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Unveiling the Effects of Fennel (*Foeniculum vulgare*) Seed Essential Oil as a Diet Supplement on the Biochemical Parameters and Reproductive Function in Female Common Carps (*Cyprinus carpio*)

Hamidreza Ahmadniaye Motlagh [1,*], Yoshifumi Horie [2], Hediye Rashid [1], Mahdi Banaee [3,*], Cristiana Roberta Multisanti [4] and Caterina Faggio [4,*]

[1] Department of Fisheries, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad 9177948974, Iran; rashid.hediye@gmail.com
[2] Research Center for Inland Seas (KURCIS), Kobe University, Fukaeminami-Machi, Higashinada-ku, Kobe 658-0022, Japan
[3] Aquaculture Department, Faculty of Natural Resources and the Environment, Behbahan Khatam Alanbia University of Technology, Behbahan 6361663973, Iran
[4] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98166 Messina, Italy
* Correspondence: ahmadnia@um.ac.ir (H.A.M.); mahdibanaee2@gmail.com (M.B.); cfaggio@unime.it (C.F.)

Abstract: The present study aimed to investigate the effect of *Foeniculum vulgare* essential oil on the health of the common carp (*Cyprinus carpio*). A total of 120 healthy fish were provided with feed containing 200, 400, and 600 mg/kg of *F. vulgare* oil for 60 days. Findings revealed that the oral administration of 200 mg/kg of *F. vulgare* oil significantly increased final weight, weight gain, and specific growth rate compared to the control group ($p < 0.05$). Plasma biochemical enzyme levels remained unchanged in 200 and 400 mg/kg treatments, whereas they were found to be significantly increased in treatments with 600 mg/kg. Although no significant alterations in glucose, triglyceride, and cholesterol levels were observed, the treated groups exhibited significant increases in total protein, albumin, globulin, and creatinine levels. Results also indicated significantly decreasing levels of glutathione peroxidase, whereas superoxide dismutase activity increased. The gonadosomatic index showed notable improvement in the 200 and 400 mg/kg groups. Furthermore, plasma concentrations of estradiol and testosterone were significantly affected by doses of 400 and 200 mg/kg. Findings suggest that, following the administration of *F. vulgare* extract, the reproductive and general health of the fish appears to be improved. Nevertheless, it is recommended to supplement fish diets with up to 200 mg/kg of *F. vulgare* extract to improve their reproductive and general health. Concentrations above this limit can potentially cause harm.

Keywords: *Cyprinus carpio*; fennel extract; phytoestrogen; reproduction

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1. Introduction

Inappropriate environmental conditions in captivity can impede the normal reproductive abilities of numerous aquatic species [1]. This is primarily attributed to the absence of environmental cues necessary for initiating the reproductive process in breeding conditions. In this context, natural or synthetic hormones are commonly used to overcome this issue and produce high-quality gametes [2].

Studies showed that aquatic animals' reproductive systems and other organs are susceptible to damage due to the prolonged presence of hormones. According to Lange et al. [3], natural and synthetic steroids have differing effects on ovarian tissue, growth, and maturation. Synthetic steroids tend to have greater efficacy due to their resistance to degradation during digestion, which slows down their excretion compared to natural hormones [2,4].

Conversely, several plant-based compounds have been studied for their protective and performance-enhancing effects on fishes [5]. For instance, plant-based steroid compounds in biological equilibrium do not accumulate in the body like synthetic steroids and therefore do not cause harmful effects [6]. This is why plants containing phytoestrogens are a better alternative to industrial estrogens, as they offer a safer option [7]. Phytoestrogens are plant-derived compounds that have a similar structure to the hormone estrogen found in humans and animals. Phytoestrogens can be found in various plant-based foods, including soybeans, flaxseeds, chickpeas, lentils, and fennel [8,9]. Phytoestrogens can exert weak estrogenic or anti-estrogenic effects in the body, depending on factors such as the type of phytoestrogen and the amount consumed. They can bind to estrogen receptors in the body and mimic some of the actions of estrogen. Some studies have shown that they can help balance, regulate, and increase levels of the luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are important hormones for ovulation [10].

There is some evidence to suggest that phytoestrogens may have a positive effect on reproductive health in female fishes, including their potential to induce ovulation. Phytoestrogens can have diverse effects on female fishes, depending on the species, the dose, and other factors. In some cases, phytoestrogens may benefit a female fish's health. However, exposure to high levels of phytoestrogens or prolonged exposure over time can disrupt the normal endocrine function in a female fish, leading to adverse effects such as impaired fertility, altered fecundity, and increased risk of ovarian degeneration. Furthermore, because phytoestrogens are structurally similar to the hormone estrogen, they can interfere with the action of natural estrogen in the body, potentially leading to hormonal imbalances and related health issues [11]. Therefore, more research is needed to understand this relationship fully.

In certain fish species, phytoestrogens have been reported to stimulate the production of endogenous estrogens, which can help induce ovulation. Studies showed that feeding a female African catfish a diet containing a soybean meal rich in phytoestrogens resulted in higher levels of endogenous estrogens and increased ovulation rates [12]. However, the effects of phytoestrogens on fish reproduction can be complex and depend on various factors, such as the species of fish, the dose and duration of exposure to the phytoestrogen, and the timing of administration concerning the reproductive cycle.

Fennel (*Foeniculum vulgare*) contains phytoestrogens, plant compounds that mimic the hormone estrogen in the body. *F. vulgare* contains a variety of bioactive compounds, including phenolic compounds (flavonoids, phenolic acids, and tannins), essential oils (anethole, fenchone, and estragole), terpenes (limonene, pinene, and myrcene), coumarins (scopoletin and umbelliferone), and alkaloids (anabasine and nicotine) [13]. The primary phytoestrogen found in fennel is called anethole. Anethole is believed to have weak estrogenic activity, meaning it can bind to estrogen receptors in the body and exert some estrogen-like effects [8]; however, the extent of these effects is relatively mild compared to those of actual estrogen.

Fennel has undergone extensive research revealing its numerous benefits, which encompass antimicrobial, antifungal, anti-inflammatory, antioxidant, anxiolytic, cardioprotective, and potential hormonal properties [13]. Notably, fennel acetone extract shows promise in menstrual cycle regulation [14], and it may affect hormone levels by increasing the follicle-stimulating hormone while decreasing the luteinizing hormone and testosterone [15]. Moreover, fennel essential oils notably influence oocyte maturation and sexual maturity in fishes [16,17].

Although phytoestrogens such as *F. vulgare* may potentially induce ovulation in some fish species, further research is needed to fully understand their effects and how they could be used in aquaculture or other applications. This study primarily aims, for the first time, to examine the impact of fennel essential oil on the growth performance, reproductive indices, and liver enzymes of the female common carp (*Cyprinus carpio*), as it ranks as the fourth most economically valuable aquaculture species.

2. Materials and Methods

2.1. Preparing Fennel Essential Oil and Determining the Amount of Effective Substance

In this study, the fennel essence was extracted by mixing 100 g of washed and dried fresh fennel seeds with 1000 milliliters of distilled water in a Clevenger apparatus for four hours. The resulting essential oil was stored in a dark container with a lid in the refrigerator, which was covered completely with aluminum foil to avoid any light exposure. The essential oil accounted for nearly three percent of the seed weight. In order to identify the active component present in the essential oil, the trans-anethole content was measured using gas chromatography–mass spectrometry (Agilent GC-Mass 6890N, Agilent Co., Santa Clara, CA, USA). The analysis displayed that around 33.40% of the essential oil was composed of trans-anethole (Table 1). This study was designed as a completely randomized design in three experimental treatments of 200, 400, and 600 mg/kg of fennel extract in diet and a control treatment (without essence) in three repetitions.

Table 1. Volatile compounds present in essential oil of *Foeniculum vulgare*.

Compounds	Essential Oil (%)	KI
α-Pinene	1.96	914
β-Pinen	0.95	969
Limonen	7.31	1036
1,8 Cineol	7.54	1042
Trepinen	0.81	1093
Fenchone	7.05	1101
Camphor	0.65	1152
4-Terpineol	0.33	1168
α-Terpineol	7.78	1168
Estragloe	13.25	1242
E-Anethole	33.4	1276
2,4-Decadienal	18.12	1326
Germacrene	0.85	1493

2.2. Rearing Condition and Fish Feed

During the experiment, the physicochemical parameters of rearing water, including temperature, pH, dissolved oxygen, and total hardness, were measured (27 ± 1.5 °C; 7.3 ± 0.21; 7.12 ± 0.33 mg/L; and 220.68 ± 42.9 mg/L). The light conditions were set as natural light. Feedstuffs composition and proximate chemical composition of the basal diet are presented in Table 2.

2.3. Design and Procedure

One hundred twenty pre-productive healthy females of the same weight and same size (78.66 ± 11.10 g and 22.70 ± 3.80 cm) were purchased from a local farm. After passing 14 days of adaptation, they were randomly divided into 12 aquariums. The fish were fed a basic diet during the adaptation period. Food was prepared as follows: the extract was mixed with the basal diet according to the desired amount, dried at room temperature, packed, and stored in the refrigerator. Feeding was completed at the rate of 2–3% of body weight daily for 60 days.

2.4. Sampling

To evaluate the essence's impact on the growth performance, the fish were fasted for 24 h at the end of the feeding trial. After anesthetizing the fish with clove powder (150 mg L^{-1}), their weight was measured with a digital scale (ACZET, mp300, Piscataway, NJ, USA) with an accuracy of 0.01 g [18,19]. Three fish were chosen randomly from each repetition and euthanized employing 2 g L^{-1} of clove powder. The ovaries were

extracted, weighed, and blood samples were taken using a 2 cc heparin syringe. Growth and reproduction indices were subsequently computed utilizing the following Formulas [18]:

$$Specific\ growth\ rate\ (SGR)(\%body\ weight\ day-1) = \left[\frac{(lnWf - lnWi)}{t}\right] \times 100$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Feed\ consumed}{Weight\ gain}$$

$$Weight\ gain\ (\mathrm{g}) = (final\ weight - initial\ weight)$$

$$Gonadosomatic\ index\ (GSI)\ (\%) = \frac{gonad\ weight}{fish\ total\ weight - gonad\ weight} \times 100$$

Table 2. Feedstuffs composition and chemical characteristics of the basal diet.

Feed Items	Percentage (%)			
	Basic Diet	Experimental Diet 1	Experimental Diet 2	Experimental Diet 3
Fish meal	33	33	33	33
Meat meal	9	9	9	9
Wheat gluten	10	10	10	10
Hydrolyzed yeast	4	4	4	4
Wheat flour	34	34	34	34
Rice bran	3.5	3.5	3.5	3.5
Fish oil	2	2	2	2
Mineral supplement	1.5	1.5	1.5	1.5
Vitamin supplement	1.5	1.5	1.5	1.5
Bentonite	0.5	0.48	0.46	0.44
Sodium chloride	0.5	0.5	0.5	0.5
Antifungal	0.5	0.5	0.5	0.5
Fennel extract	0	0.02	0.04	0.06
Chemical Composition (% of Dry Matter)				
Dry matter	94.5	94.68	94.78	94.87
Crude protein	32.5	32.56	32.63	32.59
Crude fat	6.5	6.51	6.52	6.52
Ash	4.5	4.49	4.5	4.49
Crude fiber	7.5	7.48	7.53	7.54
Nitrogen free extract	49	49	49	49
Gross energy (kcal/kg)	3656	3663	3670	3667

2.5. Plasma Biochemical Indices

The prepared blood samples were centrifuged at 6000× *g* for 5 min using a refrigerated centrifuge. The plasma was then separated using a sampler and kept in a freezer at −80 °C until the test.

The activity levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) enzymes were measured using the kits provided by the Pars Azmoun Company and Unico UV/VIS spectrophotometer (Suite E Dayton, NJ, USA) 2100 [19].

The glucose was measured using the enzyme method of glucose oxidase, while cholesterol and triglycerides were measured using CHO-PAP [20,21]. Total plasma protein was measured based on a biuret reaction at a wavelength of 540 nm [19,21]. The Bromocresol Green method was employed to measure plasma albumin levels at 540 nm. Globulin level was also calculated based on the difference between total protein and albumin levels. The JAFFE method was used to measure creatinine levels at 510 nm, utilizing picric acid in an alkaline environment [19,21].

2.6. Oxidative Biomarkers

Catalase (CAT) activity was evaluated based on Góth's procedure [22], which utilized hydrogen peroxide as a substrate and ammonium molybdate to halt the reaction. On the other hand, superoxide dismutase, glutathione peroxidase, and glutathione reductase activities were measured with Biorex biochemical kits following the manufacturer's instructions [23].

2.7. Measurement of Sexual Hormones

The concentration of sex steroid hormones (testosterone and estradiol) was measured through a radioimmunoassay following the guidelines. In order to do so, 50 to 100 microliters of plasma samples, controls, or standard solutions were mixed with rat polyclonal antibodies coated tubes. Hormones labelled with iodine 125, such as estradiol (COAT-A-COUNT, Diagnostic Products Corporation, Los Angeles, CA, USA, DPC®) or 17 alpha hydroxyprogesterone (COAT-A-COUNT, Diagnostic Products Corporation, Los Angeles, CA, USA, DPC®) were added to all test tubes. The mixture was then incubated in a water bath and washed with phosphate buffer before reading the level of radioactive activity using a gamma counter.

2.8. Statistical Analysis

The percentage data were converted to Arcsin before conducting a statistical analysis through SPSS version 18. After ensuring that the data met the two primary requirements of parametric tests, namely homogeneity of variance and normality, a one-way analysis of variance test was conducted to explore the main factors and their mutual influence. Furthermore, Duncan's multiple range test was conducted at a significance level of 5% to check for differences in means. Charts and tables were prepared using Excel version 2013.

3. Results

The results of the growth performance of *C. carpio* following a 60-day feeding study are shown in Table 3. There was no significant difference in initial weight. The experimental groups had a significantly greater final weight and weight gain ($p < 0.05$) compared to the control group. The highest reported weight gain and final weight were in the 200 mg/kg group.

Table 3. Growth parameters of *C. carpio* fed experimental diets containing varying quantities of *F. vulgare* essence over 60 days (mean ± SD, $n = 3$).

	***F. vulgare* Essence Levels (mg/kg Feed)**			
	0	**200**	**400**	**600**
Initial weight	79.25 ± 12.34	77.20 ± 12.08	78.25 ± 12.80	79.93 ± 14.32
Final weight	89.20 ± 16.32 ^{a}	93.81 ± 18.20 ^{c}	91.10 ± 15.71 ^{b}	90.85 ± 10.4 ^{b}
Weight gain	09.87 ± 0.21 ^{a}	16.40 ± 0.54 ^{c}	12.84 ± 1.96 ^{b}	10.92 ± 1.24 ^{b}
FCR	1.86 ± 0.11 ^{bc}	1.43 ± 0.25 ^{a}	1.55 ± 1.14 ^{ab}	2.00 ± 0.07 ^{c}
SGR	0.25 ± 0.17 ^{a}	0.48 ± 0.05 ^{b}	0.41 ± 0.10 ^{ab}	0.29 ± 0.05 ^{ab}

Notes: Significant differences exist between means with different letters in the same row (ANOVA, $p < 0.05$). FCR: feed conversion ratio; SGR: specific growth rate.

In addition, the results indicate that oral treatment of 200 mg/kg *F. vulgare* essence considerably lowered the FCR. The 600 mg/kg group exhibited the highest FCR value, with a significant increase compared to the other two experimental groups ($p < 0.05$). SGR was significantly higher than the control group ($p < 0.05$). Table 4 displays the mean liver enzyme activity of the *C. carpio* that were fed experimental meals, including *F. vulgare* essence for 60 days. AST in the control group did not differ substantially from the lowest treatment (200 mg/kg). However, this value increased significantly ($p < 0.05$) in the 400 and 600 mg/kg treatments compared to the control group. The lowest ALT activity was then detected at 200 mg/kg, although the difference was not significant compared to the control.

The maximum ALT activity was observed after the final treatment (600 mg/kg). Regarding ALP, no significant change was noticed between the control and other treatments. LDH activity increased significantly at 600 mg/kg compared to the control, but there was no significant difference between the control and the other treatments. Table 4 represents the mean plasma biochemical parameters of the *C. carpio* that were fed the experimental diets for 60 days. The results showed that glucose and triglyceride did not change significantly compared to the control and the treated groups. Total protein was significantly increased in the 400 and 600 mg/kg treatments compared to the control ($p < 0.05$). The highest and lowest albumin contents were reported in the 600 and 200 mg/kg treatments, respectively ($p < 0.05$). No significant difference was observed between the albumin content of the control group and the 400 mg/kg treatment. A significant increase in globulin value was detected at 400 mg/kg compared to the control ($p < 0.05$). Other treatments had no significant difference from the control. Cholesterol content was not significantly changed and remained unaffected. The plasma creatinine content, only at the highest level of 600 mg/kg, showed a significant increase compared to the control ($p < 0.05$).

Table 4. Plasma biochemical parameters of *C. carpio* that were fed the experimental diets containing different levels (0, 200, 400, and 600 mg/kg) of *F. vulgare* essence for 60 days (mean ± SD, $n = 3$).

	***F. vulgare* Essence Levels (mg/kg Feed)**			
	0	**200**	**400**	**600**
AST (U/L)	48.57 ± 6.14 ^{a}	53.22 ± 4.65 ^{ab}	55.71 ± 2.71 ^{b}	58.86 ± 3.75 ^{b}
ALT (U/L)	15.92 ± 1.15 ^{ab}	13.16 ± 0.55 ^{a}	14.92 ± 0.98 ^{b}	16.39 ± 0.94 ^{c}
LDH (U/L)	416.68 ± 19.64 ^{a}	441.14 ± 39.76 ^{ab}	462.78 ± 78.47 ^{ab}	484.22 ± 27.27 ^{b}
ALP (U/L)	184.71 ± 8.35	181.35 ± 10.71	193.42 ± 14.48	190.48 ± 13.89
Glucose (mg/dL)	64.94 ± 4.33	65.03 ± 10.72	60.99 ± 5.59	67.11 ± 10.21
Total protein (g/dL)	2.71 ± 0.14 ^{a}	2.23 ± 0.50 ^{a}	3.45 ± 0.60 ^{b}	3.53 ± 0.64 ^{b}
Albumin	2.00 ± 0.15 ^{b}	1.24 ± 0.19 ^{a}	1.86 ± 0.08 ^{b}	2.48 ± 0.46 ^{c}
Globulin (g/dL)	0.71 ± 0.11 ^{a}	1.00 ± 0.39 ^{a}	1.59 ± 0.60 ^{b}	1.05 ± 0.53 ^{a}
Cholesterol (mg/dL)	73.80 ± 4.87 ^{ab}	80.18 ± 5.55 ^{b}	67.57 ± 8.41 ^{a}	69.80 ± 6.05 ^{a}
Triglycerides (mg/dL)	185.9 ± 17.25	173.75 ± 12.06	168.34 ± 15.52	171.33 ± 14.43
Creatinine (mg/dL)	0.31 ± 0.04 ^{a}	0.36 ± 0.11 ^{a}	0.40 ± 0.11 ^{a}	0.53 ± 0.10 ^{b}

Notes: Significant differences exist between means with different letters in the same row (ANOVA, $p < 0.05$). AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase.

The mean plasma biochemical parameters of *C. carpio* that were fed the experimental diets for sixty days are shown in Table 4. Glucose and triglyceride levels did not differ significantly between the control and experimental groups. The 400 mg/kg and 600 mg/kg treatments significantly enhanced total protein compared to the control ($p < 0.05$). The treatments with the highest and lowest albumin levels were 600 mg/kg and 200 mg/kg, respectively ($p < 0.05$). The difference between the control group and the 400 mg/kg treatment was not statistically significant. The group with 400 mg/kg exhibited a substantial rise in globulin levels compared to the control ($p < 0.05$). Other treatments did not differ significantly from the control. The cholesterol content did not change considerably and stayed unchanged. Only at the highest dosage, 600 mg/kg, did plasma creatinine levels increase significantly compared to the control ($p < 0.05$).

As shown in Table 5, plasma CAT activity remained unaltered with experimental diets; however, SOD activity decreased significantly in the 600 mg/kg group compared to the control group ($p < 0.05$). Compared to the control, the plasma GPx activity of the treated groups significantly decreased ($p < 0.05$).

Table 5. Antioxidant enzyme activity of *C. carpio* that were fed the experimental diets containing different levels (0, 200, 400, and 600 mg/kg) of *F. vulgare* essence for 60 days (mean ± SD, $n = 3$).

	***F. vulgare* Essence Levels (mg/kg Feed)**			
	0	**200**	**400**	**600**
CAT (KU/mg protein)	0.15 ± 0.01	0.15 ± 0.02	0.14 ± 0.03	0.14 ± 0.03
SOD (U/mg protein)	0.78 ± 0.25 ^{b}	0.90 ± 0.24 ^{b}	0.70 ± 0.32 ^{b}	0.36 ± 0.07 ^{a}
GPx (U/mg protein)	7.80 ± 1.06 ^{c}	4.45 ± 1.45 ^{b}	2.51 ± 0.75 ^{a}	2.66 ± 0.46 ^{a}

Note(s): Significant differences exist between means with different letters in the same row (ANOVA, $p < 0.05$). CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase.

Estradiol levels in female *C. carpio* that were fed 400 mg/kg were substantially greater than the control (Figure 1a, $p < 0.05$), although the other treatments were unchanged. The investigation of testosterone concentration, on the other hand, revealed that the highest concentration was found in carps that were fed 200 mg/kg *F. vulgare* essence, which was significantly greater than the control (Figure 1b, $p < 0.05$). Compared to the control, the higher inclusion levels had no significant impacts.

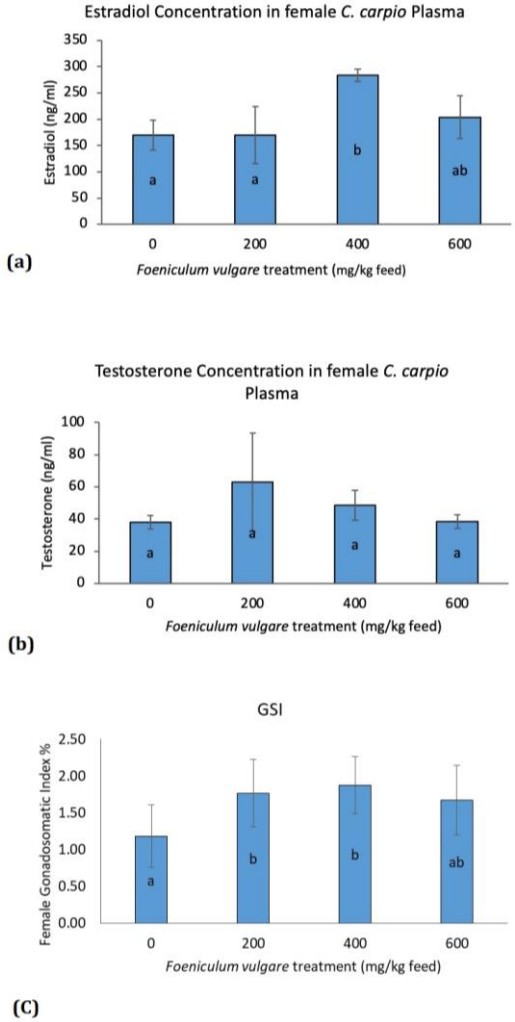

Figure 1. Estradiol (**a**) and testosterone (**b**) concentration in plasma and a gonadosomatic index (**c**) in *C. carpio* that were fed the experimental diets containing different levels (0, 200, 400, and 600 mg/kg) of *F. vulgare* essence for 60 days (mean ± SD, $n = 3$). Different letters indicate significant differences ($p < 0.05$).

Figure 1c displays the GSI of *C. carpio* that were fed varying concentrations of *F. vulgare* essence. After 60 days of therapy, the GSI of the experimental groups (200 and 400 mg/kg) increased significantly compared to the control group ($p < 0.05$). No significant differences were identified between the tested groups. The highest treatment (600 mg/kg) represented no significant increase compared to the control.

4. Discussion

Phytoestrogens are plant-derived compounds that can mimic the effects of estrogen in animals. Some studies have explored the use of phytoestrogens in inducing fish to ovulate, particularly in species where spawning can be difficult to induce. However, it is important to note that the use of phytoestrogens in aquaculture is still a relatively new area of research, and there are concerns about the potential environmental impacts of increased phytoestrogen levels in aquatic ecosystems. Additionally, the effectiveness of phytoestrogens in inducing ovulation may vary depending on fish species and other factors, so further studies are needed to fully understand their potential applications in aquaculture.

Phytoestrogens may be excreted, absorbed, or broken into stronger compounds after entering the digestive tract. Isoflavones become active during digestion as well as absorption in human and animal bodies, where this conversion is processed by bacteria in the small intestine as follows: the sugar part is separated from the molecules by bacteria and converted into an active form. Then, the activated form of isoflavones is absorbed from the small intestine, most of which is taken up by the liver after entering the body. A small amount is excreted by the kidneys and through urine [24]. Absorbed phytoestrogens and isoflavones can interfere with the expression of growth-related genes such as IGF1 and promote growth [25]. Since fennel essence has antimicrobial properties [26], the presence of this compound in the intestines of the treated fish will change the microbial balance of the intestines in favour of the host and will help the growth of the host by enhancing the production of digestive enzymes. Such results were also observed in the use of other herbal compounds such as peppermint extract [27] and Iranian shallot extract [28].

Based on the results, fennel essential oils improved growth, FCR, and SGR in common carp (*C. carpio*). Many studies revealed the positive effects on fish growth with the oral administration of plant-based essential oils. For instance, according to Kesbic et al. [29], the administration of a Monterey Cypress (*Cupressus macrocarpa hartw*) leaf essential oil as a dietary supplement of *C. carpio*'s diet was found to be a suitable growth promotor. Moreover, similar results to our findings were obtained in the treatment of *Oreochromis niloticus* and *Poecilia reticulate* with fennel [17,30]. Studies have shown that a Bergamot (*Citrus bergamia*) peel oil supplementation in fish diets was able to optimize growth performance, feed utilization, and general health status [31,32]. *Vitex agnus* extract, which has phytoestrogen properties, was used to produce feminization, with the results indicating that 15 g/kg of the extract also increased growth [33].

Phytoestrogens can react with enzymes and receptors, and due to their stable structure and low molecular weight, they can pass through cell membranes [34,35]. There is evidence confirming that phytoestrogens affect fat metabolism. Since fats play an essential role in supplying the nutrients in the eggs, phytoestrogens can likely help improve the storage of nutrients in the developing ovaries and ultimately enhance the GSI as well as accelerate the sexual maturation of fish. The results of our study also showed that fennel essence significantly contributed to the development of gonads in the treatments of 400 and 200 mg/kg. There are various studies indicating the positive effect of phytoestrogens in boosting vitellogenin production and improving GSI. For example, researchers who investigated the effect of fennel essential oils and phytoestrogen genistein in *Cichlasoma nigrofasciatum* and *Huso huso*, respectively, achieved similar results at the level of 125 mg/kg and 0.4 g/kg, respectively [6,16].

Phytoestrogens bind to estrogen receptors in the body with less affinity than estradiol; they are weakly bound to ERα receptors and strongly bound to ERβ receptors with specific and anti-estrogenic effects, in such a way that they have agonist effects in some tissues

and have antagonistic effects in others. On the other hand, phytoestrogens that bind to ERβ induce the transcription of estrogen target genes more than when they are bound to ERα [33] and can leave estrogen agonistic plus antagonistic effects. In this study, it was also found that fennel seed essence did not influence increasing testosterone production, but it significantly elevated estradiol in the 400 mg/kg treatment. There are several studies indicating the effect of plant compounds containing phytoestrogens, such as vitex [31], *Tribulus terrestris* [34,35], garlic [36], aloe vera [37], and *Matricaria recutita* [7], on the sexual performance of fishes. They have revealed that these plants can exert effective hormone-like effects on the fish in certain amounts due to the presence of estrogen-like compounds. The effect of fennel in enhancing the secretion of sex hormones in *P. reticulata* and rats [17,38] has also been proven.

In general, it can be stated that the activity of phytoestrogens in the body depends on factors such as the concentration of estrogens in the body, the state of saturation of estrogen receptors, the duration of binding of phytoestrogens to estrogen receptors, and the time it takes until the phytoestrogen is broken down and enters the blood circulation. When the concentration of estrogen in the blood is low, phytoestrogens show pro-estrogen effects, while on the contrary, when the concentration of estrogen in the blood is high, they have anti-estrogenic effects and impact the growth of estrogen-dependent cells plus the sexual cycle [38]. The results of this study also indicated that fennel seed essence at the highest level (600 mg/kg ration) both reduced the amount of estradiol and prevented gonad growth. Similar results were obtained in the study of the effect of this plant on the development of the testes of common carp, where the excessive dietary administration of the extract caused a decline in GSI and plasma testosterone concentration [39].

An increase in the activity of liver enzymes is a sign of unfavourable rearing conditions and vital organ tissue damage such as the liver and kidney, so measuring the activity of liver enzymes has always been mentioned as an indicator of fish health, and their investigation is recommended in research related to medicinal plants. Fennel seed essence has been reported to have hepatoprotective activity [8]. The results of a biochemical analysis of plasma compounds showed that the treatment of carp with fennel seed essence had no significant effect on liver enzymes, and only ALT activity was significantly reduced in the treatment of 200 mg/kg. These results suggest that fennel essential oil in the amounts that were used did not damage the tissue but protected it.

Reactive oxygen species (ROS) are key signalling molecules that play an important role in healing inflammatory disorders and help clear pathogens as well as foreign particles. However, high levels of reactive oxygen species can also destroy normal cells [40], while antioxidant enzymes can defend against this excessive increase in these molecules [13]. One of the reasons for using medicinal plants in the ratio of aquatic animals is to boost the antioxidant capacity of the body and improve the ability to cope with adverse environmental conditions, which ultimately enhances survival and production in aquaculture. SOD, CAT, Gr, and GPx are the main antioxidant enzymes that catalyze reactive oxygen species [41,42]. Despite the strong antioxidant activity in fennel seed extract [43,44], there have not been many studies on the effect of this plant on the antioxidant activity of aquatic animals. In the present experiment, the results confirmed the increase in SOD enzyme activity, while glutathione peroxidase decreased significantly in the experimental treatments. Similar to our findings, the activity of antioxidant enzymes and the expression level of SOD plus CAT genes increased significantly in the liver of *Micropterus salmoides* treated with fennel essence [13].

In this experiment, the amount of total protein, albumin, globulin, and cholesterol increased due to treatment with fennel seed essence, and no change was reported in glucose and triglyceride status. The biochemical parameters of blood reflect the health, nutritional, and environmental status of the fish, where the change in the biochemical variables of blood is probably the result of increasing the non-specific immune response of fish [45], since globulin, albumin, and total protein are known as important components of the innate immune system of fish [23]. Consistent with our findings, Gulec et al. [46] reported that

the administration of fennel seed essential oils to the *Oncorhynchus mykiss* diets elevated plasma biochemical indices, including total protein, albumin, cholesterol, triglycerides, and bilirubin.

There are various studies that confirmed the reduction in glucose due to the use of fennel [42,44] and other plants containing phytoestrogens such as Vitex [29], while the glucose level remained constant in our experiment. Glucose reduction can be due to increased insulin secretion or increased fish metabolism because of drug treatments [24], whereas fennel apparently did not show such an effect. Cholesterol, as a precursor of steroid hormones, plays an important role in the biosynthesis of these hormones and the acceleration of sexual maturation. The increase in the level of blood cholesterol as well as the increase in the secretion of estradiol hormone both confirm the positive effects of fennel on the sexual activity of carps.

Creatinine is a breakdown product of creatine phosphate resulting from protein metabolism, which is released by the body at a constant rate [47]. Blood creatinine is an important indicator of kidney health, as it is an easily measurable byproduct of muscle metabolism excreted by the kidneys without any change [47]. In the present study, the amount of blood creatinine in the treatments of 200 and 400 mg/kg was not significantly different from the control group, indicating that this level of essence did not negatively affect the kidney and vital organs, but the 600 mg/kg treatment was associated with a notable increase in the creatinine content. Studies by Abdel Rahman et al. [48] showed that in the case of aflatoxin poisoning in *Oreochromis niloticus*, fennel essential oils can significantly lower the level of blood creatinine, which was elevated due to poisoning.

It is crucial to mention that phytoestrogens can negatively impact species and aquatic ecosystems that are not the intended target. Studies showed that phytoestrogens could have multiple effects on aquatic ecosystems. Phytoestrogens could disrupt the endocrine system in aquatic animals, alter their behaviours, and change population dynamics. Moreover, waterborne phytoestrogens could impact non-target species and cause shifts in their community structure [49,50]. Therefore, it is recommended to investigate its unwanted environmental effects before prescribing any phytoestrogen.

5. Conclusions

The findings from our research demonstrated that the inclusion of fennel essential oil in the dietary regimen of *C. carpio* not only enhanced their reproductive capabilities but also had positive effects on their overall health and antioxidant status. A noteworthy aspect of this study was the careful consideration given to the amount of medicinal plant compounds utilized in the aquatic diet. The results indicated that excessive use of these products could exert undue pressure on vital organs such as the liver and kidneys, potentially leading to adverse effects on reproduction. Hence, it is important to strike a balance. Considering all factors, a recommended dosage of 200 mg/kg of fennel seed essential oil is suggested for optimal carp breeding outcomes.

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