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Original Article

Effects of dietary *Cirsium arvense* essential oil on growth performance, digestive enzymes, and antioxidant parameters in rainbow trout, *Oncorhynchus mykiss*

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Abstract: The present study assessed the effects of dietary supplementation with *Cirsium arvense* essential oil (CAEO) on growth and antioxidant responses in rainbow trout, *Oncorhynchus mykiss*. Four diets were prepared containing 0 (CTR), 2 (CAEO2), 4 (CAEO4), and 6 (CAEO6) mL/kg CAEO and offered to fish (31.31±0.15 g) in triplicate for 60 days. Dietary CAEO significantly increased growth, feed efficiency, intestinal chymotrypsin, trypsin, serum superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Dietary CAEO significantly decreased serum malondialdehyde. The highest growth rate, gut chymotrypsin, and serum GPx were observed in CAEO6, whereas the highest feed efficiency, intestinal trypsin, serum SOD, and CAT, and lowest serum MDA were observed in CAEO4 and CAEO6. In conclusion, CAEO can be used as a feed additive for the aquaculture of rainbow trout, as it improves the production and antioxidant of the fish at a concentration of 6 mL/kg.

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Introduction

Thanks to its fast growth rate and capacity to tolerate intensive rearing culture, rainbow trout, *Oncorhynchus mykiss*, is an important species in the aquaculture industry. According to FAO (2023), the total annual production of rainbow trout worldwide exceeded 952,000 metric tons in 2021. This is around a 50% increase in trout production within a decade (2001-2021). However, disease outbreaks are one of the major obstacles to decreasing global trout production (Duman et al., 2023). Opportunistic pathogens are common agents of disease outbreaks in trout farms when fish are reared under unfavorable conditions and have weak immune defenses (Hoseini et al., 2022a). Prophylactic practices are superior to curing treatments because of economic and environmental considerations. Besides biosecurity, which reduces the risk of pathogens' entrances to trout farms, boosting the welfare of the fish helps prevent disease outbreaks (Taheri Mirghaed et al., 2023).

Functional feeds are useful tools to boost fish health and welfare. They provide micronutrients, bioactive compounds, or useful microorganisms that target different fish organs and physiological pathways, leading to diverse benefits such as growth promotion, stress resistance, immune-boosting, and antioxidant capacity elevation (Encarnação, 2016). The application of functional feeds in aquaculture has gained significant attention because most are multifunctional and simultaneously improve growth performance and fish welfare.

Essential oils are one of the classes of feed additives known best for their antioxidant effects in fishes. They also showed growth-promoting effects in fish, even at low concentrations (Souza et al., 2019). Essential oils contain various bioactive compounds, attracting aquaculturists due to their natural origin and safety. Numerous studies have approved the Functionality and benefits of herbal essential oils [see Sutili et al. (2018) for review].

Abundant in the northern hemisphere, *Cirsium arvense* naturally grows in grasslands and contains an

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essential oil (Tiley, 2010). α-Bisabolol and δ-Cadinene are two main bioactive compounds of *C. arvense* essential oil (CAEO) (Dehjurian et al., 2017). These sesquiterpenes were found to have antioxidant, anti-microbial, anti-cancer, anti-inflammatory, and neuroprotective effects in humans and animals, either *in vitro* or *in vivo* (Pérez-López et al., 2011; Kim et al., 2013; Hui et al., 2015; Eddin et al., 2022). Despite these potentials, there is no data regarding the benefits of CAEO in fish. Thus, this study aimed to investigate the benefits of dietary CAEO on growth performance, digestive enzymes, and antioxidant capacity in rainbow trout.

Materials and Methods

CAEO extraction: CAEO was extracted using a Clevenger apparatus. First, *C. arvense* was dried by a blower and crushed using an electric mill. Then, the mill was mixed with water at a 1:3 ratio (w:v) and heated for five hours in a Clevenger apparatus. The volume of extracted CAEO was determined and transformed to mass based on its density (0.858 g/mL). Sodium sulfite was used to dry CAEO, and the dried CAEO was kept at 2°C until use.

Preparation of diets: Four diets containing 0 (CTR), 2 (CAEO2), 4 (CAEO4), and 6 (CAEO6) mL/kg CAEO (corresponding to 0, 1.6, 3.2, and 4.8 g per kg) were used in this experiment. These concentrations were chosen based on a preliminary study. The procedure for the feed preparation included mixing feed ingredients (Table 1), creating a paste by adding 0.35 L/kg water to the ingredient mixture, pelleting using a meat grinder, and drying overnight.

Fish rearing conditions: All animal experiments were conducted at the Research Institute of Integrated Fish Farming (Moscow region, Noginsk district, Russian Federation) and were approved by the Ethics Committee of the Peoples' Friendship University of Russia (RUDN University). Rainbow trout (with an average weight of 31 g) were placed in a 2000-L tank for a two-week acclimatization period, during which they were fed the control diet (CTR). After acclimatization, the fish were randomly distributed into 12 tanks, each with a capacity of 300 L, containing 15 fish per tank. These tanks were filled with dechlorinated tap water and continuously aerated to maintain optimal conditions.

Table 1. Composition of the control diet.

Ingredients (g/kg)	CTR	CAEO2	CAEO4	CAEO6
Corn meal	100	98.4	96.8	95.2
Wheat meal	160	160	160	160
Soybean meal	150	150	150	150
Soybean oil	65	65	65	65
Fishmeal[1]	172	172	172	172
Poultry byproduct[2]	340	340	340	340
Vitamin premix[3]	5	5	5	5
Mineral premix[3]	5	5	5	5
Methionine	1	1	1	1
Lysine	2	2	2	2
CAEO	0	1.6	3.2	4.8
Proximate composition				
Moisture	91.0	90.8	90.2	91.1
Crude protein	411	410	408	409
Crude fat	159	158	161	160
Crude ash	79.2	78.9	78.4	79.3

1 Crude protein 64%; crude fat 10%, 2 Crude protein 54%; crude fat 22%, and 3 Amineh Gostar Co. (Tehran, Iran).

Over 60 days, each diet was administered to three tanks at a daily feeding rate of 5%, divided into three meals. Half the water in each tank was replaced daily with clean water to ensure acceptable water quality. Throughout the rearing period, environmental conditions were carefully controlled, maintaining a photoperiod of 14:10 (light: dark), a water temperature of 15.3±0.30°C, a pH of 7.33 ± 0.30, and dissolved oxygen levels of 6.88±0.20 ppm. The biomass in each tank was measured biweekly to adjust feed quantities appropriately.

Evaluating fish growth performance: After 60 days, the fish growth and nutrition parameters were determined as follows (Hoseini et al., 2016):

Weight gain (WG%) = 100 × (final weight-initial weight)/initial weight

Specific growth rate (SGR%/d) = [(Ln(final weight)-Ln(initial weight)/days] × 100

Feed conversion ratio (FCR) = feed intake/(final weight-initial weight)

Sampling procedure: At the end of the rearing period, the fish underwent a 24-hour fasting period (Hoseini et al., 2019) before sampling to assess the

activity of digestive enzymes and to analyze antioxidant and biochemical parameters in serum. From each treatment group, fifteen fish were randomly selected and anesthetized using clove extract at a concentration of 150 ppm. Blood samples were collected from the caudal vein using 2 mL non-heparinized syringes. Following collection, the samples were allowed to clot at room temperature for 30 min, after which they were centrifuged at 3000 rpm for 10 min. The resulting serum was carefully transferred to new tubes and stored at -20°C for subsequent analysis.

After blood sampling, the fish were euthanized, and their abdominal cavities were opened. The whole gut was dissected, washed with cold distilled water, and frozen in liquid nitrogen. The samples were then thawed at room temperature, weighed, and homogenized with a five-volume (w:v) NaCl solution (200 mM). After centrifugation (1000 g; 30 min), the supernatant was collected in a new tube and kept at -80°C until analysis.

Analysis of gut digestive enzymes: The gut amylase activity was determined using 1% starch as substrate, while 3,5-dinitrosalicylic acid was used to detect the formed maltose at 540 nm (Bernfeld, 1955). The gut trypsin activity was determined using Benzoyl DLarginine-p-nitroanilide as substrate at 410 nm (Liang et al., 2022). The gut chymotrypsin activity was determined using –benzoyl-L-tyrosine ethyl ester as substrate at 256 nm based on Villanueva-Gutiérrez et al. (2020). Soluble protein contestations of the enzyme extracts were measured according to Bradford (1976), and the activities of the enzymes were expressed as units per mg protein × min.

Analysis of serum antioxidant and biochemical parameters: The activities of superoxide dismutase (SOD) and catalase (CAT) were detected based on the reduction of cytochrome C and decomposition of hydrogen peroxide, respectively, using commercial kits (Zellbio GmBH Co., Lonsee, Germany). Glutathione peroxidase (GPx) activity was measured based on converting glutathione to glutathione disulfide using a commercial kit (Zellbio GmBH Co., Lonsee, Germany). Malondialdehyde content was measured based on the reaction of serum aldehyde with thiobabituric acid at 95°C using a commercial kit (Zellbio GmBH Co., Lonsee, Germany). The activities of ALP, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in the serum samples were measured using commercial kits (Parsazmun Co., Tehran, Iran) and based on the protocol provided by the company.

Statistical analysis: This study employed a fully randomized design to ensure the integrity of the results. Before analysis, the data was subjected to one-way ANOVA after confirming normality with the Shapiro-Wilk test and assessing homogeneity of variances using the Levene test. The Tukey test was applied for pairwise comparisons between treatments, with a significance threshold established at $P<0.05$.

Results

Final weight, weight gain, SGR, and feed intake significantly increased along with the elevation in dietary CAEO concentration, and the highest growth performance was observed in the CAEO6 treatment. On the other hand, FCR significantly decreased along with the elevation in dietary CAEO concentration, and the lowest FCR was found in the CAEO4 and CAEO6 treatments. There was no significant difference in fish survival among the treatments (Table 2).

Dietary CAEO levels showed no significant effects on gut amylase activity. Chymotrypsin activity in the fish gut significantly increased along with the elevations in dietary CAEO concentration, and the highest activity was found in the CAEO6 treatment. The highest gut trypsin activity was observed in the CAEO4 and CAEO6 treatments, whereas the lowest activity was related to the CTR and CAEO2 treatments (Table 3).

The treatments had no significant differences in serum ALT, AST, and ALP activities. The highest serum SOD and CAT activities were observed in CAEO4 and CAEO6 treatments, but the lowest activities were found in CTR and CAEO2 treatments. Serum GPx significantly increased in CAEO6 compared to the other treatments. There was no significant difference in serum GPx activity among

Table 2. Growth performance, feed efficiency, and survival of rainbow trout fed diets supplemented with 0 (CTR), 2 (CAEO2), 4 (CAEO4), and 6 (CAEO6) mL/kg CAEO. Significant differences among the treatments are shown by different superscripted letters (P<0.01).

	CTR	CAEO2	CAEO4	CAEO6
Initial weight (g)	31.9±1.3	30.8±1.8	31.2±1.5	31.4±1.4
Final weight (g)	64.4±2.7^{d}	71.3±3.1^{c}	79.2±1.5^{b}	85.3±1.4^{a}
Weight gain (%)	101.8±6.2^{d}	131.4±5.5^{c}	153.8±10.4^{b}	171.6±15.5^{a}
Specific growth rate (%/d)	1.17±0.06^{d}	1.39±0.04^{c}	1.55±0.02^{b}	1.66±0.04^{a}
Feed conversion ratio	1.41±0.03^{a}	1.32±0.05^{b}	1.21±0.04^{c}	1.19±0.02^{c}
Feed intake (g)	33.4±1.2^{c}	34.2 ±2.1^{c}	38.5±1.5^{b}	42.3±1.4^{a}
Survival (%)	95.5±1.5	95.2±2.2	98.5±2.5	98.5±1.5

Table 3. Gut digestive enzymes of rainbow trout fed diets supplemented with 0 (CTR), 2 (CAEO2), 4 (CAEO4) and 6 (CAEO6) mL/kg CAEO. Significant differences among the treatments are shown by different superscripted letters (P<0.01).

	CTR	CAEO2	CAEO4	CAEO6
Amylase (U/mg protein)	0.18±0.04	0.16±0.03	0.2±0.05	0.21±0.04
Chymotrypsin (U/mg protein)	2.9±0.12^{d}	3.2±0.1^{c}	3.9±0.11^{b}	4.2±0.15^{a}
Trypsin (U/mg protein)	6.2±0.17^{b}	6.1±0.15^{b}	9.1±0.12^{a}	8.8±0.15^{a}

Table 4. Serum enzymatic activities of rainbow trout fed diets supplemented with 0 (CTR), 2 (CAEO2), 4 (CAEO4) and 6 (CAEO6) mL/kg CAEO. Significant differences among the treatments are shown by different superscripted letters (P<0.01).

	CTR	CAEO2	CAEO4	CAEO6
SOD (U/mL)	25.8±1.3^{b}	28.7±1.8^{b}	34.4±1.5^{a}	35.6±1.4^{a}
CAT (U/ mL)	85.5±7.2^{b}	91.2±6.5^{b}	125.4±11.4^{a}	136.3±12.5^{a}
GPx (U/ mL)	2.3±0.3^{b}	2.21±0.4^{b}	2.12±0.3^{b}	3.15±0.1^{a}
ALT (U/L)	30.2±3.4	32.3±6.5	33.7±3.4	31.2±4.5
AST (U/L)	22.4±5.8	25.2±4.1	19.6±6.2	20.4±7.1
ALP (U/L)	16.1±6.2	18.1±6.2	23.2±4.2	19.2±6.3

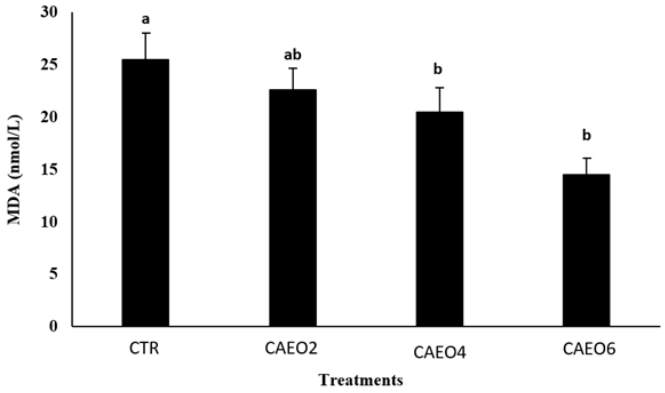

Figure 1. Serum MDA concentrations of rainbow trout fed diets supplemented with 0 (CTR), 2 (CAEO2), 4 (CAEO4), and 6 (CAEO6) mL/kg CAEO. Different letters above the bars show significant differences among the treatments (P<0.01).

CTR, CAEO2, and CARO4 treatments (Table 4). Serum MDA showed significant decreases in CAEO4 and CAEO6 treatments compared to CTR (Fig. 1).

Discussions

This is the first study evaluating the benefits of CAEO in fish. Dietary CAEO supplementation significantly boosted growth performance and feed efficiency in the fish. This can be due to the boosted feed digestion processes as supported by the elevations in proteolytic enzymes' activity in the fish gut. Essential oils can boost fish gut health by controlling harmful microbes (Al-Sagheer et al., 2018) and improving structural development (Lopes et al., 2020), and these health benefits may support better digestion processes. The present results align with those of Hajirezaee et al. (2024), who reported improvements in digestive enzymes' activity and growth performance of rainbow trout following dietary supplementation with 5 g/kg of *Juniperus communis* essential oil. Moreover, dietary supplementation with 0.125-3 mL/kg *Origanum onites* essential oil (Diler et al., 2017) or 0.5-1.5 g/kg sage or thyme essential oils (Sönmez et al., 2015) significantly improved growth performance of rainbow trout.

ALT, AST, and ALP are abundant cytosolic enzymes in the hepatobiliary system of the fish. They are not functional in circulation but are indicators of the hepatobiliary system health (Hoseini et al., 2022b). There were no significant effects of dietary CAEO on ALT, AST, and ALP activities in the fish serum, suggesting similar health status of the hepatobiliary

system among the treatments, as reported by other researchers who used different essential oils (Abdel-Latif et al., 2020; Chung et al., 2021). Contrariwise, it has been found that essential oils of some plants such as peels of *Citrus sinensis* (Mohamed et al., 2021) and *Citrus limon* (Mohamed et al., 2021) and leaves of *Thymus vulgaris* (Ghafarifarsani et al., 2022) decrease ALT and AST in fish blood; whereas, essential oil of *Ocimum basilicum* (de Souza et al., 2019) and tea tree (Liu et al., 2022) resulted in elevations in the activity of these enzymes in fish blood.

Essential oils are best known for their antioxidant capacity thanks to their phenolic compounds. There is no report on CAEO antioxidant activity, although *C. arvense* extract has been found to have H_2O_2- and superoxide-scavenging capacity at about 3-4 folds higher concentrations (Hossain et al., 2016). Such an antioxidant capacity of CAEO can be related to the presence of high concentrations of α-Bisabolol and δ-Cadinene, as these sesquiterpenes have great radical-scavenging capacities (Kundu et al., 2013; Ramazani et al., 2022). Here, we found elevated antioxidant enzyme activity in the fish-fed CAEO-supplemented diets. Superoxide molecules are constantly formed in living cells as the byproduct of the electron transfer chain, and SOD is responsible for neutralizing them to protect the cells against oxidation. Upon neutralization by SOD, superoxide is converted to hydrogen peroxide, which must be neutralized by CAT (at high hydrogen peroxide concentrations) or GPx (at low hydrogen peroxide concentrations). Thus, CAEO could improve enzymatic antioxidant capacity that reduced lipid peroxidation, characterized by decreases in MDA concentration. The present results are in line with previous studies on other essential oils, such as *Lippia alba* (Saccol et al., 2013) and *J. communis* (Hajirezaee et al., 2024) essential oils, where fish showed improvements SOD, CAT, and GPx activities and decrease in lipid peroxidation up on dietary intake of the essential oils.

In conclusion, this study showed that dietary CAEO potentiates boost growth rate, digestive enzymes, and antioxidant capacity in rainbow trout. According to the present study, a 6 mL CAEO per kg trout diet is adequate to excrete the abovementioned benefits.

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