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Research Article

Exploring benefits of black seed on the meat quality of grass carp

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ABSTRACT

Nigella sativa or black cumin seed growing worldwide as annual herb having significant medicinal importance. Current investigations revealed the efficacy of black cumin at selected levels of concentrations (0%, 1%, 2%, 3% and 4%) in fish feed effecting muscles proximate and composition of fatty acids in grass carp (*Ctenopharyngodon idella*). A total of 150 fingerlings consisting average body weight of 16.2±0.1g and divided into five groups, each having five replications fed with experimental diets using 4% of body weight two times in a day for 63 days. Consequently, muscles protein, crude fat, ash and moisture content in grass carp were increased significantly using proximate analysis at 3% concentration of black cumin. Fatty acid profile including both saturated and polyunsaturated fatty acids were also checked because of dietary black cumin; it was determined that saturated fatty acids reduced. However, polyunsaturated fatty acids increased at 3% of dietary black cumin. Herein, it is proved that, the 3% concentration level of supplemented diet (black seed) has potential to maximize the fish production acknowledging the high nutritional values.

Keywords: *Nigella sativa*; grass carp; fatty acids; nutritional values; fish meat; medical importance; feed additives.

INTRODUCTION

Aquaculture or blue revolution is currently lauded as fast-expanding food production industry having subsequent historical background aiming to food security and was primarily practiced in Egypt and China (Parker, 2011; Anderson et al., 2017). Globally, this industry has also been proven as a significant supply of food mainly to the ongoing increase of human population (Khanjani et al., 2022).

Acknowledging the considerable benefits of fresh water ecosystem, significant contributions and services including nutrient retentions, habitat availability, water purification and climate regulation are being provided to mankind due to this ecosystem (Sierra et al., 2021).

In Pakistan, grass carp is an herbivorous fish species with high commercial value and contributing in economy due to its high production and exportation to various countries (Nusrat, 2021). It was primarily farmed in polyculture systems with other carps. Having wide range of tolerance to environmental stressors, hypoxia, and disease, grass carp found as a suitable model species for analysis of dietary interventions (Yang et al., 2013). Phytobiotics in aquaculture has attracted a lot of attention globally; therefore, it has become a subject of active scientific investigations (Awad & Awaad, 2017). To date, a subsequent work on the usefulness of food supplements in aquaculture industry have been discussed by various authors likewise, Yuangsoi et al. (2014) presented the outstanding work on the efficacy of pumpkin seed on fresh water fishes and observed the meaningful results. Similarly, Yousif (2020) described the effectiveness of moringa seed as a diet in aquaculture

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industry. Wang et al. (2020) acknowledged the importance of faba bean in fish production. Saleh (2020) compiled the impressive results of olive extract as a potential fish diet. Maqbool et al. (2021) provided the healthy impact of black seed as a fish feed. Rahman et al. (2021) determined the nutritive impact of okara in fish when consumed as a diet supplement. Similarly, following the observations of dietary effects of plant based diet in fish health, Voss et al. (2021); Koskela et al. (2021); Gopan et al. (2021); Sezgin and Aydın (2021); Hazreen-Nita et al. (2022); Peng et al. (2022) and Hekamatpour (2023) presented the significant outline of these diet for the better health of fresh water fishes.

All these plants contained numerous bioactive compounds having medicinal important consequently (Faheem et al., 2020). Moreover, growth, immunity level, stress and antioxidant capacity of fishes can also be improved using these bioactive compounds as a nutritive diet. *Nigella sativa* is a well-known economically important medicinal plant with therapeutic properties containing protein, carbohydrates, lipids, ash and fiber and broadly used as diet supplement throughout the world (Botnick et al., 2012). However, unsaturated fatty acid was also observed with composition of essential fatty acid as compared to saturated fatty acids (Bourgou et al. 2008). Knowledge on the dietary influences due to black seed on fresh water fishes is poorly studied including Eskandari et al. (2015); Joshi (2022); Maqbool et al. (2021); Aydin (2021); Latif et al. (2021); Yousefi et al. (2021) and Akter et al. (2024), herein the presented work was planned for investigation of black seed impact on the qualitative traits of grass carp.

MATERIALS AND METHODS

Aquaria Preparation

A total of twenty-five glass tanks, each with a volume of 40 litters of water were used for the experiment and placed under controlled environmental parameters (e.g., temperature, lighting). Research work was performed in quintuplicate condition during the experiment duration (63 days). Tanks disinfection was performed using $KMnO_4$ solution then washed with chlorinated water and placed for drying purpose in sun light for three days.

Stocking of Fish and Acclimatization

Fish samples were obtained from a Public Fish Seed Hatchery, Fatehpur Kamal, District Rahim Yar Khan, Pakistan. Obtained fish samples were transferred to the laboratory using methodology discussed by Joshi (2022). A total of 150 healthy individuals with an average body weight (16.2 ±0.1g) irrespective of male and female individuals were used in experiments. Herbivorous grass carp specimens were placed in glass aquaria for acclimatization under laboratory conditions for the period of two weeks before experimental observations. Keeping them unfed for 24 hours, basal diet was used at the rate of 4% bodyweight with proper aeration facilities, where they acclimatized before experimentation. The studied fish from the acclimatized stocks of 150 individuals were stocked with the density of six fishes per aquaria. The aquaria were labelled properly. All tanks were connected to a central air compressor for proper aeration. Chlorine-free aerated tap water was used in all experiments.

Experimental Design

A total of 150 fish individuals were divided into five experimental groups. Five replications were made followed by sub-division of each group. Each replication was contained six fish individuals. Five experimental treatments (Test Diet I-V), each comprising 30 fish (six per subgroup), were randomly distributed. The first group kept as the control (G0) and provided with basic feed during experimental process, whereas the remaining groups (G1, G2, G3, and G4) were provided the same diet contained with *Nigella sativa* at concentrations of 1%, 2%, 3%, and 4% per kg of fish feed for the same duration.

Supplemented diet with 4% of their body weight was provided to studied fish in a regular fashion twice a day. Quantity of feed was fixed biweekly as per original weight of each treatment group. Calculated feed was given to fish on daily basis and unconsumed pellets were collected after two hours of feeding in order to prevent degradation.

Diet Formulation

The feed ingredients with their corresponding percentage composition are given in Table (2). Feed was prepared using standard available procedure with the help of most related literature. The raw materials were selected based on their nutritional value. Major ingredients were Soybean (source of protein), wheat flour (source of carbohydrate), fish oil and sunflower (source of lipid) along with other ingredients as vitamins premix and mineral sources were included in supplemented diet. In the experimental work, the concentrations of *Nigella sativa* added to the basal diet followed by Bektas et al. (2019). Proximate composition of prepared feed calculated using AOAC (1995) the standard procedure to determine the crude fat, protein, total ash and moisture contents (Table 1).

Feed ingredients were individually weighed, oven-dried, finely ground, and passed through a mesh of 0.5 mm in order to ensure uniform particle size. The processed material was mixed with some water leads to dough consistency. The prepared consistent material shaped into pellets (usually 2 mm) using a manual food grinder. The pellets were sun-dried for three consecutive days and subsequently stored at −20°C to prevent microbial growth and nutrient degradation, following the methodology of Rajan and Rohini (2021).

Table 1. Proximate compositions and fatty acid profile of black cumin seeds (BCS).

Proximate composition	(% age)
Crude protein	27.51
Crude lipid	14.82
Dry matter	90.24
Ash	6.51
Myristic (C14:0)	0.2
Stearic acid (C18:0)	0.29
Palmitic acid (C16:0)	12.2
Palmitoleic acid (C16:1)	0.2
Oleic acid (C18:1)	23.9
Linoleic acid (C18:2)	56.5
Linolenic acid (C18:3)	0.3
Eicosadienoic acid (C20:2)	2.8
Cis-10-Pantadecanoic acid(C15:1)	0.2

Table 2. Feed formulation and proximate composition of the tested diets (Total ingredients=100%).

Ingredients	Test Diet-I (control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V
Sunflower meal	0	15	25	35	50
Fish meal	50	35	25	15	0
Soybean meal	15	15	15	15	15
Wheat flour	17	16	15	14	13
Rice polish	8	8	8	8	8
Fish oil	6	6	6	6	6
Vitamin Premix	1.0	1.0	1.0	1.0	1.0
Mineral Premix	1.0	1.0	1.0	1.0	1.0
Ascorbic acid	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0
Black Cumin (*Nigella sativa*)	0 %	1%	2%	3%	4%
Proximate composition					
Crude protein	42.72	43.45	44.56	45.75	46.21
Crude lipid	18.17	18.34	18.62	19.81	19.21
Dry matter	91.41	90.99	90.72	90.45	90.03
Ash	10.45	9.95	9.71	9.40	9.01

Physio-chemical Analyses of Water Sample

Various water parameter such as temperature, pH, dissolved oxygen, total hardness and alkaline nature were analyzed on weekly schedule by following standard methodologies.

Table 3. Methods used for analyses of physio-chemical parameters of water sample.

Water parameters	Optimal range	Instruments/ Methods used	References
Dissolved oxygen (DO)	6.4 mg/ L	Water quality testing kit (HACH kit FF-2)	Akter et al., 2024
PH	7.8	Digital pH meter	Apha, 2005
Water temperature	27ºC	Celsius thermometer	Akter et al., 2024
Total hardness	140 mg/ L	TDS Tester	
Total alkalinity	121 mg/ L	Titration method	Apha, 2005

Sampling

Upon completion of the experimental trial, fish were randomly sampled and anesthetized immediately using clove oil at a concentration of 75 µL/L (Aydın and Barbas, 2020). The fish were dissected to collect the muscle tissues from the epaxial trunk region of fish, were stored at −20°C for subsequent proximate and fatty acids analyses.

Biochemical Analysis

Muscles proximate analysis

Dorsal muscles were observed to calculate the proximate composition. This analysis was completed through Association of Official Analytical Chemists (AOAC) standard procedures.

Determination of crude fat

The crude fat in meat samples of fish was determined with the help of Soxhlet method, a standard crude fat analysis method. Following formula was used to calculate the percent crude fat in the meat of grass carp.

$$\text{Crude fat (\%)} = (\text{W2} - \text{W1}) \times \frac{100}{\text{Weight of sample}}$$

W1= Weight of empty flask.

W2= Weight of flask and extracted fat.

Estimation of protein content

One-gram powder of dried fish sample was taken for the estimation of protein content using standard Kjeldhal methodology. The amount of protein content was calculated using the given formula:

$$(\%)\ \text{N} = \frac{\text{Volume of N/(10 H2SO2 used)} \times 0.0014 \times \text{volume of sample dilution}}{\text{Weight of sample} \times \text{Volume of sample solution used (10 ml)}} \times 100$$

Crude protein (%) = % Nitrogen×6.25

Moisture

Moisture was calculated by oven drying of fish body meat at 105°C to a constant weight using following formula.

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Total ash

Dry ash was calculated by using the muffle furnace apparatus at 550°C using following formula.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

Fatty acid profile

Samples were used for the analysis fatty acid and lipids extractions using chloroform. Subsequently, for the preparation of Fatty Acid Methyl Easter through Trans methylation with 2M KOH in methanol and n-heptane followed by the standard methodology of Ichibara et al. (1996). Extracted oil was mixed in two mili litter of n heptane using 4 mL methanolic potassium hydroxide. Tube vortexed for two minutes and placed in room temperature. For obtaining of gas chromatography-mass spectrometry, centrifugation of solution was performed at the rate of 4000 rpm for ten minutes.

Gas chromatographic condition

The fatty acid profile was analyzed by gas chromatography clarus 500 with auto sampler (Perkin Elmer, USA) equipped with a flame ionization detector and fused silica capillary sue column. The oven temperature was held at 140ºC for 5 minutes, raised to 200ºC at the rate of 4ºC minute-1 and held at 240ºC at the rate of 1ºC min-1, while the temperature of injector and detector fixed at 240ºC and 280ºC respectively (Sezgin and Aydın, 2021). Fatty Acid Methyl Esters was used to identify fatty acid by considering the retention time. This experiment was repeated twice to obtain the results.

Statistical Analysis

For the determination of differences between treatment groups, one-way ANOVA was used. Similarly, homogeneity of variances and the normality observation of ANOVA, Levene's and the Kolmogorov-Smirnov test were used respectively. Tukey's Honestly Significant Difference was used to check the post hoc multiple comparisons. Overall, different statistical software such as IBM Corp., Armonk, NY, USA's SPSS software (version 20.0) were used.

RESULTS AND DISCUSSION

Proximate Composition of Muscles

The muscles proximate composition of grass carp treated with black cumin and analyzed to observe the nutation (Table 4). It was noted that, the crude protein (CP) was 16.51%, 17.53%, 17.35%, 18.47%, and 18.51%, respectively. Statistical analysis of data showed that dietary black cumin had highly significant ($p<0.001$) effect on protein content. The crude lipid levels in muscles tissue was found to be increased from 4.51% to 5.51% following dietary black seed

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supplementation in comparison with untreated group. In the study, the moisture content varied between 71.84% and 70.24%, with the highest value observed in control group and the lowest observed in the group 5. There was also a slight increase in the ash contents among dietary treatments.

Table 4. Muscles proximate composition of grass carp. Values are shown as mean ± standard error.

Parameters	Diet-I (G0)	Diet-II (G1)	Diet-III (G2)	Diet-IV (G3)	Diet-V (G4)
Crude protein (%)	16.51±0.09C	17.53±0.09B	17.35±0.07B	18.47±0.08A	18.51±0.08A
Crude fat (%)	4.51±0.09B	4.53±0.09A	5.35±0.07A	5.47±0.08A	5.51±0.08A
Moisture (%)	71.84±0.04A	71.45±0.03 B	71.04±0.03 C	70.64±0.18D	70.24±0.04E
Ash (%)	2.25±0.04E	2.44±0.04D	2.56±0.02C	2.75±0.04B	2.90±0.01A

Total Fatty Acids Profile of Muscle

Based on the fatty acids composition, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) in grass carp fed dietary black cumin were evaluated. Three major saturated fatty acids in the studied fish were stearic acid (C18:0), myristic acid (C14:0), and palmitic acid (C16:0). Notably, there was a significant reduction in myristic acid (C14:0), ranged from 5.67 % in Diet 1 to 4.79 % in Diet 4. Similarly, stearic acid (C18:0) levels declined from 5.30 % to 3.48% with increased level of inclusions. However, palmitic acid (C16:0) showed modest decrease from 51.60% to 50.08% with dietary levels (Diet 2 to Diet 4). As presented in Table 5, stearic acid and myristic acid content in fish fed with NS- diets were lower than those of the unfed group ($P < 0.05$). Statistical analysis of the presented data indicated that dietary black cumin had no significant effect on SFAs. However, the progressive reduction was recorded in SFA across increased level of dietary black cumin. Monounsaturated fatty acids such as palmitoleic acid and oleic acid had significantly higher content at 3% and 4% levels of NS in comparison to the other samples ($p < 0.05$). Whereas the erucic acid levels were substantially higher in dietary group 2 and 4 than in the other samples ($p < 0.05$).

As opposed, polyunsaturated fatty acid (PUFA) analysis of five experimental diets showed highly significant difference in linoleic acid (C18:2), linolenic acid (C18:3), and eicosapentaenoic acid (EPA; C20:5) contents. These findings demonstrated that a supplemented diet enhances the lipid profile, with the experimental diets having a higher PUFA content and a lower SFA level in fish meat.

Present study confirmed the hypothesis that optimal level of black cumin could contribute in the better health as well as meat quality of grass carp. To this end, various studies demonstrated the body composition and nutritional values of fish generally impacted by various supplemented diet (Gümüs¸ and Aydın, 2013; Li et al., 2020). Furthermore, Rasmussen (2001), Özden and Erkan (2008) have found that the nutritional values also differ based on the feeding schedule, feed composition, harvest season, sex, size, and environmental factors. The results of our investigation are comparable with already published work and gave similarities such as effect of black seed extraction as a useful feed additive. Consuming black seed leads to the quality of fish health and longevity. Moreover, the result of present study was close to Latif et al. (2020). In other word, present study indicated that, black cumin enhanced crude protein, ash content and dry matter consequently. Acknowledging the use of N. sativa as organic preservative, generally it may result in positive changes in fat as well as protein contents of rohu. Focusing the key changes in fat and protein content, our findings were similar to Saadat et al. (2024). Likewise, Ozpolat and Duman (2017) performed an outstanding work to check the beneficial aspects of black seed on fish meat. They found that selected concentration of 3% and 4% of supplemented diet can increase the nutritional values. This result could be explained by the fact that plant protein effectively replaces fish meal without altering the proximate composition of meat, offering a sustainable alternative in aquaculture feed. A similar study was held by Acar and his colleagues in 2018 also comparable and having promising results in fish meal quality using dietary lupin meal as a supplemented diet. Recent published literature based on plant supplements also have common results for meat quality indifferent fish species (Voss et al., 2021; ZeinEddine et al., 2021). Similarly, another study, performed by Oz et al. (2018) is also comparable to our work having similar dietary impacts like high contents of polyunsaturated fatty acid due to black seed consumption and low level of saturated fatty acid. Maqbool et al. (2021) studied the dietary impact of black cumin oil on fatty acid profile of Labeo rohita. They observed a progressive reduction in level of saturated fatty acids methyl stearate and methyl heptadedecanoate

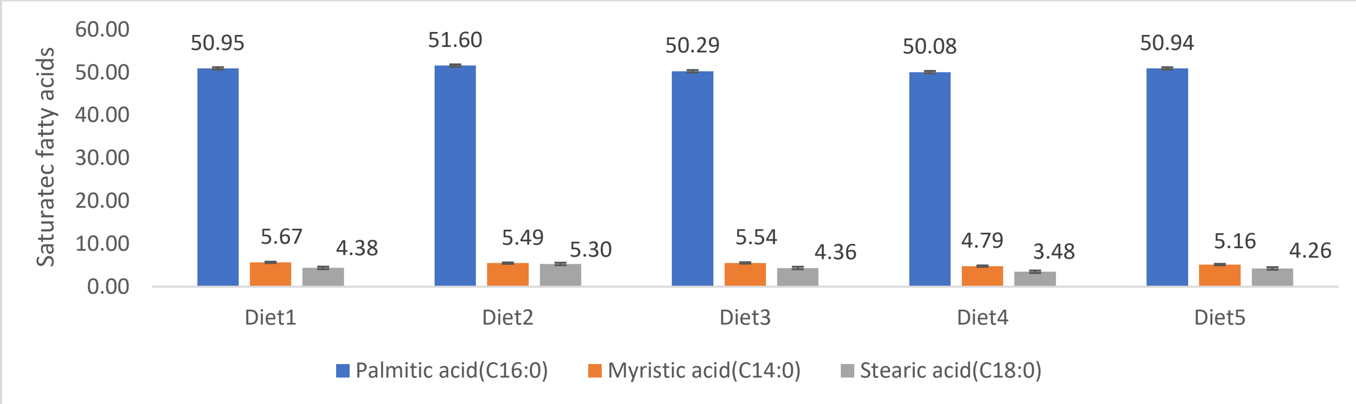

Figure 1. Saturated fatty acid analysis using experimental diets.

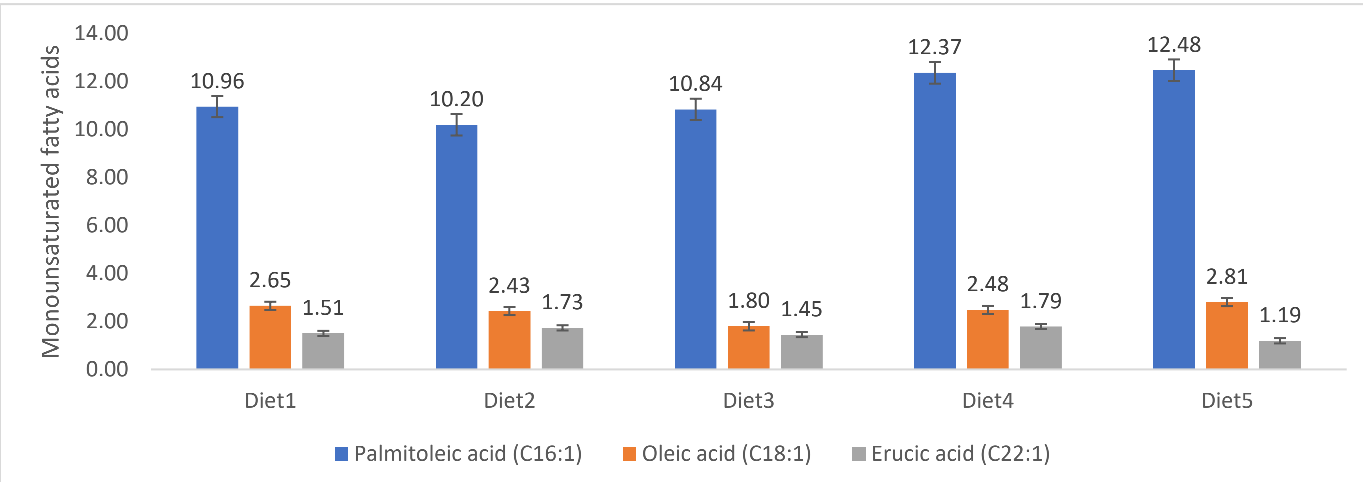

Figure 2. Monounsaturated fatty acid analysis using experimental diets.

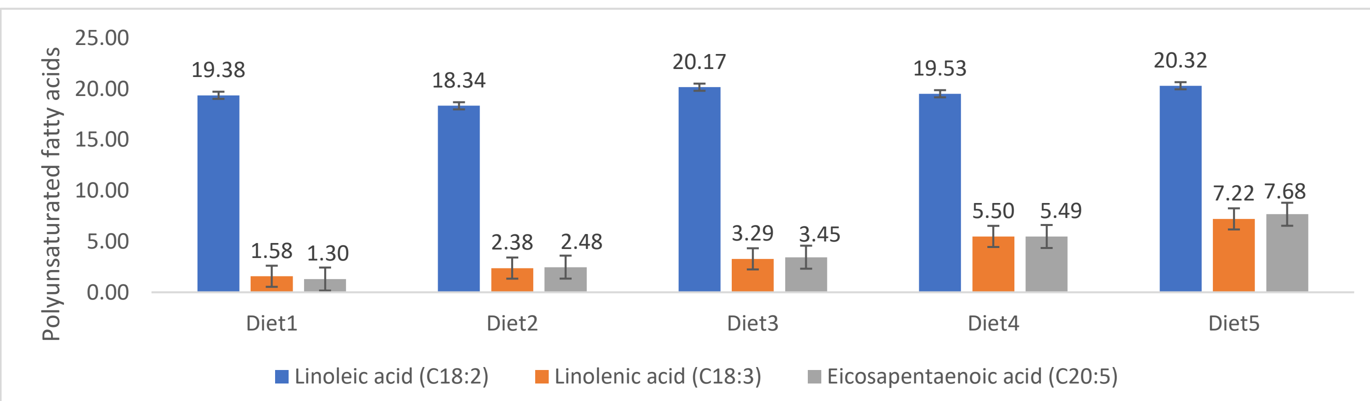

Figure 3. Polyunsaturated fatty acid analysis using experimental diets.

Table 5. Fatty acids composition of grass carp.

Parameters	Diet-I (G0)	Diet-II (G1)	Diet-III (G2)	Diet-IV (G3)	Diet-V (G4)
Myristic (C14:0)	5.67±0.04A	5.49±0.06A	5.54±0.04A	4.79±0.11C	5.16±0.06B
Stearic acid (C18:0)	4.38±0.08B	5.30±0.10A	4.36±0.12B	3.48±0.15C	4.26±0.04B
Palmitic acid (C16:0)	50.95±0.28AB	51.60±0.43A	50.29±0.43B	50.08±0.49B	50.94±0.31AB
∑SFAs	61.00±0.40	62.39±0.59	60.19±0.58	58.36±0.75	60.36±0.40
Palmitoleic acid (C16:1)	10.96±0.22B	10.20±0.09C	10.84±0.19B	12.37±0.25A	12.48±0.25A

Oleic acid (C18:1)	2.65±0.10AB	2.43±0.05B	1.80±0.04C	2.48±0.09B	2.81±0.10A
Erucic acid (C22:1)	1.51±0.05B	1.73±0.10A	1.45±0.04B	1.79±0.07A	1.19±0.03C
∑MUFAs	15.12±0.37	14.37±0.24	14.09±0.27	16.64±0.41	16.47±0.38
Linoleic acid (C18:2)	19.38±0.12B	18.34±0.05C	20.17±0.29A	19.53±0.03B	20.32±0.24A
Linolenic acid (C18:3)	1.58±0.07E	2.38±0.21D	3.29±0.25C	5.50±0.18B	7.22±0.25A
Eicosapen-taoic acid (C20:5)	1.30±0.03E	2.48±0.14D	2.45±0.13C	5.49±0.13B	7.68±0.21A
∑PUFAs	22.25±0.22	23.20±0.4	26.91±0.67	30.51±0.34	35.21±0.7

because black cumin dietary fatty acid profiles and increased unsaturated fatty acids especially gamma linolenic acid (C18:3n6), methyl cis 5,8,11,14 eicosatetraenoic acids, which enhanced the meat quality of fish. Results of these studies totally aligned with present work. Our findings were also supported by Omolo et al. (2017), who found that when rohu were fed a supplemented diet of Spirulina platensis algae, the amount of saturated fatty acids in their meat decreased when compared to the control group. At the same time, the poly-unsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid significantly increased because tilapia is capable of converting dietary alpha-linolenic acid to poly-unsaturated omega-3 fatty acids. In the context of our findings, the role of black cumin is particularly remarkable and the results are comparable to those of other reported studies.

CONCLUSION

Current study recognized that *Nigella sativa* diet to the grass carp notably helped in fish health as well as flesh quality. The recommended concentration (3%) was used in our experiment and found to be useful for the development of fish muscles. It was observed that number of healthy fats linolenic acid and eicosapentaenoic acid with increasing trend, while saturated fatty acids were found decreased throughout. The increased unsaturated and decreased saturated fatty acid behavior leads to healthy fish which can be suitable for human consumption also. Briefly, unsaturated fatty acid plays important role to improve the meat quality in fishes. In conclusion, addition of black seed with concentration of 3% is recommended as fish diet which is considered to improve and protect the fish health allover.

AUTHOR'S CONTRIBUTION

A Majeed conducted the research experiment. ZH Arslan Planned and supervised the research project. MT Rasheed provided the statistics analysis

FUNDING

No funding was available for this project.

AVAILABILITY OF DATA AND MATERIAL

The data supporting the findings of this research is available from the corresponding author, upon measured proposal.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the research experiments were conducted according to standard ethical rules and regulations adopted by the ethical committee of KFUEIT-RYK

CONSENT FOR PUBLICATION

Not relevant. This study does not use any individual's data in any way. All authors have approved to its publication.

CONFLICT OF INTERESTS

All authors attest to the validity of manuscript contents and agree for submission.

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