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Effect of Different Levels of Dried *Azolla pinnata* in Grass Carp (*Ctenopharyngodon idella* , Val .1844) Diets on the Fatty Acid Profile of Muscles

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ABSTRACT

This research assessed the impact of varying proportions of dried Azolla in the diets of grass carp (*Ctenopharyngodon idella*) fingerlings and examined its influence on fatty acid concentrations in the muscles of both the anterior and posterior sections of the fish. Fish were randomly allocated to glass tanks (40×60×40) cm throughout three treatments (T1, T2, and T3). The experimental fish were fed three experimental diets for eight weeks, each containing a specific percentage of Azolla. The first diet was designated as the control diet and was free of dried azolla. Dried azolla was added at 15% and 30% to the second and third diets, designated T2 and T3 respectively.

The results of the current study showed differences in the percentages of fatty acids in the muscles of the anterior regions R1 and posterior regions R2 of the studied fish. These percentages ranged between (0.7- 50) % in the anterior muscles during the time periods and for the different concentrations used in the study. While the percentages of fatty acids ranged between (1.4 -52.5) % in R1 during the time periods and for the concentrations used in the study. Palmitic acid recorded a constant appearance, as it was recorded in all concentrations and periods for the two studied regions. The highest percentage appeared in R2, especially at a concentration of 30%, reaching 52.5%. While Enanthic acid appeared at 0% in all treatments and for the three periods in the two studied regions. The statistical results proved the existence of significant differences ($p<0.05$) in the percentages of fatty acids in the anterior and posterior muscles during the time periods and for the studied concentrations.

1. Introduction

Fish meat is one of the most widely consumed food sources due to its high protein, unsaturated fatty acids, and numerous minerals and vitamins. Fish is a major source of polyunsaturated fatty acids, particularly Eicosapentaenoic acid and Docosahexaenoic acid [1][2]. Polyunsaturated fatty acid content varies between freshwater and saltwater fish. The unsaturated fat content of marine fish ranges from 40% to 60% of the total fat. Freshwater fish contain lower levels, ranging from 20% to 40% [3]. Freshwater fish contain lower levels of C20, C22, and n-3 polyunsaturated fatty acids. In

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comparison, they contain lower levels of C18 and n-6 polyunsaturated fatty acids compared to saltwater fish [4].
The farming of fish beneficial to human health, such as carp, is of great importance to fisheries professionals and researchers [5]. The grass carp is of great interest to aquaculture practitioners [6] due to its high growth and production rates [7]. The fatty acid composition of fish muscles is directly affected by the quality and composition of the feed on aquatic plants, including Azolla pinnata [8]. Azolla is one of the high-biomass aquatic plants that can be used as direct feed for fish. It can double its biomass in (2-5) days. Therefore, fish farmers strive to incorporate ingredients with high nutritional value, local availability, and low cost, such as aquatic plants that provide high nutrient content [9].

2.Materials and Methods

2.1. Experimental fish

Grass carp fingerlings were used in the experiment. Fingerlings weighed between 10.5 and 2g. About 200 fishes were collected between October 2024 and February 2025 from the fish farming ponds of the Marine Science Center at the University of Basrah. The fish were transported in special plastic containers to the Laboratory of Ecology in the Department of Biology, College of Education, Al-Qurna, University of Basra. The experiment included three groups (2replicates/group) as follows:
1. The control group T1:it was fed a diet devoid of azolla
2. The second group T2: was fed a diet containing 15% azolla
3. The third group T3: had a 30% azolla diet
The fish were acclimatized for ten days. They were then fed the experimental diet (Table 1) twice daily (8:00 a.m. and 2:00 pm.). Two regions of skeletal muscle were prepared from the fish's body. The anterior region (R1) represented the trunk region, and the posterior region (R2) represented the proximal caudal peduncle. The muscle tissues were dried in an oven at 60°C for 48 h until constant weight was reached. The dried samples were ground using an electric grinder to obtain a fine powder. The samples were stored in tightly closed plastic bottles until used in the extraction process

Table 1 . Components of experimental feeds provided to grass carp

Component	T1	T2	T3
Dried Azolla	0%	15%	30%
Fish meal	50%	35%	20%
Flour	25%	25%	25%
Wheat bran	23%	23%	23%
Vitamins	1%	1%	1%
Vegetable oil	1%	1%	1%
Total	100%	100%	100%

2.2. Lipid Extraction

Fat was extracted from dried - ground samples of the studied muscle areas. The Soxhlet extraction method was used, which involved placing (5-10) g of the sample in a porous tube made of filter paper. This tube was inserted into the extraction chamber of the Soxhlet apparatus. Hexane was used as the extraction solvent and placed in a 250 ml boiling flask. The apparatus was set to (65-68)°C, and the flask was heated until the solvent evaporated. It was then transferred via a side tube to the condenser, where it re-liquefied in the extraction chamber. Once the solvent was filled, it returned to the flask via a siphon tube, carrying the lipids with it. The process continued until the filter paper became transparent. This indicates that the extraction process is complete. The process takes (24-6) hours. The extract was transferred to a glass vial. The vial was then placed in a 45°C oven overnight to evaporate the remaining solvent and obtain a pure lipid extract [10].

2.3. Fatty acid analysis

Fatty acids were analyzed directly by GC-MS. Fats were extracted and converted to fatty acid methyl esters (FAME) via esterification using sulfuric acid, then identified by comparing the results with a standard reference mixture.

3. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 22. The data were analyzed using one-way analysis of variance (ANOVA), followed by Fisher's Least Significant Difference (LSD) test for multiple comparisons. Differences were considered statistically significant at ($p \leq 0.05$).

4. Results

Table 2 shows the recording of 12 saturated and monounsaturated fatty acids, distributed as follows: (9 saturated) (4 monounsaturated) in the R1 anterior muscles during the first week of feeding, which included a certain percentage of azolla. At T1, four saturated fatty acids were identified: (Palmitic acid, Valeric acid, Pelargonic acid, and Stearic acid) at concentrations of 40.7%, 11.7%, 2.92%, and 7.8%, respectively. Meanwhile, saturated acids (Caproic acid, Enanthic acid, Caprylic acid, Myristic acid, Palmitamide acid) did not record any percentage at T1. (Palmitoleic acid, Oleic acid) were recorded at percentages of 6.0%, 30.8% respectively. In contrast, Elaidic acid did not record any percentage at T1. When the results were statistically analyzed for the percentages of fatty acids within one concentration, significant statistical differences were recorded, ($p < 0.05$), Table (2) .

While saturated fatty acids recorded different percentages at T2, palmitic acid had the highest percentage of 42.1%. In comparison, Pelargonic acid recorded the lowest percentage of 2.19%. In contrast, the acids (Stearic, Caproic, Enanthic, Myristic, Palmitamide) did not record any percentages. In comparison, the monounsaturated fatty acids (Elaidic, Palmitoleic) recorded percentages of (26.2%, 5.3%), respectively. At the same time, Table (2) shows that Oleic acid had no percentage (0%). Significant statistical differences were noted ($p<0.05$) when the data were statistically examined at T2 as shown in Table (2).

At T3, saturated fatty acids recorded different percentages. Palmitic acid (40.4%), Stearic acid (4.5%), and Caproic acid (30.2%), while saturated fatty acids (Pelargonic, Valeric, Enanthic, Caprylic, Myristic, Palmitamide) did not record any percentage (0.0%) Table (2). Monounsaturated fatty acid Elaidic recorded a percentage of (24.9)%, while Palmitoleic and Oleic acids did not record any percentages (0.0%) Table (2). When analyzing the statistical results of fatty acid ratios within T3, significant differences were recorded ($p < 0.05$) Table (2). When comparing the statistical results of fatty acid ratios in the first week of muscle tissue in the anterior region R1, significant differences were shown ($p < 0.05$) between the concentrations of each fatty acid for the studied treatments concentrations Table (2). Table (3) shows the fatty acid ratios in the posterior muscles R2 within the first week and within the concentrations used in the study. Palmitic acid recorded the highest percentages of (52.5, 44.8, 38.4)% within T3, T2,T1, respectively, within the saturated fatty acids. While Pelargonic acid recorded the lowest concentrations of (5.7, 0.0, 5.3) % in T3, T2, and T1, respectively. Stearic acid recorded percentages of 4.8% in T1, 6.8% in T2, and 7.6% in T3. When the results of the fatty acid percentages mentioned above were statistically analyzed, significant differences were recorded ($p < 0.05$), Table (3). While the monounsaturated fatty acids Elaidic Acid, Palmitoleic Acid, and Oleic Acid recorded varying percentages at T3 , T2, and T1, Oleic Acid recorded the highest percentage of 34.1% in T3. While Elaidic Acid recorded 28.1% in 15% concentration and 17.1% in T1, Palmitoleic acid recorded the lowest percentage of 5.2% in T1. In contrast, no percentage was recorded in T3 and T2 , which was(0.0)% Table (3). When the results were statistically analyzed, whether within the same concentration or the difference in fatty acid percentages between different concentrations, significant differences were shown ($p<0.05$) Table (3). Table (4) shows the fatty acid percentages in the anterior muscles R1 in the fourth week, within the concentrations used for the Azolla plant. Palmitoleic acid recorded the highest percentage (49.7, 50.0, 40.4)% in T3, T2, T1 respectively. In comparison, Valeric acid recorded a percentage of 11.9% at 0% concentration. In contrast, no percentages were recorded at T3 and T2. Stearic acid

recorded percentages of (8.6, 9.6, 7.8)% at T3 ,T2 and T1 respectively. While Pelargonic acid recorded a percentage of 2.9% at T1, and no percentages were recorded at T3 and T2, Table (4). When the results were analyzed statistically, they showed significant differences ($p<0.05$) within the fatty acid percentages in Azolla plant concentrations and also between fatty acids within the same concentration, Table (4).

Monounsaturated fatty acids recorded a clear difference in percentages between the used concentrations. Elaedic acid recorded percentages of 41.6% and 40.4% at T3 and T2, respectively. In contrast, no percentage was recorded at T1 Table (4). Palmitoleic acid and Oleic acid recorded percentages of 31.0% and 6.0%, respectively, at T1 .When the results of the acids mentioned above were statistically analyzed, significant differences appeared ($p < 0.05$), Table (4). The percentages of fatty acids in the background region R2 are shown in Table (4). Palmitic acid appeared as the highest percentage of saturated fatty acids. It reached 41.3%, 49.5%, and 42.4% at T3 ,T2 and T1, respectively. In comparison, Valeric acid recorded a percentage of 8.9% at T1 only. Stearic acid recorded different percentages at T3 ,T2 and T1, reaching 8.1%, 10.3%, and 8.8%, respectively. While the percentages of other fatty acids recorded were 0.0%, Table (4). As for monounsaturated fatty acids, Oleic Acid showed the highest percentages, 38.9%, 40.2%, and 33.4%, at T3 ,T2 and T1 respectively, Table (5). In contrast, Palmitoleic acid had percentages of 6.6% at a T1 and 5.7% at T3, Table (5). When analyzing the results of fatty acid percentages, it was shown that there were significant differences ($p < 0.05$) between fatty acids at the same concentration, in addition to comparing them at the concentrations used in Table (5). Tables (6,7) show the difference in fatty acid percentages in the anterior muscles R1 and posterior muscles R2 at T3,T2 and T1. In the eighth week, Palmitic acid recorded the highest percentages among the recorded fatty acids, reaching (42.0, 43.1, 32.9) % at T3 ,T2 ,T1, respectively, Table (6). While in the posterior region, R2 percentages were recorded, reaching 37.9%, 43.9%, 35.5% at T3 ,T2 and T1 Table (7). While saturated fatty acids recorded lower percentages, reaching 0.7% Caprylic acid, 1.2% Myristic acid, 2.0% Pelargonic acid, 6.3% Stearic acid, and 21.4% Caproic acid at T1 Table (6). While (2.4% Pelargonic acid, 6.8% Stearic acid, 14.7% Valeric acid) were recorded at T1 in the muscles of R2 Table (7). In contrast, Stearic acid recorded a percentage of 7.8% at T2 in the muscles of the R1 Table (6) and a percentage of 8.2% for the same acid in R2 Table (7) Regarding T3, the acid mentioned above recorded percentages of R1 (9.7%) and R2 (7.1%), as shown in Tables (6) and (7), respectively. As for monounsaturated fatty acids, they showed different percentages in the targeted areas and concentrations in the study. Oleic Acid recorded percentages of (48.3, 44.1, 30.9)% at T3 , T2 and T1 respectively, in R1, Table 6. Also, percentages of (42.4%, 47.9%, 35.9%) were recorded for the same acid at T3 , T2 , T1 respectively, in R2, Table (7). In contrast, Elaidic Acid did not record any percentages in the three concentrations of Azolla plant in both studied areas, as it appeared at T1 in Tables (6) and (7). The statistical results revealed significant differences ($p < 0.05$) when analyzed statistically, as shown in Tables (6) and (7).

Table 2. The percentage of fatty acids in R1 at week1.

Fatty acids%		R1(Week 1)			*p* value
		T1(Mean±SD)	T2(Mean±SD)	T3(Mean±S)	
SFAs	Palmitic acid	$(40.7^{a} \pm 0.3^{A})$	$(42.1^{b} \pm 0.1^{A})$	$(40.4^{a} \pm 0.1^{A})$	0.0001
	Valeric acid	$(11.7^{a} \pm 0.2^{C})$	$(17.3^{b} \pm 0.07^{C})$	$(0.0^{a} \pm 0.0^{E})$	0.0001
	Pelargonic acid	$(2.92^{a} \pm 0.1^{F})$	$(2.9^{a} \pm 0.1^{F})$	$(0.0^{b} \pm 0.0^{E})$	0.0001
	Stearic acid	$(7.8^{a} \pm 0.1^{D})$	$(0.0^{b} \pm 0.0^{G})$	$(4.5^{c} \pm 0.1^{D})$	0.0001
	Caproic acid	$(0.0^{a} \pm 0.0^{G})$	$(0.0^{a} \pm 0.0^{G})$	$(30.2^{b} \pm 0.2^{B})$	0.0001
	Enanthic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{E})	
	Caprylic acid	$(0.0^{a} \pm 0.0^{G})$	$(6.2^{b} \pm 0.1^{D})$	$(0.0^{a} \pm 0.0^{E})$	0.0001
	Myristic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{E})	
	Palmitamide	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{E})	
MSFAs	Elaidic Acid	$(0.0^{a} \pm 0.0^{G})$	$(26.2^{b} \pm 0.15^{B})$	$(24.9^{c} \pm 0.2^{C})$	0.0001
	Palmitoleic acid	$(6.0^{a} \pm 0.3^{E})$	$(5.3^{b} \pm 0.2^{E})$	$(0.0^{c} \pm 0.0^{E})$	0.0001
	Oleic Acid	$(30.8^{a} \pm 0.3^{B})$	$(0.0^{b} \pm 0.0^{G})$	$(0.0^{b} \pm 0.0^{E})$	0.0001
	***p* value**	0.0001	0.0001	0.0001	

Lowercase letters (different) represent significant differences in fatty acid concentrations between different Azolla plant concentrations at the 0.05 probability level. Capital letters (different) represent significant differences in fatty acid concentrations within the same concentration at the 0.05 probability level.

Table 3. The percentage of fatty acids in R2 at week1.

Fatty acids%		R2(Week 1)			*p* value
		T1(Mean±SD)	T2(Mean±SD)	T3(Mean±SD)	
SFAs	Palmitic acid	$(38.4^{a} \pm 0.1^{A})$	$(44.8^{b} \pm 0.1^{A})$	$(52.5^{c} \pm 0.1^{A})$	0.0001
	Valeric acid	(0.0 ± 0.0^{F})	(0.0 ± 0.0E)	(0.0 ± 0.0^{E})	
	Pelargonic acid	$(5.3^{a} \pm 0.1^{D})$	$(0.0^{b} \pm 0.0^{E})$	$(5.8^{c} \pm 0.1^{D})$	0.0001
	Stearic acid	$(4.8^{a} \pm 0.1^{E})$	$(6.8^{b} \pm 0.1^{D})$	$(7.6^{c} \pm 0.1^{C})$	0.0001
	Caproic acid	$(29.2^{a} \pm 0.2^{B})$	$(20.3^{b} \pm 0.1^{C})$	$(0.0^{c} \pm 0.0^{E})$	0.0001
	Enanthic acid	(0.0 ± 0.0^{F})	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{E})	
	Caprylic acid	(0.0 ± 0.0^{F})	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{E})	
	Myristic acid	(0.0 ± 0.0^{F})	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{E})	
	Palmitamide	(0.0 ± 0.0^{F})	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{E})	
MSFAs	Elaidic Acid	$(17.1^{a} \pm 0.2^{C})$	$(28.1^{b} \pm 0.1^{B})$	$(0.0^{c} \pm 0.0^{E})$	0.0001
	Palmitoleic acid	$(5.2^{a} \pm 0.1^{D})$	$(0.0^{b} \pm 0.0^{E})$	$(0.0^{b} \pm 0.0^{E})$	0.0001
	Oleic Acid	$(0.0^{a} \pm 0.0^{F})$	$(0.0^{a} \pm 0.0^{E})$	$(34.1^{b} \pm 0.3^{B})$	0.0001
	***p* value**	0.0001	0.0001	0.0001	

Lowercase letters (different) represent significant differences in fatty acid concentrations between different Azolla plant concentrations at the 0.05 probability level. Capital letters (different) represent significant differences in fatty acid concentrations within the same concentration at the 0.05 probability level.

Table 4. The percentage of fatty acids in R1 at week4.

Fatty acids%		R1(Week 4)			*p* value
		T1(Mean±SD)	T2(Mean±SD)	T3(Mean±SD)	
SFAs	Palmitic acid	$(40.4^{a} \pm 0.2^{A})$	$(50.0^{b} \pm 0.1^{A})$	$(49.7^{c} \pm 0.1^{A})$	0.0001
	Valeric acid	$(11.9^{a} \pm 0.1^{C})$	$(0.0^{b} \pm 0.00^{D})$	$(0.0^{b} \pm 0.0^{D})$	0.0001

	Pelargonic acid	$(2.9^{a} \pm 0.1^{F})$	$(0.0^{b} \pm 0.0^{D})$	$(0.0^{b} \pm 0.0^{D})$	0.0001
	Stearic acid	$(7.8^{a} \pm 0.1^{D})$	$(9.6^{b} \pm 0.1^{C})$	$(8.6^{c} \pm 0.1^{C})$	0.0001
	Caproic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{D})	
	Enanthic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{D})	
	Caprylic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{D})	
	Myristic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{D})	
	Palmitamide	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{D})	
MSFAs	Elaidic Acid	$(0.0^{a} \pm 0.0^{G})$	$(40.4^{b} \pm 0.2^{B})$	$(41.6^{c} \pm 0.2^{B})$	0.0001
	Palmitoleic acid	$(6.0^{a} \pm 0.3^{E})$	$(0.0^{b} \pm 0.0^{D})$	$(0.0^{b} \pm 0.0^{D})$	0.0001
	Oleic Acid	$(31.0^{a} \pm 0.1^{B})$	$(0.0^{b} \pm 0.0^{D})$	$(0.0^{b} \pm 0.0^{D})$	0.0001
	p value	0.0001	0.0001	0.0001	

Lowercase letters (different) represent significant differences in fatty acid concentrations between different Azolla plant concentrations at the 0.05 probability level. Capital letters (different) represent significant differences in fatty acid concentrations within the same concentration at the 0.05 probability level.

Table 5. The percentage of fatty acids in R2 at week4.

Fatty acids%		**R2(Week 4)**			***p* value**
		T1(Mean±SD)	**T2(Mean±SD)**	**T3(Mean±SD)**	
SFAs	Palmitic acid	$(42.4^{a} \pm 0.2^{A})$	$(49.5^{b} \pm 0.2^{A})$	$(41.3^{c} \pm 0.1^{A})$	0.0001
	Valeric acid	$(8.9^{a} \pm 0.1^{C})$	$(0.0^{b} \pm 0.0^{D})$	$(0.0^{b} \pm 0.0^{F})$	0.0001
	Pelargonic acid	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{F})	
	Stearic acid	$(8.8^{a} \pm 0.1^{C})$	$(10.3^{b} \pm 0.1^{C})$	$(8.1^{c} \pm 0.1^{C})$	0.0001
	Caproic acid	$(0.0^{a} \pm 0.0^{E})$	$(0.0^{b} \pm 0.0^{D})$	$(0.0^{c} \pm 0.0^{F})$	
	Enanthic acid	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{F})	
	Caprylic acid	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{F})	
	Myristic acid	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{F})	
	Palmitamide	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{D})	(6.0 ± 0.1^{D})	
MSFAs	Elaidic Acid	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{F})	
	Palmitoleic acid	$(6.6^{a} \pm 0.1^{D})$	$(0.0^{b} \pm 0.0^{D})$	$(5.7^{c} \pm 0.2^{E})$	0.0001
	Oleic Acid	$(33.4^{a} \pm 0.1^{B})$	$(40.2^{b} \pm 0.1^{B})$	$(38.9^{c} \pm 0.1^{B})$	0.0001
	p value	0.0001	0.0001	0.0001	

Lowercase letters (different) represent significant differences in fatty acid concentrations between different Azolla plant concentrations at the 0.05 probability level. Capital letters (different) represent significant differences in fatty acid concentrations within the same concentration at the 0.05 probability level.

Table 6. The percentage of fatty acids in R1 at week8.

Fatty acids%		**R1(Week 8)**			***p* value**
		T1(Mean±SD)	**T2(Mean±SD)**	**T3(Mean±SD)**	
SFAs	Palmitic acid	$(32.9^{a} \pm 0.1^{A})$	$(43.1^{b} \pm 0.1^{B})$	$(42.0^{c} \pm 0.1^{B})$	0.0001
	Valeric acid	(0.0 ± 0.0^{I})	$(0.0^{b} \pm 0.00^{E})$	$(0.0^{b} \pm 0.0^{D})$	
	Pelargonic acid	$(2.0^{a} \pm 0.1^{F})$	$(0.0^{b} \pm 0.0^{E})$	$(0.0^{b} \pm 0.0^{D})$	0.0001
	Stearic acid	$(6.3^{a} \pm 0.1^{D})$	$(7.8^{b} \pm 0.1^{C})$	$(9.7^{c} \pm 0.2^{C})$	0.0001

	Caproic acid	$(21.4^{a} \pm 0.1^{C})$	$(0.0^{b} \pm 0.0^{E})$	$(0.0^{b} \pm 0.0^{D})$	0.0001
	Enanthic acid	(0.0 ± 0.0^{I})	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{D})	
	Caprylic acid	$(0.7^{a} \pm 0.2^{H})$	$(0.0^{b} \pm 0.0^{E})$	$(0.0^{b} \pm 0.0^{D})$	0.0001
	Myristic acid	$(1.2^{a} \pm 0.1^{G})$	$(0.0^{b} \pm 0.0^{E})$	$(0.0^{b} \pm 0.0^{D})$	0.0001
	Palmitamide	(0.0 ± 0.0^{I})	(0.0 ± 0.0^{E})	(0.0 ± 0.0^{D})	
MSFAs	Elaidic Acid	(0.0 ± 0.0^{I})	(0.0 ± 0.2^{B})	(0.0 ± 0.0^{D})	
	Palmitoleic acid	$(4.6^{a} \pm 0.3^{E})$	$(5.0^{b} \pm 0.12^{D})$	$(0.0^{c} \pm 0.0^{D})$	0.0001
	Oleic Acid	$(30.9^{a} \pm 0.1^{B})$	$(44.1^{b} \pm 0.1^{A})$	$(48.3^{c} \pm 0.1^{A})$	0.0001
	p value	0.0001	0.0001	0.0001	

Lowercase letters (different) represent significant differences in fatty acid concentrations between different Azolla plant concentrations at the 0.05 probability level. Capital letters (different) represent significant differences in fatty acid concentrations within the same concentration at the 0.05 probability level.

Table 7. The percentage of fatty acids in R2 at week8

Fatty acids%		**R2(Week 8)**			***p* value**
		T1(Mean±SD)	**T2(Mean±SD)**	**T3(Mean±SD)**	
SFAs	Palmitic acid	$(35.5^{a} \pm 0.1^{B})$	$(43.9^{b} \pm 0.1^{B})$	$(37.9^{c} \pm 0.1^{B})$	0.0001
	Valeric acid	$14.7^{a} \pm 0.1^{C})$	$(0.0^{b} \pm 0.0^{D})$	$(6.6^{C} \pm 0.1^{D})$	0.0001
	Pelargonic acid	$(2.4^{a} \pm 0.0^{F})$	$(0.0^{b} \pm 0.0^{D})$	$(0.0^{b} \pm 0.0^{G})$	0.0001
	Stearic acid	$(6.8^{a} \pm 0.2^{D})$	$(8.2^{b} \pm 0.2^{C})$	$(7.1^{c} \pm 0.1^{C})$	0.0001
	Caproic acid	$(0.0^{a} \pm 0.0^{G})$	$(0.0^{b} \pm 0.0^{D})$	$(0.0^{c} \pm 0.0^{G})$	
	Enanthic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{G})	
	Caprylic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{G})	
	Myristic acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(1.4 ± 0.1^{F})	0.0001
	Palmitamide	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{G})	
MSFAs	Elaidic Acid	(0.0 ± 0.0^{G})	(0.0 ± 0.0^{D})	(0.0 ± 0.0^{G})	
	Palmitoleic acid	$(4.7^{a} \pm 0.1^{E})$	$(0.0^{b} \pm 0.0^{D})$	$(4.6^{a} \pm 0.1^{E})$	0.0001
	Oleic Acid	$(35.9^{a} \pm 0.1^{A})$	$(47.9^{b} \pm 0.1^{A})$	$(42.4^{c} \pm 0.1^{A})$	0.0001
	p value	0.0001	0.0001	0.0001	

Lowercase letters (different) represent significant differences in fatty acid concentrations between different Azolla plant concentrations at the 0.05 probability level. Capital letters (different) represent significant differences in fatty acid concentrations within the same concentration at the 0.05 probability level.

5. Discussion

Lipids and their main constituents of fatty acids, in addition to proteins, represent the largest organic components of muscles, appearing in different types and percentages that directly influence fish fleshes quality [11]. Fish meat provides essential elements for the human diet due to its richness in both saturated fatty acids (SFA) and unsaturated fatty acids (UFA), which impart fish oil its characteristics as a primary of health source lipids for body [12].

Fatty acids are classified according to the number of carbon atoms, their degree of saturation, and the type of bond. Saturated fatty acids (SFA) contain no double bonds and a higher number of hydrogen atoms compared with unsaturated fatty acids (UFA). Unsaturated fatty acids may be monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA) [13]. The concentration and type of fatty acids in the different body tissues of fish are influenced by several factors, the most important of which are diet, season, and physiological status [14].

Accordingly, in the present study, three levels nutritional from Azolla (30%, 15%, and 0%) were used to evaluate the effect of their inclusion in the diet of grass carp fingerlings on the proportions and concentrations of fatty acids in the anterior (R1) and posterior (R2) muscle regions. The results demonstrated clear effects leading to differences in the types and contentes of fatty acids in the studied muscle regions across the three levels and experimental periods (1, 4, and 8 weeks). A total of 12 fatty acids were identified and divided into two groups: the first group comprised nine saturated fatty acids (Palmitic acid, Valeric acid, Pelargonic acid, Stearic acid, Caproic acid, Enanthic acid, Caprylic acid, Myristic acid, and Palmitamide acid), while the second group included three monounsaturated fatty acids (Elaidic acid, Palmitoleic acid, and Oleic acid).

Several studies investigating fatty acid composition in fish muscles, such as the work of Zhang [15] on 22 species of bony fish from different families, have reported that fish oil typically contains Palmitic acid, Stearic acid, and Myristic acid. In the present study, a marked increase was observed in the concentration of fatty acids, particularly Palmitic acid, in both types of muscle throughout the experimental periods. This fatty acid was consistently detected across all levels and periods in both R1 and R2, with the highest proportion recorded in posterior muscles (R2) at T3 inclusion. This indicates that Palmitic acid is the most stable saturated fatty acid present, reflecting its importance in enhancing the nutritional value of fish flesh, with reported effects in reducing liver inflammation, protecting pancreatic cells, and playing a key role in strengthening muscle fibers and consequently supporting the skeletal system [16].

These variations can be attributed to differences in the proportions of red and white muscle fibers between the two regions (R1 and R2) of the same fish, as well as differences in locomotor activity and the functional role of the muscle fibers [17] [18]. Additionally, the chemical composition of muscles is affected by several factors including fish species, muscle type, anatomical location, function, diet, and locomotor activity [19].

As for Enanthic acid, it was absent across all treatments, time periods, and studied muscle regions. This can be explained by the fact that short- and medium-chain fatty acids are rapidly absorbed and metabolized in tissues such as the liver, where they are used as an energy source rather than being stored in muscle tissue [20].

6. Conclusions

The current results demonstrated that Azolla had a clear effect on the disappearance of certain fatty acids in the studied muscles. They also showed that palmitic acids had the highest percentage of fatty acids compared to other fatty acids. Enanthic acid was 0% in all treatments and across the three periods in the two studied areas. This reflects the plant's impact on fatty acid levels and its effect on muscle growth.

Conflict of Interest

The researchers confirm that there is no conflict of interest in conducting the research

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تأثير احلال نسب مختلفة من نبات الازولا المجفف في علائق اسماك الكارب العشبي على نسب الاحماض الدهنية في العضلات *Ctenopharyngodon idella* (Val.1844,

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معلومات البحث

الاستلام 11 تشرين أول 2025
المراجعة 12 تشرين ثاني 2025
القبول 12 تشرين ثاني 2025
النشر 31 كانون أول 2025

الكلمات المفتاحية

نبات الازولا ، الكارب العشبي ، عضلات الأسماك ، احماض دهنية.

الملخص

تناولت الدراسة الحالية تقييم استخدام نسب مختلفة من نبات الازولا المجففة في العلائق المقدمة لاصبعيات اسماك الكارب العشبي *Ctenopharynogodon idella* (Val. 1844) وتأثيرها على نسب الاحماض الدهنية في انسجة عضلات المنطقتين الامامية والخلفية للاسماك قيد الدراسة. وزعت الاسماك عشوائيا على احواض زجاجية (40 x60 x40) cm على ثلاث معاملات (T2,T1 و T3), غذيت الأسماك لمدة ثمان أسابيع على ثلاث علائق تجريبية تضمنت كل منها نسبة معينة من نبات الازولا الجاف خصصت العليقة الأولى للمعاملة الأولى T1 (مجموعة السيطرة)وكانت خالية من مسحوق الازولا بينما اضيف مسحوق الازولا نسبة 15% و 30% في المعاملتين الثانية T2 والثالثة T3 على الترتيب.
أظهرت نتائج التحليل الاحصائي للدراسة الحالية وجود اختلاف نسب الاحماض الدهنية في عضلات المنطقتين الامامية R1 و الخلفية R2 للاسماك المدروسة ، والتي تراوحت بين (0.7 - 50) % في العضلات الامامية خلال الفترات الزمنية و للتراكيز التغذوية المستخدمة في الدراسة ، بينما تراوحت نسب الاحماض الدهنية بين (1.4 - 52.5)% في العضلات الخلفية خلال الفترات الزمنية و للتراكيز المستخدمة في الدراسة .سجل Palmitic acid ثباتاً في الظهور اذ سُجل في جميع التراكيز و الفترات للمنطقتين المدروستين وظهرت اعلى نسبه في العضلات الخلفية R2 وخاصة في المعاملة T3 اذ بلغت (52.5)% ، بينما ظهر Enanthic acid بنسبة (0.0%) في جميع المعاملات وللفترات الثلاث للمنطقتين المدروستين .اثبتت النتائج الإحصائية وجود اختلافات معنوية ($p \leq 0.05$) في نسب الاحماض الدهنية في العضلات الامامية والخلفية خلال الفترات الزمنية و للتراكيز المدروسة.

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