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Research Article

The Effect of Mixture of Rapeseed Meal, White Lupin Seed, and Pea Seed in Rabbit Diets on Performance Indicators and Fatty Acid Profile of Meat and Fat

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Abstract

Quality of rabbit's carcasses is largely determined by the composition of the lipid fraction. The objective of this study was to determine the effect of partial or complete substitution of soybean meal with a combination of rapeseed meal, white lupin seed and pea seed on the production results and fatty acid profile of meat and fat in rabbits. Ninety New Zealand White rabbits were divided into three feeding groups, 30 in each group: Control - C (mean protein source - 15% soybean meal in the diet), Experimental 1 - E1 (7.5% soybean meal, 5% rapeseed meal, 4% white lupin seed, 3% pea seed) and Experimental 2 - E2 (0% soybean meal, 10% rapeseed meal, 8% white lupin seed, 6% pea seed). No significant differences were determined in the final body weights of rabbits. Feed efficiency was better in both of the E groups than in group C. Dressing percentage was higher in group E2 than in group C. Also, protein content in thigh muscle was higher in groups E than in group C. As the dietary proportion of soybean decreased, the proportion of SFA in meat and in perirenal fat decreased, and that of PUFA increased. The obtained results indicate that soybean meal may be successfully replaced in rabbit diets by the combination of rapeseed meal, white lupin seed, and pea seed.

Keywords: *Brassicaceae, Fabaceae, Fatty acid profile, Growth performance, Meat composition, Rabbit feeding*

Tavşan Diyetlerinde Kolza Tohumu, Beyaz Acı Bakla Tohumu ve Bezelye Tohumu Karışımının Et ve Yağ Performans Göstergeleri ve Yağ Asidi Profili Üzerine Etkisi

Öz

Tavşan karkaslarının kalitesini büyük ölçüde lipit fraksiyonunun kompozisyonu belirler. Bu çalışmanın amacı, kolza tohumu küspesi, beyaz acı bakla tohumu ve bezelye tohumu kombinasyonu ile kısmen veya tam ikame edilmiş soya fasulyesi küspesinin tavşanlarda et ve yağın üretim sonuçları ve yağ asidi profili üzerindeki etkisini belirlemektir. Doksan Yeni Zelanda Beyaz tavşanı, her biri 30 tavşan içeren üç besleme grubuna ayrıldı: Kontrol - C (ortalama protein kaynağı - diyette %15 soya küspesi), Deneme 1 - E1 (%7.5 soya küspesi, %5 kolza tohumu küspesi, %4 beyaz acı bakla tohumu, %3 bezelye tohumu) ve Deneme 2 - E2 (%0 soya küspesi, %10 kolza tohumu küspesi, %8 beyaz acı bakla tohumu, %6 bezelye tohumu). Tavşanların final vücut ağırlıklarında anlamlı bir fark saptanmadı. Yemden yararlanma her iki E grubunda da C grubuna göre daha iyiydi. Tüylenme yüzdesi E2 grubunda C grubuna göre daha yüksekti. Ayrıca, but kasındaki protein içeriği E gruplarında C grubuna göre daha yüksekti. Soya fasulyesinin diyet oranı azaldıkça, et ve perirenal yağdaki SFA oranında azalmaa ve PUFA oranında artış görülmekteydi. Elde edilen sonuçlar, soya fasulyesi ununun kolza tohumu küspesi, beyaz acı bakla tohumu ve bezelye tohumu kombinasyonu ile tavşan diyetlerinde ikame olarak başarıyla değiştirilebileceğini göstermektedir.

Anahtar sözcükler: *Turpgiller, Baklagiller, Yağ asidi profili, Büyüme performansı, Et kompozisyonu, Tavşan besleme*

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INTRODUCTION

Complete pelleted mixtures are used in intensive rabbit production. As much as 19% of crude protein with an appropriate amino acid composition has to be supplied to the rabbits to ensure good fattening results. The most important limiting amino acids in rabbit nutrition are lysine, tryptophan, cystine, and methionine [1,2]. Soybean meal is currently the most common source of protein in the diets of rabbits from large-scale farms. However, soybean is also used in the countries where soybean is not grown and has to be imported. To become independent of soybean imports, research is being conducted to replace it with other components in rabbit diets. These include by-products of biofuel production, mainly rapeseed meal and rapeseed cake as well as dried distillers grains with solubles - DDGS [3-6]. Products such as sunflower cake and sunflower meal are also used for replacement [7-9]. Moreover, attempts to use the seed of white lupin [7,9-11], pea [12-14], and other plants of the *Fabaceae* family have been made [15,16]. All these products come from plants that have for years been grown in the transitional temperate climate zone. It was shown that even complete replacement of dietary soybean meal with a combination of rapeseed meal and the seeds of the pea and white lupin did not have a negative effect on production results, digestibility of nutrients nitrogen retention, and functioning of the gastrointestinal tract in rabbits [17,18].

However, regardless of the production parameters of rabbits, an important consideration is the quality of carcasses used for consumption, which is largely determined by the composition of the lipid fraction. Today, efforts are made to reduce the amount of saturated fatty acids and to increase the amount of unsaturated fatty acids in animal fat deposits [19]. There are plenty of studies indicating that rabbit nutrition has an effect on the fatty acid composition of the carcasses [5,20-22]. Therefore, when studying the usefulness of a component in rabbit nutrition, evaluation of production results should be followed by the investigation of its effect on fatty acid profile in muscle and adipose tissue.

It was hypothesized that replacement of soybean meal in the diets of meat rabbits with a mixture of *Brassicaceae* and *Fabaceae* plants seeds and by-products will have no adverse effect on the fatty acid profile of the carcasses. Thus, the objective of the study was to determine the effect of partial or complete substitution of soybean meal with a combination of rapeseed meal, white lupin seed and pea seed on the production results and fatty acid profile of meat and fat in New Zealand White rabbits.

MATERIAL and METHODS

Animal Care

The animal protocol used in this study was approved by the Local Institutional Animal Care and Use Committee in Olsztyn (Number: 24/2015), and the study was carried out in accordance with EU Directive 2010/63/EU for animal experiments.

Experimental Factor

The experimental factor was the contribution of rapeseed meal (RSM), white lupin seed (WLS) and pea seed (PS) in pelleted feed mixtures. The chemical composition and energy value of these components and of the soybean meal (SBM) are presented in *Table 1*. The control feed mixture (C group) contained 15% extracted soybean meal (SBM). In the first experimental group (E1), the diet contained 7.5% SBM, which was partially substituted with a mixture of RSM, WLS and PS. In the second group (E2), soybean meal was completely substituted with RSM, WLS and PS in pelleted feed mixtures. The formulation, chemical composition and energy value of feed mixtures are presented in *Table 2*, and the fatty acid content of these mixtures is given in *Table 3*. All rations met the nutritional requirements of growing rabbits [1].

Animals and Treatments

Ninety New Zealand White (NZW) rabbits were selected from 18 litters for the experiments. They were divided into 3 groups, 30 rabbits in each, being analogous in terms of origin, proportion of sexes, and body weight. The experiment was carried out from September to November and started when rabbits were weaned at 35 d of age (average body weight - 926.23±6.08) and terminated when they reached 90 d of age. Rabbits were kept in a closed experimental pavilion, in wire net flat deck cages (0.5´0.6´0.4 m; 2 animals each), and were fed pelleted diets *ad libitum*. They were kept under standard conditions: temperature of 18-20°C and relative air humidity of 60-75%, intensive ventilation of rooms, and regulated photoperiod (16-h lighting and 8-h darkness).

Table 1. *Chemical composition (%) and measured energy content of soybean meal, rapeseed meal, white lupine seed and pea seed*

Specification	SBM	RPM	WLS	PS
Dry matter	90.11	91.02	88.79	88.14
Crude ash	6.26	7.24	4.09	3.37
Crude protein	45.19	34.57	41.09	22.26
Ether extract	1.85	3.63	2.02	0.85
NDF	14.12	23.93	27.89	11.26
ADF	6.24	13.31	19.64	6.96
ADL	5.01	5.84	5.32	4.71
Lysine	2.38	1.67	1.37	1.51
Methionine + cystine	1.01	1.45	0.75	0.60
Threonine	1.37	1.64	1.12	0.84
Tryptophan	0.41	0.44	0.27	0.22
Gross energy (MJ/kg)	17.53	15.91	16.67	16.60

SBM: *soybean meal;* ***RPM:*** *rapeseed meal;* ***WLS:*** *white lupine seed;* ***PS:*** *pea seed;* ***NDF:*** *neutral detergent fibre;* ***ADF:*** *acid detergent fibre;* ***ADL:*** *acid detergent lignin*

Table 2. *Ingredients, chemical composition and measured energy content of feed mixtures*

Specification	Group		
	C	E1	E2
Ingredients (%)			
Soybean meal (48% CP)	15.0	7.5	-
Rapeseed meal	-	5.0	10.0
White lupine seed	-	4.0	8.0
Pea seed	-	3.0	6.0
Barley	14.5	12.5	10.5
Wheat	6.0	7.5	9.0
Corn	16.0	12.5	9.0
Dried alfalfa (18% CP)	23.0	23.0	23.0
Wheat bran	11.0	11.0	11.0
ARBOCEL*	6.0	5.5	5.0
Beet molasses	2.0	2.0	2.0
Skimmed milk powder	2.0	2.0	2.0
Dried brewer's yeast	1.0	1.0	1.0
Calcium carbonate	1.0	1.0	1.0
Dicalcium phosphate	1.0	1.0	1.0
Mineral-vitamin premix†	1.0	1.0	1.0
NaCl	0.5	0.5	0.5
Chemical composition (%)			
Dry matter	90.66	91.00	90.56
Crude ash	6.58	6.71	6.16
Crude protein	17.40	17.95	17.95
Ether extract	2.28	2.90	3.37
NDF	22.57	22.45	24.16
ADF	13.14	13.91	15.48
ADL	3.22	3.16	3.28
Lysine	0.78	0.85	0.79
Methionine + cystine	0.73	0.74	0.82
Threonine	0.70	0.79	0.77
Tryptophan	0.16	0.16	0.15
Gross energy (MJ/kg)	16.86	16.91	16.85

NDF: *neutral detergent fibre;* ***ADF:*** *acid detergent fibre;* ***ADL:*** *acid detergent lignin; * Crude fibre concentrate; † Composition mineral-vitamin premix 1 kg: Vit. A: 3.500.000 IU, Vit. D_3: 200.000 IU, Vit. E: 28.000 mg, Vit. K_3: 200 mg, Vit. B_1: 1.500 mg, Vit. B_2: 2.800 mg, Vit. B_6: 2.800 mg, Vit. B_{12}: 20.000 mcg, Folic acid: 200 mg, Niacin: 10.000 mg, Biotin: 200.000 mcg, Calcium pantothenate: 7.000 mg, Choline: 30.000 mg, Fe: 17.000 mg, Zn: 2.000 mg, Mn: 1.000 mg, Cu (copper sulfate x $5H_2O$, 24.5%): 800 mg, Co: 1.000 mg, I: 100 mg, Methionine: 150 g, Ca: 150 g, P: 100 g*

Table 3. *Fatty acid content in feed mixtures (% of total fatty acid pool)*

Fatty Acids	Group		
	C	E1	E2
SFA			
C12:0 (lauric)	0.43	0.35	0.35
C14:0 (myristic)	0.48	0.43	0.44
C16:0 (palmitic)	17.73	15.10	14.50
C18:0 (stearic)	2.99	3.00	2.94
C20:0 (arachidic)	0.51	0.97	1.23
Other SFA	0.32	0.34	0.33
Total SFA	22.46	20.19	19.79
MUFA			
C16:1 (palmitoleic)	0.22	0.27	0.34
C18:1 (oleic)	23.36	24.44	24.99
Other MUFA	0.71	0.94	1.17
Total MUFA	24.29	25.65	26.50
PUFA			
C18:2 (linoleic)	46.50	46.12	45.62
C18:3 (α-linolenic)	6.13	7.57	7.41
C20:4 (arachidonic)	0.40	0.27	0.48
C20:5 (EPA)	0.21	0.20	0.19
Other PUFA	0.00	0.00	0.01
Total PUFA	53.24	54.16	53.71

SFA: *saturated fatty acids;* ***MUFA:*** *monounsaturated fatty acids;* ***PUFA:*** *polyunsaturated fatty acids*

The rabbits were weighed individually on an electronic scale on days 35, 63 and 90. These data allowed calculating daily body weight gains (BWG) of the rabbits and the feed efficiency (FE) [BWG (g)/feed intake (g)]. At the end of the production trial, after 24-h fasting, the animals were weighed and killed according to the accepted recommendations for euthanasia of experimental animals (rabbits were stunned and bled, and the whole procedure took about 2 min). After the slaughter, the animals were skinned and eviscerated. After cooling the carcasses (for 24 h, at 4°C), tissue samples were taken for chemical analyses, and dressing percentage was calculated as follows:

DP (%) = Chilled carcass weight without head and giblets (kg)/Live weight (kg) x 100%

In addition, the percentage content of the primal cuts: forepart, loin and hind part, was calculated in the carcass. The carcasses were divided into the head (cut through the craniovertebral joint), the fore part (cut between the 7th and 8th thoracic vertebrae), the loin (cut between the 6th and 7th thoracic vertebrae) and the hind part (carcass section remaining after separation of the loin from the front, comprising the hindquarters and hind limbs). For fatty acids in the muscles and in adipose tissue, the saturation index (S/P) was calculated using equations presented by Peiretti and Meineri [21] and Volek and Marounek [9]:

$$S/P = (C14:0 + C16:0 + C18:0)/\sum MUFA + \sum PUFA$$

Chemical Analyses

The nutrient content of feed was determined by AOAC [23] standard methods in duplicate samples. Dry matter content (method 978.10) was determined in a laboratory drier, at

103°C. Crude ash content (method 942.05) was estimated by sample mineralization in a muffle furnace (Czylok, Poland) at 600°C. Total nitrogen content (method 984.13) was determined by the Kjeldahl method, in the FOSS TECATOR Kjeltec 2200 Auto Distillation Unit. Ether extract content (method 920.39) was estimated by the Soxhlet method, in the FOSS SOXTEC SYSTEM 2043. NDF (neutral detergent fiber), ADF (acid detergent fiber) and ADL (acid detergent lignin) were estimated in the FOSS TECATOR Fibertec 2010 System. NDF was determined according to the procedure proposed by Van Soest et al.[24]. ADF and ADL were determined according to procedures of AOAC [23] (methods 973.18 and 973.18D, respectively). The levels of amino acids in diets (method 982.30) were determined using the Biochrom 20 plus amino acid analyzer and Biochrom amino acid analysis reagents (Biochrom Ltd., Cambridge, England). Gross energy content was determined using a bomb calorimeter (IKA® C2000 basic, Germany).

Fat from ground samples of feed and animal tissues were extracted by the Soxhlet extraction procedure [23]. To determine fatty acid composition, all fat samples were methylated by the modified Peisker method [25] (1.5 cm^3 of a methanol:chloroform:concentrated sulfuric acid mixture, 100:100:1 v/v, was added to ca 150 mL fat, thermostat -80°C, 3 h), and fatty acid methyl esters were obtained. Fatty acids were separated and determined by gas chromatography: VARIAN CP-3800 gas chromatograph-Netherlands, flame-ionization detector (FID), capillary column (length - 50 m, f = 0.25 mm, film d = 0.25 µm), split injector, split ratio 50:1, 1 µL sample, detector temperature -250°C, injector temperature - 225°C, column temperature –200°C, carrier gas - helium, flow rate - 1.2 cm^3/min. Fatty acids were identified by comparing the retention times of individual fatty acid methyl ester standards (Sigma-Aldrich) and the retention times of peaks in the analyzed samples. The relative content of each fatty acid was expressed as percent of the total peak area of all fatty acids in the sample.

Statistical Analysis

Calculations were made with Statistica software [26]. Cage was considered the experimental unit. Data are expressed as means ± standard error of the mean (SEM). The results were processed statistically using least squares means in GLM procedures. For comparison of data, the $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + \varepsilon_{ijk}$ model was used, where µ is the general mean, α_i is the effect of diet, β_j is the effect of sex, $\alpha_i\beta_j$ is the interaction effect between diet and sex, and ε_{ijk} is the random error. Analyses did not reveal significant effects of sex or significant interactions between fixed effects, therefore they are not reported in the tables.

RESULTS

Body weights and daily BWG of rabbits showed no significant differences among groups *(Table 4)*. Rabbits fed a control diet (C) consumed overall more feed (107.53 g/d) compared to rabbits fed E1 and E2 diets (99.91 g/d and 92.69 g/d, respectively), and the difference between groups C and E2 was statistically highly significant. Calculated FE parameter achieved more favorable values in E1 and E2 groups (3.38 g/g and 3.30 g/g, respectively) than in C group (3.78 g/g). DP was higher in group E2 compared with groups C and E1. The experimental factor appeared to have no impact on the content of the primal cuts (forepart, loin and hind part) in the carcass among rabbits' groups. Dry matter content (%) in thigh muscle did not differ between the groups, but crude protein content (%) was higher in both of the experimental groups than in the control group. A reverse trend was observed for ether extract, which was more abundant in the muscles of rabbits receiving 15% soybean meal in the diet compared to the experimental groups.

Table 5 shows the proportion of fatty acids in thigh muscle of the rabbits. Total SFA content was higher in the meat of rabbits from group C compared to those of groups E1 and E2. Among these fatty acids, the proportion of myristic

Table 4. *Productivity parameters of rabbits (mean±SEM)*

Specification	Group		
	C	E1	E2
BW at 35 d (g)	926.20±10.78	928.74±5.53	923.75±1.92
BW at 63 d (g)	1705.45±12.45	1699.92±16.82	1714.46±17.02
BW at 90 d (g)	2490.77±113.19	2554.42±134.36	2468.63±138.41
BWG 35-63 days od age (g/d)	27.83±3.86	27.54±3.75	28.24±4.14
BWG 63-90 days od age (g/d)	29.09±4.09	31.65±4.20	27.93±3.96
BWG 35-90 days od age (g/d)	28.45±3.92	29.56±4.46	28.09±4.41
FI 35-63 days od age (g/d)	96.05±11.53^{a}	89.59±10.87	83.71±10.34^{b}
FI 63-90 days od age (g/d)	119.43±13.26^{a}	110.61±12.09^{b}	101.99±11.70^{b}
FI 35-90 days od age (g/d)	107.53±12.83^{A}	99.91±11.52	92.69±11.18^{B}
FE 35-63 days od age (g/g)	3.45±0.04^{A}	3.25±0.03	2.97±0.03^{B}
FE 63-90 days od age (g/g)	4.10±0.05^{A}	3.50±0.04^{B}	3.65±0.05^{B}
FE 35-90 days od age (g/g)	3.78±0.05^{A}	3.38±0.03^{B}	3.30±0.04^{B}
DP (%)	45.33±0.28^{B}	46.39±0.47^{b}	48.46±0.41^{Aa}
Forepart (%)	36.65±0.18	35.52±0.16	35.37±0.18
Loin (%)	26.21±0.13	27.40±0.11	27.04±0.12
Hind part (%)	37.14±0.16	37.08±0.14	37.59±0.14
DM (%) in thigh muscle	28.68±0.37	28.02±0.21	28.43±0.13
CP (%) in thigh muscle	21.19±0.18^{B}	22.31±0.19^{A}	22.26±0.19^{A}
EE (%) in thigh muscle	4.96±0.54^{a}	3.58±0.46^{b}	3.78±0.32^{b}

SEM: *standard error of the mean;* ***BW:*** *body weight;* ***BWG:*** *body weight gains;* ***FI:*** *feed intake;* ***FE:*** *feed efficiency;* ***DP:*** *dressing percentage;* ***DM:*** *dry matter;* ***CP:*** *crude protein;* ***EE:*** *ether extract;* ^{a,b} *Values with different superscripts are significantly different at P<0.05;* ^{A,B} *Values with different superscripts are significantly different at P<0.01*

Table 5. *Fatty acid content in thigh muscle (% of total fatty acid pool; mean±SEM)*

Fatty Acids	Group		
	C	E1	E2
SFA			
C12:0 (lauric)	0.26±0.04	0.30±0.02	0.27±0.01
C14:0 (myristic)	$3.42±0.10^{Aa}$	$3.06±0.11^{b}$	$2.81±0.13^{B}$
C16:0 (palmitic)	$30.01±0.51^{Aa}$	$27.53±0.42^{Ab}$	$26.35±0.48^{B}$
C18:0 (stearic)	$5.94±0.17^{B}$	$6.63±0.31^{b}$	$7.01±0.15^{Aa}$
C20:0 (arachidic)	$0.10±0.00^{B}$	$0.11±0.00^{b}$	$0.13±0.00^{Aa}$
Other SFA	$0.19±0.03^{Bb}$	$0.32±0.04^{A}$	$0.39±0.03^{Aa}$
Total SFA	$39.92±0.53^{Aa}$	$37.95±0.40^{b}$	$36.95±0.53^{B}$
MUFA			
C16:1 (palmitoleic)	$4.57±0.28^{A}$	$4.45±0.24^{A}$	$3.14±0.22^{B}$
C18:1 (oleic)	25.85±0.49	25.64±0.45	24.97±0.38
Other MUFA	$0.02±0.00^{A}$	$0.01±0.00^{B}$	$0.01±0.00^{B}$
Total MUFA	$30.44±0.73^{a}$	$30.10±0.52^{a}$	$28.12±0.57^{b}$
PUFA			
C18:2 (linoleic)	$21.39±0.67^{B}$	$22.61±0.55^{b}$	$24.36±0.52^{Aa}$
C18:3 (α-linolenic)	$3.85±0.07^{Bb}$	$4.52±0.12^{a}$	$4.53±0.10^{Aa}$
C20:4 (arachidonic)	$3.49±0.34^{Bb}$	$3.80±0.23^{Ba}$	$4.81±0.35^{A}$
C20:5 (EPA)	0.21±0.01	0.24±0.01	0.23±0.01
Other PUFA	$0.70±0.04^{b}$	0.78±0.05	$1.00±0.07^{a}$
Total PUFA	$29.64±1.06^{B}$	$31.95±0.70^{b}$	$34.93±0.95^{Aa}$
PUFA 6/3	5.31±0.22	5.08±0.10	5.11±0.09
S/P	$0.66±0.06^{a}$	$0.60±0.05^{b}$	$0.57±0.05^{b}$

SEM: *standard error of the mean;* ***SFA:*** *saturated fatty acids;* ***MUFA:*** *monounsaturated fatty acids;* ***PUFA:*** *polyunsaturated fatty acids;* ***S/P:*** *saturation index;* ^{a,b} *Values with different superscripts are significantly different at P<0.05;* ^{A,B} *Values with different superscripts are significantly different at P<0.01*

Table 6. *Fatty acid content in loin (% of total fatty acid pool; mean±SEM)*

Fatty Acids	Group		
	C	E1	E2
SFA			
C12:0 (lauric)	0.22±0.03	0.29±0.03	0.22±0.02
C14:0 (myristic)	$3.53±0.07^{Aa}$	$3.20±0.13^{b}$	$2.92±0.10^{B}$
C16:0 (palmitic)	$30.15±0.55^{Aa}$	$28.55±0.49^{Ab}$	$26.36±0.42^{B}$
C18:0 (stearic)	5.64±0.13	5.98±0.26	6.38±0.22
C20:0 (arachidic)	$0.10±0.00^{Bb}$	$0.11±0.01^{Ba}$	$0.16±0.00^{A}$
Other SFA	$0.20±0.02^{B}$	$0.39±0.03^{Aa}$	$0.26±0.03^{b}$
Total SFA	$39.84±0.57^{A}$	$38.52±0.60^{a}$	$36.30±0.46^{Bb}$
MUFA			
C16:1 (palmitoleic)	$4.09±0.26^{A}$	$3.94±0.32^{A}$	$2.59±0.23^{B}$
C18:1 (oleic)	27.42±0.48	26.64±0.47	26.56±0.32
Other MUFA	$0.03±0.01^{A}$	$0.01±0.00^{B}$	$0.03±0.01^{A}$
Total MUFA	$31.54±0.58^{a}$	30.59±0.50	$29.18±0.41^{b}$
PUFA			
C18:2 (linoleic)	$21.77±0.77^{B}$	$23.20±0.87^{b}$	$25.79±0.79^{Aa}$
C18:3 (α-linolenic)	$4.23±0.12^{Bd}$	$4.82±0.22^{bc}$	$5.40±0.14^{Aa}$
C20:4 (arachidonic)	1.81±0.14	2.13±0.42	2.45±0.15
C20:5 (EPA)	$0.13±0.01^{B}$	$0.21±0.02^{A}$	0.16±0.02
C22:6 (DHA)	$0.03±0.01^{A}$	$0.03±0.01^{A}$	$0.00±0.00^{B}$
Other PUFA	0.66±0.07	0.50±0.06	0.73±0.07
Total PUFA	$28.63±0.84^{Bb}$	$30.89±0.68^{Ba}$	$34.53±0.76^{A}$
PUFA 6/3	5.40±0.23	5.04±0.12	5.09±0.09
S/P	$0.65±0.05^{a}$	0.61±0.05	$0.56±0.04^{b}$

SEM: *standard error of the mean;* ***SFA:*** *saturated fatty acids;* ***MUFA:*** *monounsaturated fatty acids;* ***PUFA:*** *polyunsaturated fatty acids;* ***S/P:*** *saturation index;* $^{a,b;c,d}$ *Values with different superscripts are significantly different at P<0.05;* ^{A,B} *Values with different superscripts are significantly different at P<0.01*

and palmitic acids was highest in group C, lower in E1, and lowest in E2. An opposite trend was found for stearic and arachidic acids, the proportions of which were highest in group E2 and lowest in group C. On the other hand, total MUFA proportion was higher in C and E1 than in E2 group. A similar relationship occurred for palmitoleic acid. The proportions of oleic acid did not differ significantly among the groups. Meanwhile, total PUFA showed high levels in group E2, insignificantly lower in E1, and significantly lowest in C group. The same relationship between the groups was observed for the two most important PUFA: linoleic and α-linolenic acids, in which highly significant differences between groups E2 and C were noted. The proportion of arachidonic acid was significantly higher in rabbit meat of group E2 compared to the other groups, and significantly higher in E1 than in C. There were no significant differences between the groups in the amount of EPA (eicosapentaenoic acid). Also, differences in PUFA 6/3 ratio were only numerical and statistically not significant. The S/P ratio was higher in group C than in both experimental groups.

Table 6 presents the proportions of fatty acids in the loin of the studied rabbits. Overall, the loin showed similar trends for fatty acids as did the thigh. The highest SFA content was noted in the loin of rabbits from group C, followed by groups E1 and E2, which was largely due to the differences in the amounts of palmitic acid and to a lesser extent in the amounts of myristic acid. However, the proportions of arachidic acid were lowest in group C and highest in group E2. Total MUFA was higher in group C than in group E2. In groups C and E1 there was more palmitoleic acid than in group E2. The proportions of PUFA were highest in the group of animals receiving no soybean, lower in the group fed 7.5% soybean, and lowest in the loin of animals from group C. This tendency was in relation to the proportions of linoleic and α-linolenic acids, for which the same intergroup relationships were observed. No significant differences between the groups were observed in the PUFA 6/3 ratio, just like in the thigh muscle. The S/P ratio was highest in group C and lowest in group E2, with a statistically significant difference between these groups.

Table 7. *Fatty acid content in perirenal fat (% of total fatty acid pool; mean±SEM)*

Fatty Acids	Group		
	C	E1	E2
SFA			
C12:0 (lauric)	0.36±0.07	0.52±0.06	0.35±0.03
C14:0 (myristic)	4.96 ± 0.14^{Aa}	4.38 ± 0.18^{b}	4.04 ± 0.12^{B}
C16:0 (palmitic)	32.68 ± 0.66^{Aa}	30.62 ± 0.44^{Ab}	28.32 ± 0.35^{B}
C18:0 (stearic)	5.25±0,10	5.61±0.29	5.83±0.26
C20:0 (arachidic)	0.03±0.00	0.04±0.01	0.07±0.01
Other SFA	0.25 ± 0.04^{Bb}	0.63 ± 0.07^{A}	0.38 ± 0.04^{Ba}
Total SFA	43.53 ± 0.79^{A}	41.80 ± 0.64^{A}	38.99 ± 0.29^{B}
MUFA			
C16:1 (palmitoleic)	4.88 ± 0.41^{A}	4.46 ± 0.44^{a}	2.93 ± 0.24^{Bb}
C18:1 (oleic)	25.94±0.35	25.81±0.34	26.50±0.31
Other MUFA	0.02±0.01	0.01±0.00	0.01±0.00
Total MUFA	30.84±0.61	30.28±0.58	29.44±0.40
PUFA			
C18:2 (linoleic)	20.82 ± 0.76^{Bb}	22.49 ± 0.70^{a}	25.22 ± 0.60^{A}
C18:3 (α-linolenic)	3.93 ± 0.10^{B}	4.43 ± 0.25^{Ab}	5.10 ± 0.08^{Aa}
C20:4 (arachidonic)	0.33±0.02	0.40±0.05	0.45±0.02
C20:5 (EPA)	0.03 ± 0.00^{b}	0.04±0.01	0.05 ± 0.01^{a}
Other PUFA	0.52 ± 0.07^{b}	0.56±0.08	0.75 ± 0.10^{a}
Total PUFA	25.63 ± 0.84^{Bd}	27.92 ± 0.97^{bc}	31.57 ± 0.61^{Aa}
PUFA 6/3	4.84±0.15	4.69±0.12	4.57±0.09
S/P	0.76 ± 0.07^{Aa}	0.70 ± 0.06^{bc}	0.63 ± 0.05^{Bd}

SEM: *standard error of the mean;* ***SFA:*** *saturated fatty acids;* ***MUFA:*** *mono-unsaturated fatty acids;* ***PUFA:*** *polyunsaturated fatty acids;* ***S/P:*** *saturation index;* $^{a,b;\ c,d}$ *Values with different superscripts are significantly different at P<0.05;* ^{A,B} *Values with different superscripts are significantly different at P<0.01*

The proportion of fatty acids in perirenal fat of the studied rabbits is given in *Table 7*. Total SFA was higher in groups C and E1 than in group E2. Similar to the case of thigh muscle and loin, the proportion of myristic acid in perirenal fat was highest in the control group, lower in group E1, and lowest in group E2. Also, the proportions of palmitic acid in the studied rabbits reflected the situation observed in the muscles: they were highest in group C and lowest in group E2. No differences were found between the groups in total MUFA, although the proportion of palmitoleic acid was highest in the control group, lower in group E1, and lowest in group E2. In turn, total PUFA was highest in group E2 and lowest in the control group. Similar tendencies were observed for individual PUFA. Perirenal fat of the rabbits fed diets E1 and E2 contained more linoleic and α-linolenic acids compared to the rabbits fed the control diet. In addition, there was more EPA in group E2 than in group C. However, for the fatty acid profile, no differences between the groups were found for PUFA 6/3 ratio. The S/P ratio was highest in group C, lower in group E1, and lowest in group E2.

DISCUSSION

In our experiment we found that partial or complete replacement of soybean meal with the experimental components did not cause differences in body weight between the studied groups of rabbits, but it considerably improved feed efficiency. The experimental diets (E1 and E2) contained rapeseed meal, white lupin seed and pea seed. The proportions of barley, wheat and corn were manipulated to balance the diets. The content of protein, including lysine, methionine + cystine, and threonine in the experimental diets was higher than in the control diet and this probably caused differences in protein content in thigh muscle, the highest DP in group E2, and the lowest DP in the control group. The obtained content of dry matter, protein and ether extract in meat is characteristic of broiler rabbits. Chełmińska and Kowalska [27] observed a similar content of dry matter and protein to ours, and a slightly lower fat content in the meat of NZW rabbits. Also, the range of DP obtained in our study is consistent with the findings of Daszkiewicz et al.[28] and Chełmińska and Kowalska [27]. The BW of NZW rabbits in our experiment were slightly higher than those obtained by the previous studies. However, BWG of the rabbits investigated by Chełmińska and Kowalska [27] were similar to ours. Similar BWG in NZW rabbits, although calculated for the age range of 30-80 days, were reported by Cardinali et al.[29]. It is worth emphasizing that rabbits' body weights and BWG may differ according to the subject breed. In our previous experiment, Californian rabbits achieved body weights of 2291-2371 g at age 98 days, with BWG of 24.6-26.2 g [4]. Today, commercially bred hybrid rabbits may reach over 3000 g on day 84 of age [6,30].

Compared to the meat of other livestock species, rabbit meat is characterized by a low content of intramuscular fat, which has a beneficial composition of fatty acids for the consumer [19]. The diet of rabbits has a major effect on the subsequent fatty acid composition of the carcasses, because most fatty acids supplied to rabbits with the diet is not modified during the digestion and they quickly enter the fat depots with minor modifications [31,32]. Our results confirm this observation for total SFA and PUFA, although a different trend for total MUFA was observed. Nowadays, attempts are being made to reduce SFA levels and increase MUFA and PUFA levels in the human diet. In our study, relatively most PUFA in the thigh muscle of the rabbits was found in group E2, in which soybean meal was completely removed from the ration. Among PUFA, methylene-interrupted polyenes are considered particularly beneficial. Among these, special attention should be given to n-3 α-linolenic acid, which is usually consumed in inadequate amounts by humans. As reported by Dalle Zotte [19], its content in the loin of rabbits is 3.14% compared to 2.98% in the thigh muscle. The content of this acid in the thigh muscle of the rabbits studied by us ranged from 3.85 to 4.53% and it was much more favorable

in groups E1 and E2. A similar situation was found for the proportion of α-linolenic acid in perirenal fat, where the most favorable amount was noted in group E2, followed by E1, and the lowest amount in group C. This tendency is generally consistent with the proportions of this acid observed in the dietary mixtures, although in diet E1 it was slightly higher than in group E2. The dietary n-6/n-3 PUFA ratio is another important consideration. In most European countries, this ratio in the human diet is too high, at 10-20:1. The optimal n-6/n-3 PUFA ratio in the human diet should not be higher than 5-6:1. As stated by Newton [33], excessive consumption of n-6 fatty acids disrupts the metabolism of n-3 acids and the physiological balance of the compounds synthesized from these acids. In the present study, the n-6/n-3 PUFA ratio was relatively low in all carcasses, ranging from 5.08 to 5.31 and from 4.57 to 4.84 in thigh muscle and in perirenal fat, respectively; however, it did not differ statistically between groups. By way of comparison, in rabbits fed 10% of maize DDGS, the n-6/n-3 ratio was 6.51 [27]. We also calculated the simple saturation index (S/P) in the analyzed tissues *(Table 5, 6, 7)*. In general, both in muscles and in adipose tissue, this index was lower in groups E1 and E2 than in group C. Therefore, human consumers would benefit from eating rabbits in whose diets soybean meal was replaced with a combination of rapeseed meal, white lupin seed, and pea seed.

Based on the results obtained in our experiment, it may be concluded that substitution of soybean meal with white lupin and pea seeds improved the feed efficiency as well as dressing percentage and crude protein content of the carcasses. Importantly for human consumers, as the dietary proportion of soybean decreased, the proportion of SFA in meat and in perirenal fat decreased, and that of PUFA increased. As a result, the saturation index was more beneficial in the groups supplemented with rapeseed meal, white lupin seed and pea seed compared to group C. This indicates that soybean meal in rabbit diets may be replaced by the mixture of the above components.

Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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