence) and polyphenols. Flavonoids are the major polyphenols in chamomile that have antioxidant properties (Srivastava and Gupta, 2009). The use of antioxidants reduces the rate of oxidation. The detection of toxicity and carcinogenicity of synthetic antioxidants has drawn the attention of scientists to the exploration of natural antioxidants (Ghanavati et al., 2008). In addition to flavonoids, coumarins, terpenoids, and bisabolol are the most important compounds of chamomile flowers that are medically considerable. Chamomile extract has antioxidant, disinfectant, antibacterial, sedative, antispasmodic, anti-diabetic, analgesic and anti-cancer activities, and protective effects on the gastrointestinal tract, nervous system and immunogenic modification (Izadi et al., 2013).

Over the past decades, the global trade in ornamental fish has significantly grown (Ranjan et al., 2017). One of the farmers' favorite ornamental fish is zebra fish (*Danio rerio* Hamilton, 1822) (Hill et al., 2011). Because of simplicity of reproduction method and omnivorous diet, zebra fish has known as an experimental model in laboratories to answer important research questions in various fields like biomedical, genetics, growth biology, pharmacology, toxicology, and evolutionary physiology (Vascotto et al., 1997; Sundin et al., 2019). Regarding the importance of zebra fish in aquaculture and its value as a research model on the other hand, this species was selected as a target fish in the present study. Therefore, the present study was aimed to evaluate the effect of chamomile aqueous extract on growth and antioxidant parameters of zebra fish, *D. rerio*.

Materials and Methods

Experimental conditions: The experiment was conducted in the Gorgan-Mahi Company (Gorgan, Golestan Province, Iran). Fish were stocked in experimental aquariums (40x25x25 cm) and to adapt, they were fed with a control diet (32% protein and 8% lipid) for two weeks. After that, 600 fish (with a mean

weight of 0.20 ± 0.02 g) were randomly assigned into 12 aquaria, 50 fish per aquarium with three replicates per treatment. Fish were fed with the experimental diets, until satiety, three times a day (08:00, 12:00, and 16:00 h) for 8 weeks. During the experiment, the water temperature, dissolved oxygen, and pH were recorded at 23°C, 6.5 mg. l⁻¹, and 7-8.5, respectively, by portable apparatus (Hach HQ40d, Loveland, Colorado, USA).

Preparation of chamomile extract and experimental diets:

As the dietary additive, a commercially available chamomile powder (Giahine Industrial Co., Ltd. Isfahan, Iran) was used. First, 10 g of chamomile powder was boiled in 200 ml of the boiled water for 2 hours (Wu et al., 2010), then, it was filtered by filter paper. The required concentrations, including 0 (control), 0.5, 1.0, and 2.0% was added to the control diet to prepare treatments. The control diet was formulated according to Table 1 containing 32% protein and 8% fat (O'Brine et al., 2015). After that, the ingredients were powdered and passed through a mesh (0.5 mm), and mixed thoroughly well and then, water, soya oil, and chamomile aqueous extract (in exchange the water) were added to the mixture. The obtained dough was passed through a mesh (1 mm) to form sticks which were allowed to dry for 24 h (at room temperature) and finally stored at 4°C until use.

Growth performance: For growth assessment, individuals (n15/tank) were randomly selected to measure the weight and length, at least 12 h after the last feeding (Akrami et al., 2013, 2015). Weight gain (WG; g) and (WG; %), specific growth rate (SGR; % day⁻¹), condition factor (CF), survival rate (%), and biomass gain (BG; g) were calculated on the basis of the following formulas (Htun-Han, 1978; Tacon, 1990; Watanabe et al., 1993; Kamali-Sanzighi et al., 2019):

$$WG (g) = FW - IW$$

$$WG (\%) = 100 (FW - IW) / IW$$

$$SGR = 100 (\ln FW - \ln IW) / T$$

$$CF = 100 FW / L^3$$

$$SR = 100 (\text{Number of fish at the end of the experiment} / \text{Number of fish at the beginning of the experiment})$$

Table 1. Foodstuffs and chemical composition of the control diet (% dry matter basis).

Ingredients	Amounts (%)
Fishmeal ^a	21
Soybean meal ^b	25
Wheat flour	43.6
Meat meal ^c	5
Soybean oil	4
Mineral mix ^d	0.5
Vitamin mix ^e	0.5
Lysine ^f	0.2
Methionine ^g	0.2
Total	100
Proximate composition (%)	
Crude protein	32.40
Crude lipid	8.63
Crude fiber	3.13
Crude ash	5.66

^a 66.21% protein; 13.59% lipid; ^b Gorgan Soya Co., Gorgan, Iran (45% protein; 1.5% lipid); ^c 40% protein; 22.61% lipid; ^d The premix provided following amounts per kg of diet: Mg: 350 mg; Fe: 13 mg; Co: 2.5 mg; Cu: 3 mg; Zn: 60 mg; NaCl: 3 g; Dicalcium phosphate: 10 g; ^e The premix provided following amounts per kg of feed: A: 1000 IU; D3: 5000 IU; E: 20 mg; B5: 100 mg; B2: 20 mg; B6: 20 mg; B1: 20 mg; H: 1 mg; B9: 6 mg; B12: 1 mg; B4: 600 mg; C: 50 mg; ^f Sigma, St. Luise, MO, USA; ^g Mad Tiour Co., Sanandaj, Iran.

BG = FB – IB.

Sampling and measurement of antioxidant status: At the end of trial, due to the small size of fish, the activity of antioxidant enzymes was measured using the whole body. For this purpose, 3 fish were taken from each tank and anesthetized with clove powder (500 ppm). After that, the head and fins were separated and homogenized (Holbech et al., 2001) and then centrifuged (10,000 rpm) in 3 stages at 4°C. The high phase was finally used to measure antioxidant activities. The superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) enzymes were evaluated using commercial kits (Navand, Tehran, Iran). SOD level was estimated by measuring the rate of cytochrome C reduction (McCord and Fridovich, 1969), Catalase level was measured based on Góth (1991) method by measuring the rate of hydrogen peroxide decomposition, and glutathione peroxidase level was measured by estimating the glutathione oxidation rate (Yousefi et al., 2018).

Statistical analysis: Growth performance and

antioxidant status were analyzed using one-way analysis of variance (One-Way ANOVA) with a probability of 5% error in a completely randomized experimental design. The significance of the difference between the mean of different factors, with Duncan's follow-up and multi-domain tests was done at $P < 0.05$ using SPSS 13 software.

Results

The effect of the dietary chamomile aqueous extract levels on weight gain (WG g), percentage of weight gain (WG%), specific growth rate (SGR), biomass gain (BG), condition factor (CF), and survival rate (SR) are shown in Table 2. Fish fed with the 2% chamomile diet demonstrated a significantly higher BG and WG (g) compared to fish fed with the 1% chamomile and control group ($P < 0.05$). WG (%) and SGR significantly improved as dietary chamomile levels increased from 0.5 to 2% ($P < 0.05$). Although there was no significant difference of CF among the control, 0.5, and 2% treatments, CF recorded a significant increase in 2% treatment compared to 1%

Table 2. Ingredients and chemical composition of the experimental diets (% dry matter basis) containing different levels of chamomile aqueous extract.

Parameter	Dietary chamomile extract (%)			
	Control	0.5	1	2
IW (g)	0.21±0.003 ^a	0.20±0.012 ^a	0.18±0.006 ^a	0.21±0.016 ^a
FW (g)	0.30±0.01 ^b	0.34±0.01 ^{ab}	0.29±0.01 ^b	0.36±0.01 ^a
WG (g)	0.09±0.015 ^c	0.13±0.006 ^{ab}	0.11±0.005 ^{bc}	0.15±0.005 ^a
WG (%)	42.13±7.07 ^b	65.66±1.01 ^a	59.96±1.62 ^a	71.26±6.53 ^a
SGR (% day ⁻¹)	0.58±0.08 ^b	0.84±0.01 ^a	0.79±0.01 ^a	0.89±0.05 ^a
BG (g)	4.5±0.76 ^c	6.67±0.33 ^{ab}	5.5±0.28 ^{bc}	7.5±0.28 ^a
CF	1.02±0.05 ^{ab}	1.08±0.02 ^{ab}	0.99±0.04 ^b	1.14±0.03 ^a
SR (100)	100	100	100	100

Data are represented as mean±SE. The values of means in columns were significantly different ($P<0.05$).

Table 3. Changes in activities of glutathione peroxidase (GPx), sodium dismutase (SOD), and catalase (CAT) of zebra fish fed diets containing dietary chamomile extract for 8 weeks.

Parameter	Dietary chamomile extract (%)			
	Control	0.5	1	2
GPx (U/mg)	4.89±0.07 ^a	4.95±0.15 ^a	4.92±0.02 ^a	5.05±0.21 ^a
SOD (U/mg)	4661.22±375.51 ^a	3416.32±48.98 ^a	3181.62±459.18 ^a	3010.20±606.11 ^a
CAT (U/mg)	0.36±0.024 ^a	0.35±0.008 ^a	0.35±0.041 ^a	0.37±0.059 ^a

Data are represented as mean ± SE. The values of means in columns were significantly different ($P<0.05$).

one ($P<0.05$). survival rate also was 100% in all groups.

After the 8-week trial, the glutathione peroxidase (GPx) and catalase (CAT) activities improved in those fish fed with chamomile 2% diet over the control group ($P>0.05$). However, there were no significant differences between the treatments compared with the control group (Table 3). Also, there was no significant difference in activity of superoxide dismutase in all treatments compared to the control group ($P<0.05$) (Table 3).

Discussions

The present study confirmed that short feeding on a diet rich in chamomile (2%) led to a significant increase in growth indices of zebra fish. This result is consistent with the previous studies in Nile tilapia (*Oreochromis niloticus*) (Abdel-Wahhab et al., 2001; Abdel-Maksoud et al., 2002; Khalafalla et al., 2009; Zaki et al., 2012) and African catfish (*Clarias gariepinus*) (Abdelhadi et al., 2010). In addition, administration of different levels of chamomile powder (*Matricaria chamomilla* L.) showed a

significant increase of biomass and improved growth performance in hybrid red tilapia (*Oreochromis* sp.) (Nordin, et al., 2017). Medicinal plants contain phytochemicals, including alkaloids, flavonoids, pigments, phenols, terpenes, steroids, polysaccharides, and essential oils which involved in different activities such as growth, nutrition, immune and anti-stress stimulation, and antimicrobial function (Citarasu et al., 2010; Chakraborty and Hancz, 2011). Additionally, bioactive compounds such as polysaccharides and terpenes regulate the immune system and act as prebiotics, which increase growth in fish (Wang et al., 2009; Ringo et al., 2010). Also, monoterpene phenols, including thymol and carvacrol act as growth promoters in Channel catfish (*Ictalurus punctatus*) (Zheng et al., 2009). As potential bioactive substances of chamomile i.e. alkanoids, coumarins, and triterpenoids can affect the digestion process and increase the activity of digestive enzymes to improve digestion, food absorption, and growth in fish (Ell, 1965; Samy et al., 2008; Immanuel et al., 2009; Citarasu et al., 2010; Kaleeswaran et al., 2010; Abdel-Tawab et al., 2010; Hashemi and Davoodi, 2011).

Moreover, essential fatty acids, including linoleic, linolenic and arachidonic acids in the composition of plant additives, were detected to be essential to growth (Murray et al., 1991; Abdel-Latif et al., 2004).

In the present work, chamomile treatment's failure to significantly affect antioxidant activities may be explained by the method of extract preparation, i.e. boiling chamomile in boiled water. It is likely that heating chamomile is accountable for not affecting antioxidant activities, since Abaee et al. (2018) documented that although the polyphenols of chamomile aqueous extract were resistant to heat at temperatures of 70-120°C for 5 and 15 min, the antioxidant activities significantly decreased, even at a temperature of 70°C for 5 min. Similarly, Davidov-Pardo et al. (2011) found that the antioxidant activity of grape seed extract decreased by heating. Svehlikova et al. (2004) also reported that while increasing temperature, thermal degradation of flavonoids increased in chamomile extract. Chamomile's antioxidant activity is mainly attributed to flavonoids and especially to 7-glycoside apigenin and its derivatives. Therefore, the destruction of flavonoids decreases antioxidant activity. At higher heat, more damage is introduced to the structure of flavonoid which decreases the antioxidant activities (Srivastava et al., 2009). Comparing the findings related to the total content of polyphenol compounds and antioxidant activity revealed that although heating chamomile extract does not affect the total content of phenolic compounds, it reduces the antioxidant activity. The likely reason for this phenomenon was attributed to the fact that not all phenolic compounds of the extract behave similarly under heating conditions (Abaee, 2018). In previous works, the content of phenolic compounds in galangal - a spice in the ginger family – increased by heating at 100°C for 10-30 min, while their values decreased in red pepper. Heating degraded and hydrolyzed some flavonoids and converted their glycoside forms to aglycone ones (Ayusuk et al., 2009). In addition to the aforementioned points, other factors such as method of fish sampling, using the whole body instead of blood, doses used, short duration of administration,

and environmentally suitable conditions may be reasons for not observing differences in antioxidant parameters in the present study.

In conclusion, as a considerable medicinal herb, chamomile requires further investigation to be determined the effective doses of chamomile to improve the antioxidant defense. The results indicated that chamomile aqueous extract had a significant, potential efficacy on growth parameters in zebra fish. So, with respect to the absence of adverse effects of chamomile on the activity of antioxidant enzymes and its positive effect on growth performance, the use of this supplement in the diet of zebra fish is recommended.

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