Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(1): 1489 – 1508 (2024)
www.ejabf.journals.ekb.eg

Impact of Caraway Essential Oil on Growth, Feed Utilization, and Flesh Quality of the Nile Tilapia, *Oreochromis niloticus*

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ARTICLE INFO

Article History:
Received: Jan. 17, 2024
Accepted: Feb. 13, 2024
Online: Feb. 15, 2024

Keywords:
Tilapia,
Essential oil,
Feed additive,
Histology,
Muscle

ABSTRACT

The use of medicinal plants or their essential oils (EOs) as feed additives for fish has become a more useful tool in the aquaculture sector than traditional chemicals. Therefore, the current study was conducted to evaluate the effects of the graded levels (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5g/ kg diet) of caraway essential oil (CEO) on the growth performance, feed utilization, somatic indices, the chemical composition of the body and muscles, as well as histological and histometric parameters of dorsal muscles, alongside flesh quality parameters of the Nile tilapia *Oreochromis niloticus* for 90 days. The obtained findings revealed that fish fed with 0.1g CEO/ kg diet significantly increased growth performance, feed utilization, histological and histometric parameters of dorsal muscles, and flesh quality parameters, among other levels of CEO. The high level of CEO, up to 0.1g/ kg diet, hurts the above-tested criteria. Thus, it could be concluded that the judicious use of CEO for *O. niloticus*, especially at a level of 0.1g CEO/ kg diet, proved to be beneficial..

INTRODUCTION

Tilapias are one of the most widespread fish in the world. The Nile tilapia, *Oreochromis niloticus*, is widely accepted in all markets since it is considered one of the most important species within all tilapia species due to its rapid growth, good survival in high-density culture, and disease tolerance **(El-Sayed, 2006)**. Globally, tilapia farming has grown extremely fast in the last decade to meet the human need for animal protein and reduce the nutritional gap. They are cultured worldwide with an annual growth rate of about 12.2% **(Wang & Lu, 2016)**. The last decades have witnessed an outstanding global extension of *O. niloticus* culture almost throughout 100 countries in Asia and Africa **(Gu *et al.*, 2017)**. Tilapia is the second most farmed fish worldwide, and its production has quadrupled over the past decade due to its suitability for aquaculture, marketability, and stable market prices **(FAO, 2022)**.

In the current situation, the whole world is witnessing climate change, natural disasters, wars, and international conflicts over water and food resources; therefore, finding new alternatives or more efficient methods to solve the food crisis that has spread in various societies is needed **(FAO, 2015)**. Among those alternatives are the use of cheap foodstuffs with high protein content or the use of some feed additives. There is an increased interest in using natural feed additives from the whole or extracts of some herbs and edible plants as safe supplements instead of chemically produced compounds **(Done *et al*., 2015)**. Thus, several attempts are widely accepted to use medicinal plants as feed additives **(Elumalai *et al*., 2020)**. Caraway, *Carum carvi,* belonging to the Apiaceae family, has been widely used as a food spice, vegetable preservative, and herbal medicine. It is an important medicinal plant that has been cultivated for a long time in the north and center of Europe, Egypt, Iran, Australia, and China **(Rasooli & Allameh, 2016)**. The *C. carvi* seeds have antibacterial and fungicidal properties, and they are significant in pharmaceutical applications and human and veterinary medicine **(Sedlakova *et al*., 2001)**. Therefore, numerous studies evaluated the beneficial effects of caraway seed meal (CSM) as a feed additive on *Oreochromis* spp., such as hybrid red tilapia, *O. niloticus* × *O. aureus* **(El-Dakar, 2004)**, and *O. niloticus* **(Khalafalla**, **2009; Abbass *et al*., 2010; Ahmad & Abdel-Tawwab, 2011)**, as well as the effects of other medicine plant meal (as a feed additive) on *O. niloticus*, such as oregano **(Seden *et al*., 2009)**, green tea **(Abdel-Tawwab *et al*., 2010)**, cinnamon **(Ahmad *et al*., 2011)**, dried garlic lobes (DGL) **(Mehrim *et al*., 2014)**, rocket leaves or seeds **(Khalil *et al*., 2015)**, and chamomile powder **(Nordin *et al*., 2017)**.

Throughout the world, essential oils (EOs) are one of the most used natural products derived from different parts of a plant, viz. fruits, seeds, leaves, bark, flowers, peels, buds, etc., which are used not only as a feed additive for farm animals but for humans as well **(Panda *et al*., 2022)**. Plant extracts, especially EOs, are a new class of feed additives, and knowledge regarding their modes of action and aspects of application is still rather rudimentary. The use of EOs in enhancing the productivity may have promising effects as growth and health promoters, improving physiological and immune responses, and increasing resistance against pathogens in aquaculture **(Burt, 2004; Zeng *et al*., 2015)**, where their chemical composition and concentration are the main factors or variables. Countless attempts also confirmed the positive effects of different types of EOs on growth, feed utilization, chemical composition, and physiological and immune responses, such as in *O. niloticus* fed fennel and garlic EOs **(Hassaan & Soltan, 2016)**, fed clove basil and ginger EOs **(Brum *et al*., 2017)**, and fed sweet orange EOs **(Mohamed *et al*., 2021)**. These positive effects of EOs were also recorded in different fish species, such as *O. mossambicus* **(Baba *et al*., 2016)** fed *Citrus limon* peel EO, rainbow trout (*Oncorhynchus mykiss*) **(Gültepe, 2018)** & common carp (*Cyprinus carpio*) fed orange EO **(Abdel-Latif *et al*., 2020)**, and *C. carpio* fed bitter orange (*C. aurantium*) EO **(Acar *et al.*, 2021)**, as well as Caspian roach, *Rutilus caspicus* **(Ghafarifarsani *et al*., 2022**) fed savory EO. Regarding the caraway EO (CEO), no study has been done till now on its effects on fish productivity. Therefore, the current study was conducted to evaluate the effects of the graded levels of CEO extracted from seeds (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5g/ kg diet) on growth performance, feed utilization, somatic indices, the chemical composition of the body and muscles, as well as histological and histometric parameters of dorsal muscles, alongside the flesh quality parameters of *O. niloticus*, for 90 days.

MATERIALS AND METHODS

1. The procedures and management of experimental fish

The present study was carried out at the Fish Research Unit, Faculty of Agriculture, Mansoura University, Egypt. Four hundred and fifty Nile tilapia fingerlings (with an average initial body weight of 36.64± 1.2g and 13.82± 0.25cm total length) were brought from a private farm in El-Manzala, Al Dakahlia Governorate, Egypt. Fish were acclimated to laboratory conditions for two weeks. During this period, fish were daily fed a commercial feed containing 29.86% of crude protein by hand. Water was changed twice a week at a rate of ~50% of the total volume. After the adaptation period, fish were randomly distributed among six treatments (three replicates per treatment) and stocked at 25 per tank (total volume: 600L). Each tank was supplied with an air stone connected to an air pump.

2. The physiochemical parameters of water

The water quality parameters such as temperature, dissolved oxygen (DO), and pH were monitored during the study. The water temperature was routinely measured by a thermometer; DO was measured by a Milwaukee MW600 PRO portable DO-meter, USA, and pH was measured by HI98129 waterproof pH & temperature testers, Hungary. All the above water quality parameters were weekly measured during the experimental period. The average of these water quality parameters during the experimental period was 25.45± 2.12°C for water temperature, 8.2± 0.75 for pH, and 5.0– 6.4mg/ L for DO. All water quality parameters measured in the present study are within the acceptable limits for rearing *O. niloticus* fingerlings according to **Boyd and Tucker (1998)**.

3. The gas chromatography-mass spectrometry (GC-MS) analysis

The analysis of the CEO of seeds was carried out using the gas chromatography-mass spectrometry (GC-MS) instrument at the Department of Medicinal and Aromatic Plants Research, National Research Center, Egypt, according to **Sedlakova *et al*. (2001)**. The CEO was stored in dark glass bottles in a cold condition at 4°C until usage.

4. Preparation of the experimental diets

A commercial diet was produced by Haid Factor, Al Dakahlia Governorate, Egypt. The chemical analysis of a commercial diet is shown in Table (1) according to the guidelines outlined by **AOAC (2016)**. The diet was thoroughly milled and mixed well with the CEO. The CEO obtained it from the local hydro-distillation factory in El Aayad, Giza Governorate, Egypt. The graded levels of CEO were added to the experimental diet at 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5g/ kg diet. Due to the small amount of CEO added to the diets, it was mixed with 10 mL of corn oil per kg of diet. The mixture was then thoroughly mixed with the addition of tap water (50%). Subsequently, the diets were extruded through a commercial mincing machine into pellets of 3mm diameter. The obtained pellets were air-dried for 48 hours at room temperature (28°C) and stored at -20°C in plastic bags until used. Fish were fed the experimental diets at a rate of 4% of their total body weight. The feed quantity was readjusted biweekly based on the actual average body weight of the fish in each treatment. Fish were fed by hand twice a day at 9.00 am and 2.00 pm for 90 days.

Table 1. Chemical composition of the experimental diet

Nutrient	**Percentage**
Moisture (%)	7.82
Protein (%)	29.86
Ether extract (EE, %)	5.82
Ash (%)	10.48
Crude fiber (%)	5.49
NFE[1]	48.35
Gross energy[2]	1857.2

[1]Nitrogen-free extract (NFE) = 100 - (protein + EE + ash + fiber).
[2]Gross energy (MJ/100g DM) was calculated by multiplication of the factors 17.11, 23.64, and 39.54MJ GE/ kg DM for carbohydrate, protein, and EE, respectively **(NRC, 2011)**.

5. The experimental measurements

5.1. Growth performance and feed utilization parameters

At the end of the experiment, fish in each tank were weighed to calculate the growth performance parameters according to **Lovell (2001)**, following the equations below:

- Total weight gain (TWG, g) = FW - IW
- Average daily gain (ADG, g/fish/day) = (FW – IW)/ Ti

Where, FW: Final weight (g); IW: Initial weight (g) & Ti: Time (days)

Feed intake (FI, g) was recorded, and feed conversion ratio (FCR) was calculated according to the following equations;

- Feed conversion ratio (FCR) = FI / TWG
- Protein efficiency ratio (PER) = live weight gain (g) / protein intake (g).
- Protein productive value (PPV, %) = [retained protein (g) / protein intake (g)] × 100.
- Energy utilization (EU, %) = [retained energy (Kcal) / energy intake (Kcal)] × 100.

5.2. Somatic indices

To calculate the condition factor (CF) and internal organ indices, fish were individually weighed, and their total body length (TBL) was measured. Then, fish were dissected to obtain internal organs that were weighed, such as viscera, liver, spleen, and intestine, to calculate the somatic indices following these equations:

- CF (%) = [fish weight/(fish length)^3] × 100
- Viscera somatic index (VSI, %) = (viscera weight / fish weight) × 100

- Hepato-somatic index (HSI, %) = (liver weight / fish weight) × 100
- Spleen-somatic index (SSI, %) = (spleen weight / fish weight) × 100
- Intestine-somatic index (ISI, %) = (intestine weight / fish weight) × 100

5.3. Chemical composition of the fish's whole body and dorsal muscles

The chemical analyses of the fish's whole body and their dorsal muscles as a percent of dry matter weight in each treatment were done according to the methods of **AOAC (2016)**. While the determination of chemical analysis of the commercial floating diet was conducted through New Hope Egypt Aquatic Feed (the Chinese company for aquatic feed), Damietta Governorate, Egypt.

5.4. Flesh quality parameters

To estimate water holding capacity (WHC), individual dorsal muscle fillet portions (*n* = 6/treatment) were separated, weighed individually, and put between two filter papers, then placed a weight of 3.5kg on it for 15 minutes. The WHC is estimated as a percentage by dividing the difference between the two weights by the fresh weight. Dorsal muscle samples (*n* = 6/treatment) were kept at 4 and –20°C for 24 hours to calculate stored loss (SL) and frozen leakage rate (FLR), respectively, as the percentage of initial weight dropped **(Lingqiao *et al*., 2014)**. The additional samples of the dorsal muscle (*n* = 6 per treatment) were held at 4°C for 72 hours to determine drip loss (DL) according to **Bosworth *et al*. (2004)**, which is defined as the percentage of original weight lost.

5.5. Histological characteristics and histometric parameters of dorsal muscles

The dorsal muscle samples (*n* = 3 /treatment) were taken and fixed in a 10% neutralized formalin solution. Before use, the samples were washed with tap water. Then, samples were dehydrated using various alcohol grades (70, 85, 96, and 99%). Subsequently, xylene was used to clean the samples before they were embedded in paraffin wax. According to **Roberts (2001)**, wax blocks were sectioned into six microns and stained with hematoxylin and eosin stains (H & E) to provide histological slides for evaluation. The histometric parameters were measured according to **Radu-Rusu *et al*. (2009)**. Photomicrographs of the samples were captured at a magnification of 200× (scale bar = 50µm) using a Leica DM 500 phase-contrast microscope and an ICC50W digital camera, UK.

6. Statistical analysis

All numerical data were subjected to one-way analysis of variance (ANOVA) using the SAS® software version 9.1.3 for Windows **(SAS, 2006)** to detect the overall effects of treatments (T_1, T_2, T_3, T_4, T_5, and T_6). All ratios and percentages were arcsine-transformed before statistical analyses. The differences between the means of treatments were compared using *Tukey's post hoc* significant test, and differences were considered statistically significant based on a probability level of $P \leq 0.05$.

RESULTS

1. Growth performance and feed utilization parameters

Data in the present study showed a significant ($P \leq 0.05$) increase in growth performance (FBW, TWG, and ADG) (Fig. 1), and significant improvements in feed utilization (FCR, PER, PPV, and EU) (Table 2) parameters of *O. niloticus* were fed with 0.1g/ kg diet of CEO among all tested levels at the end of the experimental period (90 days).

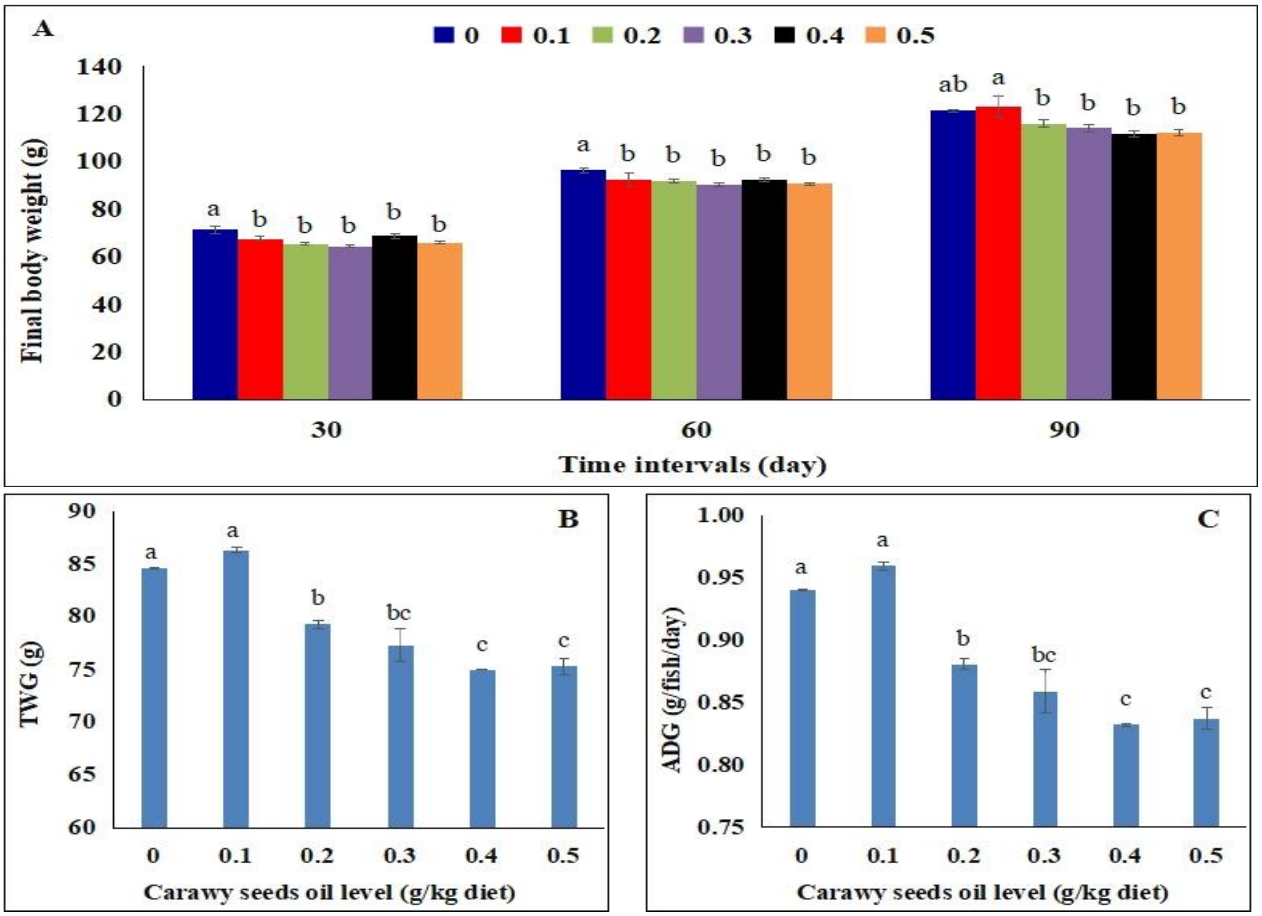

Fig. 1. Growth performance parameters such as (A) Body weight during interval times, (B) TWG, and (C) ADGs of the Nile tilapia fed different levels of caraway essential oil. Vertical bars indicate standard error; means with an asterisk indicate significant differences ($P \leq 0.05$)

Table 2. Effect of dietary caraway essential oil on feed utilization parameters of the Nile tilapia

CEO level (g/kg diet)	FI (g)	FCR	PER	PPV (%)	EU (%)
0	174.2±2.71^{a}	2.06±0.03^{c}	1.63±0.02^{b}	20.33±0.31^{b}	12.31±0.18^{b}
0.1	166.5±4.13^{b}	1.93±0.04^{d}	1.74±0.04^{a}	21.95±0.48^{a}	13.61±0.30^{a}
0.2	163.3±0.55^{b}	2.06±0.02^{c}	1.63±0.01^{b}	15.08±0.13^{d}	10.03±0.09^{c}
0.3	160.9±0.27^{b}	2.09±0.04^{bc}	1.61±0.03^{b}	17.25±0.38^{c}	12.09±0.26^{b}
0.4	166.0±0.25^{b}	2.21±0.00^{a}	1.51±0.00^{c}	7.87±0.01^{f}	5.94±0.01^{d}
0.5	162.2±1.05^{b}	2.15±0.01^{ab}	1.55±0.01^{bc}	16.23±0.05^{e}	10.36±0.04^{c}
P-value	0.009	0.0003	0.0004	<.0001	<.0001

Means in the same column having different small letters are significantly different ($P \leq 0.05$). CEO: Caraway essential oil, FI: Feed intake, FCR: Feed conversion ratio, PER: Protein efficiency ratio, PPV: Protein productive value, EU: Energy utilization.

2. Somatic indices

Data in Table (3) show that fish in the 0.4 treatment displayed a significantly increased SSI (%) among all treatments, ($P \leq 0.05$). However, there are insignificant increases between all (K- factor – HSI (%) – VSI (%) – ISI (%)) treatments at $P \leq 0.05$.

Table 3. Effect of dietary caraway essential oil on body and organs indices of the Nile tilapia

CEO level (g/kg diet)	CF (%)	HSI (%)	VSI (%)	SSI (%)	ISI (%)
0	1.71±0.04	3.15±0.39	11.25±0.72	0.29±0.02	4.90±0.19
0.1	1.73±0.06	2.60±0.27	9.10±0.57	0.23±0.04	3.98±0.16
0.2	1.77±0.04	2.42±0.16	8.66±0.50	0.21±0.03	4.14±0.48
0.3	1.74±0.04	2.34±0.08	10.21±0.63	0.28±0.02	4.53±0.78
0.4	1.76±0.06	2.80±0.24	11.26±1.51	0.37±0.06	4.88±0.83
0.5	1.78±0.05	2.98±0.38	11.07±0.75	0.31±0.07	4.84±0.54
P-value	0.8885	0.287	0.1381	0.0786	0.76

Means in the same column having different small letters are significantly different ($P \leq 0.05$). CEO: Caraway essential oil, CF: Condition factor, HSI: Hepatosomatic index (%), VSI: Viscera somatic index (%), SSI: Spleen somatic index (%), ISI: Intestine somatic index (%).

3. Chemical composition of the whole fish body and muscles

In the present study, *O. niloticus* that were fed with 0.1g/ kg diet of CEO showed a significant ($P \leq 0.05$) increase in DM, CP, and ash contents of the whole body among other levels. While, fish fed with 0.4g/ kg diet of CEO showed a significant ($P \leq 0.05$) increase in EE and EC among other levels (Table 4). Regarding the chemical composition of *O. niloticus* muscles, DM, ash, and CP contents significantly ($P \leq 0.05$) increased in fish fed high levels of CEO at 0.3 and 0.5g/ kg diet, respectively. Meanwhile, EE and EC were significantly increased in fish fed with low levels of CEO at 0.1 and 0.2g/ kg diet, respectively (Table 4).

4. Flesh quality parameters

The flesh quality parameters of *O. niloticus* fed a supplemented diet with different levels of CEO are presented in Table (5). There are no significant ($P \geq 0.05$) differences in WHC, SL, and DL among all treatments. Regarding the percentage of FLR after freezing periods of 0, 1, and 2 hours of *O. niloticus* fed with 0.1g/ kg diet of CEO, it significantly ($P \leq 0.05$) decreased among other CEO levels.

5. Histological and histometric parameters of dorsal muscles

Data in Table (6) and Fig. (2) show the histometric parameters and histological properties, respectively, of dorsal muscles of the Nile tilapia fed with different levels of CEO. Where fish fed with 0.1g CEO/kg diet revealed the best histometric parameters (Table 6; $P \leq 0.05$**)** and histological properties (Fig. 2) among other levels of CEO.

Table 4. Effect of dietary caraway essential oil on the chemical composition of the whole body and muscles of the Nile tilapia

CEO level (g/kg diet)	DM (%)	As a percentage on DM basis (%)			
		Protein	EE	Ash	EC (MJ/100g DM)
The whole body					
0	20.70±0.08^{b}	58.32±0.28^{a}	22.29±0.20^{e}	19.39±0.08^{ab}	2260±1.45^{c}
0.1	21.20±0.06^{a}	57.43±0.36^{ab}	22.87±0.19^{e}	19.71±0.23^{a}	2262±4.63^{c}
0.2	17.82±0.03^{e}	55.29±0.05^{c}	24.88±0.03^{c}	19.83±0.06^{a}	2291±1.76^{b}
0.3	19.89±0.03^{c}	54.55±0.03^{c}	26.62±0.05^{b}	18.84±0.03^{b}	2342±1.35^{a}
0.4	13.47±0.05^{f}	53.03±0.22^{d}	27.48±0.16^{a}	19.50±0.10^{a}	2340±2.33^{a}
0.5	18.87±0.04^{d}	56.47±0.09^{b}	23.86±0.01^{d}	19.67±0.08^{a}	2279±1.68^{b}
P-value	<.0001	<.0001	<.0001	0.0464	<.0001
The muscle					
0	11.66±0.01^{b}	88.72±0.09^{b}	4.92±0.00^{b}	6.36±0.09^{a}	2292±2.00^{ab}
0.1	10.77±0.05^{d}	87.74±0.11^{c}	5.47±0.15^{a}	6.80±0.04^{b}	2290±3.17^{ab}
0.2	13.28±0.02^{e}	88.28±0.09^{b}	5.33±0.10^{ab}	6.39±0.02^{b}	2298±1.86^{a}
0.3	13.23±0.07^{a}	87.63±0.09^{c}	5.40±0.11^{ab}	6.96±0.01^{a}	2285±1.95^{b}
0.4	10.59±0.11^{d}	89.76±0.06^{a}	3.86±0.01^{c}	6.38±0.05^{b}	2274±1.02^{c}
0.5	11.16±0.02^{c}	89.77±0.13^{a}	4.18±0.08^{c}	6.05±0.07^{c}	2288±1.58^{ab}
P-value	<.0001	<.0001	<.0001	0.0002	0.0099

Means in the same column having different small letters are significantly different ($P \leq 0.05$). CEO: Caraway essential oil, DM: Dry matter, EE: Ether extract, EC: Energy content.

Table 5. Effect of dietary caraway essential oil on flesh quality parameters of the Nile tilapia

Level of CEO (g/kg diet)	WHC (%)	SL (%)	DL (%)	FLR (%)		
				0h	1h	2h
0	5.22±0.25	2.25±0.26	4.50±0.39	1.59±0.24[ab]	2.77±0.34[b]	3.99±0.38[c]
0.1	4.96±1.04	2.40±0.07	4.31±0.13	1.50±0.14[b]	2.41±0.05[b]	3.84±0.23[c]
0.2	4.96±0.85	2.26±0.13	3.83±0.14	1.57±0.32[ab]	2.70±0.34[b]	3.88±0.40[c]
0.3	5.36±0.55	2.21±0.17	4.02±0.24	2.26±0.23[a]	3.89±0.35[a]	5.35±0.45[a]
0.4	5.38±1.23	2.04±0.10	3.60±0.28	2.02±0.22[ab]	3.07±0.32[ab]	4.30±0.40[ab]
0.5	5.50±0.85	2.26±0.13	4.25±0.28	2.03±0.19[ab]	3.00±0.17[b]	4.18±0.26[b]
P-value	0.8145	0.7237	0.1933	0.0199	0.0234	0.0155

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). CEO: Caraway essential oil, WHC: Water holding capacity, SL: Stored loss, DL: Drip loss, FLR: Frozen leakage rate, h: Hour.

Table 6: Effect of dietary caraway essential oil on histometric characteristics of the Nile tilapia dorsal muscles

CEO level (g/kg diet)	Smallest diameter (µm)	Largest diameter (µm)	Mean diameter (µm)	Smallest/ largest ratio	Intensity of muscular bundles (mm^{-2})	Percentage of muscular bundles area (%)*	Percentage of connective tissue area (%)**
0	42.57±0.80[a]	60.25±1.33[ab]	51.91±0.83[ab]	0.706±0.03[b]	273.5±12.1[ab]	56.66±1.72[b]	43.34±1.72[b]
0.1	43.40±0.88[a]	63.37±1.60[a]	53.38±0.90[a]	0.758±0.03[a]	288.4±11.2[a]	61.80±1.69[a]	38.20±1.69[c]
0.2	36.19±0.77[b]	56.90±1.35[b]	46.54±0.72[c]	0.692±0.02[c]	262.9±13.8[b]	60.02±1.65[ab]	39.98±1.65[c]
0.3	36.14±0.70[b]	57.99±1.20[ab]	47.07±0.72[c]	0.655±0.02[c]	226.2±10.2[c]	56.62±1.47[b]	43.38±1.47[b]
0.4	36.67±0.78[b]	55.19±1.63[b]	45.43±0.90[c]	0.664±0.03[c]	215.0±9.1[d]	50.24±1.36[c]	49.76±1.36[a]
0.5	37.95±0.76[b]	58.62±1.43[ab]	48.29±0.77[b]	0.647±0.02[c]	189.0±12.5[e]	49.76±1.25[c]	50.24±1.25[a]
P-value	<.0001	0.0018	<.0001	0.0013	<.0001	<.0001	<.0001

Mean in the same row having different letters are significantly different ($P \leq 0.05$). CEO: Caraway essential oil.

* Percentage of muscular bundles area (PMBA)= $[3.14 \times (\text{mean diameter}/2)^2] \times$ intensity of muscular bundles (mm^{-2}) ×100,

Whereas, the muscular bundles were appeared as circularity shape approximately.

** Percentage of connective tissue area (PCTA, mm^{-2}) = (1− muscular bundles area) × 100.

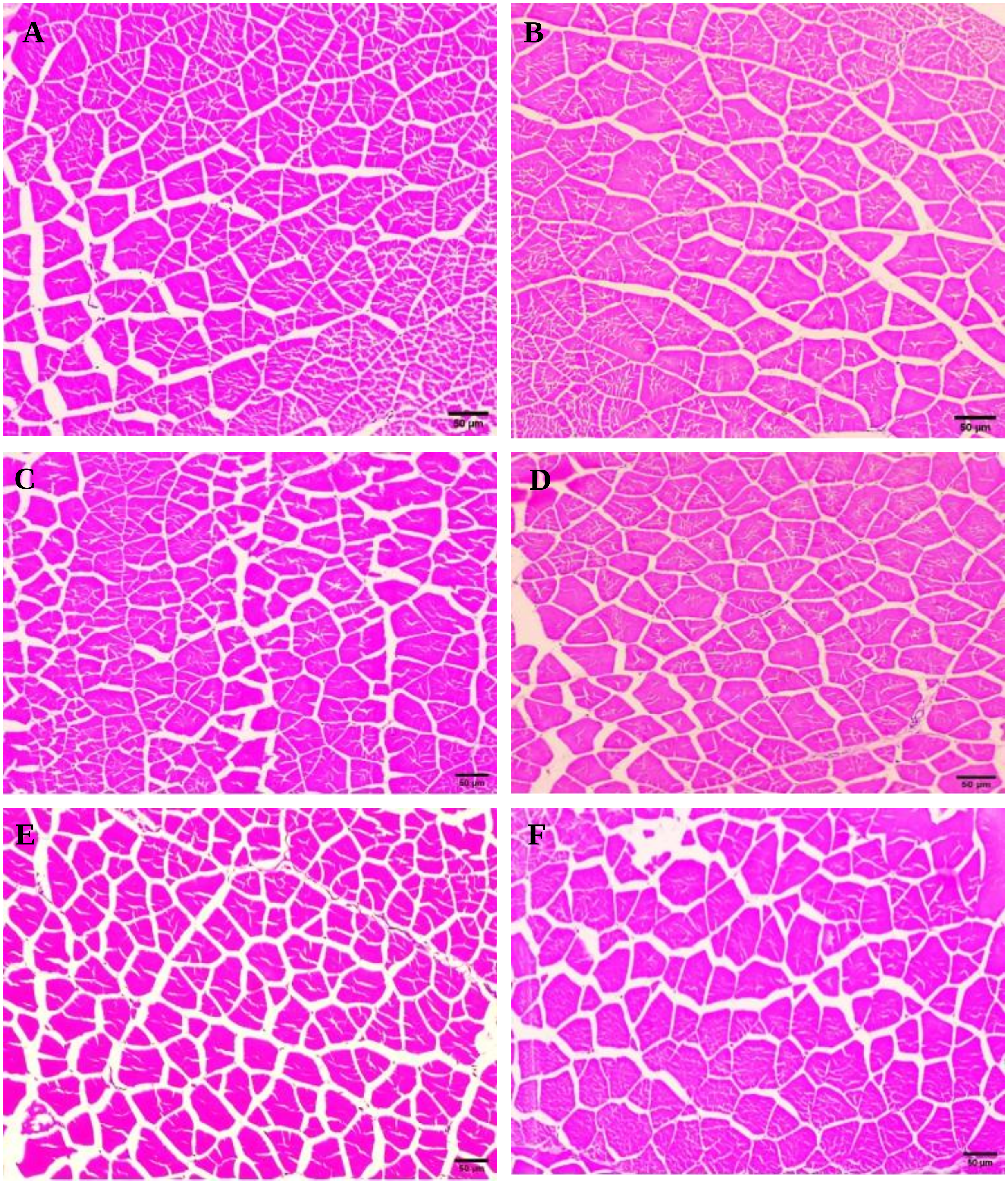

Fig. 2. Effect of dietary caraway essential oil (CEO) on histological properties of dorsal muscles of the Nile tilapia (A) Fed with 0.0CEO g/ kg diet, (B) Fed with 0.1CEO g/ kg diet, (C) Fed with 0.2CEO g/ kg diet, (D) Fed with 0.3CEO g/ kg diet, (E) fed with 0.4CEO g/ kg diet, (F) Fed with 0.5CEO g/ kg diet. Scale bar = 50µm

DISCUSSION

Aromatic herbs and their EOs are often requested to improve the flavor and palatability of feeds, thus increasing voluntary feed intake and resulting in improved weight gain **(Zeng *et al*., 2015)**. The present study is the first application of the effects of the dietary addition of CEO on the growth performance and feed utilization parameters of *O. niloticus*. In the present study, a significant increase in growth performance and significant improvements were detected in feed utilization parameters of *O. niloticus* fed with 0.1g/ kg diet of CEO among all tested levels at the end of the experimental period (90 days). In harmony with the current findings, the significant increase in BWG and SGR of hybrid tilapia, *O. niloticus* × *O. aureus* fingerlings fed with 5g/ kg diet of caraway seed meal (CSM), was reported in the study of **El-Dakar (2004)**. Moreover, **Khalafalla (2009)** concluded that the use of a 1% CSM-supplemented diet enhanced growth performance and consequently saved about 21% of the feeding cost per unit for *O. niloticus*, and the author considered using 1% CSM to be economically superior for *O. niloticus* among other tested diets. The same findings were also reported in *O. niloticus* fed with 10g/ kg diet of CSM **(Abbass *et al*., 2010; Ahmad & Abdel-Tawwab, 2011)**.

This enhancement in growth performance of *O. niloticus* fed with CSM may be due to the high inclusion levels of crude protein, total lipid, nitrogen-free extract, and the rich content of -7% EO and different bioactive components in CSM, which have been essential for growth **(Chandra *et al*., 2013)**. In addition, this could be due to the over-activation of CSM, and a condition similar to "immune fatigue" has occurred. The same trend was found in the previous studies of *O. niloticus* fed with 10g/ kg diet of oregano, *Origanum vulgar* **(Seden *et al*., 2009)**, fed with 10g fennel seed meal/ kg diet **(Abd El Hakim *et al*., 2010)**, fed with 0.5g green tea/ kg diet **(Abdel-Tawwab *et al*., 2010)**, fed with 10g cinnamon/ kg diet **(Ahmad *et al*., 2011)**, or fed with 10g DGL/ kg diet **(Mehrim *et al*., 2014)**, as well as in red hybrid tilapia, *Oreochromis* sp. fed with 6% chamomile powder **(Nordin *et al*., 2017)**. On the other hand, the present study recorded the negative effects of CEO up to a 0.1g/ kg diet on both growth and feed utilization parameters. Similarly, **Abbass *et al*. (2010)** and **Ahmad and Abdel-Tawwab (2011)** reported that the high CSM level (up to 10g/ kg) had negative effects on the growth and feed utilization of *O. niloticus*. They also suggested that high levels of CSM may have an anti-nutritional factor that reduced the utilization of the experimental diet. On the other hand, previous studies on the impact of dietary EOs extracted from medicinal plants on fish growth performance have been conducted. In agreement with the obtained results herein, the same findings were previously reported in *O. niloticus* fed with EOs of fennel and garlic **(Hassaan & Soltan, 2016)**, fed with clove basil and ginger EOs **(Brum *et al*., 2017)**, and fed with sweet orange EO **(Mohamed *et al*., 2021)**. Similarly, the positive effects were found in different fish species like *O. mossambicus* **(Baba *et al*., 2016)** fed with *C. limon* peel EO, rainbow trout (*O. mykiss*) **(Gültepe, 2018)** fed with orange EO, common carp (*C. carpio*) **(Abdel-Latif *et al*., 2020)**, and *C. carpio* fed with bitter orange

(*C. aurantium*) EO **(Acar *et al.*, 2021)**, as well as Caspian roach *R. caspicus* **(Ghafarifarsani *et al*., 2022)** fed with savory EO.

In the present study, no significant ($P \geq 0.05$) differences were detected in all tested somatic indices (CF, HSI, VSI, SSI, and ISI) among all treatments. This means that there are no drastic effects according to the addition of CEO to the *O. niloticus* diet. This means the safety addition of the CEO to the *O. niloticus* diet. The same observation was detected by **Toutou *et al*. (2018)**, who recommended that lemon (*C. aurantifolia*) peel is acceptable, safe, and healthy for *O. niloticus*. The obtained findings are in full harmony with those obtained by **Mohamed *et al*. (2021)**, who stated that CF, HSI, and VSI of *O. niloticus* are not affected by dietary supplementation of OEO and/or LEO compared to the control group.

In the present study, the increase of DM and CP in the bodies of fish fed with 0.1g/ kg diet of CEO is in harmony with the positive effects of this level on the growth performance and feed utilization of treated fish, among other tested levels. These positive effects may be reflected in the important role of the CEO or its bioactive compounds in the synthesis of protein in the body of fish. While, the EE and EC in the fish body were significantly increased by increasing the additional levels of CEO; the same trend is found in the study of **Ahmad and Abdel-Tawwab (2011)** considering the body of *O. niloticus* fed with high levels of CSM. This fat accumulation in fish bodies may be due to the balance between dietary absorbed fat, the synthesis of fatty acids (lipogenesis), and fat catabolism (lipolysis). Moreover, **Weerasingha *et al*. (2022)** stated that the body protein of tropical hybrid carp larvae, fed with crude palm oil, was significantly higher than that of larvae fed with canola oil, sunflower seed oil, and soybean oil. Inversely with the current findings, no significant differences were detected in the chemical composition parameters of the body of hybrid tilapia, *O. niloticus* × *O. auraus* fingerlings fed with different levels of CSM **(El-Dakar, 2004)**. The same findings were obtained by **Khalafalla (2009)** and **Abbass *et al*. (2010)** who reported no significant differences in dry matter, protein, lipid, or ash contents in the body of *O. niloticus* fed with a supplemented diet, with different levels of CSM. The same trend was also confirmed in *O. niloticus* fed with different levels of anise seeds **(Sakr, 2003)**, fed fenugreek seeds **(Shalaby, 2004)**, or cinnamon **(Ahmad *et al*., 2009)**. In the same vein, **Hassaan and Soltan (2016**) stated that the dietary addition of 1mL of fennel EO/ kg diet and 1mL of garlic EO/ kg diet had nonsignificant effects on the all-whole body chemical composition parameters of *O. niloticus*. **Abdel-Latif *et al*. (2020)** also demonstrated that dietary oregano EO did not significantly affect the moisture, CP, lipids, and ash contents of the whole body in common carp (*C. carpio*). Recently, the same findings were reported in Indian shrimp (*Penaeus indicus*) fed with sweet basil oil **(Abdel-Tawwab *et al*., 2021)** or those fed chamomile oil **(Abdel-Tawwab *et al*., 2022)**. The differences between the current findings and the above studies are not surprising since there are variable factors between them, such as fish species, age, stage of life, type of medicinal herbs, or their

EOs, and the amount of the bioactive compounds in each type of EO, their additional levels, the feeding periods, and the experimental management.

Inversely to the current findings, **Qiu *et al*. (2003)** found that there were no significant differences in moisture and CP contents in the muscle of crucian carp fed with diets supplemented with or without traditional Chinese medicines (TCM) as a feed additive, however, the muscle of fish fed with TCM-supplemented diet has a significantly higher lipid content. In this regard, **Lin *et al*. (2006)** found that the apparent digestibility coefficients of lipids increased as dietary TCM levels increased for white shrimp, *Litopenaeus vannamei*. Moreover, the composition of the muscles of growing rabbits was not significantly affected when fed with rosemary EO and ginger EO-supplemented diets **(Elazab *et al*., 2022)**, or those fed diets supplemented with oregano extract, rosemary extract, or a combination **(Cardinali *et al*., 2015)**, and those fed diets containing remnants of mint, fennel, basil, and anise **(Mohamed *et al*., 2016)**. Generally, it could be noted that an unclear trend among all tested levels of CEO affected the chemical composition of fish muscles. This unclear trend among all treatments may be due to the addition of the pure CEO, which contains high levels of bioactive compounds that may cause some distributions in the metabolism process of the body that negatively affect the muscle composition of treated fish. In this regard, **Acar *et al*. (2021)** stated that there are many advantages to feeding fish medicinal plants; however, there are often dangers from their active additives and overdosing.

Improving meat quality and aquaculture productivity depends on several variables, including stocking density, the quality of the water and feeds, as well as additional environmental variables, such as temperature, dissolved oxygen, and salinity level of water **(Shakya, 2017)**. To our knowledge, there have been no previous studies conducted to evaluate the effects of EOs, including CEO, on flesh quality or histometric parameters of fish muscles. Therefore, the present work is the first study that estimates the effect of graded levels of CEO on flesh quality and the histometric and histological properties of the muscles of *O. niloticus*. The current findings revealed that fish fed with the low-level of 0.1g CEO/ kg diet showed an improvement in flesh quality parameters, especially FLR, and a significant increase in the histometric and histological properties of fish muscles among all tested levels of CEO. These positive effects are confirmed by the significantly increased growth performance and feed efficiency of fish fed with a low level of CEO (0.1g/ kg diet). In harmony with the current findings, the positive effects on histometric parameters of dorsal muscles were previously reported in *O. niloticus* fed jojoba meal supplemented with methionine and Biogen® at 0.6 and 2g/ kg diet, respectively, to replace 25% fish meal **(Khalil *et al*., 2009)**, fed with 3g Biogen® (as a commercial probiotic)/ kg diet **(Mehrim, 2009)**, fed 400μg chromium picolinate/ kg diet **(Mehrim, 2014),** or those fed with different sources and levels of some biological additives **(Abdelhamid *et al*., 2014)**. Regarding the flesh quality, similary to the obtained findings herein, **Refaey *et al*. (2023)** recently concluded that the dietary replacement of

20% fresh green Azolla or less significantly improved the flesh quality parameters of *O. niloticus*. At the same time, these improvements in both the flesh quality and histometric and histological properties of the muscles of *O. niloticus* may be due to the inclusion of the major bioactive substances in CEO, such as carvacrol, carvone, α-pinene, limonene, γ-terpinene, linalool, carvenone, p-cymene, carveol, camphene, and fenchen **(Seidler-Lozykowska *et al*., 2010)**. These substanaces have been associated with beneficial effects in relieving gastrointestinal symptoms associated with antiulcerogenic effects **(Zheng *et al*., 1992)** and dyspepsia **(Thomson Coon & Ernst, 2002)**. Due to its antiulcerogenic **(Khayyal *et al*., 2001)**, immunomodulatory **(Raphael & Kuttan, 2003)**, and antioxidative **(Lado *et al*., 2004)** properties, it may have a positive effect on the intestinal flora, improving digestion, and enhancing nutrient utilization, resulting in improvements in growth performance and flesh quality as well.

CONCLUSION

In conclusion, the obtained results of this study demonstrate that dietary CEO levels form an important factor in a practical diet for the Nile tilapia, *O. niloticus*. A level of 0.1g CEO/ kg diet provided the highest significant fish performance, feed efficiency, histometric parameters, and histological properties of muscles, along with improving the flesh quality of *O. niloticus*, among other levels of CEO. This means that the appropriate inclusion level of CEO in a practical diet for *O. niloticus* should be 0.1g/ kg or less since EOs have harmful consequences at higher doses. Consequently, more attempts should be required to understand the mechanism or mode of action of the CEO on the development of internal organs, the chemical composition, the histological properties of muscles, the physiological and immune responses, antioxidant status, and flesh quality in treated fish, which may help to seriously determine the optimum level of CEO not only for *O. niloticus* but also for different fish species.

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