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Effect of feeding sunflower and linseed oil in pelleted mixtures on chemical composition of carp meat and fatty acid profile

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Abstract. Experiment was conducted during the growing season in two commercial ponds, each measuring 1.2 ha and 1.2 m deep on average. Each pond was stocked on 27 March with 1250 two-year-old carps with an average body weight of about 180 g. Throughout the feeding season (May – September), fish in the control pond received standard Aller Clasic pellets, and fish in the experimental pond were fed the same pellets supplemented with a 6% mixture of sunflower oil and linseed oil (80%:20%). In the feed fatty acid profile, the gross energy and amino acid content were determined. In meat samples basic nutrients, fatty acid profile and total cholesterol content were measured. It has been shown that the level of C_{20} and C_{22} acids in carp meat increased with their age. These acids were also found to increase in the meat of carp as a result of feeding diets enriched with a mixture of sunflower and linseed oil. The results show that the feeding is an important factor, which enable the modification of meet fat making fish meat of higher dietetic and health-promoting value, because of reduced total cholesterol content.
Key Words: sunflower oil, linseed oil, carp meat, fatty acids, cholesterol.

Streszczenie. Doświadczenie przeprowadzono w sezonie wegetacyjnym w dwu stawach towarowych o powierzchni 1.2 ha i średniej głębokości 1.2 m. Każdy ze stawów obsadzono (27 marca) 1250 szt. kroczka karpia o średniej masie ciała 180 g. Podczas całego sezonu odrostowego (maj-wrzesień) ryby w stawie kontrolnym karmione były standardową mieszanką granulowaną Aller Classic, natomiast w stawie doświadczalnym tym samym granulatem z dodatkiem 6% mieszaniny oleju słonecznikowego i lnianego (80%:20%). W paszy określono profil kwasów tłuszczowych, energię całkowitą i zawartość aminokwasów. W próbkach mięsa ryb oznaczono zawartość podstawowych składników, profil kwasów tłuszczowych i zawartość cholesterolu. Wykazano, że poziom kwasów C_{20} i C_{22} w mięsie karpi wzrasta wraz z ich wiekiem oraz jako wynik stosowania diety wzbogaconej mieszanina olejów słoneczcnikowego i lnianego. Wyniki te wskazują, iż żywienie jest ważnym czynnikiem, który pozwala modyfikować zawartość tłuszczów w mięsie oraz zmniejszać w nim zawartość cholesterolu, przez co możliwe jest zwiększanie dietetycznych i prozdrowotnych walorów mięsa karpia.
Słowa kluczowe: olej słonecznikowy, olej lniany, mieso karpia, kwasy tłuszczowe, cholesterol.

Rezumat. Experimentul s-a desfășurat în timpul sezonului de creștere, în două bazine folosite la producerea peștelui în scop comercial, fiecare pond măsurând în medie 1,2 ha și 1,2 m adâncime. Bazinele de pământ au fost populate cu pește pe data de 27 martie, cu 1250 bucăți de puiet în vârstă de doi ani și o greutate corporală medie de 180g. Pe toată durata sezonului de hrănire (lunile mai – septembrie), peștii lotului martor au primit furaj standard Aller Clasic, iar cei din bazinul experimental au fost hrăniți cu același tip de furaj, dar suplimentat cu 6% amestec de ulei de floarea soarelui și in (în raportul de 80%:20%). S-au determinat profilul acizilor grași, energia brută și conținutul în aminoacizi al furajelor utilizate. În ceea ce privește probele de carne, s-au măsurat nutrienții de bază, profilul acizilor grași și colesterolul total. S-a arătat că nivelul de acizi C_{20} și C_{22} din carnea de crap cresc odată cu vârsta. S-a mai constatat faptul că conținutul în acești acizi cresc în carnea de crap ca rezultat al hrănirii cu furaj suplimentat cu uleiuri de floarea soarelui și in. Rezultatele demonstrează că hrănirea este un factor important care permite modificarea grăsimii din carne, ducând la obținerea unei cărni de pește cu o valoare superioară din punct de vedere dietetic și al conținutului total redus de cholesterol, cu importanță asupra sănătății consumatorului.
Cuvinte cheie: ulei de floarea soarelui, ulei de in, carne de crap, acizi grași, colesterol.

Introduction. The quality of food eaten by humans is receiving increasing attention around the globe. The notion of "health foods" and "organic foods", produced without exposure to mineral fertilizers or pesticides, is very popular while being an excellent marketing phrase. It requires no proving that humans cannot do without animal-origin food, including fat, mainly polyunsaturated fatty acids and cholesterol. Numerous data on the role of fatty acids, including fish fatty acids, can be found in papers by Gertig & Przysławski (1994), Kulasek & Bartnikowska (1994), Sargent (1997) and Vesa et al (2009). A review of literature on fatty acids and cholesterol in meat (Bieniarz & Kołdras 2000) points to limited amounts of data on fatty acids and cholesterol in the meat of freshwater fish, especially in those found in Polish waters. The first more extensive report on this subject was not published until 2001 by Bieniarz et al (2001b).

Carp are a dominant species in Polish aquaculture, Poland occupying the first place in terms of the volume of carp production in Europe. A recent downward trend, resulting from unfavorable epizootic situation in carp farms, was reversed in 2008, when the volume of commercial carp production showed a slight increase, with market demand estimated at 18,000-20,000 tons (Lirski & Myszkowski 2009). The demand for carp meat varies. It is highest in December, when the meat of this fish species is prepared in many ways for the Christmas table. Carp meat is nutritious and has considerable dietetic benefits (Bieniarz & Kołdras 2000). In recent years, consumers are looking for meat (including fish meat) that has the highest nutritive value and health-promoting properties, with special emphasis on the high content of essential unsaturated fatty acids (EUFA) and low cholesterol content. These requirements are met by sea fish and, to a smaller degree, by freshwater fish such as carp. However, they contain much lower amounts of EUFA (Kulasek & Bartnikowska 1994; Bieniarz et al 2001b). One of the ways for increasing the dietetic value of carp meat is through nutrition. Feeding diets with appropriate supplements (Bieniarz et al 2001a; Epler et al 2009) may provide carp fat with higher amounts of EUFA, which enhances human health by reducing lifestyle diseases (cerebral strokes, hypertension, heart attacks) (Sargent 1997). To this end, research is performed with many species of farm animals (Micek et al 2004; Pisulewski et al 2001; Sosin et al 2006; Szewczyk et al 2006), including carp, although to a lesser extent. Relevant studies have shown a positive effect of feeding some oils on increasing the EUFA content of carp meat fat and limiting the amount of cholesterol (Epler et al 2009). Therefore, we carried out this study under production conditions to show the possibility of altering the fatty acid and cholesterol profile of carp meat.

Materials and Methods. A feeding trial was conducted during the growing season of 2008 in two commercial ponds, each measuring 1.2 ha and 1.2 m deep on average. The ponds were supplied with water from the Rudawa River through the Młynówka watercourse. After filling with water, the ponds were stocked on 27 March with two-year-old carp. Each pond had the same stocking rate of 1250 two-year-old fish with an average body weight of about 180 g. Throughout the feeding season (May – September), fish in the control pond received standard Aller Clasic pellets, and fish in the second pond were fed the same pellets supplemented with a 6% mixture of sunflower oil and linseed oil (80%:20%). Fish were fed three times a week using feeding tables. During the season, about 1700 kg of the pellet per pond was fed. In October, at the end of the feeding season, fish were harvested, counted and weighed.

Samples of the pellets were taken for chemical analysis to determine the content of basic nutrients, the profile of fatty acids, gross energy and amino acid content. Fish were sampled for analysis three times: 10 carp at the beginning of the season (at stocking), 10 carp from each pond in July, and 30 carp from each pond at harvesting in October.

After killing, a muscle slice was excised from the left side of each fish. The slice was subjected to chemical analysis to determine basic nutrients, the fatty acid profile of meat and total cholesterol content. In the feed and meat samples, basic nutrients were analyzed according to AOAC procedures (1995). The gross energy content of pelleted feeds was determined using a bomb calorimeter, model KL-10. NDF (neutral-detergent fiber), ADF (acid-detergent fiber) and ADL (acid-detergent lignin) content of feed were

determined by the method of Goering & Van Soest (1970) using an Ankom 220 apparatus. Amino acid content was determined by the ninhydrin method after 6n HCl hydrolysis using an AAA-400 amino acid analyser (Ingos).

Samples of feeds and meat were prepared for analysis of fatty acid profile using the method of Folch et al (1975). Fatty acid profile was determined by Varian 3400CX gas chromatograph with an FID detector and CP-WAX column (50m × 0.53mm). Working conditions were the following: carrier gas – argon, injector temperature – 200°C, detector temperature – 240°C (12 min.), column temperature – 220°C (20 min.). Total cholesterol content of meat fat 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 (2002). Prior to analysis of variance, normality of data distribution and equality of variance between the analyzed 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.

Tabular results designated with letters a, b and c differ significantly ($P\leq0.05$) and those designated with letters A, B and C differ highly significantly ($P\leq0.01$).

Results and Discussion. Fish were caught at the end of the feeding season. A total of 790 carp with an average body weight of 1355 g were harvested from the control pond, and 830 carp whose body weight averaged 1338 g were caught from the experimental pond, in which they were fed a pelleted diet containing a 6% mixture of sunflower and linseed oil. At stocking, each pond was filled with 1250 two-year-old carp. For this reason, survival of fish was 63.2% in the control pond and as much as 66.4% in the experimental pond. Increasing the energy value of feed and the amount of unsaturated fatty acids increased the weight of fish produced with comparable consumption of feed per growing season. These production results do not differ from those reported in other studies conducted under production conditions (Bieniarz et al 2001a).

The chemical composition of the pelleted feeds are shown in Table 1. The mixture of oils caused a slight increase in the crude fat content of the experimental feed and in gross energy content. Crude fibre, NDF, ADF and N-free extractives showed a decrease. Other dietary components were at a similar level. These results are comparable to those reported by other authors (Micek et al 2004; Sosin et al 2006; Epler et al 2009) who used fat additives in concentrate mixtures for animals. The addition of fat to the concentrate mixture increased its content of mono- and polyunsaturated fatty acids and decreased the amount of saturated acids (Table 2). Thus, the intended result of increasing the UFA content of the feed for experimental carp was achieved in accordance with the methodology. This is also consistent with the results of other authors (Kulasek & Bartnikowska 1994; Pisulewski et al 2001; Micek et al 2004), who found that vegetable fats added to feeds increase their EUFA content.

The feeds given to carp were analyzed for amino acid content to obtain full information on their nutritive value (Table 3). The oil supplement had no significant effect on the amino acid content of the analyzed pellets. The values obtained fell within the range of such feeds having the components used. These amounts completely met the amino acid requirement of fish (Filipiak 1998).

The chemical composition of meat from carp killed in three periods showed some variation in the content of some components (Table 4). Both the age of fish and the type of feed either increased or decreased the content of the components analyzed. The content of dry matter, crude protein and N-free extractives increased with the age of fish, especially at the end of rearing. Fat content decreased slightly in relation to the initial sample, while the type of feed had a varying but non-significant effect.

When analyzing the total cholesterol content of the meat samples, it was found that it decreased with age and decreased significantly when feeding pellets with a higher UFA content through the addition of oil mixture. This result is of considerable value as it supports the hypothesis that feeding unsaturated fatty acids reduces the total cholesterol content of fish meat, thus improving its nutritive and health value (Kulasek & Bartnikowska 1994; Sargent 1997). This relationship is consistent with the results of

studies involving carp and other species of animals (Micek et al 2004; Szewczyk et al 2006; Epler et al 2009), which reduced the cholesterol content of meat, milk and eggs as a result of using vegetable oils in the diet.

Table 1

Chemical composition of pelleted feed for carp (%)

Ingredients	Pelleted feed	
	Control	Experimental
Dry matter	91.58	89.99
Crude ash	6.89	6.98
Organic matter	84.69	83.01
Crude protein	29.06	29.13
Crude fibre	4.14	3.81
NDF	14.44	13.44
ADF	7.50	6.69
ADL	2.64	2.72
Crude fat	5.27	6.52
N-free extractives	46.22	43.55
Gross energy (MJ)	18.57	19.42

Table 2

Profile of fatty acids (% of total acids) in pelleted feeds

Type of acid	Pelleted feed	
	Control	Experimental
$C_{14:0}$	1.24	0.53
$C_{16:0}$	16.56	9.67
$C_{16:1}$	1.72	0.95
$C_{18:0}$	4.02	3.54
$C_{18:1}$	42.92	50.08
$C_{18:2}$	23.27	26.16
$C_{18:3}$	6.88	6.59
$C_{20:1}$	1.91	1.73
Unidentified acids	1.48	0.75
Total saturated acids	21.82	13.74
Total monounsaturated acids	46.55	52.76
Total polyunsaturated acids	30.15	32.75

Table 3

Amino acid content of pelleted feeds (g/kg)

Type of amino acid	Pelleted feed	
	Control	Experimental
Asp	26.83	27.25
Thr	9.75	9.41
Ser	11.98	11.98
Glu	46.65	46.66
Pro	15.72	15.77
Gly	12.97	12.93
Ala	13.18	13.27
Val	15.31	15.35
Isoleu	10.03	9.73
Leu	21.38	21.43
Tyr	6.98	6.68
Phe	13.94	14.00
His	9.60	10.02
Lys	17.29	16.71
Arg	19.08	18.36
Cys	2.93	2.93
Met	4.89	4.64

The addition of sunflower and linseed oil (80%:20%) to the pelleted mixture had an effect on the fatty acid profile of carp meat. The age of carp also caused differences in the profile of fatty acids in the carp meat studied. $C_{18:0}$ was found to increase in the final period of rearing. This saturated acid is classified as having a desirable hypocholesterolemic effect in humans (Bartnikowska & Kulasek 1994). Large differences were found in the profile of $C_{18:2}$ and $C_{18:3}$ acids, and both the age of fish and the addition of oils had an effect on these changes. There was a marked increase in $C_{18:2}$ (linoleic acid) together with the growth of carp and this acid was found to increase significantly as a result of using the mixture of oils. A similar relationship occurred for the content of $C_{18:3}$ (linolenic acid), but the dietary factor became noticeable at the end of rearing. These changes in the profile of $C_{18:2}$ and $C_{18:3}$ acids are considered beneficial because these acids have a very positive influence on the body of fish in addition to the body of humans eating meat with a higher proportion of these acids (Bartnikowska & Kulasek 1994; Salter et al 2002; Staples et al 2002). $C_{18:2}$ and $C_{18:3}$ are the precursors of the synthesis of C_{20} and C_{22} exogenous acids that serve important functions in the organisms of fish and humans. They serve as building blocks of nerve cells, are the precursors of biologically active substances such as prostaglandins and leukotrienes, and contribute to the vision process (Abayasekara & Wathes 1999). Our findings demonstrated that the level of C_{20} and C_{22} acids in the analyzed samples of carp meat increased with their age. These acids were also found to increase in the meat of carp as a result of feeding diets enriched with a mixture of sunflower an linseed oil. A positive effect of the dietary factor on the EUFA content of carp meat was obtained in line with the methodology. Therefore, the meat of such fish is considered to be of higher dietetic and health-promoting value, because it not only contains more PUFA but also has reduced total cholesterol content.

Table 4

Chemical composition and fatty acid profile of carp meat (% of total acids)

Item	Group[1]					SE[2]	Orthogonal contrasts			
	T	K1	K2	D1	D2		T vs K1 and K2	K1 vs K2	K1 vs D1	K2 vs D2
n	10	10	30	10	30					
Dry matter, %	20.7	22.1	23.2	21.9	23.7	0.19	0.10	**0.01**	0.28	0.06
Crude ash, %	1.24	1.19	1.17	1.20	1.20	0.01	0.69	0.59	**0.02**	**0.03**
Crude protein, %	16.9	17.8	18.3	17.9	18.4	0.07	0.21	**< 0.001**	0.62	**<0.001**
Crude fat, %	2.7	2.0	1.8	1.3	2.0	0.10	**0.05**	0.91	0.56	0.09
Cholesterol, mg/100g	109.6	89.4	95.0	87.3	82.1	1.83	0.79	0.20	**0.001**	**0.004**
C_{12}	0.06	0.06	0.04	0.06	0.04	0.01	0.12	**0.003**	0.66	0.88
C_{14}	0.89	0.91	1.03	0.98	1.15	0.01	0.40	**< 0.001**	**0.003**	0.72
$C_{14:1}$	0.06	0.09	0.11	0.10	0.12	0.01	0.85	**0.002**	0.51	**0.03**
C_{15}	0.13	0.15	0.32	0.17	0.34	0.01	**< 0.001**	**< 0.001**	**0.05**	0.35
C_{16}	19.79	17.32	17.18	17.74	18.04	0.14	0.89	0.09	**0.005**	**< 0.001**
$C_{16:1}$	5.66	5.51	5.14	6.16	5.53	0.09	**0.05**	0.95	0.07	0.70
C_{18}	3.90	4.35	5.16	4.20	5.62	0.10	**0.007**	**< 0.001**	**0.01**	**0.16**
$C_{18:1}$	53.79	42.40	38.38	40.52	33.30	0.69	**0.003**	**< 0.001**	**< 0.001**	**< 0.001**
$C_{18:2}$	10.24	11.71	13.33	14.40	16.21	0.24	0.28	**< 0.001**	**< 0.001**	**< 0.001**
$C_{18:3}$	1.08	3.43	5.42	3.49	6.39	0.20	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
C_{20}	0.16	0.31	0.22	0.26	0.17	0.01	0.58	**< 0.001**	**0.01**	**< 0.001**
$C_{20:1}$	1.67	2.55	2.75	2.41	2.30	0.04	0.92	**0.04**	**< 0.001**	**< 0.001**
$C_{20:2}$	0.66	0.44	0.35	0.43	0.40	0.01	0.91	0.30	**< 0.001**	**< 0.001**
$C_{20:3\ n=6}$	1.25	1.21	0.42	1.23	0.43	0.05	**< 0.001**	**< 0.001**	0.93	0.66
$C_{20:4}$	nd	0.20	1.09	0.21	1.04	0.05	-	**< 0.001**	0.92	0.33
$C_{20:3\ n=3}$	0.26	1.68	0.26	1.82	0.31	0.07	**< 0.001**	**< 0.001**	0.08	**< 0.001**
$C_{20:5}$	0.12	0.21	1.94	0.14	1.72	0.09	**< 0.001**	**< 0.001**	**0.02**	0.57
$C_{22:5}$	0.16	0.33	0.54	0.63	0.48	0.03	**0.007**	0.08	0.29	0.08
$C_{22:6}$	0.31	1.24	1.76	1.59	1.85	0.08	0.84	**0.008**	0.56	**< 0.001**
$C_{22:1}$	nd	0.11	0.30	0.12	0.30	0.01	**-**	**< 0.001**	0.82	**0.81**

[1]T – group slaughtered at the beginning of the trial (March, background); K1 – control group slaughtered in July; K2 – control group slaughtered in October; D1 – group receiving fat supplement and slaughtered in July; D2 – group receiving fat supplement and slaughtered in October; [2]standard error of the mean.

Conclusions

1. Carp feeding is an important factor as it may have favourable effects on meat fat, making it more healthy and increasing its nutritive value.
2. The use of a mixture of sunflower and linseed oil (80%:20%) at 6% of the diet increased EUFA content and decreased total cholesterol content in carp meat.

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