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# Effect of adding rapeseed and fish oils to the diet of rabbits on the fatty acid composition of saddle fat and the degree of carcass fatness

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**The aim of the study was to modify the fatty acid composition of rabbit fat using complete pelleted diets with 2% rapeseed or fish oil. Sixty male New Zealand White rabbits were assigned to three groups receiving complete diets: a standard diet or a diet supplemented with rapeseed oil or fish oil. The use of rapeseed and fish oils in the diet had a favourable effect on the final body weight of the animals (90 days of age). The carcasses of rabbits fed the diet supplemented with rapeseed oil had the lowest proportions of shoulder fat, kidney fat and abdominal fat, with significant (P≤0.01) differences with respect to the other groups. Targeted supplementation of the diet with rapeseed and fish oils had no effect on the level of protein and intramuscular fat in the *longissimus dorsi* muscle. As a result of supplementation with fish oil, the *n-6*/*n-3* PUFA ratio in the adipose tissue of the rabbits decreased significantly (P≤0.01), which is beneficial for the consumers of this meat. The use of rapeseed oil in the diet reduced the content of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids in the adipose tissue and improved the atherogenic index and saturation. There was a considerable improvement in the thrombogenic index after adding fish oil to the rabbit diets.**

**KEY WORDS: rabbit fat / rapeseed oil / fish oil / fatty acids**

Restricting consumption of animal fats is important because they are considered one of the main factors contributing to the occurrence and development of numerous diseases, including atherosclerosis, cardiovascular disease, cancer, and obesity. Most of these fats have an unfavourable fatty acid profile (dominance of saturated fats) in terms of health promotion. This problem also pertains to rabbit fat, which has a relatively high proportion of palmitic acid (C16:0). Although rabbit fat, like the fats of ruminants, contains conjugated linoleic acid, its level is too low to exhibit biologically beneficial properties [11].

One way to improve unfavourable proportions of fatty acids of varying degrees of saturation in meat is to feed animals vegetable and animal fats rich in unsaturated fatty acids. In the case of ruminants, due to the specific structure of their gastrointestinal tract and the microflora of the rumen, most unsaturated fatty acids supplied by this route undergo biohydrogenation to saturated fats, and in this form are deposited in fat tissues. Rabbits are monogastric animals, so the fatty acid profile in their tissues can be modified via their diet to a greater degree than in ruminants.

Vegetable oils used to supplement animal feeds differ in their content of metabolic energy, fatty acid profile, and digestibility. Soybean oil has the highest energy value (37 MJ ME/kg), closely followed by rapeseed oil (36.5 MJ ME/kg). Rapeseed oil is an excellent source of unsaturated fatty acids, including oleic acid (C18:1), which lowers LDL (low-density lipoprotein) in the blood. As unsaturated acids are more easily digested than saturated acids, rapeseed oil is also the most easily assimilated oil. Its digestibility is about 96%. It also has a high content of tocopherols, antioxidants essential to maintaining proper antioxidant status [15].

Animal products with high content of eicosapentaenoic acid (EPA, C20:5), docosapentaenoic acid (DPA, C22:5), and especially docosahexaenoic acid (DHA, C22:6), supplying substances performing health-promoting functions in the human body, have been recognized as functional. Natural synthesis of these unsaturated fatty acids in the body is a slow process and its scope is limited.

In contrast with vegetable oils, fish fats contain considerable quantities of EPA, DPA and DHA in a form that requires no conversion. Therefore they are a useful tool in production of meat enriched with *n-3* acids. Although in comparison with rapeseed oil fish fat has a higher proportion of total saturated fatty acids, comparable content of polyunsaturated fatty acids and lower content of unsaturated fatty acids, it contains a considerable quantity of the long-chain unsaturated *n-3* acids EPA and DHA. The *n-6* to *n-3* ratio is also smaller, and thus far more beneficial than in vegetable oils [12].

The aim of the study was to evaluate the results obtained following modification of the fatty acid composition of rabbit fat by means of a diet of pelleted complete mixed rations containing 2% rapeseed oil or fish oil.

## Material and methods

The study on animals and the tissues obtained from them was carried out in the spring of 2014 on a rabbit farm belonging to the National Research Institute of Animal Production in Aleksandrowice. All analyses were performed at the Central Laboratory of the National Research Institute of Animal Production (statutory activity).

The experimental material consisted of 60 male New Zealand White rabbits, which after being weaned from their mothers (35th day), weighed, and individually marked

(tattooing), were kept in metal wire cages, four animals per cage, in a closed, heated room. Zootechnical and technological conditions were in compliance with general principles for this type of production.

During the period from 35 to 90 days of life the rabbits (20 individuals per group) were fed *ad libitum* pelleted complete mixed rations:

- group I – standard feed mixture used on the farm
- group II – feed mixture supplemented with rapeseed oil (2%)
- group III – feed mixture supplemented with fish oil (2%)

The standard feed mixture contained dried alfalfa (25%), wheat bran (18.6%), barley meal (24%), maize meal (14%), soybean extraction meal (14%), Pollac milk substitute (2%), feed phosphate (1%), NaCl (0.4%), and a vitamin and mineral supplement (1%), i.e. premix for rabbits with a coccidiostat (robenidine).

The mixture for group II was supplemented with rapeseed oil from Zakłady Tłuszczowe 'Kruszwica' S.A. in Kruszwica, with 27.6% linoleic acid (C18:2 *n-3*) and 10.2% linolenic acid (C18:3 *n-3*) guaranteed by the producer. The fish oil used to supplement the feed mixture for group III was from Agro-fish Sp. z o.o. in Gniewino and contained the following fatty acids: linolenic (C18:3 *n-3*) – 3.9%, EPA (C20:5 *n-3*) – 8.4%, DHA (C22:6 *n-3*) – 13.6%, and DPA (C22:5 *n-3*) – 0.9%.

The feed mixtures were balanced according to experimental procedures and the content of feed components was calculated on the basis of 'Dietary recommendations and nutritional value of animal feeds' [22]. The amount of protein and fibre was kept at a constant level in all groups, while the level of fat was left without interference. From each batch of feed subsamples were taken from 7 places to determine the content of dry matter (SOP M.O11 – gravimetric method), crude protein (SOP M.007a – Kjeldahl method), crude fat (SOP M.013), crude ash (SOP M.014 – gravimetric method), and crude fibre (SOP M.012) – Table 1.

**Table 1**
Results of basic analysis of balanced, complete, pelleted feed mixtures (%)

| Group | Dry matter | Crude ash | Total protein | Crude fat | Crude fibre | N-free extractives |
|---|---|---|---|---|---|---|
| I | 88.62 | 6.17 | 14.82 | 3.04 | 12.53 | 52.06 |
| II | 88.21 | 6.22 | 14.68 | 4.01 | 12.81 | 50.49 |
| III | 89.12 | 6.42 | 14.45 | 4.21 | 11.94 | 52.10 |

After the rearing period (90th day of life), 10 rabbits were randomly selected from each group and slaughtered after 24-hour fasting. Slaughter was carried out according to currently binding methodology (Council Regulation (EC) No. 1099/2009), in identical technological conditions for all groups.

After slaughter carcass analysis was carried out according to the method described by Bieniek [1]. Dressing percentage was calculated as the ratio of the hot carcass weight including the head to the weight of the animal before slaughter, according to the following equation:

$$\text{WR (\%)} = \frac{\text{MT x 100}}{\text{MC}}$$

where:

WR – dressing percentage (%)

MT – carcass weight (g) with head, excluding edible internal organs (liver, kidneys, lungs and heart)

MC – body weight before slaughter (g)

The carcasses were divided into three parts: the anterior part, with the cut made at the height of the last rib; the saddle, cut at the height of the last lumbar vertebra; and the posterior part, including the hind legs. A scalpel was used to separate the subcutaneous fat from the shoulders in the anterior part, organ fat from around the kidneys and stomach in the middle part (saddle), and subcutaneous inguinal fat in the posterior part.

Both *longissimus dorsi* muscles were removed. Intramuscular fat content was determined by the Soxhlet method according to PN-ISO 1444:2000 [18] and protein by the Kjeldahl method according to PN-75/A-04018 [17].

Content of fatty acids was determined in the freeze-dried samples of the *longissimus dorsi* muscle. They were extracted with a solution of chloroform and methanol according to Folch et al. [7]. Methyl esters of fatty acids were prepared according to ISO 12966-2:2011 [10]. The fatty acid profile of the methyl esters was determined by gas chromatography using a VARIAN 3400 gas chromatograph with a flame ionization detector (FID), at an injector temperature of 250ºC, using an Rtx 2330 column (105 m × 0.32 mm ×0.2 µ). The carrier gas was helium with a 3 ml/min flow rate and the size of the injected samples was 0.7 µl. Acid standards from Larodan Fine Chemicals AB were used to determine CLA, and standards from Sigma-Aldrich for the remaining acids.

The atherogenic index (AI), thrombogenic index (TI) and saturation were calculated using formulas given by Ulbricht and Southgate [21]:

$$\text{AI} = \frac{\text{C12:0} + 4\text{C14:0} + \text{C16:0}}{\text{PUFA}_{n\text{-}3} + \text{PUFA}_{n\text{-}6} + \text{MUFA}}$$

$$\text{IT} = \frac{\text{C14:0} + \text{C16:0} + \text{C18:0}}{0{,}5\text{MUFA} + 05\text{PUFA}_{n\text{-}6} + 3\text{PUFA}_{n\text{-}3} + \frac{\text{PUFA}_{n\text{-}3}}{\text{PUFA}_{n\text{-}6}}}$$

$$\text{S:P} = \frac{\text{C14} + \text{C16:0} + \text{C18:0}}{\text{MUFA} + \text{PUFA}}$$

The results obtained were analysed statistically by one-way analysis of variance (ANOVA). Significance of differences between means in groups was estimated by Duncan's multiple range test using Statistica 8 software (StatSoft, USA, 2008).

## Results and discussion

Carcass analysis (Tab. 2) revealed that both the rapeseed oil and fish oil introduced to the feed mixtures had a significant effect ($p \leq 0.01$) on the final body weight, lean meat content, and dressing percentage of the rabbits. The lowest proportion of shoulder, kidney and stomach fat was observed in the rabbits whose feed was supplemented with rapeseed oil (9.22% and 22.0%); these differences proved significant ($p \leq 0.01$) with respect to the values noted for groups I (14.1% and 38.2%) and III (12.4% and 32.6%).

Fat deposition in the body is influenced by the degree of saturation of fatty acids contained in food. Fats with a low level of saturation may result in lower fat cover. Another cause of low fat cover may be the stimulatory effect of polyunsaturated fatty acids on enzymes causing breakdown of fatty acids—β-oxidation [2, 9].

Protein content in the *longissimus dorsi* muscle was similar in all groups, at 21.8-22.2% (Tab. 3). The results obtained are lower than the values reported for the same muscle by Szkucik and Libelt [19] – 23.91%, Szkucik and Pyz-Łukasik [20] – 23.9%,

**Table 2**
Results of rabbit carcass analysis

| Item | Group | | |
|---|---|---|---|
| | I | II | III |
| Body weight (g) | $2420.1^{A} \pm 25.3$ | $2590.8^{B} \pm 28.2$ | $2610.1^{B} \pm 19.8$ |
| Hot carcass weight (g) | $1220.0^{A} \pm 18.4$ | $1358.2^{B} \pm 16.8$ | $1390.2^{B} \pm 16.5$ |
| Giblets weight (g) | 117.3 ±2.81 | 111.0 ±3.62 | 107.6 ±4.11 |
| Inedible parts weight (g) | 1024.6 ±22.4 | 1084.2 ±18.6 | 1061.3 ±16.2 |
| Inguinal fat weight (g) | 6.21 ±0.99 | 6.14 ±0.85 | 5.98 ±0.91 |
| Shoulder fat weight (g) | $14.1^{A} \pm 1.23$ | $9.22^{B} \pm 0.85$ | $12.4^{A} \pm 1.56$ |
| Kidney and abdominal fat weight (g) | $38.2^{A} \pm 2.22$ | $22.0^{B} \pm 1.85$ | $32.6^{A} \pm 2.11$ |
| Carcass muscle weight (g) | $945.2^{A} \pm 18.4$ | $1086.2^{B} \pm 21.2$ | $1120.2^{B} \pm 22.3$ |
| Dressing percentage (%) | $50.4^{A} \pm 1.05$ | $52.4^{B} \pm 1.08$ | $53.3^{B} \pm 2.01$ |

Values denoted by different letters in rows differ statistically significantly: A, B – $P \leq 0.01$, a, b – $P \leq 0.05$

and Cygan-Szczegielniak et al. [3] – 23.6%, but similar to values given by Pla et al. [16] – 22.1%. Differences in protein content in rabbit meat depend on the breed and age of the animals, the composition of their feed, the anatomical part of the carcass, and preparation for slaughter.

Similarly, no significant differences were found in the content of intramuscular fat, which ranged from 1.87% to 1.98%, with a downward tendency in the group receiving the feed with rapeseed oil. Growth and fattening of meat animals is linked to the order of the increase in fat deposition, i.e. first subcutaneous and organ fat, and then intramuscular fat [4]. Different rabbit breeds having similar content of subcutaneous and organ fat may differ substantially in the level of intramuscular fat. This suggests that the site of fat deposition in the carcass is determined by genetic factors [5]. A lower content of intramuscular fat in the *longissimus dorsi* muscle than in the present study was reported by Pla et al. [15] – 1.20%, Łapa [13] – 1.71%, Maj et al. [14] – 1.60% and Szkucik and Libelt [19] – 1.12%. Similar values were obtained by Kowalska et al. [12]. An optimal content of intramuscular fat gives meat a suitable flavour, juiciness and tenderness. A level below 1% is considered unacceptable, as it is likely to reduce the flavour qualities of the meat, which becomes dry and tough when cooked.

**Table 3**
Content of protein (%) and intramuscular fat (%) in rabbit meat

| Specification | Group | | |
|---|---|---|---|
| | I | II | III |
| Protein | 21.8 ±1.10 | 22.0 ±1.31 | 22.2 ±1.21 |
| Fat | 1.8 ±0.11 | 1.87 ±0.13 | 1.96 ±0.11 |

No statistically significant differences

The present study, in which compound feed for rabbits was supplemented with rapeseed oil (group II) or fish oil (group III), found a significant decrease ($P \leq 0.01$) in lauric acid (C12:0) with respect to group I. The lowest percentage of palmitic acid (C16:0) was noted in the group receiving the feed supplemented with rapeseed oil. The addition of rapeseed oil significantly increased the amount of linoleic (C18:2) and erucic (C22:1) acids with respect to the other groups, while fish oil increased the content of linolenic acid (C18:3), EPA (C20:5) and DHA (C22:6). The highest level of polyunsaturated fatty acids of the *n-3* family in the fat of the meat and the lowest ratio of *n-6/n-3* PUFA was noted in the case of supplementation of the rabbit feed with fish oil (Tab. 4)

There have been many studies dealing with the choice of a suitable addition of plant and animal oils to rabbit feeds such that the dietary modification would have no detrimental effect on the welfare of the animals or on the sensory characteristics of the meat and its

**Table 4**
Content of selected fatty acids (%) in rabbit meat fats

| Fatty acids | Group | | |
|---|---|---|---|
| | I | II | III |
| C12:0 | $0.38^{A} \pm 0.02$ | $0.19^{B} \pm 0.01$ | $0.17^{B} \pm 0.03$ |
| C14:0 | $3.27^{a} \pm 0.52$ | $3.07^{ab} \pm 0.44$ | $2.96^{b} \pm 0.52$ |
| C16:0 | $32.78^{A} \pm 2.55$ | $28.79^{B} \pm 2.48$ | $31.58^{AB} \pm 4.21$ |
| C16:1 | $3.29^{a} \pm 0.13$ | $3.45^{a} \pm 0.14$ | $2.24^{b} \pm 0.11$ |
| C18:0 | $6.54^{a} \pm 1.21$ | $6.00^{b} \pm 1.11$ | $5.99^{b} \pm 0.96$ |
| C18:1 | $25.99 \pm 2.56$ | $25.74 \pm 2.11$ | $25.79 \pm 2.09$ |
| C18:2 *n-6* | $25.19^{A} \pm 1.96$ | $27.88^{B} \pm 2.04$ | $25.06^{A} \pm 2.32$ |
| C20:0 | $0.11^{a} \pm 0.01$ | $0.07^{b} \pm 0.01$ | $0.15^{a} \pm 0.02$ |
| C18:3 *n-3* | $4.05^{A} \pm 0.42$ | $4.32^{A} \pm 0.22$ | $6.03^{B} \pm 0.28$ |
| C20:4 *n-6* | $2.12 \pm 0.22$ | $2.98 \pm 0.31$ | $2.10 \pm 0.11$ |
| C22:1 | $0.02^{A} \pm 0.00$ | $0.07^{B} \pm 0.00$ | $0.02^{A} \pm 0.01$ |
| C20:5 *n-3* (EPA) | $0.12^{A} \pm 0.01$ | $0.13^{A} \pm 0.01$ | $0.41^{B} \pm 0.02$ |
| C22:6 *n-3* (DHA) | $0.03^{A} \pm 0.00$ | $0.07^{A} \pm 0.01$ | $2.01^{B} \pm 0.03$ |
| SFA | $43.21^{Aa} \pm 2.33$ | $39.18^{Bab} \pm 2.45$ | $40.85^{ABb} \pm 3.01$ |
| UFA | $61.37^{A} \pm 4.45$ | $64.75^{B} \pm 4.11$ | $63.80^{B} \pm 5.03$ |
| MUFA | $29.31 \pm 1.98$ | $29.28 \pm 2.05$ | $28.10 \pm 2.11$ |
| PUFA | $32.06^{A} \pm 2.14$ | $35.54^{B} \pm 2.45$ | $35.69^{B} \pm 3.04$ |
| PUFA *n-6* | $27.46^{A} \pm 2.11$ | $30.91^{B} \pm 3.32$ | $27.20^{A} \pm 2.98$ |
| PUFA *n-3* | $4.21^{A} \pm 0.56$ | $4.53^{A} \pm 0.48$ | $8.47^{B} \pm 1.01$ |
| PUFA *n-6/n-3* | $6.52^{A} \pm 1.03$ | $6.82^{A} \pm 0.99$ | $3.22^{B} \pm 0.85$ |

Values denoted by different letters in rows differ statistically significantly: A, B – $P \leq 0.01$, a, b – $P \leq 0.05$

suitability for processing. An excessively high level of the supplement may lead to unfavourable changes in the flavour and aroma of the meat and the consistency of the fat, in addition to negatively influencing its shelf-life.

Kowalska [11] found that even 1% fish oil introduced to a compound feed can significantly alter the fatty acid content of rabbit meat lipids. Among *n-3* acids the author observed a significant increase in linolenic acid (C18:3), EPA (C20:5) and DHA (C22:6). El-Moghazy et al. [6] showed a positive effect of supplementation of rabbit diets with fish oil in the amount of 0.5, 1.0 and 1.5 ml per day/kg body weight. A substantial decrease in the amount of cholesterol and triglycerides was observed in the blood of all groups, as well as beneficial changes in the fatty acid profile. At the same time, no detrimental effect was observed on the histological structure of the liver and kidneys of the rabbits.

Gigaud and Combes [8] studied the effect of adding palm oil and rapeseed oil to reduce the ratio of *n-6* to *n-3* PUFA. Rabbit meat was obtained with the optimum *n-6* to *n-3* ratio for the consumer, i.e. 4.8.

By lowering the proportion of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids in the fat tissue of the rabbits receiving feed supplemented with rapeseed oil, significantly lower values for the atherogenic index were obtained as compared to the control ($P \leq 0.01$) and the group receiving feed supplemented with fish oil ($P \leq 0.05$). The differen-

ce between groups I and III was also significant (P≤0.05). The thrombogenic index was lowest in the fat of the rabbits receiving feed supplemented with fish oil, and differed significantly from the control (P≤0.01) and the group receiving feed supplemented with rapeseed oil (P≤0.05) – Table 5. The ratio of polyunsaturated to saturated fatty acids (P:S) was lowest in the meat of the rabbits receiving feed supplemented with rapeseed oil.

**Table 5**
Value of atherogenic (AI) and thrombogenic (TI) indices and saturation of rabbit meat fat

| Specification | Group | | |
|---|---|---|---|
| | I | II | III |
| IA | $0.59^{Aa} \pm 0.03$ | $0.49^{Bb} \pm 0.02$ | $0.54^{b} \pm 0.03$ |
| IT | $1.03^{A} \pm 0.04$ | $0.86^{Ba} \pm 0.04$ | $0.75^{Bb} \pm 0.02$ |
| S:P | $0.69^{a} \pm 0.02$ | $0.58^{b} \pm 0.03$ | $0.63^{b} \pm 0.03$ |

Values denoted by different letters in rows differ statistically significantly: A, B – P≤0.01, a, b – P≤0.05

The significant decrease in the atherogenic and thrombogenic indices and in saturation in the rabbits whose feed was supplemented with rapeseed or fish oil substantially improved the dietary quality of the rabbit meat.

It can be concluded from the study that the use of rapeseed and fish oil in the rabbits' diet had a beneficial effect on their final body weight. The carcasses of the rabbits whose feed was supplemented with rapeseed oil had the lowest proportion of shoulder, kidney and abdominal fat, and the differences were significant (P≤0.01) with respect to the other groups. Supplementation of the feed ration with rapeseed or fish oil did not affect the level of protein or intramuscular fat in the *longissimus dorsi* muscle. Supplementation with fish oil resulted in a significant (P≤0.01) reduction in the *n-6/n-3* PUFA ratio in the adipose tissues of the rabbits, which is beneficial to consumers of the meat. The use of rapeseed oil in the rabbits' diet reduced the proportions of lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids in their adipose tissues and improved the atherogenic index and fat saturation. A significant improvement in the thrombogenic index was obtained by adding fish oil to the feed mixture.

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