

EFFECT OF ADDING A MIXTURE OF OILS TO CARP PELLETS ON FATTY ACID PROFILE AND AMINO ACID CONTENT OF MEAT

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Abstract

The aim of the study was to determine the effect of enriching a pelleted mixture with a mixture of sunflower and rapeseed oils on fatty acid profile and cholesterol and amino acid content of carp meat. The experiment was performed under production conditions using two commercial ponds, each having an area of 1.2 ha. After filling the ponds with water, they were stocked in spring with two-year-old carp weighing an average of 330 g at a stocking rate of 1250 fish per pond. The control group (pond I) was fed from May to end of September with standard Aller Classic pellets. The experimental group (pond II) received the same pelleted feed supplemented during production with a 6% mixture of sunflower and rapeseed oils at a 1:1 ratio. Feeding was terminated at the end of September when carp were harvested, counted and weighed. Chemical analyses were performed using 20 two-year-old carp randomly chosen after carp stocking as well as 30 carp from pond I (group C) and 30 carp from pond II (group E) after harvesting. After the fish were slaughtered, a muscle slice was excised from the left flank for chemical analysis, which included determination of the basic components, fatty acid profile, and cholesterol and amino acid content. Pellets were also analysed chemically for basic nutrients, amino acids, and fatty acid profile. The results obtained showed that the addition of a mixture of sunflower and rapeseed oils increased the content of unsaturated fatty acids and decreased the content of saturated fatty acids, cholesterol and some amino acids in carp meat.

Key words: carp, sunflower and rapeseed oils, fatty acids, cholesterol, amino acids

Society's growing awareness of well-balanced nutrition has forced producers of foodstuffs and food products to increase their efforts at marketing top-quality products that have high nutritive value and health-promoting benefits (Pisulewski et al., 2001). This particularly concerns the content of fats, including the amount of fatty acids. Research has shown that eating food products with a high fat content and a high proportion of saturated fatty acids can induce many lifestyle-related diseases

such as hypertension, heart attacks, cerebral stroke and other life-threatening diseases (Bartnikowska and Kulasek, 1994; Connor et al., 1992; Dobryniewski et al., 2007).

Fish meat is a food product of high nutritive value (Seremak-Bulge, 2009), but its composition shows considerable differences in protein, amino acid and fat content and in fatty acid profile depending on fish species (Bieniarz et al., 2000; Bieniarz et al., 2001; Sikorski, 1997).

Sea fish, such as herring, halibut and sprat are a rich source of polyunsaturated fatty acids (DHA, EPA) that have health-promoting properties (Drevon, 1992; How, 1995; Shirai et al., 2002). Freshwater fish, including carp, are rich in amino acids but have much less essential unsaturated fatty acids compared to sea fish (Bieniarz et al., 2000; Steffens and Wirth, 2005, 2007; Viola and Lalav, 1991).

Efforts are therefore made to find ways of increasing the amount of these acids. The relevant studies (Bieniarz et al., 2001; Epler et al., 2009 a, 2009 b) have shown that the addition of vegetable oils to feed may increase the amount of fatty acids in fish meat and reduce the cholesterol content. From the physiological point of view, not only the amount of unsaturated fatty acids in food products but also the ratio of n-6:n-3 fatty acids are important for the human body health. These acids should have a ratio of 4–10:1. A higher proportion of n-6 acids may lead to a deficiency of n-3 acids, which causes visual disturbances and electroretinogram abnormalities (Connor et al., 1992; Sorgent, 1997). For this reason, mixtures of oils are often used as feed supplements to obtain the desired n-6:n-3 acid ratio in animal products (Epler et al., 2009 b).

In this connection, a study was carried out under production conditions to investigate the effect of adding a mixture of sunflower and rapeseed oils (1:1) to carp pellets on the content of basic nutrients in their meat as well as the amino acid and cholesterol content and fatty acid profile.

Material and methods

The experiment investigating the use of sunflower and rapeseed oil supplements in carp pellets was conducted during the growing season in 2009. Two commercial ponds, each with an area of 1.2 ha and average depth of 1.2 m were used. In early spring, the ponds were filled water from the Rudawa River through the Młynówka watercourse. These ponds were stocked with two-year-old carp with an average body weight of 330 g at a stocking rate of 1250 fish per pond. Fish were fed from May to the end of September with Aller Classic pellets. Fish in the control pond (C) received standard Aller Classic pellets, and those in the second experimental pond (E) were fed the same Aller Classic feed supplemented with a 6% mixture of sunflower and rapeseed oils (50%:50%). Feeds were provided three times a week (Monday, Wednesday, Friday). During the season, 2 tons of feed per pond were fed. Feeding was terminated in late September when fish were harvested, counted and weighed.

Chemical analyses of fish meat were performed using 20 two-year-old carp randomly chosen during spring stocking as well as 30 carp from pond C and 30 carp from pond E during the autumn harvesting. After killing, a muscle slice was excised from the left flank of each fish for chemical analysis.

In the pellet and meat samples, basic nutrients were analysed according to standard AOAC procedures (2005). The gross energy content was determined using a bomb calorimeter, model KL-10. NDF, ADF and ADL content of feed was determined by the method of Goering and Van Soest (1970) using an Ankom 220 apparatus. Amino acid content in feeds and meat was determined by the ninhydrin method after hydrolysis. Acidic amino acids were determined by 6N HCl hydrolysis and sulphur amino acids by oxidizing hydrolysis with formic acid and perhydrol. The determinations were made using an AAA-400 amino acid analyser (Ingos) with an Ostion LG ANB ion exchange column (370 mm long). Samples of pelleted feeds and carp meat were prepared for analysis of fatty acid profile using the method of Folch et al. (1975). Fatty acid profile in carp meat and feeds was determined by Varian 3400CX gas chromatograph with an FID detector and CP-WAX column (50 m × 0.53 mm). Working conditions were as follows: carrier gas – argon, injector temperature – 200°C, detector temperature – 240°C (12 min.), column temperature – 220°C (20 min.). Total cholesterol content of meat was determined using the method described by Korzeniowski et al. (1992).

Statistical analysis

Results were subjected to one-way analysis of variance using the GLM procedure of the SAS software, ver. 9.1. Prior to analysis of variance, normality of data distribution and equality of variance between the analysed experimental groups were tested using Levene's test. The results were interpreted based on planned comparisons (contrasts). A probability value at $P < 0.05$ was considered to be significant, and results at $P < 0.10$ were reported as trends.

Results

Nutrient content of the feeds is shown in Table 1. Supplementation of the pellets for carp from the experimental group with 6% sunflower and rapeseed oils only increased fat content and gross energy. The content of other macronutrients were at a comparable level in pellets. The addition of the oil mixture also decreased the sum of saturated fatty acids and increased the sum of unsaturated fatty acids, including polyunsaturated fatty acids in the experimental diet (Table 2). The amino acid content of the pellets was comparable, although slightly higher in the experimental diet, which was reflected in the higher biological value of this mixture (Table 3). The limiting amino acids were tyrosine in both diets, methionine in the control diet and cystine in the experimental diet.

Table 1. Chemical composition of pelleted diets for carp (%)

Ingredients	Pelleted mixture (C)	Pelleted mixture (E)
Dry matter	89.44	89.66
Crude ash	6.23	6.27
Organic matter	83.21	83.39
Crude protein	27.63	28.00
Crude fibre	13.43	12.78
Crude fat	6.00	8.12
N-free extractives	36.15	34.49
NDF*	12.20	13.04
ADF**	6.64	6.74
ADL***	2.28	2.20
Gross energy (MJ)	17.15	18.63

*NDF – neutral detergent fibre.

**ADF – acid detergent fibre.

***ADL – acid detergent lignin.

Table 2. Profile of fatty acids (% of acids in total acids)

Type of acid	Pelleted mixture (C)	Pelleted mixture (E)
C ₁₂	0.03	0.04
C ₁₄	2.52	0.84
C _{14:1}	0.50	0.04
C ₁₅	0.19	0.12
C ₁₆	12.60	10.85
C _{16:1}	2.68	1.31
C ₁₈	2.73	4.09
C _{18:1}	41.25	41.75
C _{18:2}	21.24	30.50
C _{18:3}	5.61	4.56
C ₂₀	0.44	0.45
C _{20:1}	2.43	1.42
C _{20:2}	0.10	0.10
C _{20:4}	0.18	0.19
C _{20:5}	3.38	1.54
C _{22:1}	0.36	0.37
C _{22:5}	0.57	0.37
C _{22:6}	1.63	1.12
Unidentified acids	1.66	0.34
Total saturated acids	18.51	16.39
Total monounsaturated acids	47.22	44.89
Total polyunsaturated acids	32.61	38.38

Table 3. Amino acid content in pelleted feeds ($\text{g}\cdot\text{kg}^{-1}$ feed)

Amino acids	Pelleted mixture (C)	Pelleted mixture (E)
ASP	20.17	23.80
Thr	7.40	8.62
Ser	10.36	11.63
Glu	40.74	46.12
Pro	14.27	15.11
Gly	9.96	11.32
Ala	9.65	10.73
Val	11.36	13.46
Ile	7.89	9.44
Leu	17.02	18.90
Tyr	5.46	6.12
Phe	10.02	11.32
His	8.01	9.21
Lys	12.35	13.81
Arg	10.45	11.43
Cys	3.66	3.40
Met	4.84	6.20
Σ AA	203.61	230.62
Chemical score	methionine, tyrosine	tyrosine, cystine
Essential amino acid index	58.52	64.63

In the autumn when the feeding season ended, 1136 carp with an average weight of 1250 g were harvested from the control pond and 1200 carp weighing an average of 1275 g were harvested from the experimental pond. Comparison of the number of fish harvested with the number of fish after pond stocking showed that survival rate was 90.88% for the control pond and 96.00% for the experimental pond. This suggests that adding the mixture of oils to the pellets improved fish survival by about 5%, thus increasing fish mass production with comparable feed conversion (kg/kg gain) for growing season, which was 1.54 kg (C) and 1.60 kg (E), respectively.

The samples of meat obtained in spring from two-year-old carp and in autumn from commercial carp were analysed for basic chemical composition, total cholesterol content, gross energy and fatty acid profile, and the results are presented in Table 4. The presence of a 6% mixture of sunflower and rapeseed oils in carp pellets significantly decreased the crude ash content of carp meat. The content of other components was similar regardless of the age in carp and feeds provided. However, there was a significant reduction in total cholesterol content in carp receiving pellets supplemented with oils. The gross energy content in the analysed muscle tissues was similar in all fish (about $5.0 \text{ MJ}\cdot\text{kg}^{-1}$ meat).

Table 4. Content of basic components of carp meat and fatty acid profile

Item	Group ¹			SE ²	Orthogonal contrasts	
	back-ground	control	experimental		background vs. control	control vs. experimental
n	20	30	30			
Basic analysis						
Dry matter (%)	20.91	21.16	21.48	0.09	0.10	0.24
Crude ash (% d.m.)	1.25	1.18	1.16	0.01	0.16	<0.001
Crude protein (% d.m.)	17.65	17.67	17.99	0.06	0.03	0.90
Crude fat (% d.m.)	1.78	1.60	1.56	0.08	0.83	0.35
Cholesterol (mg/100 g)	65.30	72.64	68.56	1.02	0.14	<0.001
Gross energy (MJ · kg ⁻¹)	5.03	4.62	4.64	0.15	0.91	0.15
Fatty acid profile						
C ₁₂	0.11	0.15	0.28	0.01	<0.001	0.33
C ₁₄	1.46	1.99	1.29	0.07	<0.001	0.001
C _{14:1}	0.07	0.09	0.12	0.13	0.003	0.20
C ₁₅	0.44	0.24	0.27	0.01	0.10	<0.001
C ₁₆	20.12	19.22	18.37	0.22	0.09	0.10
C _{16:1}	4.77	4.55	4.17	0.12	0.14	0.45
C ₁₈	7.90	6.07	5.95	0.15	0.61	<0.001
C _{18:1}	39.78	38.84	35.29	0.45	<0.001	0.36
C _{18:2}	10.48	13.88	16.85	0.37	<0.001	<0.001
C _{18:3}	2.06	2.55	2.91	0.09	0.07	0.03
C ₂₀	0.21	0.16	0.17	0.01	0.67	0.003
C _{20:1}	2.01	1.47	1.55	0.05	0.22	<0.001
C _{20:2}	0.34	0.50	0.66	0.02	0.002	<0.001
C _{20:3} ⁿ⁶	0.46	0.22	0.39	0.02	<0.001	<0.001
C _{20:3} ⁿ³	0.09	0.08	0.13	0.01	0.01	0.67
C _{20:4}	2.06	0.98	1.51	0.08	<0.001	<0.001
C _{22:1}	-	0.06	0.07	0.01	0.08	-
C _{20:5}	1.02	1.74	2.22	0.11	0.06	0.004
C _{22:5}	0.45	0.49	0.63	0.02	0.01	0.46
C _{22:6}	1.80	2.06	3.03	0.11	0.70	<0.001
Unidentified acids	4.37	4.66	4.14			
Total saturated acids	30.24	27.83	26.33			
Total monounsaturated acids	46.63	45.01	41.20			
Total polyunsaturated acids	18.76	22.50	26.33			

¹ background – two-year-old carp,
control – commercial carp,
experimental – commercial carp, group receiving dietary fat supplement,

² standard error of the mean.

In the meat intended for consumption, not only fat content but also the content and type of fatty acids is important. Table 4 shows the results of chemical analysis of meat from two-year-old and commercial carp for the profile of fatty acids. This analysis revealed a significant effect of the age of carp on the fatty acid profile of meat fat. As carp grew older, the content of saturated fatty acids in meat decreased and polyunsaturated fatty acids fat increased. It was found that feeding pellets with a mixture of oils for about 6 months had a significant effect on the fatty acid profile of commercial carp. Differences occurred in many acids. It is worth noting the increase in essential unsaturated fatty acids (EUFAs), i.e. $C_{18:2}$, $C_{18:3}$, $C_{20:3}$, $C_{20:5}$ (EPA) and $C_{22:6}$ (DHA) in carp fed pellets containing a mixture of sunflower and rapeseed oils. These differences in fatty acid profile had an influence on the sum of individual acids. The sum of saturated fatty acids in meat fat decreased and that of polyunsaturated fatty acids increased, which is considered very favourable from the perspective of consumers eating this meat. It should also be emphasized that compared to the meat of two-year-old carp, the meat of older carps had less saturated acids and a much greater amount of polyunsaturated acids.

Table 5. Amino acid content of carp meat ($g \cdot kg^{-1}$)

Item	Group ¹			SE ²	Orthogonal contrasts	
	background	control	experimental		back-ground vs. control	control vs. experimental
Asp	17.37	19.66	19.05	0.18	0.09	<0.001
Thr	7.02	8.21	7.68	0.09	0.004	<0.001
Ser	6.21	7.46	6.97	0.11	0.02	<0.001
Glu	24.41	26.35	26.18	0.10	0.70	<0.001
Pro	5.45	5.82	5.47	0.07	0.02	0.03
Gly	8.70	9.15	8.95	0.08	0.30	0.03
Ala	9.24	10.05	9.65	0.09	0.05	<0.001
Val	8.27	8.63	9.05	0.08	0.01	0.05
Ile	7.23	7.67	7.98	0.07	0.04	0.009
Leu	12.74	13.89	13.63	0.11	0.28	<0.001
Try	4.50	5.20	5.14	0.07	0.61	<0.001
Phe	7.00	7.40	7.53	0.06	0.35	0.01
His	5.04	6.29	6.35	0.09	0.66	<0.001
Lys	15.45	16.29	16.07	0.12	0.43	0.007
Arg	10.97	11.18	11.29	0.10	0.63	0.40
Cys	1.34	1.29	1.21	0.01	0.005	0.15
Met	3.38	3.28	3.21	0.03	0.38	0.22
∑ AA	154.32	167.82	165.41			
Chemical score	cystine, methionine	cystine, methionine	cystine, methionine			
Essential amino acid index	66.21	69.39	67.73			

¹ background – two-year-old carp,
control – commercial carp,
experimental – commercial carp, group receiving dietary fat supplement,

² standard error of the mean.

Products intended for consumption should have a high content of nutrients, including protein. However, in terms of nutritional value, not only the protein content but also the composition of its amino acids (especially essential amino acids) is important. The chemical analysis of carp meat protein (Table 5) showed that the amino acids were abundant and much higher in the meat of older fish, which must have had an effect on total amino acids (Σ AA) in the carp meat protein studied. These differences were significant. There were also significant differences between the control and experimental groups in the amino acid content of meat, which indicates that the oil mixture added to the pellets caused differences in the amino acid composition of the carp meat protein.

The calculated chemical score (CS) showed that in the meat of two-year-old and commercial carp the limiting amino acids were cystine and methionine, which are sulphur amino acids. The calculated essential amino acid index (EAAI) showed that carp meat protein had a high biological value, with higher values obtained for meat protein from older (commercial) carp. However, this index was lower for carp from the group receiving the oil diet.

Discussion

Feeds used in carp nutrition provide necessary nutrients and energy components as well as biologically active substances. Feeds can be used in animals, including carp, to influence not only production volume but also the nutrient content of the meat obtained (Bieniarz et al., 2001; Epler et al., 2009 b; Puchała and Pilarczyk, 2007). Some feed ingredients may be directly deposited in tissues, thus improving the nutritive and health-promoting value of the meat, while others are used by the body to synthesize components that are deposited in meat tissues (Hadjinikolova, 2004; Kushik, 1995; Steffens and Wirth, 2005, 2007). These are fatty acids (linoleic and linolenic). They are precursors for the synthesis of EPA and DHA fatty acids, which have very important and multiple functions (Sorgent, 1997). Vegetable oils are a rich source of C₁₈ fatty acids (Borowiec et al., 2001; Epler et al., 2009 b). For this reason, in the present study we used a mixture of sunflower and rapeseed oils fed at 6% of the pelleted mixture. This supplement increased the amount of unsaturated fatty acids (including C₁₈ acids) and the gross energy content. A similar result was obtained by other researchers (Epler et al., 2009 b; Steffens and Wirth, 2005).

The addition of the oil mixture to the pelleted diet increased its amino acid content to a small extent, possibly due to the increased concentration of true protein (including amino acids) in the pellets.

The chemical analysis of the carp meat showed that the mixture of sunflower and rapeseed oils had no effect on the content of basic nutrients between the control and experimental groups. The values obtained were similar between the groups and close to the values reported by other authors (Bieniarz et al., 2001; Puchała and Pilarczyk, 2007).

Because of cardiovascular diseases, which are counted among lifestyle-related diseases, it is important to be aware of the animal products consumed by hu-

mans and the cholesterol content, which is the main factor causing these diseases (Dobryniewski et al., 2007). Studies by some authors (Bieniarz et al., 2000, 2001; Epler et al., 2009 b) and our own study with carp have shown that giving feeds containing unsaturated fatty acids significantly reduced the cholesterol content of fish meat, which is considered highly beneficial because of the health-promoting effects. The lower amount of cholesterol in the carp meat consumed has a direct effect on decreasing the possibility of circulatory disturbances in humans (Sorgent, 1997). Also consumption of products containing n-3 essential unsaturated fatty acids reduces liver triglyceride synthesis in humans. It is believed that n-3 EUFA inhibit the esterification of other fatty acids and reduce the activity of DGAT, an enzyme that takes part in the final stage of triglyceride synthesis (Connor et al., 1992).

The present study demonstrated that the use of a mixture of sunflower and rapeseed oils caused a significant increase in EUFA in carp meat. Likewise, other studies (Steffens et al., 1995; Steffens and Wirth 2005, 2007; Vacha et al., 2007) with carp and other species of freshwater fish showed that EUFA increased after using diets rich in these acids. This suggests the possibility of modifying fish meat by enriching it with EUFA through nutritional factors. Drobna et al. (2006) reported that the amount of oil added to fish diets should be strictly defined to ensure good sensory quality of the meat. For African catfish, this amount is 2.5%. Steffens and Wirth (2007) reported that adding different oil types to carp and trout feeds at 10% had a positive effect on enriching fish fat with EUFA while having no adverse effect on meat quality.

An important component that has a direct effect on the nutritive value of meat is its protein content and composition of amino acids, especially essential amino acids. The amount of protein in the analysed meat of carp was close to the values reported by Epler et al. (2009 b) and Puchała and Pilarczyk (2007). It should be stressed that the protein content of fish meat is not stable but varies according to species, type of food consumed, age and water environment conditions (Puchała and Pilarczyk, 2007; Vacha et al., 2007; Zuraini et al., 2006). Our study failed to show a direct effect ($P > 0.05$) of adding the oil mixture to feed on the crude protein content in carp. However, certain differences were found in the content of some amino acids in the meat of carp according to age and the feed supplement used. Some amino acids were less abundant in meat protein from two-year-old carp ($P < 0.01$) compared to commercial carp. Perhaps the rapid rate of growth in different months of life reduced the number of protein-bound amino acids in older fish. Such a relationship was reported by Shirai et al. (2002) who investigated the content of some free amino acids in the meat of sardines, in which the amount of amino acids decreased in the autumn months compared to the summer months. When analysing the amount of amino acids in commercial carp from groups C and E, it should be pointed out that adding a mixture of sunflower and rapeseed oils reduced the content of some amino acids in carp meat. Although the values were very similar between the groups, the differences turned out to be statistically significant. It is therefore noted that fatty acids, including unsaturated fatty acids, take part in metabolic processes (including protein metabolism) occurring in the body (Shirai et al., 2002; Zuraini et al., 2006).

However, this requires further research with regard to biochemical indicators in a dynamic system.

In conclusion, it can be assumed that the composition of carp meat can be modified in a way that provides health benefits by increasing the content of essential unsaturated fatty acids, thus decreasing the cholesterol content. This can be obtained by supplementing carp diets with a mixture of sunflower and rapeseed oils. However, a slight decrease in some amino acids in carp meat protein must be accounted for when feeding such diets.

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Wpływ dodatku mieszaniny olejów w mieszkach granulowanych dla karpi na profil kwasów tłuszczowych i zawartość aminokwasów w mięsie

STRESZCZENIE

Celem podjętych badań było wykazanie wpływu wzbogacenia mieszanki granulowanej mieszaniną oleju słonecznikowego i rzepakowego, na profil kwasów tłuszczowych oraz zawartość cholesterolu i aminokwasów w mięsie karpi.

Doświadczenie wykonano w warunkach produkcyjnych. Do badań wykorzystano dwa stawy towarowe, każdy o powierzchni 1,2 ha. Po nawodnieniu stawów wiosną obsadzono je krocziem o średniej masie ciała 330 g, w ilości 1250 szt. na staw. Żywnienie ryb prowadzono od maja do końca września mieszanką granulowaną „Aller Clasic” standardową – grupa kontrolna (K) (I staw). Grupa doświadczalna (D) (II staw) otrzymywała również mieszankę granulowaną, do której podczas produkcji zastosowano 6% dodatek mieszaniny oleju słonecznikowego i rzepakowego w stosunku 1:1. Pod koniec września zakończono okres żywienia. Karpie odłowiono, policzono i zważono.

Do analiz chemicznych wybrano losowo 20 sztuk kroczka po zarybianiu stawów oraz po 30 sztuk karpia z grupy K (I staw) i D (II staw) po odłowieniu ryb.

Po uboju ryb wycinano z lewego boku płat mięśniowy, który poddano analizie chemicznej oznaczając w nim zawartość podstawowych składników, profil kwasów tłuszczowych, zawartość cholesterolu i aminokwasów. Mieszanki granulowane również poddano analizie chemicznej na zawartość podstawowych składników pokarmowych, aminokwasów i profil kwasów tłuszczowych.

Uzyskane wyniki wykazały, że dodatek mieszaniny olejów: słonecznikowego i rzepakowego wpłynął na zwiększenie zawartości nienasyconych kwasów tłuszczowych i obniżenie zawartości kwasów nasyconych i cholesterolu oraz niektórych aminokwasów w mięsie karpi.