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Meat quality of rabbits fed a diet supplemented with fish oil and antioxidant*

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The aim of the study was to determine the relative percentage of individual fatty acids and cholesterol content in the muscles of the rabbit hind leg as well as to estimate the efficiency of transfer of fatty acids from feed to tissue as affected by supplementation the diet with fish oil and vitamin E.
Three groups of New Zealand White rabbits were used (one control and two experimental) with 40 animals per group. The animals of group I (control) were fed complete standard pelleted diet, of group II – control diet supplemented with 3% fish oil and those of group III – control diet with 3% fish oil and vitamin E. The content of the latter was increased by 100% as related to control (*i.e.* with 50 mg for 100 mg control diet). Fish oil introduced into rabbit diet had a beneficial effect on the composition of lipid fraction of meat. Relative share of n-3 PUFA increased and the decrease in the n-6/n-3 PUFA ratio occurred, both being beneficial from the dietetic point of view. A reduction (P≤0.05) of cholesterol content was shown in meat of animals from groups II and III. Fish oil made the fat of freeze-stored (-8°C for 14 or 90 days) meat slightly more susceptible to oxidation, but the vitamin E supplement prevented from that process. The study confirmed that it is beneficial to add dietary antioxidants to rabbit feed containing fats rich in UFA. The fish oil supplement had a beneficial effect on reducing carcass fatness and improving the juiciness and tenderness of rabbit meat.

KEY WORDS: fatty acids / feeding / fish oil / rabbits / vitamin E

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Modern nutritional methods of altering the functional properties of meat consist mainly of modifying the composition of fatty acids in depot and intramuscular fat aiming at increasing the proportion of mono- and polyunsaturated fatty acids (MUFA and PUFA) while reducing the share of undesirable saturated fatty acids (SFA). When growing animals are given vegetable oils and fish oils rich in MUFA and PUFA, they use them to synthesize their own adipose tissue [Hańczakowski 2003]. Relatively much attention has been given to modifying the composition of porcine fat in line with modern feeding recommendations [Sawosz *et al*. 2000]. Mention should also be made of recent studies that test the functionality of modified meat or meat products in humans [Achremowicz and Szary-Sworst 2005].

Long-chain n-3 PUFA with a beneficial effect on human health include eicosapentaenoic acid – EPA ($C_{20:5\ n-3}$), docosapentaenoic acid – DPA ($C_{22:5\ n-3}$) and docosahexaenoic acid – DHA ($C_{22:6\ n-3}$). In the body, long-chain fatty acids give rise to specific tissue hormones (eicosanoids) such as thromboxanes and prostacyclins, which have antithrombotic and anti-inflammatory properties [Simopoulos 1991, 1999].

Rabbits rank fifth in the world production of meat, but the importance of rabbit meat is increasing because of its high dietetic, taste and pro-health value. Compared to meat of other species it is characterized by low cholesterol level and high level of linolenic acid, and should, therefore, be included in the human diet [Bielański *et al*. 2002].

The aim of the study was to outline the fatty acid profile and determine the cholesterol content of muscle tissue in the rabbit hind leg as well as recognize the efficiency of transfer of acids from feed to tissue as affected by adding 3% fish oil to the diet and increasing the diet's vitamin E content by 100% related to the control.

Material and methods

Three groups of New Zealand White rabbits (40 animals per group) were arranged maintained from weaning on day 35 to day 90 of life. Rabbits were kept in tiered cages (0.77 m long × 0.54 m wide × 0.37 m high) made from spot-welded wire (four females or four males per cage).

Based upon the fact that dietary long-chain fatty acids of fish oil enrich the animal tissues with n-3 PUFA (particularly EPA, DPA and DHA) in the physiologically most efficient way [Świątkiewicz and Koreleski 2003] the feeds were supplemented with fish oil. Vitamin E (DL-α-tocopherol acetate, BASF), which protects PUFA from oxidation by regulating lipoperoxidase production, was used as a natural antioxidant.

Rabbits were fed as follows:

Group I (control) – complete standard pelleted diet;

Group II – complete standard pelleted diet supplemented with 3% fish oil;

Group III – complete standard pelleted diet supplemented with 3% fish oil and vitamin E in proportion by 100% higher than in groups I and II (*i.e*. with 50 mg per 100 mg).

The complete standard pelleted diet offered to the control rabbits contained grass meal (26%), wheat bran (18.6%), ground barley (25%), ground maize (18%), soybean meal (8%), milk powder (2%), phosphate (1%), NaCl (0.4%) and a mineral-vitamin supplement (1%).

The fish oil added to the complete diets of both experimental groups was a by-product obtained during production of fish meal from herrings, sprats, silver carp, mackerels and cods and contained 3.9% of $C_{18:3n-3}$, 8.4% of $C_{20:5n-3}$ (EPA), 13.6% of $C_{22:6n-3}$ (DHA) and 0.9% of $C_{22:5n-3}$ (DPA) and was a product of Agro-Fish Ltd., Kartoszyno, Poland. Oil was added to the standard complete diet during pelleting, accounted for 3% in relation to air-dry diet and reduced the proportion of ground maize from 18% to 16% and of grass meal from 26% to 25%. Rabbits received thier respective diets throughout rearing period, *i.e.*from day 35 to day 90 of life.

The study was carried out on a K-001 rabbit farm belonging to the National Research Institute of Animal Production in Balice, Poland. Feed and meat samples were analysed at the Institute's Main Laboratory.

Individual feed samples were withdrawn from seven different points to make a bulk sample. Bulk sample of feed was made and the representative sample (0.5 kg) was withdrawn after thorough mixing to make the following determinations:

– proximate analysis (dry matter, crude protein, crude fat, crude ash, crude fibre and N-free extractives [AOAC 1990];
– composition of higher fatty acids in the form of methyl esters using a Varian 3400 gas chromatograph on an Rtx 2330 column (105 m × 0.32 mm × 0.2 micron).

At the age of 90 days, six animals were randomly selected from each group. After 24 h fasting they were slaughtered in accordance with the procedure used for rabbits. Data were recorded of empty body weight, weight of edible parts (carcass without head, liver, heart, kidneys and lungs), slaughter waste (fur, blood, legs, digestive tract), weight of head, and slaughter losses. Dressing percentage was calculated as a hot carcass weight with head to body slaughter weight ratio.

Meat was analysed for physico-chemical traits, sensory properties, higher fatty acids composition of meat lipids (hind leg), total cholesterol, vitamin E and the degree of fat oxidation – malonolialdehyde TBA-RS after 14 and 90 days of storage using the reaction of secondary oxidation products with thiobarbituric acid (TBA-RS).

Sensory evaluation of meat was performed on the *longissimus dorsi* muscle. The muscle was matured at 4°C for three days. Samples were heated in 0.6% NaCl solution to mild boiling (one part of muscle to two parts of solution) to an internal temperature of 85°C. Cooking was chosen as the meat preparation method because it is best for extracting flavour from meat while showing whether the fish oil used changes meat flavour, or not. The thermal treatment method is best in evoking the intense and typical aroma of rabbit meat.

The panel of evaluators did not object to the thermal treatment, which was conducted in these conditions on the grounds that the samples were "undercooked".

Thermally treated meat was cooled under cover to room temperature, sliced and evaluated.

Sensory analysis included the evaluation of meat aroma, juiciness, tenderness and palatability on a 5-point scale [Tilgner 1957]. The evaluation was performed by a panel of 5 assessors with previous sensory evaluating experience.

The composition of higher fatty acids in meat was determined with a Varian 3400 gas chromatograph using acids in the form of methyl esters and total cholesterol was determined according to Rhee *et al*. [1982]. Vitamin E was determined with HPLC.

The degree of meat fat oxidation was measured based on secondary oxidation products (thiobarbituric acid-reactive substances – TBA-RS) using frozen meat after two weeks *vs*. three months of storage. Meat samples were thawed in the open air.

The numerical data obtained were analysed statistically using ANOVA in the SAS package. Analysis of variance was performed using the following model:

$$X_{ij} = \mu + a_i + e_{ij,}$$

where:

X_{ij} – observation of a trait;

μ – overall mean;

a_i – effect of i-th group (1,2,3);

e_{ij} – effect of random factors (random error).

Because data in tables refer to six rabbits slaughtered per group, one standard error of mean (SEM) is given.

The experiment was conducted according to the guidelines of the Local Animal Experimentation Ethics Committee.

Results and discussion

Table 1 shows the results of proximate analysis of complete standard pelleted diets for all three groups. As expected, the diets for groups II and III contained twice as much crude fat than the diet for group I, with almost the same level of protein and slightly higher level of dry matter.

Table 1. Results of proximate analysis of complete standard pelleted diets (%)

Group	Dry matter	Crude ash	Crude protein	Crude fat	Crude fibre	N-free extractives
I	87.15	5.44	16.20	2.51	11.30	57.10
II	87.80	5.47	16.31	5.05	12.00	54.44
III	88.70	5.30	16.30	5.04	11.91	55.46

Table 2 presents the profile of higher fatty acids of feeds. The use of fish oil in both experimental diets (groups II and III) caused changes in the content of long-chain PUFA, mainly n-3 acids.

Table 2. Composition of fatty acids of complete standard pelleted diets (g per 100 g of all acids determined)

Fatty acid	Group I	Group II	Group III
$C_{14:0}$	0.175	0.527	0.764
$C_{16:0}$	16.266	11.549	12.193
$C_{16:1}$	0.221	0.757	0.975
$C_{18:0}$	2.132	2.006	1.921
$C_{18:1}$	20.265	30.512	33.789
$C_{18;2n-6}$	52.830	36.513	37.229
$Gamma_{18:3n-6}$	0.000	0.009	0.010
$C_{20:0}$	0.568	0.442	0.577
$C_{18:3n-3}$	6.745	15.142	9.040
CLA $_{c9t11}$	0.064	0.070	0.118
CLA $_{t10c12}$	0.045	0.085	0.098
CLA $_{c9c11}$	0.000	0.000	0.000
CLA $_{t9t11}$	0.088	0.024	0.035
$C_{22:0}$	0.497	0.319	0.432
$C_{20:4n-6}$	0.000	0.000	0.000
$C_{22:1}$	0.103	0.316	0.398
$C_{20:5\ n-3}$ (EPA)	0.000	0.669	0.914
$C_{22:6\ n-3}$ (DHA)	0.000	1.060	1.507
SFA	19.638	14.843	15.887
UFA	80.362	85.157	84.113
MUFA	20.589	31.585	35.162
PUFA	59.773	53.572	48.952
$PUFA_{n-6}$	52.830	36.522	37.239
$PUFA_{n-3}$	6.745	16.871	11.461
UFA/SFA	4.712	6.790	6.160
MUFA/SFA	1.048	2.128	2.213
PUFA/SFA	3.044	3.609	3.081
$PUFA_{n-6/n-3}$	7.832	2.165	3.249
CLA	0.197	0.179	0.251

Table 3 shows the fatty acids profile of the lipids of hind leg muscles in rabbits slaughtered at the age of 90 days. In groups II and III, the level of SFA was found to be highly significantly lower than in control group I.

The fish oil supplement caused a highly significant increase in n-3 PUFA of muscle lipids. Differences also occurred between groups II and III, showing that the antioxidant used was efficient. The increase in the level of DHA and sum of n-3 PUFA observed in group III, was probably due to reduced susceptibility of these acids to the oxidative processes. In groups II and III, the decrease in the n-6/n-3 PUFA ratio ($P \leq 0.01$) was beneficial from the viewpoint of modern human dietetics.

Table 3. Composition of fatty acids of the muscle tissue lipids in the rabbit hind leg (g per 100 g of all acids determined)

Fatty acid	Group I	Group II	Group III	SEM
$C_{14:0}$	3.589^{b}	2.587^{a}	3.289^{b}	0.18
$C_{16:0}$	29.714^{b}	22.874^{a}	19.954^{a}	0.99
$C_{16:1}$	3.497^{a}	3.299^{a}	4.220^{b}	0.54
$C_{18:0}$	5.574^{B}	7.574^{C}	4.481^{A}	0.27
$C_{18:1}$	26.430^{B}	25.527^{B}	17.943^{A}	0.94
$C_{18;2n-6}$	23.629^{A}	22.858^{A}	35.133^{B}	1.04
$Gamma_{18:3n-6}$	0.047^{A}	0.085^{B}	0.145^{C}	0.01
$C_{20:0}$	0.097^{b}	0.065^{a}	0.058^{a}	0.01
$C_{18:3n-3}$	2.817a	3.349a	3.953b	1.74
CLA $_{c9t11}$	0.382^{B}	0.058^{A}	0.057^{A}	0.01
CLA $_{t10c12}$	0.000^{A}	1.407^{C}	0.087^{B}	0.01
CLA $_{c9c11}$	0.049^{B}	0.930^{C}	0.000^{A}	0.01
CLA $_{t9t11}$	0.019^{A}	0.012^{A}	1.323^{B}	0.01
$C_{22:0}$	0.588^{C}	0.264^{B}	0.002^{A}	0.01
$C_{20:4n-6}$	2.192^{B}	2.575^{B}	0.881^{A}	0.08
$C_{22:1}$	0.145	0.104	0.060	0.08
$C_{20:5\ n-3}$ (EPA)	0.052^{A}	1.162^{B}	1.706^{C}	0.02
$C_{22:6\ n-3}$ (DHA)	0.064^{A}	4.482^{B}	6.389^{C}	0.04
SFA	40.705^{C}	33.662^{B}	28.097^{A}	0.84
UFA	59.294^{A}	66.337^{B}	71.902^{C}	0.84
MUFA	30.073^{B}	28.930^{B}	22.224^{A}	1.40
PUFA	29.221^{A}	36.987^{B}	49.677^{C}	2.16
$PUFA_{n-6}$	25.868^{A}	25.518^{A}	36.160^{B}	1.08
$PUFA_{n-3}$	2.930^{A}	9.000^{B}	12.050^{C}	1.81
UFA/SFA	1.459^{A}	1.976^{A}	2.577^{B}	0.16
MUFA/SFA	0.739	0.861	0.794	0.03
PUFA/SFA	0.720^{A}	1.102^{B}	1.783^{C}	0.18
$PUFA_{n-6/n-3}$	8.849^{B}	2.835^{A}	3.008^{A}	2.16
CLA	0.419^{A}	2.408^{C}	1.467^{B}	0.03

SEM – standard error of mean.
[aA...]Within rows means bearing different superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

The transformation of dietary fatty acids into tissue fatty acids may be affected by the content of fatty acids, especially those which compete with one another (PUFA and MUFA, n-3 PUFA and n-6 PUFA, arachidonic acid and eicosapentaenoic acid) in dietary fat [Christensen and Hoy 1997].

The efficient transformation of fish oil FAs into muscle tissue FAs is evident from the fact that long-chain fatty acids from fish oil enrich the tissue with n-3 PUFA in a physiologically efficient manner. Sawosz *et al*. [2000] and Swiatkiewicz and Koreleski [2003] have shown that despite a high content of α-linolenic acid, linseed oil (which is the richest source of n-3 acids) causes these acids to be deposited in muscle tissue in a manner comparable to fish oil.

Table 4. Total cholesterol (mg/100g), vitamin E (mcg/g) and TBA-RS (mg/kg) content of meat

Item	Group I	Group II	Group III	SEM
Total cholesterol	66.26^{b}	59.99^{a}	62.72^{a}	3.41
Vitamin E	1.81^{A}	2.19^{A}	3.76^{B}	0.42
TBA-RS_{14}*	0.501^{a}	0.540^{b}	0.480^{a}	0.21
TBA-RS_{90}**	0.702^{a}	0.882^{b}	0.662^{a}	0.20

SEM – standard error of mean.
*After 14 days of storage.
**After 90 days of storage.
$^{aA...}$Within rows means bearing different superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

Table 4 shows the levels of meat total cholesterol, vitamin E and TBA-RS, the latter after two periods of freeze storage. Mean cholesterol content of feeze-stored meat occurred lower (P≤0.05) in group II and III than in group I.

Studies on the effect of increased proportion of fat in balanced diets for rabbits [Xiccato and Trocino 2003] showed that the use of essential unsaturated fatty acids with appropriate relations between individual types of acids may reduce the level of total cholesterol in muscles and depot fat. This effect takes place through stimulation or inhibition of the hepatic activity of HMG CoA reductase, an enzyme that regulates cholesterol synthesis.

After increasing the level of dietary vitamin E, the deposition of the vitamin in meat increased markedly. This is important to consumers because for the proper assimilation of PUFA, the acids should be consumed together with vitamin E as a natural antioxidant. The recommended dose is 0.4 mg of α-tocopherol per g of PUFA in the diet (1 mg of α-tocopherol = 1 IU vitamin E) – Simopoulos *et al*. [1999], Gibney *et al*. [2002].

Analysis of the susceptibility of rabbit meat to oxidation showed that FBA-RS after 14 or 90 days of storage tended to increase when the addition of fish oil was used. The rancidity process of fat in meat, which takes places during storage and processing, is detrimental to the meat quality by negatively affecting stability, aroma, flavour and nutritive value, by reducing the PUFA content and by increasing the amount of fat and cholesterol-oxidation products. The vitamin E supplemented to the diet of group III at 100 mg/kg had a beneficial effect as it protected lipids from oxidation.

Table 5 presents the results of slaughter analysis and the muscling and fatness traits of rabbit carcasses. Feed conversion (kg/kg gain) was the same for all the groups (3.7 kg, figures not shown in Tables). Supplementing of feeds with oil was found to have a significant effect on carcass fatness. Fat deposition in the body is affected by the degree of saturation of dietary fatty acids [Hanczakowski 2003]. Low saturated fats can have an effect on lower fatness. Low fatness may also result from the stimulating effect of polyunsaturated acids on enzymes that degrade fatty acids (β-oxidation). The addition of fish oil had no significant effect on the muscling of animals.

Table 5. Slaughter value indicators of rabbit carcasses

Indicator	Group I	Group II	Group III	SEM
Body weight of rabbit (g)	2473.0	2568.0	2356.0	85.2
Hot carcass weight (kg)	1188.0	1247.5	1188.5	66.9
Total edible parts (g)	1311.0	1374.0	1291.0	75.8
Total slaughter waste (g)	1001.0	1015.0	910.0	15.3
Dressing percentage	54.54	55.47	57.02	4.8
Cooled carcass weight (g)	1153.5	1227.0	1155.5	39.4
Weight of carcass muscles (g)	904.0	982.5	930.5	30.3
Weight of carcass bones (g)	202.0	220.0	204.5	13.9
Weight of carcass fat (g)	47.5^{B}	25.0^{A}	20.5^{A}	5.8

SEM – standard error of mean.
^{AB}Within rows means bearing different superscripts differ significantly at P≤0.01.

Table 6. Sensory traits of rabbit meat (pts.)

Trait	Group I	Group II	Group III	SEM
Aroma intensity	4.6^{B}	4.3^{A}	4.2^{A}	0.07
Aroma quality	4.6^{B}	4.1^{A}	4.1^{A}	0.09
Tenderness	4.2^{A}	4.8^{B}	4.9^{B}	0.05
Juiciness	4.1^{A}	4.6^{B}	4.6^{B}	0.05
Taste intensity	4.3	4.2	4.2	0.08
Taste quality	4.7^{a}	4.5^{b}	4.4^{b}	0.06
Total sensory quality	4.41	4.42	4.40	0.07

SEM – standard error of mean.
$^{aA...}$Within rows means bearing different superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

Table 6 presents the results of sensory evaluation of meat, with tenderness as an important criterion. The main factors that affect tenderness assessed by sensory analysis or mechanically (e.g. using shear and penetration force), are the properties of muscle fibres and the quantity and quality of the connective tissue. When describing the role of connective tissue in shaping meat tenderness, the greatest importance is attributed to collagen [Purslow 2005].

In the present study, highly significant differences in meat tenderness and juiciness were found between the control (I) and groups II and III. The results obtained were validated by additional analysis of tenderness by means of a shear force test (TA.XT Plus texture analyser) using a Warner-Bratzler cutting blade. Shear force expressed in kg was 4.11 in group I and from 3.21 to 3.24 (group II and III, respectively). Figures are not shown in Tables.

It is concluded that the fish oil added to rabbit diets had a beneficial effect on the composition of the meat lipid fraction. Relative deposition of n-3 PUFA increased and

the decrease in the n-6/n-3 PUFA ratio was beneficial from the standpoint of modern human dietetics. A significant ($P \leq 0.05$) reduction occurred in meat cholesterol in animals from both experimental groups, *i.e*. those receiving fish oil.

Fish oil made the fat of stored meat slightly more susceptible to oxidation, but the dietary vitamin E supplement protected lipids from oxidation. The study confirmed that it is purposeful to add dietary antioxidants in rabbit feeds containing fats rich in unsaturated fatty acids. The fish oil supplement had a beneficial effect on reducing carcass fatness and improving the juiciness and tenderness of rabbit meat.

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Jakość mięsa królików żywionych mieszanką z dodatkiem oleju rybiego i przeciwutleniacza

S t r e s z c z e n i e

Celem badań było określenie zawartości kwasów tłuszczowych i cholesterolu w tkance mięśnia tylnej nogi króliczej oraz efektywności transformacji kwasów tłuszczowych z paszy do mięsa w wyniku wzbogacenia paszy 3% dodatkiem oleju rybiego oraz zwiększonym o 100% dodatkiem witaminy E (z 50 mg do 100 mg).

Doświadczenie przeprowadzono na 120 królikach rasy białej nowozelandzkiej, żywionych granulowanymi mieszankami pełnoporcjowymi, wzbogacając mieszankę dla grupy II 3% dodatkiem oleju rybiego, a dla grupy III 3% dodatkiem oleju rybiego i zwiększonym o 100% udziałem witaminy E.

Stosowany dodatek oleju rybiego do paszy korzystnie wpływał na udział kwasów tłuszczowych we frakcji lipidowej mięsa. Stwierdzono zwiększenie względnego odłożenia kwasów wielonienasyconych n-3 oraz zawężenie proporcji n-6/n-3 tych kwasów, w kierunku korzystnym z punktu widzenia współczesnej dietetyki. Uzyskano istotne ($P \leq 0,05$) zmniejszenie średniej zawartości cholesterolu w mięsie grupy II i III. Tłuszcz rybi dodawany do paszy nieznacznie zwiększał podatność tłuszczu mrożonego mięsa (przechowywanego w -8°C przez 90 dni) na utlenianie, ale procesom oksydacji lipidów zapobiegał dodatek do paszy witaminy E. Badania potwierdziły celowość stosowania przeciwutleniaczy paszowych w żywieniu królików paszą z udziałem tłuszczów o wysokim poziomie kwasów nienasyconych. Dodatek oleju rybiego do paszy korzystnie wpływał na zmniejszenie otłuszczenia tuszek króliczych oraz poprawiał soczystość i kruchość mięsa.