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FATTY ACID PROFILE IN DIFFERENT AGE CATEGORIES OF FARMED RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM, 1792)

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ABSTRACT
The objective of this study was to compare the fatty acid profile in different age categories of farmed rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) collected from the aquaculture facility—cold-water fish farm "Trofta"—located in the Republic of Kosovo.
Considering the results of the fatty acid profile, SFA participated with 25.76 % (I age group), followed by 25.67 (II), 18.28 % (III), and 18.46 % (IV). There is an evident decrease in the SFA content with increased fish age. Of those, the most dominant are palmitic fatty acids (16.13; 16.31; 10.68; 10.69, respectively). The content of MUFA is rapidly increasing with the ages (31.28 % - I; 29.14 % - II; 46.01 % - III and 46.39 % - IV). Of those, the most dominant is an oleic fatty acid with a double increase from the 8 - 9 to 12 -14-month-old fish (20.03 %; 18.21 %; 39.41 %; 40.48 %, respectively). PUFA participates with an average of 40.00 % in total fatty acid content, from which, the most dominant at the 8- and 9-month-old fish is cervonic fatty acid (27.68 and 30.40 %, respectively), with an evident decrease at 12- and 14-months fish (10.53 and 8.52 %, respectively). The biggest increase is determined in linoleic acid, starting with 4.95 and 4.22 % at 8 and 9-month-old fish, while the content at 12 and 14-month-old fish was enormously higher (14.73 and 16.70 %, respectively).

1. Introduction

Nutritional and health benefits achieved by the consumption of fish (Burger and Gochfeld, 2009), the content of high biological value proteins, low-fat, and low cholesterol content (Conor, 2000), as well as valuable quantities of essential fatty acids (Sidhu, 2003), increased the demand of fish in the human diet.

According to Özoğul *et al*. (2007), freshwater and marine fish species are good sources of essential fatty acids. Variations in fatty acid compositions within fish species depend on many factors such as food availability, season, environmental condition, geographic location, sex, diet, and age (Gorgun and Akpinar 2007; Petterson *et al.*, 2009; Sicuro *et al.*, 2010). Due to its positive impact on health, fatty acids in fish lipids have been a subject of great interest (Wang *et al.*, 2006; Riediger *et al.*, 2009).

Salmonids are one of the most farmed fish in aquaculture due to their greater resistance to diseases compared to cyprinid fish, the excellent and balanced composition of fish meat, and the favorable content of polyunsaturated fatty acids.

Oncorhynchus mykiss (Walbaum, 1792), commonly known as rainbow trout, is a species of trout native to cold-water tributaries of the Pacific Ocean in Asia and North America. Sarma *et al.* (2013) considered that it is widely used in aquaculture because of its rapid growth and high market value due to its flesh quality, especially the content of significant amounts of essential fatty acids.

Rainbow trout is currently a significant agricultural product preferred in the global market, fresh, frozen, or processed (mainly smoked). Usually, rainbow trout in the market is requested in portion sizes of 200 - 400 gr, but depending on the situation, purpose, and many factors, it can be requested in other sizes as well. Thanks to the favorable amount of polyunsaturated fatty acids (especially omega 3 and omega 6) compared to saturated fatty acids, it is good to know in which fish age categories this amount is the highest to prefer certain consumption sizes.

The objective of this study was to compare the fatty acid profile in different age categories of farmed rainbow trout (Oncorhynchus mykiss Walbaum, 1792) collected from the aquaculture facility—cold-water fish farm "Trofta"—located in the Republic of Kosovo.

2. Materials and methods

A total of 46 samples of rainbow trout were collected from an intensive fish farm located in Istog, Republic of Kosovo (Figure 1). Examinations were performed on rainbow trout of four different age categories, as follows: I group - 8 months old (14.80 g weight and 9.33 cm length); II group - 9 months old (34.11 g weight and 11.07 cm length); III group - 12-month-old (273.14 g weight and 23.71 cm length) and IV group - 14-month-old (444.30 g weight and 27.12 cm length) (all of these are average values).

The fish farm Trofta is supplied with water from the river Istog. In this fish farm, pelleted food from manufacturer Aller Aqua is used, with the following content (per specification):

- Aller Futura (2 mm): fish meal, wheat, rapeseed oil, soya protein concentrate, hemoglobin meal (porcine) and fish oil. Besides the main components, the feed contains the following components (per specification): crude protein (47%), crude fat (25%), fiber (1.4%), crude ash (7.1 %), total Ca (1%), total Na (0.4%), total P (1%), Vitamin A (10000 IU/kg), Vitamin D3 (1250 IU/kg), Calcium iodate (3 mg/kg), Manganese chelate (12 mg/kg), Zinc chelate (50 mg/kg), Cooper chelate (5 mg/kg), Antioxidant BHT (E321)(70 mg/kg) and Antioxidant BHA (E320)(45 mg/kg).

- Aller Amber (3 mm): soy oil, soya bean meal, wheat, pap 65% (poultry), hemoglobin, fish meal LT 70%, hydrolyzed poultry feather meal, fish oil, DDGS, mono ammonium phosphate, diamol, lysin, vit-mineral premix, methionine, choline-chloride 60%. Besides the main components, the feed contains the following components (per specification): protein (min 40%), fat (min 28%), moisture (max 8 %), fiber (max 2.4%), ash (max 5.6 %), Ca (0.9%), Na (0.2%), total P (1%), Vitamin A (6000 kJ/kg), Vitamin D3 (2000 kJ/kg), Vitamin E (200 mg/kg), Vitamin C (150 mg/kg), Lizin (min 2%) and Methionine + Cistin (min 1%);

- Aller Amber (4.5 mm): rapeseed oil, wheat, fishmeal, poultry meal, soya meal, sunflower protein concentrate, hemoglobin meal (porcine), hydrolyzed feather meal, fish oil, distiller`s grains. Besides the main components, the feed contains the following components (per specification): crude protein (40%), crude fat (28%), fiber (1.8%), ash (6.2 %), Ca (0.7%), Na (0.2%), total P (1%), Vitamin A (10000 IU/kg), Vitamin D3 (1250 IU/kg), Calcium iodate (3 mg/kg), Manganese chelate (12 mg/kg), Zinc chelate (50 mg/kg), Cooper chelate (5 mg/kg), Antioxidant BHT (E321)(70 mg/kg) and Antioxidant BHA (E320)(45 mg/kg).

- Aller Amber (6 mm): rapeseed oil, wheat, fish meal, poultry meal, soya meal, sunflower protein concentrate, hemoglobin meal (porcine), hydrolyzed feather meal, fish oil, and distiller`s grains. Besides the main components, the feed contains the following components (per specification): crude protein (40%), crude fat (28%), fiber (1.8%), ash (6.2 %), Ca (0.7%), Na (0.2%), total P (1%), Vitamin A (10000 IU/kg), Vitamin D3 (1250 IU/kg), Calcium iodate (3 mg/kg), Manganese chelate (12 mg/kg), Zinc chelate (50 mg/kg), Cooper chelate (5 mg/kg), Antioxidant BHT (E321)(70 mg/kg) and Antioxidant BHA (E320)(45 mg/kg).

In this fish farm rainbow trout are hatched, raised, and produced for the local market as well as exported to neighboring countries for retailers, restaurants, and other clients. This

is a modern farm, producing about 800 tons of trout per year. They distinguish themselves in the region by the high quality of their fish, and the fingerlings which are also exported abroad.

Gas chromatography - AOAC method 996.06 was used to determine the fatty acid composition.

Data were presented as mean ± standard deviation (SD) and subjected to analysis of variance (ANOVA).

3. Results and discussions

Considering the results of the fatty acid profile of the rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) from fish farm Trofta (Istog, Kosovo), we`ve obtained the following results (Table 2, Figure 2):

Figure 1 Fish farm Trofta (Istog, Republic of Kosovo)
https://www.aller-aqua.com/media/593390/new-co-trofta-kosovo.jpg

Table 1. Fatty acid profile of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) from fish farm Trofta (Istog, Kosovo) in different age categories

Lipid numbers	Name	Type	I	II	III	IV
C8:0	Caprilic acid	SFA	0.48	0.36	0.60	0.76
C14:00	Myristic acid	SFA	3.90	3.75	1.84	1.85
C15:0	Pentadeclic acid	SFA	0.28	0.29	0.16	0.14
C16:0	Palmitic acid	SFA	16.13	16.31	10.68	10.69
C18:0	Stearic acid	SFA	3.84	3.82	3.85	4.03
C20:0	Arachidic acid	SFA	1.13	1.13	1.15	0.98
TOTAL SFA (saturated fatty acid)			**25.76**	**25.67**	**18.28**	**18.46**
C16:01	Palmitoleic acid	MUFA	4.00	3.64	1.79	1.64
C18:1	Oleic acid	MUFA	20.03	18.21	39.41	40.48
C20:1	Gondoic acid	MUFA	3.91	3.89	3.15	3.02
C22:1	Erucid acid	MUFA	3.34	3.40	1.66	1.25
TOTAL MUFA (monounsaturated fatty acid)			**31.28**	**29.14**	**46.01**	**46.39**
C18:2 n-6 c	Linoleic acid	PUFA	4.95	4.22	14.73	16.70
C18:3 n-6	α- linolenic acid	PUFA	0.16	0.13	0.75	0.75
C18:3 n-3	α- linolenic acid	PUFA	1.82	1.77	4.48	4.47

C20:2 n-6	Eicosadienoic acid	PUFA	0.52	0.50	0.88	0.88
C20:3 n-6	Dihomo-γ-linoleic acid	PUFA	0.23	0.20	0.70	0.76
C20:4 n-6	Arachidonic acid (AA)	PUFA	1.03	0.98	0.88	0.80
C20:3 n-3	Eicosatrienoic acid (ETE)	PUFA	0.19	0.20	0.24	0.25
C20:5 n-3	Timnodonic acid	PUFA	6.36	6.47	2.48	1.97
C22:6 n-3	Cervonic acid	PUFA	27.68	30.40	10.53	8.52
TOTAL PUFA (polyunsaturated fatty acid)			**42.94**	**44.87**	**35.67**	**35.10**
Total SFA (saturated fatty acid)			25.76	25.67	18.28	18.46
Total UFA (unsaturated fatty acid)			74.24	74.00	81.71	81.53
Total PUFA n-6			6.37	5.54	17.06	19.01
Total PUFA n-3			36.05	38.83	17.74	15.23
n-6/n-3			0.18	0.14	1.01	1.28
n-3/n-6			5.72	7.10	1.06	0.82
UFA/SFA			2.88	2.89	4.57	4.44
PUFA/SFA			1.67	1.75	1.97	1.91
PUFA/MUFA			1.41	1.60	0.79	0.76

*I size - 8-months-old (14.80 g weight and 9.33 cm length)
II size - 9-months-old (34.11 g weight and 11.07 cm length)
III size - 12-months-old (273.14 g weight and 23.71 cm length)
IV size - 14-months-old (444.30 g weight and 27.12 cm length)

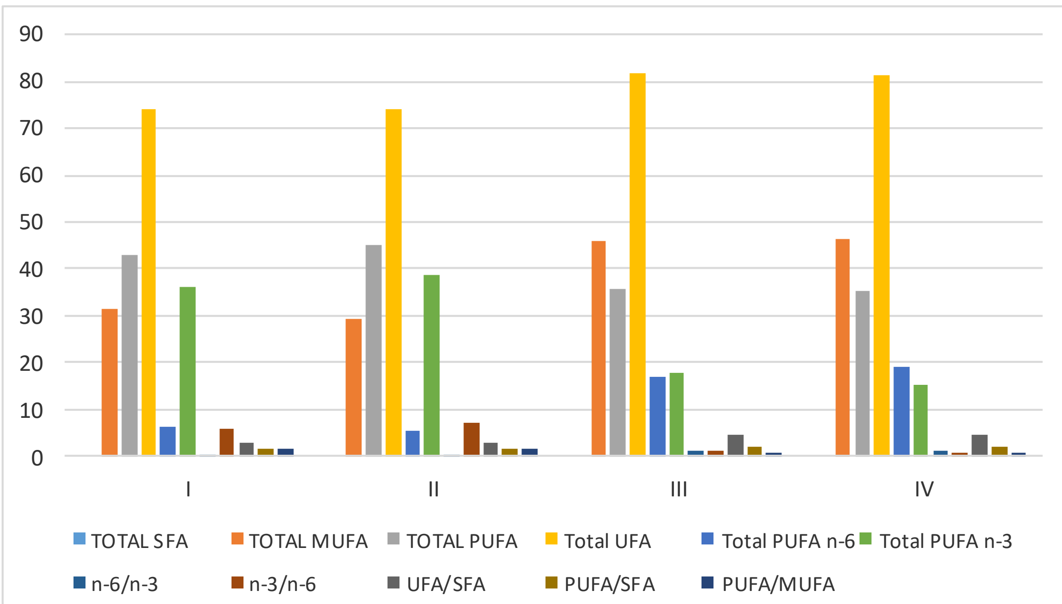

Figure 2 Comparative indicators of content and fatty acid ratio in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)

***Legend:** UFA – unsaturated fatty acid, PUFA – polyunsaturated fatty acid, SFA – saturated fatty acid, n-3 – omega 3 fatty acid, n-6 – omega 6 fatty acid

Table 2 Calculation of means and standard deviations for each age group (8, 9, 12, and 14 months) across various parameters related to fatty acids

Age months	**SFA Mean/ Std Dev**		**UFA Mean/ Std Dev**		**PUFA n-3 Mean/ Std Dev**		**PUFA n-6 Mean/ Std Dev**		**n-6/n-3 Mean/ Std Dev**		**n-3/n-6 Mean/ Std Dev**		**UFA/SFA Mean/ Std Dev**		**PUFA/SFA Mean/ Std Dev**		**PUFA/MUFA Mean/ Std Dev**	
8	25.41	0.43	74.59	0.43	37.80	5.48	6.36	0.52	0.17	0.02	5.97	0.56	2.89	0.10	1.72	0.08	1.41	0.31
9	25.66	0.50	74.34	0.50	37.35	4.27	5.74	0.63	0.16	0.03	6.55	0.81	2.97	0.13	1.79	0.18	1.52	0.25
12	17.77	2.12	82.23	2.12	15.45	3.78	16.35	1.66	1.16	0.28	0.87	0.21	4.69	0.53	2.05	0.29	0.73	0.09
14	17.34	2.27	82.66	2.27	15.41	3.75	17.36	1.80	1.13	0.26	0.88	0.19	4.69	0.43	2.04	0.25	0.76	0.06

Table 3 ANOVA tests for age-dependent variations in Dietary Fatty Acid categories and significance

Fatty Acid Category	p-value	Significance
SFA (Saturated Fatty Acid)	0.00000291	Significant Difference
UFA (Unsaturated Fatty Acid)	0.00000532	Significant Difference
PUFA n-3 (Polyunsaturated Fatty Acid Omega-3)	0.00117	Significant Difference
PUFA n-6 (Polyunsaturated Fatty Acid Omega-6)	0.21882	No Significant Difference
n-6/n-3 Ratio	0.38664	No Significant Difference

Considering the results of the fatty acid profile of the rainbow trout from the fish farm Trofta, in total fatty acid content, saturated fatty acid (SFA) participates with 25.76 % (I age group), followed by 25.67 (II), 18.28 % (III) and 18.46 % (IV). There is an evident decrease in the SFA content with the increase in fish age. Of those, the most dominant are palmitic fatty acids (16.13; 16.31; 10.68; 10.69, respectively).

The content of monounsaturated fatty acids (MUFA) is rapidly increasing with the ages (31.28 % - I; 29.14 % - II; 46.01 % - III and 46.39 % - IV). Of those, the most dominant is an oleic fatty acid with a double increase from the 8 - 9 to 12 -14-month-old fish (20.03 %; 18.21 %; 39.41 %; 40.48 %, respectively).

Polyunsaturated fatty acids (PUFA) participate with an average of 40.00 % in total fatty acid content, from which, the most dominant at the 8 and 9 months old fish is cervonic fatty acid (27.68 and 30.40 %, respectively), with an evident decrease at 12 and 14 months old fish (10.53 and 8.52 %, respectively). The biggest increase is determined in linoleic acid, starting with 4.95 and 4.22 % at 8 and 9-month-old fish, while the content at 12 and 14-month-old fish was enormously higher (14.73 and 16.70 %, respectively).

Sabetian *et al.* (2012) determined that the total SFA, MUFA, and PUFA content in rainbow trout (511.75±6.92 g weight and 35.2±0.18 cm length) from fish farms in Iran were 26.3%, 33.8% and 24.62% of total fatty acids, respectively. The ratio of n-3/n-6 fatty acids was 2.06.

N-6 (omega 6) fatty acids participate with 6.37 (I); 5.54 (II); 17.06 (III) and 19.01 (IV) %, with the higher content in older fish, while n-3 (omega 3) participate with 36.05 (I); 38.83 (II); 17.74 (III) and 15.23 (IV) %, with the lower content in older fish, and good values at younger fish.

Many authors suggested that the n-3/n-6 ratio is a good parameter to determine the nutritional value of oil present in fish meat. In our study, the n-3/n-6 ratio was very high in 8-9-month-old fish (5.72 and 7.10, respectively), with lower values in older fish (1.06 and 0.82, respectively). According to Simopoulos (2002), the n-3/n-6 ratio in freshwater fish ranges from 1 to almost 4. The n-3/n-6 ratio was appreciably higher in the study of Malenica Stavera *et al*. (2012) ranging from 8.05 - 14.77, probably due to the high n-3/n-6 ratio in the feed and environmental condition. This finding is in correlation with our results.

Simopoulos (2004) considered that excessive amounts of n-6 PUFA and a very high n-6/n-3 ratio promote cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n-3 PUFA apply suppressive effects.

In our study, the n-6/n-3 ratio is quite similar in all age categories (0.18; 0.14; 1.01, and 1.28, respectively), with a slight increase with age, as well as, PUFA/SFA ratio.

Simopoulos (2010) concluded that a ratio of 1:1 to 2:1 n-6/n-3 fatty acids should be the ideal ratio for beneficial to health. The Department of Health of the United Kingdom (1994) recommends n-6/n-3 value below 4. Consuming higher dietary quantities of n-3 PUFAs is an approach to normalizing high n-6/n-3 ratios (McDaniel *et al.*, 2013). In the study of Guler *et al.* (2017), the n-3/n-6 ratio was found to be 1.56 in cultured rainbow trout. All of these findings are in correlation with our results.

According to H.M.S.O. (1994), the ideal n-6/n-3 ratio of fatty acids is up to 4. Moreira *et al*. (2001) considered that ratios greater than this value are harmful to health and may promote cardiovascular diseases. In our research, the n-6/n-3 ratio ranged from 0.14 - 1.28, from which it can be concluded that it is favorable.

According to H.M.S.O. (1994), a minimum value of PUFA/SFA ratio is recommended as 0.45. Our findings of 1.67 - 1.97 indicate that fish meat with good nutritional quality because the obtained values are higher than the minimum (0.45) which is recommended for a healthy diet in humans.

According to AFSSA (2003), the UFA/SFA ratio of fatty acids in fish fat is very important and its value should be over 3. In our study, UFA/SFA ratio is 2.88 and 2.89 in 8 -9 months old fish, with increasing in older fish (4.57 - III and 4.44 - IV), so, favorable values are obtained by consuming 12 - 14 months old fish.

The age categories of trout displayed significant variations in SFAs content, with a significance level of $p<0.05$. In Group I (8 months), SFAs constituted 25.76% of the fatty acids. Group II (9 months) exhibited a notably higher proportion of SFAs, amounting to 25.67%. Conversely, in Group III (12 months), the SFAs proportion was 18.28%, and in Group IV (14 months), it was 18.46%. These results underline substantial alterations in SFA levels with age. Additionally, significant discrepancies in the proportions of UFAs were detected, also at $p<0.05$. In Group I (8 months), UFAs comprised 74.24% of the fatty acids. For Group II (9 months), the proportion was quite similar at 74.00%. However, in Group III (12 months), UFAs constituted 81.71% of the fatty acids. In Group IV (14 months), the proportion of UFAs reached 81.53%. Moreover, significant differences ($p<0.05$) were identified in the n-3 fatty acids content. Group III (12 months) demonstrated the highest share of n-3 fatty acids, accounting for 17.74% of the total fatty acids. In Group I (8 months), the proportion of n-3 fatty acids was 36.05%, and in Group II (9 months), it stood at 38.83%. On the other hand, in Group IV (14 months), the proportion of n-3 fatty acids amounted to 15.23%. Further differences were observed in the proportion of n-6 fatty acids. In Group III (12 months), the proportion of n-6 fatty acids was 17.06%, which was significantly higher ($p<0.05$) compared to Group II (9 months) with 5.54% and Group I (8 months) with 6.37%.

The fatty acid ratios in the trout samples indicate age-related shifts, such as a decrease in the n-6/n-3 ratio from 0.18 at 8 months to 0.14 at 9 months, and a substantial increase in the n-3/n-6 ratio from 5.72 at 8 months to 7.10 at 9 months, with notable differences observed in the UFA/SFA and PUFA/SFA ratios across age groups. The fatty acid ratios in the trout samples demonstrate age-related variations, highlighting changes in the balance of essential fatty acids as the fish mature.

Saturated Fatty Acids (SFA): Our statistical analysis indicates a significant relationship between age and the levels of saturated fatty acids in the diet. As individuals age, there is a noticeable change in the consumption of SFA. This aligns with previous research, such as the study by Mozaffarian and Wu (2010), which highlights the importance of monitoring SFA intake, especially among older adults, due to its association with cardiovascular health.

Unsaturated Fatty Acids (UFA): The statistical analysis also demonstrates a significant association between age and the levels of unsaturated fatty acids. The intake of UFA appears to vary with age. It's worth noting that UFA, including monounsaturated and polyunsaturated fats, is generally considered healthier and is linked to various health benefits (Micha *et al.,* 2017). Our findings suggest that dietary habits change as individuals grow older, potentially indicating a shift towards healthier fat choices.

Polyunsaturated Fatty Acids Omega-3 (PUFA n-3): The data reveals that the levels of PUFA n-3, often associated with heart and brain health, are significantly influenced by age. This finding is in line with research that emphasizes the importance of omega-3 fatty acids in the aging population (Dyall, 2015). It underscores the need for older individuals to maintain an adequate intake of these essential fatty acids.

Polyunsaturated Fatty Acids Omega-6 (PUFA n-6): Interestingly, our analysis did not find a substantial age-related effect on PUFA n-

6 levels. This suggests that the intake of omega-6 fatty acids remains relatively constant across different age groups. Omega-6 fatty acids, although essential, should be consumed in balance with omega-3 to avoid an imbalance that could promote inflammation (Simopoulos, 2002). The stability in PUFA n-6 levels across age groups may be attributed to consistent dietary habits.

Balance between Omega-6 and Omega-3 (n-6/n-3 Ratio): The balance between omega-6 and omega-3, as indicated by the n-6/n-3 ratio, does not exhibit significant age-related differences. This observation is noteworthy as an optimal balance is essential for overall health (Simopoulos, 2008). It's vital to maintain this equilibrium throughout life to support various physiological processes.

The fatty acid profile of fish is known to be influenced by a combination of metabolic, physiological, and environmental factors. Among these, age is a crucial determinant that plays a significant role in shaping the composition of fatty acids within the fish. Here, we will explore the metabolic and physiological reasons behind this age-related influence on fatty acid profiles:

• Metabolic Adjustments: Fish exhibit distinct metabolic patterns throughout their life stages. Younger fish are characterized by elevated metabolic rates to fuel rapid growth and development. This heightened metabolic activity influences the synthesis and utilization of fatty acids. Younger fish may favor the incorporation of specific fatty acids to support their energy and growth requirements. As fish mature, metabolic demands change, leading to a different allocation of fatty acids.

• Dietary Shifts and Prey Selection: Fish are opportunistic feeders and tend to modify their dietary preferences as they age. Younger fish often feed on different prey items, which vary in fatty acid content. The diversity in their diets during different life stages can lead to variations in fatty acid profiles. As fish grow older, their dietary choices may evolve, affecting the fatty acids they ingest.

•Physiological Development: Age-related physiological changes can significantly influence fatty acid profiles. Life stage transitions, such as larval to juvenile or juvenile to adult, are marked by shifts in organ development, particularly the liver and gonads. These changes can impact the synthesis and storage of specific fatty acids, especially those essential for reproduction and egg development in mature females. These physiological developments contribute to age-dependent variations in fatty acid composition.

•Environmental Dynamics: Fish encounter varying environmental conditions throughout their life. Changes in factors like water temperature, oxygen levels, and prey availability can alter their metabolic and physiological responses, including how they process and store fatty acids. Different seasons may present different prey options, affecting the intake of particular fatty acids.

•Stress Responses: Aging fish experience stressors such as predation threats, competition for resources, and environmental challenges. Stress responses, including the release of cortisol, can lead to alterations in fatty acid metabolism. These responses can, in turn, affect the overall balance of fatty acids within the fish.

4. Conclusions

Our statistical analysis reveals that age plays a substantial role in shaping dietary habits, especially concerning the intake of saturated and unsaturated fatty acids, as well as PUFA n-3. However, the balance between omega-6 and omega-3 fatty acids remains relatively stable across age groups. These findings underscore the importance of age-tailored dietary recommendations and interventions to ensure that individuals, particularly older adults, maintain healthy fat consumption patterns to support overall well-being.

In summary, the influence of fish age on their fatty acid profiles is a multifaceted interplay of metabolic adaptations, dietary shifts, physiological developments, environmental changes, and stress responses. Researchers keen on understanding fish nutrition and health need to consider these age-related factors when studying fatty acid composition. Recognizing the intricate relationship between age and fatty acid profiles is critical for fisheries management, aquaculture practices, and the development of age-specific nutritional interventions to promote fish health and ensure the production of fish products with desired fatty acid profiles. It underscores the importance of age as a crucial variable in fish nutrition research.

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