

## Research Article

# Preliminary Insights Into the Incorporation of Common Hop (*Humulus lupulus* L.) Into the Fish Feed: Effect on Growth Performance, Whole-Body Proximate Composition, Fillet Fatty Acid Composition, Blood Biochemical, and Antioxidant Properties of Common Carp (*Cyprinus carpio* L.)

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The present study assessed the effect of common hop (*Humulus lupulus* L.) extract on common carp (*Cyprinus carpio* L.). Fish (initial weight of  $32.22 \pm 0.65$  g) were divided into three groups and fed diets containing 0 (control), 0.75 g ( $H_{0.75}$ ), and 1.5 g ( $H_{1.5}$ ) of common hop extract per kg of isonitrogenous, isolipidic, and isoenergetic commercial diet for 8 weeks. No significant differences in growth performance across treatments were observed ( $p > 0.05$ ). Protein content in whole-body proximate composition increased significantly in  $H_{1.5}$  compared to the control ( $p < 0.05$ ). The  $H_{0.75}$  showed a significant increase in lipid peroxidation (LPO) and reduced glutathione (GSH) levels compared to the control ( $p < 0.05$ ). While  $H_{1.5}$  had higher acetylcholinesterase (AChE) than group  $H_{0.75}$  ( $p < 0.05$ ), no significant change was observed in AChE, catalase (CAT), and superoxide dismutase (SOD) activities in treated groups compared to the control ( $p > 0.05$ ). Blood biochemical indices, alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were significantly lower in  $H_{0.75}$  and  $H_{1.5}$  than in the control ( $p < 0.05$ ), while lactate dehydrogenase (LDH) increased significantly in  $H_{1.5}$  compared to the control ( $p < 0.05$ ). Cholesterol, triglyceride (TG), and the number of white blood cells (WBCs) showed no significant differences across treatments ( $p > 0.05$ ).  $H_{0.75}$  significantly increased the proportion of polyunsaturated fatty acids (PUFAs) like  $\alpha$ -linolenic acid, docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA;  $p < 0.05$ ), and both  $H_{0.75}$  and  $H_{1.5}$  raised considerably the value of oleic acid and eicosatrienoic acid compared with the control ( $p < 0.05$ ); in contrast, eicosapentaenoic acid (EPA) was decreased significantly by  $H_{1.5}$  compared to the control ( $p < 0.05$ ). Supplementation of the common hop at 0.75 and 1.5 g kg<sup>-1</sup> positively affected farmed carp.

**Keywords:** common carp (*Cyprinus carpio* L.); common hop extract; medicinal plant; n–3 polyunsaturated fatty acid; oxidative status

## 1. Introduction

The growing demand for high-quality food has led to a rapid expansion of the aquaculture sector [1]. Aquaculture has been one of the fastest-growing animal-based food-production sectors in recent decades, providing secure food and profitable income [2]. Nevertheless, the aquaculture sector faces serious challenges related to the deterioration of the health and welfare of immunocompromised animals, resulting in a high mortality rate of farmed aquatic animals [3, 4]. These challenges and problems are triggered by introducing intensive farming systems, such as stress and high susceptibility to diseases [5, 6]. The use of immunostimulants as dietary supplements can enhance the immunity and health status of animals, providing them with higher resistance to pathogens and under high-stress conditions such as sorting, reproduction, vaccination, and transport [7, 8]. Chemical agents such as hormones, antibiotics, and antifungals have been used to promote growth, immunity, and bacterial disease treatment in fish [9, 10]. However, the use of antibiotics and other chemotherapeutic agents also has negative aspects, such as the risk of the emergence of resistant pathogens, problems with the accumulation of antibiotic residues in treated fish, and unfavorable effects on the environment [11, 12].

Therefore, the demand for natural additives in aquaculture has increased, and medicinal plants have been focused on as safe and eco-friendly alternatives to chemicals [13, 14]. Medicinal plants are rich in bioactive components such as flavonoids, phenols, terpenes, alkaloids, tannins, saponins, quinones, and lecithin [15–17]. Various studies have widely confirmed their functions in farmed fish in recent decades. They exhibit diverse properties such as antioxidant [18, 19], anti-inflammatory [20], antidiabetic [21], anti-stress [22, 23], immunostimulant [24], antimicrobial [25, 26], growth promoter [27, 28], resistance to pathogens [29, 30], and disease control [31]. They are increasingly used in aquaculture due to their low side effects, unlike chemotherapeutics, and exhibit low toxicity to the water environment. Additionally, they are cost-effective, readily accessible, and biocompatible [32, 33], offering the aquaculture industry a new opportunity to improve fish health and production.

The common hop (*Humulus lupulus* L.) has been recognized to have important health promoting properties [34]. Hop is a plant rich in secondary compounds, most of which are secreted in the lupulin glands, including hop resins, phenolic compounds (flavonoids and tannins), bitter acids, and essential oils [35, 36], and exerts antimicrobial activity against many bacterial species, mainly against gram-positive bacteria [37], antifungal [38], antioxidant [39], and anti-inflammatory [40] properties.

Common carp (*Cyprinus carpio* L.) is an important aquaculture species with an annual global production of over 4.1 million tonnes (about 7.7% of total fish production) [41]. As an aquaculture species, common carp are exposed to the risk of different stressors in intensive aquaculture facilities [42]. Therefore, it is beneficial to investigate the effects of herbal additives on this species' growth and health status.

According to our literature review, no report was found on the impact of dietary common hop on growth performance,

TABLE 1: Proximate composition of the commercial diet (ALTECH SUPREME-10; 4.5 mm).

| Composition of control diet (unit)       |      |
|------------------------------------------|------|
| Protein (%)                              | 49   |
| Lipid (%)                                | 10   |
| Crude fiber (%)                          | 0.9  |
| Ash (%)                                  | 11.3 |
| Gross energy (MJ kg <sup>-1</sup> )      | 19.3 |
| Digestible energy (MJ kg <sup>-1</sup> ) | 17.3 |

whole-body proximate composition, blood biochemical and antioxidant indices, and fillet fatty acids (FAs) composition of common carp. Moreover, the existing literature on the incorporation of hop as a food additive in animal diets is limited, however in a study, the inclusion of hops in broiler diets as a possible antibiotic substitute led to a significant increase in body weight as well as feed conversion ratio (FCR) and efficiency at all ages in comparison to the control treatment [43]. Growing pigs fed a diet supplemented with hop extract in two doses (500 and 1000 mg kg<sup>-1</sup> of diet) demonstrated improved meat oxidative and color stability, lower cholesterol levels, and a positive impact on the pattern of FAs [44]. Another study which has been done on feedlot cattle showed that the inclusion of hops could support gut health by reducing the risk of bacterial infection [45]. What is more, the results of a study demonstrated that orally taken hops could increase the gastric secretion volume in rats without affecting acidity [46]. To our knowledge, no comprehensive studies are available on hop as a feed supplementation in aquaculture. Thus, the main purpose of this research is to investigate the effects of hop extract on growth performance, whole-body proximate composition, plasma biochemical indices, plasma antioxidant response, number of white blood cells (WBCs), and fillet FA composition of common carp.

## 2. Materials and Methods

**2.1. Experimental Design.** The common carp juveniles with an initial weight of  $32.22 \pm 0.65$  g and initial length of  $13.64 \pm 0.12$  cm were obtained from the breeding facilities at the Faculty of Fisheries and Protection of Waters, University of South Bohemia (Vodnany, Czech Republic). For the experimental diet, we used an isonitrogenous, isolipidic, and isoenergetic formulation. The commercial diet (ALTECH SUPREME-10; 4.5 mm) with protein and lipid content of 49% and 10%, respectively, was employed as the control diet without any supplement (C) (Table 1), in addition to the inclusion of hop extract at levels 0.75 (H<sub>0.75</sub>) and 1.5 (H<sub>1.5</sub>) g kg<sup>-1</sup> of commercial diet. Commercial hop extract was purchased from company Bylinné Kapky s.r.o. in the Czech Republic. For the preparation of diets, the commercial diet was ground into powder, mixed with hop extract, blended thoroughly in a blender (400V HTS42 2 T, RM Gastro, EU), and pelletized (2 mm) using a multifunctional spiral extrusion machine (P 98 CE, Braher Internacional, Spain). Experimental diets were dried at 60°C in the oven (Nüve, type FN 400 (P), Turkey) for 18 h, kept in the zip plastic bags, and stored at 4°C until used. The present research had three groups, three

replications, and nine tanks. Fifty-four fish were randomly distributed into nine 60 L glass tanks, and each tank was stocked with six common carp juveniles. Fish were acclimated and were fed manually with the control diet twice a day for 2 weeks before the start of the experiment. At the end of the acclimation period, tanks were assigned into three groups: C, H<sub>0.75</sub>, and H<sub>1.5</sub>. Fish were fed in the dose of 3% body weight twice a day (07:00 and 15:00) manually for 8 weeks. After 4 weeks, the biometry was done to increase the feed according to weight gain. The water parameters, including temperature, pH, and dissolved oxygen, were measured daily by Oxi 315i dissolved oxygen meter (Wissenschaftlich-Technische Werkstätten GmbH, WTW, Germany), and were maintained at 20.82 ± 0.44°C, 7.50 ± 0.25, and 7.97 ± 0.94 mgL<sup>-1</sup>, respectively. The photo period was maintained with a 12:12 light/dark cycle.

**2.2. Sampling.** At the end of the 8-week feeding trial, all fish were weighed individually immediately before sampling, after 1 day of starvation, to calculate growth parameters and feed utilization. The fish in each tank were anesthetized by ethylene glycol monophenyl ether in a concentration of 0.3 mL L<sup>-1</sup> water. Two intact fish from each tank were randomly sampled and stored at -20°C to analyze whole-body proximate composition. Blood samples from two random fish per tank were collected from the caudal vein by a heparinized syringe (40 IU mL<sup>-1</sup> blood; Heparin inj., Leciva, Czech Republic), half volume of blood was transferred into 1.5 mL Eppendorf tubes coated by sodium heparin (20 U L<sup>-1</sup>) as anticoagulant on ice for WBCs counting (g L<sup>-1</sup>). The second half of the blood samples were centrifuged for 10 min at 12,000 × g at 4°C to obtain plasma and stored at -80°C for subsequent analysis of biochemical values and antioxidant parameters. For the analysis of fillet FA composition, the samples were taken from the dorsal muscle tissues of two fish per tank and stored at -20°C until they were analyzed.

**2.3. Growth Performance.** All the fish were counted, and the individual length and weight were measured. Growth and feed utilization indices, including final body weight (FBW, g), weight gain percent (WG%), specific growth rate (SGR, %day<sup>-1</sup>), FCR, survival rate (SR%), condition factor (CF), feed intake (FI, g fish<sup>-1</sup>), and protein efficiency ratio (PER), were calculated as follows [47]:

$$\text{FBW(g)} = \text{final body weight.}$$

$$\text{WG(\%)} = \frac{\text{FBW (g)} - \text{initial body weight (IBW, g)}}{\text{IBW (g)}} \times 100.$$

$$\text{SGR(\%day}^{-1}\text{)} = \frac{\text{Ln FBW (g)} - \text{Ln IBW (g)}}{\text{Days}} \times 100.$$

$$\text{FCR} = \frac{\text{Dry feed fed (g)}}{\text{Wet weight gain (g)}}.$$

$$\text{SR(\%)} = \frac{\text{Number of fish after test}}{\text{Initial number of stocked fish}} \times 100.$$

$$\text{CF} = \frac{\text{FBW (g)}}{(\text{fish length})^3(\text{cm})^3} \times 100.$$

$$\text{FI (g fish}^{-1}\text{)} = \frac{\text{Dry feed consumed (g)}}{\text{Number of fish}}.$$

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Total protien intake (g)}}.$$

**2.4. Whole-Body Proximate Composition.** Whole-body proximate composition analysis was conducted according to the standard procedures of the AOAC [48]. Moisture was measured by drying in a type FN 400P oven (NÜVE, Ankara, Turkey) at 105°C to constant weight [49]. Crude protein was analyzed using the Kjeldahl method (BUCHI Labortechnik AG, type K-360, Königswinter, Germany) [50]. Crude lipid was determined using the extraction method of Hara and Radin [51] with slight modifications [52]. Ash content was analyzed in a muffle furnace L 40/11 BO (Nabertherm GmbH, Lilienthal, Germany) at 550°C for 4 h [50].

**2.5. Blood Biochemistry and WBC Count.** The plasma biochemical indices, including triglycerides (TGs, mmol L<sup>-1</sup>), total cholesterol (T-CHO, mmol L<sup>-1</sup>), alanine aminotransferase (ALT, U L<sup>-1</sup>), aspartate aminotransferase (AST, U L<sup>-1</sup>), alkaline phosphatase (ALP, U L<sup>-1</sup>), and lactate dehydrogenase (LDH, U L<sup>-1</sup>) were measured using a VETTEST 8008 analyzer (IDEXX Laboratories Inc., USA). For individual determination of biochemical parameters, the plasma sample is pipetted using a disposable tip and analyzer pipette/probe, and the pipette automatically dispenses 10 µL of sample onto each reagent disk (slide) and begins reading. The analysis itself takes about 5–6 min [53]. According to Svobodova, Pravda, and Palackova [54], WBC counts were measured.

**2.6. Plasma Antioxidant Response.** The level of lipid peroxidation (LPO) was determined by thiobarbituric acid reactive substances (TBARS, nmol TBARS mg<sup>-1</sup>protein) according to Uchiyama and Mihara [55] modified for microplate design. Absorbance was measured at 550 nm using a spectrophotometer (Infinite M200, TECAN), and the concentrations were calculated based on a standard calibration curve generated with malondialdehyde (MDA). Total superoxide dismutase (SOD, nmol nitro blue tetrazolium (NBT) min<sup>-1</sup>mg<sup>-1</sup>protein) activity was determined according to the method of Ewing and Janero [56] based on inhibition of NBT reduction and production of superoxides by nicotinamide adenine dinucleotide and phenazine methosulfate, with absorbance measured at 560 nm. Catalase (CAT, µmol hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) min<sup>-1</sup>mg<sup>-1</sup>protein) activity was determined spectrophotometrically at 240 nm, following the decomposition rate of H<sub>2</sub>O<sub>2</sub> by CAT, as described by Aebi [57]. Reduced glutathione (GSH, nmol GSH mg<sup>-1</sup>protein) levels were assessed using the Ellman et al. [58] method, which involves the reaction of the thiol-selective Ellmann reagent (2,2-dinitro-5,5-dithiobenzoic acid) with free SH groups to form a colored product measured at 420/680 nm and was calculated according to standard calibrations. Acetylcholinesterase (AChE, nmol min<sup>-1</sup>mg<sup>-1</sup>protein) was measured according to Ellman et al. [58] with a plate reader (Infinite M200, TECAN, Switzerland).

**2.7. Fillet FA Composition.** The lipid extraction was performed according to [51] with slight modification [59]. Approximately 2 g of sample were homogenized in 10 mL of hexane-isopropanol (3:2) using an Ultra Turrax (T25, Janke and Kunkel, IKA Werke, Germany). Subsequently, 6 mL of Na<sub>2</sub>SO<sub>4</sub> (6.67%) solution (Sigma–Aldrich) was mixed with the homogenates. After centrifugation (5000 rpm, 18°C, 5 min in Megafuge 16R Thermo Scientific, Osterode am Harz, Germany), the upper lipid phase was transferred into preweighed tubes and evaporated under nitrogen. The lipid content was determined gravimetrically using Mettler Toledo XP2U Excellence Plus XP Ultra Micro Balances (2 g × 0.1 Ug, Germany). Total lipid methylation for FA analysis was conducted according to Appelqvist's [60] method. The FAME C23:0 (Dr. Ehrenstorfer GmbH) was used as an internal standard. Gas chromatography (Trace Ultra FID; Thermo Scientific, Milan, Italy) equipped with a BPX-70, 50 m fused silica capillary column (id. 0.22 mm, 0.25 µm film thickness, SGE, USA) was used for FA analysis. The temperature gradient was initiated at 70°C for 0.5 min, followed by an increase of 30°C per minute until reaching 150°C. Subsequently, the temperature ramped up to 220°C at a rate of 1.5°C per minute and held for 11 min, with the entire analysis lasting 60 min. The PVT injector and detector temperatures were maintained at 170°C and 260°C, respectively. The peaks were identified and quantified using Thermo Xcalibur 3.0.63 (Thermo Fisher Scientific Inc.) by comparing sample retention times and peak areas with those of the standard mixture in seven levels (1000–15 µg mL<sup>-1</sup>) in the Supleco 37 Component FAME mix (Sigma–Aldrich).

### 3. Statistical Analysis

Before the analysis, the data distribution characteristics and the dispersion homogeneity were evaluated using the Shapiro–Wilk and Levene's tests. Then, data with normal distribution were analyzed using a one-way analysis of variance (ANOVA) followed by Duncan's honest significant difference (HSD) test. Statistical significance was assumed at  $p < 0.05$ . Data are presented as mean ± standard deviation (SD). All analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

### 4. Results

**4.1. Growth Performance.** No significant differences were observed in growth factors, including FBW, WG, SGR, FCR, SR, CF, FI, and PER among the experimental groups H<sub>0.75</sub> and H<sub>1.5</sub> compared to the control ( $p > 0.05$ ; Table 2).

**4.2. Whole-Body Proximate Composition.** Significant differences, including moisture and crude protein contents, were observed among all groups (Table 3). Group H<sub>1.5</sub> had a significantly higher ( $p < 0.05$ ) level of crude protein content than the control group and treatment H<sub>0.75</sub>. No significant difference was observed between treatment H<sub>0.75</sub> and the control in crude protein content ( $p > 0.05$ ). The moisture levels did not change after adding hop extract compared with the control ( $p > 0.05$ ); however, treatment H<sub>1.5</sub> had significantly ( $p < 0.05$ ) lower moisture than H<sub>0.75</sub>. Crude

TABLE 2: Growth performance parameters and feed efficacy of carp fed with a common hop-supplemented diet (H<sub>0.75</sub> and H<sub>1.5</sub>) and control diet (C) for 8 weeks ( $n$  = all fish in the tanks).

| Parameter (unit)           | Control<br>(mean ± SD) | H <sub>0.75</sub><br>(mean ± SD) | H <sub>1.5</sub><br>(mean ± SD) |
|----------------------------|------------------------|----------------------------------|---------------------------------|
| IBW (g)                    | 32.53 ± 0.17           | 32.35 ± 0.42                     | 31.77 ± 1.00                    |
| FBW (g)                    | 96.00 ± 9.60           | 97.70 ± 4.17                     | 98.00 ± 5.13                    |
| WG (%)                     | 195.19 ± 30.22         | 201.99 ± 9.39                    | 208.61 ± 18.98                  |
| SGR (%day <sup>-1</sup> )  | 1.93 ± 0.18            | 1.97 ± 0.06                      | 2.01 ± 0.11                     |
| FCR                        | 0.77 ± 0.12            | 0.74 ± 0.04                      | 0.73 ± 0.06                     |
| SR (%)                     | 100.00                 | 100.00                           | 100.00                          |
| CF                         | 1.20 ± 0.03            | 1.22 ± 0.02                      | 1.20 ± 0.01                     |
| FI (g fish <sup>-1</sup> ) | 48.00                  | 48.00                            | 48.00                           |
| PER                        | 2.31 ± 0.34            | 2.38 ± 0.14                      | 2.41 ± 0.19                     |

Note: Values in the same row with no superscripts indicate no significant differences according to Duncan's multiple-range tests ( $*p < 0.05$ ).

Abbreviations: CF, condition factor; FBW, final body weight; FCR, feed conversion ratio; FI, feed intake; IBW, initial body weight; PER, protein efficiency ratio; SD, standard deviation; SGR, specific growth rate; SR, survival rate; WG%, weight gain percent.

TABLE 3: Whole-body proximate composition of carp fed with the common hop-supplemented diet (H<sub>0.75</sub>; H<sub>1.5</sub>) and control diet (C) for 8 weeks ( $n$  = 6).

| Component<br>(% dry matter) | Control<br>(mean ± SD)     | H <sub>0.75</sub><br>(mean ± SD) | H <sub>1.5</sub><br>(mean ± SD) |
|-----------------------------|----------------------------|----------------------------------|---------------------------------|
| Moisture                    | 77.74 ± 1.1 <sup>ab*</sup> | 78.37 ± 0.29 <sup>a*</sup>       | 77.00 ± 0.95 <sup>b*</sup>      |
| Protein                     | 15.20 ± 0.56 <sup>b*</sup> | 15.27 ± 0.68 <sup>b*</sup>       | 16.51 ± 0.18 <sup>a*</sup>      |
| Lipid                       | 4.07 ± 0.88                | 3.87 ± 0.20                      | 4.41 ± 0.36                     |
| Ash                         | 1.92 ± 0.20                | 1.81 ± 0.15                      | 1.89 ± 0.19                     |

Note: Values in the same row with different superscripts indicate significant differences according to Duncan's multiple-range tests ( $*p < 0.05$ ).

Abbreviation: SD, standard deviation.

lipid and ash content did not change significantly among groups ( $p > 0.05$ ).

**4.3. Blood Biochemistry and WBC Count.** LDH, ALT, and ALP activities showed significant differences ( $p < 0.05$ ) among the treatments compared to the control (Table 4). The activity of LDH in group H<sub>1.5</sub> was significantly higher ( $p < 0.05$ ) than in the control group; however, treatment H<sub>0.75</sub> showed no significant change in LDH compared to the control ( $p > 0.05$ ). The activities of ALT and ALP were significantly lower ( $p < 0.05$ ) in both experimental groups (H<sub>0.75</sub>, H<sub>1.5</sub>) compared to the control ( $p < 0.05$ ). There were no significant changes in plasma AST, T-CHO, and TG, as well as the number of WBC counts ( $p > 0.05$ ).

**4.4. Plasma Antioxidant Response.** The levels of LPO and GSH were significantly higher ( $p < 0.05$ ) in group H<sub>0.75</sub> compared to the control and group H<sub>1.5</sub>. At the same time, there was no considerable difference between treatment H<sub>1.5</sub> and the control in the levels of LPO and GSH ( $p > 0.05$ ). There were no significant changes in plasma SOD, CAT, and AChE activity in groups H<sub>0.75</sub> and H<sub>1.5</sub> compared to the control ( $p > 0.05$ ; Table 5), while group H<sub>1.5</sub> had higher AChE than group H<sub>0.75</sub> ( $p < 0.05$ ).

TABLE 4: Blood biochemistry and WBC count of carp fed with the common hop-supplemented diet (H<sub>0.75</sub>; H<sub>1.5</sub>) and control diet (C) for 8 weeks (n = 6).

| Parameter (unit)             | Control<br>(mean ± SD)        | H <sub>0.75</sub><br>(mean ± SD) | H <sub>1.5</sub><br>(mean ± SD) |
|------------------------------|-------------------------------|----------------------------------|---------------------------------|
| T-CHO (mmolL <sup>-1</sup> ) | 3.33 ± 0.32                   | 3.34 ± 0.35                      | 3.19 ± 0.39                     |
| TG (mmolL <sup>-1</sup> )    | 2.34 ± 0.34                   | 2.35 ± 0.15                      | 2.18 ± 0.20                     |
| LDH (UL <sup>-1</sup> )      | 1282.20 ± 79.66 <sup>b*</sup> | 1347.20 ± 121.93 <sup>ab*</sup>  | 1472.30 ± 110.42 <sup>a*</sup>  |
| ALT (UL <sup>-1</sup> )      | 41.50 ± 5.12 <sup>a*</sup>    | 31.50 ± 4.03 <sup>b*</sup>       | 32.00 ± 6.75 <sup>b*</sup>      |
| AST (UL <sup>-1</sup> )      | 297.50 ± 95.37                | 382.17 ± 114.37                  | 335.50 ± 74.56                  |
| ALP (UL <sup>-1</sup> )      | 15.31 ± 2.68 <sup>a*</sup>    | 11.40 ± 2.15 <sup>b*</sup>       | 11.67 ± 2.73 <sup>b*</sup>      |
| WBC (gL <sup>-1</sup> )      | 40.67 ± 8.87                  | 33.33 ± 6.15                     | 43.17 ± 8.5                     |

Note: Values in the same row with different superscripts indicate significant differences according to Duncan's multiple-range tests (\*p < 0.05).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; SD, standard deviation; T-CHO, total cholesterol; TG, triglyceride; WBCs, white blood cells.

TABLE 5: Plasma antioxidant response of carp fed with the common hop-supplemented diet (H<sub>0.75</sub>; H<sub>1.5</sub>) and control diet (C) for 8 weeks (n = 6).

| Parameter (unit)                                                                    | Control<br>(mean ± SD)     | H <sub>0.75</sub><br>(mean ± SD) | H <sub>1.5</sub><br>(mean ± SD) |
|-------------------------------------------------------------------------------------|----------------------------|----------------------------------|---------------------------------|
| SOD (nmol NBT min <sup>-1</sup> mg <sup>-1</sup> protein)                           | 0.38 ± 0.04                | 0.39 ± 0.04                      | 0.42 ± 0.06                     |
| CAT (μmol H <sub>2</sub> O <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> protein) | 0.07 ± 0.02                | 0.08 ± 0.03                      | 0.08 ± 0.03                     |
| GSH (nmol GSH mg <sup>-1</sup> protein)                                             | 7.53 ± 0.91 <sup>b*</sup>  | 10.13 ± 2.27 <sup>a*</sup>       | 6.90 ± 0.86 <sup>b*</sup>       |
| AChE (nmol min <sup>-1</sup> mg <sup>-1</sup> protein)                              | 3.45 ± 0.22 <sup>ab*</sup> | 3.19 ± 0.38 <sup>b*</sup>        | 3.72 ± 0.41 <sup>a*</sup>       |
| TBARS (nmol TBARS mg <sup>-1</sup> protein)                                         | 0.73 ± 0.05 <sup>b*</sup>  | 0.80 ± 0.04 <sup>a*</sup>        | 0.70 ± 0.04 <sup>b*</sup>       |

Note: Values in the same row with different superscripts indicate significant differences according to Duncan's multiple-range tests (\*p < 0.05).

Abbreviations: AChE, acetylcholinesterase; CAT, catalase; GSH, reduced glutathione; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; NBT, nitro blue tetrazolium; SD, standard deviation; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

**4.5. Fillet FA Composition.** The content of *n*-3 polyunsaturated FA (PUFA), *n*-6 PUFA, monounsaturated FA (MUFA), and saturated FA (SFA) significantly differed (*p* < 0.05) among experimental diets (Table 6). H<sub>0.75</sub> significantly increased the proportion of heptadecenoic acid (C17:1),  $\alpha$ -linolenic acid (C18:3*n*-3), heneicosanoic acid (C21:0), docosapentaenoic acid (DPA, C22:5*n*-6), and docosahexaenoic acid (DHA, C22:6 *n*-3) compared with the control treatment (*p* < 0.05); however, the mentioned FAs did not change significantly by H<sub>1.5</sub> (*p* > 0.05). In addition, both H<sub>0.75</sub> and H<sub>1.5</sub> raised considerably the value of oleic acid (C18:1*n*-9) and eicosatrienoic acid (C20:3*n*-3; *p* < 0.05); in contrast, octadecenoic acid (C18:1*n*-7) and eicosapentaenoic acid (EPA, C20:5*n*-3) were changed significantly by H<sub>1.5</sub> compared to the control (*p* < 0.05). At the same time, no notable difference was observed between treatments H<sub>0.75</sub> and the control (*p* > 0.05).

## 5. Discussion

The development of medicinal plants as alternative strategies in the animal diet has become an essential trend in the last decade to improve growth performance and immune system and increase resistance to environmental stress [61–63]. The present study showed that the addition of common hop to the diet of carp did not improve growth performance significantly compared with the control. In line with our results, sea bass (*Dicentrarchus labrax*) fed thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), and fenugreek (*Trigonella*

*foenum graecum*) as feed additives [64] and rainbow trout (*Oncorhynchus mykiss*) fed with different concentrations of hyssop (*Hyssopus officinalis*) [65] showed no significant change in FCR and SGR. However, including hops in broiler diets led to a considerable increase in FBW and FCR compared to the control treatment [43].

The whole-body proximate composition of fish provides a detailed breakdown of the major components of the fish's body, giving insight into its nutritional status, health, energy content, growth performance, feed efficiency, and product quality [66]. Significantly higher protein content in the whole-body proximate composition in H<sub>1.5</sub> compared to the control is in line with the findings of golden pompano (*Trachinotus ovatus*) fed with dandelion (*Taraxacum officinale*) extract [67], Nile tilapia (*Oreochromis niloticus* L.) fed with dietary curcumin [68], and rainbow trout fed with 30 g nettle (*Urtica dioica*) leaf powder [47] which indicates the fish's enhanced utilization of dietary nutrients, resulting in improved protein deposition when hop extract is included in their feed [69]. Ramezanzadeh et al. [70] reported that adding barberry root (*Berberis vulgaris*) to the diet of rainbow trout increased whole-body protein content significantly, and this result might be due to improved protein utilization, decreased amino acids catabolized for energy, and the somatic deposition of fat [70].

Plasma biochemical parameters can provide fundamental information about fish health status, stress levels, and physiological conditions [71]. Although in our study, the

TABLE 6: Fillet FA composition of carp fed with the common hop-supplemented diet (H<sub>0.75</sub>; H<sub>1.5</sub>) and control diet (C) for 8 weeks (n = 6).

| FA              | Control<br>(% mean ± SD)   | H <sub>0.75</sub><br>(% mean ± SD) | H <sub>1.5</sub><br>(% mean ± SD) |
|-----------------|----------------------------|------------------------------------|-----------------------------------|
| C12:0           | 0.16 ± 0.02                | 0.15 ± 0.01                        | 0.16 ± 0.01                       |
| C14:0           | 3.48 ± 0.17                | 3.41 ± 0.16                        | 3.42 ± 0.28                       |
| C14:1           | 0.09 ± 0.01                | 0.08 ± 0.01                        | 0.08 ± 0.01                       |
| C15:0           | 0.41 ± 0.02                | 0.40 ± 0.03                        | 0.39 ± 0.02                       |
| C16:0           | 17.07 ± 0.48               | 16.71 ± 0.49                       | 17.04 ± 1.10                      |
| C16:1           | 4.73 ± 0.25                | 4.65 ± 0.32                        | 4.80 ± 0.47                       |
| C17:0           | 0.35 ± 0.02                | 0.33 ± 0.01                        | 0.33 ± 0.01                       |
| C17:1           | 0.21 ± 0.03 <sup>b*</sup>  | 0.26 ± 0.03 <sup>a*</sup>          | 0.23 ± 0.02 <sup>b*</sup>         |
| C18:0           | 4.03 ± 0.31                | 3.90 ± 0.29                        | 4.01 ± 0.26                       |
| C18:1 n-9-trans | 0.11 ± 0.04                | 0.06 ± 0.04                        | 0.07 ± 0.04                       |
| C18:1 n-9       | 28.77 ± 0.34 <sup>b*</sup> | 30.26 ± 0.91 <sup>a*</sup>         | 30.83 ± 1.09 <sup>a*</sup>        |
| C18:1 n-7       | 3.09 ± 0.04 <sup>b*</sup>  | 3.10 ± 0.07 <sup>b*</sup>          | 3.18 ± 0.04 <sup>a*</sup>         |
| C18:2 n-6       | 15.40 ± 0.50               | 15.62 ± 0.41                       | 15.42 ± 1.10                      |
| C18:3 n-6       | 0.16 ± 0.04                | 0.14 ± 0.06                        | 0.17 ± 0.03                       |
| C18:3 n-3       | 2.34 ± 0.09 <sup>b*</sup>  | 2.54 ± 0.06 <sup>a*</sup>          | 2.34 ± 0.19 <sup>b*</sup>         |
| C20:0           | 0.25 ± 0.02                | 0.24 ± 0.03                        | 0.25 ± 0.02                       |
| C20:1 n-9       | 4.74 ± 0.19                | 4.68 ± 0.19                        | 4.80 ± 0.24                       |
| C21:0           | 0.44 ± 0.01 <sup>b*</sup>  | 0.48 ± 0.02 <sup>a*</sup>          | 0.44 ± 0.04 <sup>b*</sup>         |
| C20:3 n-6       | 0.43 ± 0.04                | 0.43 ± 0.02                        | 0.46 ± 0.05                       |
| C20:4 n-6       | 1.04 ± 0.14                | 1.01 ± 0.13                        | 0.97 ± 0.19                       |
| C20:3 n-3       | 0.17 ± 0.01 <sup>b*</sup>  | 0.19 ± 0.01 <sup>a*</sup>          | 0.19 ± 0.013 <sup>a*</sup>        |
| C20:5 n-3       | 2.75 ± 0.12 <sup>a*</sup>  | 2.79 ± 0.08 <sup>a*</sup>          | 2.49 ± 0.22 <sup>b*</sup>         |
| C22:1 n-9       | 0.26 ± 0.03                | 0.24 ± 0.02                        | 0.26 ± 0.04                       |
| C22:5 n-6       | 0.85 ± 0.03 <sup>b*</sup>  | 0.92 ± 0.05 <sup>a*</sup>          | 0.81 ± 0.08 <sup>b*</sup>         |
| C22:6 n-3       | 7.06 ± 0.55 <sup>b*</sup>  | 8.30 ± 0.73 <sup>a*</sup>          | 6.64 ± 1.26 <sup>b*</sup>         |
| ΣSFA            | 26.20 ± 5.79               | 25.63 ± 5.66                       | 26.05 ± 5.78                      |
| ΣMUFA           | 41.99 ± 9.72               | 43.32 ± 10.24                      | 44.26 ± 10.43                     |
| ΣPUFA           | 30.19 ± 5.01               | 31.95 ± 5.19                       | 29.48 ± 4.98                      |
| Σn-3            | 12.32 ± 2.88               | 13.81 ± 3.43                       | 11.66 ± 2.70                      |
| Σn-6            | 17.88 ± 6.62               | 18.13 ± 6.72                       | 17.82 ± 6.63                      |
| n-3/n-6         | 0.69 ± 0.16                | 0.76 ± 0.19                        | 0.65 ± 0.15                       |

Note: Values in the same row with different superscripts indicate significant differences according to Duncan's multiple-range tests (\**p* < 0.05). Abbreviations: FA, fatty acid; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; SD, standard deviation; SFA, saturated FA.

administration of hop extract did not induce significant changes in the T-CHO, TG, and AST activities over 8 weeks compared to the control, the supplemented groups by H<sub>0.75</sub> and H<sub>1.5</sub> led to a marked reduction in ALT and ALP enzyme activities. Our findings align with the outcomes reported by Banaee et al. [72], indicating that oral supplementation of silymarin (an extract from “milk thistle,” *Silybum marianum*) regulated the activity levels of ALT and ALP enzymes of plasma in rainbow trout. A decrease of hepatic enzymes was also observed in common carp fed with *Artemisia absinthium* L.) extract supplemented diet [73] and rainbow trout fed with oak (*Quercus brantii*) acorn alcohol extract [74].

Elevated levels of ALT (amino acid metabolism) and ALP (membrane transport activities) in the blood are often associated with hepatic injury [75]. Consequently, the decreased levels could be construed as beneficial outcomes of plant supplementation on the metabolic processes of common carp, potentially linked to markedly reduced stress levels

[17]. The total flavonoids found in hops demonstrate a direct hepatoprotective effect against induced liver injury, with xanthohumol, the primary constituent, likely playing a crucial role [76] through enhancing liver detoxification by restraining certain enzyme activities [77, 78]. In addition, this protective mechanism is believed to be linked to antioxidant and anti-inflammatory properties, which help mitigate liver oxidative stress [79].

Increased LDH as a primary liver enzyme is considered a marker of organ damage and increased metabolic activity [80]. Significantly higher activity of LDH in H<sub>1.5</sub> could be a sign of tissue disorders induced by phytochemical components in higher concentrations present in the plant. This result was obtained in common carp on day 60 fed 10 g of marshmallow (*Althea officinalis* L.) extract [81] and in the serum of juvenile hybrid sturgeon (*Acipenser baeri* Brandt ♀ × *A. schrenckii* Brandt ♂) fed 100 mg kg<sup>-1</sup> grape seed proanthocyanidins [82]. This outcome might occur because a high dose of hop may elevate the liver burden of carp since

the effect of plant extracts on aquatic organisms can vary depending on the dosage and the specific composition of the extract [83]. Zhang et al. [84] also discovered that excessive intake of polyphenols may act as antinutritional factors, potentially impacting the health of fish. Further investigation is required to determine the optimal and safe dosage of hop extract for fish, aiming to safeguard them from potential liver damage.

In its molecular form, oxygen is indispensable for numerous metabolic processes crucial to sustaining aerobic life. Like all aerobic organisms, fish are prone to the impacts of reactive oxygen species (ROS) [85]. The capacity to scavenge ROS has been linked to enzymatic and nonenzymatic antioxidant defense mechanisms [86]. CAT, GSH, and SOD represent key antioxidant enzymes within the organism. CAT and GSH catalyze the breakdown of hydrogen peroxide into water and oxygen, with GSH additionally involved in directly neutralizing lipid peroxides generated by ROS [87]. SOD serves as a significant scavenger of free radicals, catalyzing the transformation of superoxide radicals into comparatively less harmful hydrogen peroxide [79, 88]. According to our outcome in Table 6, significantly increased activities of GSH in the  $H_{0.75}$  treatment compared to the control group agree with the results found in the intestine of crucian carp (*Carassius auratus*) supplemented with curcumin [89]. In another study, dietary curcumin (393.67 and 591.46 mg kg<sup>-1</sup> diet) increased the antioxidant activities of GSH content after infection with *A. hydrophila* in grass carp (*Ctenopharyngodon idella*) [90]. Some compounds present in the hop, such as  $\alpha$ -acids,  $\beta$ -acids, and xanthohumol, exhibit substantial capabilities for scavenging free radicals, hydrogen donors, and antioxidant activities in vitro, suggesting the potential utilization of this extract as a natural antioxidant against oxidative damage [91–94]. Wang et al. [95] illustrated that hop polyphenols contain high levels of proanthocyanidins, flavonoid glycosides, and xanthohumol, which consuming hop polyphenols in the diet offer protection against oxidation both in vitro and in vivo. Several scientific reports indicated that certain flavonoids such as xanthohumol, isoxanthohumol, 8-prenylnaringenin, and triterpenoids (including humulones and lupulones) possess protective effects on the liver due to their antioxidant properties [78, 96].

The increased level of GSH correlates with enhanced LPO, which is generally considered an indicator of oxidative stress [97]. In the present study, LPO represented by TBARS significantly increased and correlated with the increased level of GSH in the plasma of the  $H_{0.75}$  group. In aerobic fish tissues, there is a constant balance between oxidant and antioxidant factors. This balance can be influenced positively or negatively by both internal and external parameters such as environmental pollutants, diseases, and dietary supplements [98, 99]. In line with our study, goldfish (*C. auratus*) fed with 1% *Ferula elaeochytris* root powder had significantly higher TBARS levels in gonads compared with the control group [100]. Although herbal extracts may possess antioxidant compounds, they could also contain pro-oxidant compounds; in addition, many of the antioxidant compounds

have dual behavior by also displaying pro-oxidant activity or inducing changes in the fish's physiology that promote lipid oxidation, consequently leading to elevated levels of TBARS [101]. Despite the increase in the activity of antioxidant enzymes observed in fish treated with hop extract containing polyphenols, it is suggested that both the anti- and the pro-oxidant activities of natural antioxidants, including phenolic compounds like flavonoids and carotenoids, depend on various factors [102], such as structure, concentration, presence of metal ions, microenvironment inside different tissues, and duration of administration [103]. For instance, under specific conditions such as higher pH, polyphenols could exhibit pro-oxidant behavior through the formation of redox complexes and direct formation of ROS, which have detrimental effects on DNA, lipids, and protein molecules [104]. Changes in oxidative status in fish tissue fed with a plant-based diet can also be attributed to excessive antioxidant intake or detrimental effects of certain compounds found in plants. The imbalanced oxidative status produces antioxidant stress [105]. In addition, some plant compounds may act as toxic agents in fish, leading to LPO within cells [106]. Furthermore, tissues with higher levels of polyunsaturated FAs, which are proved through this study, are at increased risk of LPO, which consequently plays a significant role in the production of ROS [107].

According to Keskin et al. [108], hop cone extract significantly inhibits AChE, likely attributed to its abundant polyphenolic composition, particularly phenolic acids and catechins. However, our findings showed that the AChE levels in the two groups treated with hop did not differ significantly compared with the control group.

FA composition of fillets is almost related to fish diets and feeding routines [109, 110]. In our research, hop supplementation significantly improved the proportion of MUFA and PUFA in fish muscle compared with the control treatments. In line with our results, Ramezanzadeh et al. [70] reported that the addition of barberry root (*B. vulgaris*) in the form of both powder and extract to the diet of rainbow trout significantly increased the proportion of MUFA and PUFA. The content of DHA,  $\alpha$ -linolenic acid, and  $n-3/n-6$  ratio in fish muscle was significantly higher in treatments fed with barberry root compared with the control, which indicates fish may have improved digestion and absorption of FAs [70]. The change in the proportion of FAs after adding herbal extract to the fish diet is due to flavonoids. These flavonoids can enhance the intestinal absorption of EPA and DHA and accelerate the synthesis of EPA and DHA from their precursor  $\alpha$ -linolenic acid [111]. Similar results were obtained after the consumption of garlic (*Allium sativum*) extract by sterlet sturgeon (*Acipenser ruthenus*) led to increased EPA and DHA in the whole body of fish [112]. In our study, the highest  $\Sigma n-6$  FAs,  $\Sigma n-3$  FAs, and  $\Sigma$ PUFA were observed in  $H_{0.75}$ . The lowest  $\Sigma$ SFA was observed in  $H_{0.75}$ , too, which is inconsistent with the results of common carp fed with different doses of peony (*Paeonia lactiflora*) pollen, which showed that this herbal powder could regulate lipid metabolism and FAs composition of common carp significantly [113]. Fish possess an effective antioxidant

system to preserve health and mitigate the risk of oxidation-induced damage and death. Certain dietary elements like vitamins [114], carotenoids [115], and highly unsaturated FAs (HUFAs) such as EPA, DHA, and arachidonic acid (AA, 20:4 $n$ -6) can enhance the efficacy of this system [113, 116]. The respective dietary compositions strongly influence the FA profiles of fish tissues [117].

## 6. Conclusion

The results of the present study indicated that dietary common hop extract supplementation has no growth promoting effects in carp; however, it could improve fat metabolism and deposition of protein content in whole-body composition. Hop can stimulate humoral and hepatic antioxidant capacity and boost the FA composition in fillets. Nevertheless, in our study, the effective concentration of common hop in carp's diet was found at levels 0.75 and 1.5 g kg<sup>-1</sup>; functional concentration must be selected cautiously, as its benefits vary across different doses, depending on the fish tissue. These promising results of feed supplements suggest a starting point for further in vivo investigations of its possible utilization, as well as the underlying antioxidant and anti-inflammatory mechanism of the hop in aquaculture species, to confirm the results of the present study.

## Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

## Ethics Statement

The fish sampling and protocols of this study were conducted in compliance with Czech regulations 166/1996 and 246/1992 and approved by the Departmental Expert Committee for Authorization of Experimental Projects of the Ministry of Education, Youth, and Sports of the Czech Republic (Permit MSMT-3126/2021-3).

## Conflicts of Interest

The authors declare no conflicts of interest.

## Author Contributions

Faranak Dadras: design the experiment, perform feeding trial, sampling, sample analysis, data analysis, and writing the manuscript. Hadiseh Dadras: design the experiment, perform feeding trial, sampling, and manuscript improvement. Latifeh Chupani: design the experiment, perform feeding trial, and sampling. Josef Velisek: sample analysis and manuscript improvement. Alzbeta Stara: sample analysis. Ales Tomcala: sample analysis. Eliska Zuskova: sampling, manuscript improvement, and project supervision.

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