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Fatty acid profile of meat of seasonally fed slow-growing rabbits

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The study aimed at determining fatty acid (FA) profiles of meat of seasonally fed slow-growing rabbits in relation to lipid composition of feeds and their intake. In each season, 21 weaned male rabbits (Popielno White breed) were assigned into two homogenous groups: the control (C, n=7) and seasonally fed – summer (S, n=14) or winter (W, n=14). Meat lipid, MUFA and PUFA contents were significantly higher in the S and C groups, most likely due to a higher lipid intake, while S-rabbits had two-fold higher n-3 PUFA contents than C-rabbits. This resulted in a better thrombogenic index in S- rather than in C-rabbits, similarly as it was the case for the hypocholesterolemic/ hypercholesterolemic (H/H) ratio. In relation to the other groups myristic and palmitic acid contents in meat were greater in W-rabbits due to a higher SFA intake. As compared with the C diet, seasonal diets were associated with a higher proportion of n-3 PUFA in meat. This resulted in an improvement of the n-6/n-3 ratio (3.62 on average) in meat from seasonally fed rabbits. Both seasonal diets also increased the content of odd-numbered and branched-chain acids (OBCFA; two-fold greater than in the C group). S-rabbits had greater daily body mass gains and were heavier than the W-rabbits. The content of palmitooleic acid (C16:1 n-7) in meat was high irrespective of the diet. Feeding strategies associated with outdoor rearing, adopted in small farms, favour local rabbit populations. Thus, it is recommended to promote native, slow-growing rabbit breeds, a source of valuable nutrients.

KEY WORDS: fatty acid composition / forage composition / rabbit meat / slow-growing breeds / seasonal diet /

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Rabbit meat is appreciated by consumers due to its high nutritive value and dietetic properties. That meat is low-fat and contains less cholesterol than other meats. Regarding fat composition, rabbit meat may be recommended, as the unsaturated fatty acids (FAs) represent around 60% of the total FA, while the content of polyunsaturated FA (PUFA) is much greater than in other meats, including poultry [Dalle Zotte and Szendro 2011].

Many authors reported that feeding had a greater impact on the FA profile of meat [Poławska *et al*. 2013, Horbańczuk and Wierzbicka 2016]. Some authors attempted at increasing the content of n-3 PUFA in rabbit meat by supplementing diets with fish oil and linseed [Kouba *et al*. 2008, Kowalska and Bielanski 2009]. Also pasture rearing may provide an alternative method of enhancing the content of n-3 FAs in rabbit meat [Forrester-Anderson *et al*. 2006]. Recent studies investigated the effects of dietary alfalfa and flax sprouts on rabbit meat FA composition and lipid oxidation [Capra *et al*. 2013, Dal Bosco *et al*. 2014, Dal Bosco *et al*. 2015]. Also other FAs, namely the odd-numbered straight carbon chain and methyl-branched-chain FAs (OBCFA), in recent years have been widely investigated in animals with symbiotic fermentation due to the anti-cancer properties of those acids. The OBCFA occurs in the adipose tissue and intramuscular fat of rabbits due to caecotrophy [Leiber *et al*. 2008, Papadomichelakis *et al.* 2010a]. The effects of dietary fiber and soybean oil on the profile of OBCFA in intramuscular fat of rabbit meat were studied by Papadomichelakis *et al.* [2010a] and Papadomichelakis *et al.* [2010b]. However, those studies were conducted on commercial lines of rabbits having reduced immune competence and increased susceptibility to environmental stress [McNitt *et al*. 2003]. Local breeds or populations of rabbits are highly adaptive to diverse farming conditions and feeding systems [D'Agata *et al*. 2009, Schiavone *et al*. 2013, Paci *et al*. 2014]. According to the organic farming rules adopted in Italy, slow-growing rabbits have to be used in that alternative production system [Sciavone *et al*. 2013]. D'Agata *et al.* [2009] reported that meat derived from slow-growing rabbits in an outdoor system had a lower content of saturated FA and higher content of monounsaturated FA (MUFA) than those reared indoors. Also, a recent study of Paci *et al*. [2014] confirmed that organic rabbit meat of local grey animals may appear attractive to consumers because of an improved MUFA content. Meat originating from cross-bred rabbits reared in organic farms in Spain had less MUFA and more PUFA than meat of conventionally reared animals, as a consequence of diversified diets [Pla *et al*. 2007].

In Poland, similarly as in Italy, the implemented genetic resources conservation programme includes a native rabbit breed, i.e. Popielno White. That breed is well adapted to harsh environmental conditions, is characterised by good health and longevity, disease resistance and not dependent on chemical antibiotics. Thus they are recommended for rearing in organic and extensive farms [Bielański *et al*. 2007]. Currently, the production of rabbit meat in Poland (and in other central European countries) increases in low-input and small farms (usually family farms). That system is based on locally planted forage crops and is season-dependent. Rabbits are fed

green forage supplemented with grains in the summer season and root crops, hay and grains in winter. That strategy aims at obtaining high-quality meat derived from an extensive housing system to meet consumer expectations, including those looking for higher standards of animal welfare.

The aim of this work was to study fatty acid profiles of rabbit meat of seasonally fed slow-growing rabbits in relation to the lipid composition of feeds and their intake.

Material and methods

Experimental design and sample collection

The experiment was conducted throughout two seasons: summer (starting in mid-May) and winter (starting in mid-November) in a small farm situated in the Mazovia region in Poland. At the beginning of the respective season, male rabbits (Popielno White Rabbit breed) were weaned at 35 days of age, and 21 weighing 810 g ± 10% were randomly allocated to two homogenous groups: seasonally fed (summer – S or winter – W, 14 rabbits each) and a group of 7 rabbits fed pelleted diet all the time and serving as the controls (C) for the respective seasonally fed groups.

The diets of the S and W groups were season-dependent and consisted only of forages produced in the farm where the experiment took place (Poland, Mazovia Region). The summer diet (Group S) consisted of green forage (grass mixed with fresh-cut herbage *Medicago sativa*) and coarse grains (oats and triticale, 1:1), whereas the winter diet (Group W) consisted of carrots or beets, cooked potatoes, coarse grains (similarly as in the summer) and hay. The control group (group C) was fed a complete standard pelleted diet (typical of high-input production systems). Water was provided *ad libitum.* The rabbits were kept outdoors in comfortable wooden cages (1.20 m long × 0.80 m wide × 0.75 m high). The diet was composed so as to cover optimum nutrient requirements of young and fattening rabbits. Feed consumption was monitored by weighing the feed remainders before filling the troughs in the cages for the next day. The intakes of seasonal diet components (in g per rabbit per day) were as follows:

- summer diet (S): green forage: 350-750 g, coarse grains 30-50 g (depending on the month),
- winter diet (W): hay 30-65 g, carrots 120-140 g, potatoes 50-90 g (during the first three months), fodder beets 70-120 g (during two successive months), coarse grains 30-65 g (depending on the month).

The net consumed ratios were used to estimate the lipid and FA intakes. Body mass of every rabbit was measured at the beginning and the end of the trial and the average daily body mass gain (g/day) was computed. Each seasonal experiment lasted 145 days. The rabbits were slaughtered humanely (after 12-h feed withdrawal) at a commercial slaughterhouse located next to the farm, thereby avoiding transportation stress. The slaughter procedure was in compliance with Polish national regulations for commercial slaughtering. Warm carcasses were put in a ventilated area for 30 min,

then stored at 3-5°C for 24 h. Immediately after slaughter, muscles were collected from hind legs of a half-carcass. The meat was minced in a food processor after adding 0.01% of butyl hydroxyl toluene (BHT) to avoid lipid degradation.

Chemical analysis of forages and meat samples

Samples of feed components were collected, immediately frozen and stored at -20°C until assayed. Pooled samples of fodder were analyzed for feed components (dry matter, crude protein, crude fiber) by employing standard procedures [AOAC 2007]. Lipids from meat and from forages were extracted using a chloroform : methanol (2:1 vol/vol) mixture according to Folch *et al.* [1957]. The details of the extraction procedure were published earlier [Rutkowska *et al*. 2012].

Fatty acid analysis in meat and forage lipids

Methyl esters of FA (FAME) were prepared by transmethylation of fat samples using a mixture of concentrated H_2SO_4 (95%) and methanol [AOCS Official Method Ce 2-66]. The FA composition (as FAME) in fat samples was analysed by gas chromatography using an HP-Agilent 6890N (USA) instrument. The chromatograph was equipped with a flame ionization detector (FID), a split/splitless injector and capillary column (100 m × 0.25 mm I.D.) coated with high-polarity stationary phase Rtx 2330 (0.2 μm th; Restek Corp., USA). Conditions of FAME separation were published in detail elsewhere [Rutkowska *et al*. 2012]. Peak areas were corrected by the response factors for FAME responses of FID, and area% of FAME was appropriately converted to weight of FA. Standards Supelco 37 No. 47885-U [Sigma, Aldrich, USA] and BR2, BR3 [Larodan, Sveden] were used to determine the recovery rates and correction factors for individual FAs in fat samples. The contents of individual FAs were expressed as g/100 g FA.

The amounts of FAs in meat samples were used to compute the indices of atherohenicity (AI) and thrombogenicity (TI) as proposed by Ulbricht and Southgate [1991], and the hypocholesterolemic/hypercholesterolemic ratio (H/H) as suggested by Santos-Silva, Bessa and Santos-Silva [2002].

Statistical analysis

One-way ANOVA followed by Scheffé's *post-hoc* test was applied to the data, the diets being considered fixed terms. All FAs were processed independently. The level of $P \leq 0.05$ was considered significant.

Results and discussion

The study was conducted under natural farming conditions. The nutrition of two experimental groups of rabbits was based solely on forages planted locally, i.e. green forage supplemented with small amounts of coarse grains (oats and triticale) in the summer season (S) and root crops (potatoes, carrots and fodder beets), also with an

addition of coarse grains in winter (W). The detailed compositions of forages used in groups S and W and in the control (C) are presented in Table 1.

Table 1. Mean contents (% of dry matter) of main nutrients and of major fatty acids (g/100 g FA) in forages

Fatty acid	Seasonal feeding (groups S and W)						Control group standad pelleted diet
	green forage[1]	coarse grains[2]	fodder beets	carrots	potatoes	hay	
Dry matter	20.50±0.50	82.10±1.40	21.90±0.65	17.00±0.30	25.30±0.85	85.40±2.30	89.60±0.95
Lipids	2.10±0.08	3.00±0.14	0.62±0.04	0.56±0.07	0.35±0.01	1.50±0.10	3.36±0.26
Crude protein	21.80±1.75	14.85±0.92	1.22±0.15	1.05±0.12	2.65±0.42	18.20±1.76	17.10±1.20
Crude fiber	27.15±1.10	11.90±0.74	0.80±0.10	1.85±0.65	2.23±0.40	27.90±2.30	13.50±0.35
C12:0	0.23±0.01	0.17±0.00	ND	0.78±0.02	0.46±0.01	0.86±0.06	0.18±0.01
C14:0	0.93±0.02	0.55±0.02	1.73±0.04	1.07±0.04	1.32±0.04	1.54±0.09	0.26±0.01
C16:0	13.92±0.27	16.65±0.38	30.93±0.94	22.96±0.59	21.05±0.61	33.58±0.27	15.57±0.14
C18:0	1.71±0.05	1.56±0.03	ND	3.44±0.09	5.84±0.12	3.56±0.10	1.50±0.03
SFA	16.79±0.48	18.93±0.51	32.66±1.01	28.25±0.76	28.67±0.81	38.64±2.12	17.41±0.47
C16:1[3]	1.14±0.02	0.22±0.01	4.79±0.14	0.56±0.01	0.73±0.01	0.65±0.06	0.41±0.05
*c*9C18:1	2.06±0.09	13.57±0.32	9.13±0.29	8.85±0.28	5.19±0.17	7.32±0.14	22.91±0.19
MUFA	3.20±0.10	13.79±0.29	13.92±0.42	9.41±0.30	5.02±0.19	7.97±0.21	23.32±0.84
C18:2LA	11.55±0.25	55.18±1.48	46.03±1.28	51.17±1.54	37.06±1.28	13.47±0.18	45.16±0.46
C18:3ALA	52.85±0.95	6.26±0.12	5.24±0.12	4.49±0.12	20.30±0.86	28.84±0.13	7.24±0.09
PUFA	64.40±1.31	61.44±1.72	51.27±1.43	55.66±1.52	57.36±1.78	42.32±1.43	52.40±1.45

[1]Grass 50% + *Medicago sativa* 50%; [2]Oats and triticale 1:1; [3]C16:1 n-7.
ND – not detected; S – summer diet: green forage, coarse grains; W – winter diet: fodder beets, carrots, potatoes, hay, coarse grains.

When the two seasonal feeding regimens were compared, both S and C diets resulted in a higher productive performance of rabbits (average daily growth and slaughter live weight) when compared with the W diet (Tab. 2). An average daily weight gain, comparable with S-rabbits in our experiment, was reported for slow growing rabbits (the local grey-coloured population) in Italy, reared outdoors and

receiving pelleted diet [D'Agata *et al*. 2009, Schiavone *et al*. 2013]. In contrast, neither linseed diet [Kouba *et al*. 2008] nor fresh alfalfa fed ad libitum together with the pelleted diet [Capra *et al*. 2013] significantly modified the productive performance of hybrid rabbits.

The summer diet was associated with a higher (by about 7%) lipid intake than the W diet, due to a high lipid content in green forage (2.10%) and a considerably lower content of lipids in root crops (0.51%) in the case of W rabbits (Tab. 1 and 2). It was probably the main reason that the content of intramuscular fat in the meat of W rabbits was by 46 and 60% lower than in meat derived from S and C rabbits, respectively. In this study, the intramuscular lipid content (2.02 to 3.24%) was similar to that found in hybrid rabbits reared organically [Pla 2008] or outdoors in movable cages [Cavani *et al.* 2004]. In contrast, it was much higher than in slow-growing rabbits fed *ad libitum* a commercial pelleted diet plus alfa-alfa hay, reared outdoors or indoors (1.12, 1.40 and 1.63%, respectively; D'Agata *et al*. 2009, Preziuso *et al*. 2008] or in hybrid rabbits fed commercial pelleted diet plus fresh alfa-alfa (1.39% – Capra *et al*. 2013). This might have been due to differences in the age of rabbits – 105 days in slow growing rabbits, 92 days in hybrid rabbits, and 180 days in this study.

Table 2. Growth performance and estimated intakes of lipids and of fatty acids in seasonally fed and in control rabbits (means±SD)

Variable	S diet	C diet	W diet	C diet	S/W	C/S	C/W	C/C
Slaughter body mass (g)	4661±7	4785±8	4340±7	4750±5	***	***	*	***
Hot carcass mass (g)	2670±6	2750±7	2487±5	2722±5	***	***	***	***
Average daily body mass gain (g)	26.6±0.04	27.3±0.08	24.3±0.1	27.1±0.04	***	***	***	
Estimated intakes (g)[1]								
Lipids	465±4	460±9	432±6	468±6	***	*	***	*
SFA	81±4	80±4	130±5	82±4	***		***	
MUFA	30±3	107±4	45±7	108±4	***	***	***	
PUFA	295±6	243±6	219±6	245±6	***	***	***	
C18:3 n-3[2]	178±8	69±3	83±2	72±5	***	***	***	

[1]Throughout the experiment; [2] *9c 12 c 15c* C18:3.
*p<0.05; **p<0.01; ***p<0.001.

Season-related regimens affected the FA composition of meat (Tab. 3). The results of this study confirm reports of other authors reviewed by Dalle Zotte and Szendrö [2011], who stated that the FA composition of meat products from monogastric animals, e.g. pigs, poultry and rabbits, may be easily altered by diet. Winter forages (W group) – fodder beets, potatoes and carrots, contained higher amounts of saturated FA (SFA) (on average 29.90 g/100gFA) than in the summer (S) and control (C) groups (17.86 and 17.41 g/100gFA, respectively; see Tab. 1). This resulted in a higher SFA content in fat of meat from the W group. In contrast, feeding the S diet

(containing green forage) resulted in a lower content of atherogenic palmitic C16:0 and myristic C14:0 acids in fat of meat (13 and 38%, respectively) than in the W diet (Tab. 3). These findings were at variance with Dal Bosco *et al*. [2014], who reported a

Table 3. Mean contents (±SD) of fatty acids in meat (g/100 g FA) from seasonally fed (S and W) and from control (CS, CW) rabbits

Fatty acid	Summer		Winter		Significant differences		
	summer diet (S) n = 14	control diet (CS) n = 8	winter diet (W) n = 14	control diet (CW) n = 8	S/CS	W/CW	S/W
C8:0	0.18±0.01	0.17±0.02	0.15±0.03	0.18±0.03		*	*
C10:0	0.03±0.00	0.31±0.01	0.09±0.01	0.31±0.01	***	***	***
C12:0	0.08±0.01	0.09±0.00	0.21±0.01	0.09±0.00		***	***
C14:0	2.77±0.11	2.76±0.10	3.82±0.10	2.78±0.05		***	***
C16:0	30.74±0.54	31.08±0.21	34.77±0.27	30.69±0.33		***	***
C18:0	6.34±0.21	7.44±0.20	6.72±0.09	7.57±0.25	***	***	***
C20:0	0.11±0.01	0.14±0.00	0.18±0.01	0.14±0.00	**	***	***
C22:0	0.09±0.02	0.08±0.01	0.27±0.03	0.08±0.01		***	***
iso C12:0	0.02±0.00	0.00±0.00	0.02±0.00	0.00±0.00			
C13:0	0.03±0.00	0.03±0.00	0.05±0.06	0.03±0.00		***	***
iso C14:0	0.07±0.01	0.05±0.00	0.07±0.01	0.05±0.00	*	**	**
iso C15:0	0.14±0.01	0.08±0.01	0.13±0.01	0.08±0.01	***	***	*
anteiso C15:0	0.19±0.02	0.21±0.01	0.27±0.04	0.21±0.02	*	***	***
C15:0	0.66±0.05	0.49±0.01	0.58±0.04	0.49±0.01	***	***	***
C15:1	1.75±0.21	0.38±0.04	1.81±0.11	0.39±0.03	***	***	
iso C15:1	0.17±0.02	0.14±0.00	0.22±0.02	0.14±0.01	***	***	***
iso C16:0	0.06±0.01	0.00±0.00	0.10±0.01	0.00±0.00			
anteiso C17:0	0.17±0.04	0.14±0.01	0.20±0.03	0.14±0.01	*	**	
iso C17:0	0.51±0.05	0.48±0.01	0.61±0.08	0.49±0.02			
C17:0	0.67±0.09	0.12±0.00	0.39±0.02	0.12±0.01	***	***	***
C17:1	0.24±0.02	0.30±0.01	0.37±0.02	0.29±0.02	***	***	***
C19:0	0.15±0.03	0.00±0.00	0.22±0.03	0.00±0.00	***	***	***
C10:1	0.05±0.01	0.03±0.00	0.04±0.00	0.03±0.00	***	*	*
C16:1 n-9	0.43±0.05	0.30±0.01	0.50±0.05	0.30±0.01	***	***	***
C16:1 n-7	2.62±0.37	3.50±0.13	5.02±0.37	3.45±0.19	***	***	***
C18:1 t	0.25±0.05	0.00±0.00	0.00±0.00	0.00±0.00	***		***
c9C18:1	24.89±0.62	24.31±0.32	23.02±0.41	24.26±0.32	**	**	**
c11C18:1	1.00±0.05	1.21±0.04	1.53±0.15	1.19±0.04	***	***	***
c12C18:1	0.09±0.01	0.00±0.00	0.00±0.00	0.00±0.00	***		***
c13C18:1	0.10±0.02	0.00±0.00	0.00±0.00	0.00±0.00	***		***
C20:1n-9	0.08±0.02	0.11±0.01	0.04±0.01	0.11±0.01	**	***	***
C18:2 n-6	20.01±0.59	21.18±0.19	11.84±0.29	21.30±0.16	***	***	***
c9t11 C18:2	0.09±0.01	0.00±0.00	0.08±0.00	0.00±0.00	***	***	***
C18:3 n-3	1.95±0.09	2.04±0.10	1.23±0.07	2.04±0.07	*	***	***
C18:3 n-6	0.31±0.03	0.33±0.01	0.23±0.03	0.34±0.01	*	***	***
C20:2n-6	0.04±0.01	0.04±0.00	0.13±0.01	0.04±0.00		***	***
C20:3n-3	0.16±0.01	0.11±0.01	0.13±0.01	0.11±0.01	***	***	***
C20:3 n-6	0.19±0.04	0.49±0.01	0.19±0.01	0.49±0.02		***	***
C20:4 n-6	0.04±0.01	0.00±0.00	0.03±0.01	0.00±0.00	***	***	**
C20:5n-3	2.46±0.25	0.20±0.01	1.82±0.12	0.20±0.02	***	***	***
C22:5 n-3	0.56±0.06	0.10±0.01	0.51±0.02	0.10±0.01	***	***	**
C22:6 n-3	0.11±0.01	0.00±0.00	0.10±0.01	0.00±0.00	***	***	***

*p<0.05; **p<0.01; ***p<0.001.

2.4% increase of C16:0 in the meat of rabbits fed fresh alfalfa as complementary feed, whereas the content of C14:0 decreased by 50%. Our results are more promising that those of Capra *et al*. [2013], who recorded 2.2 and 14% decreases of C14:0 and C16:0, respectively, in rabbits fed a commercial pelleted diet supplemented by fresh alfalfa fed *ad libitum*. In our study, such a phenomenon was observed also with regard to stearic acid C18:0. Significantly lower contents were found in meat from seasonally fed rabbits (S and W) – 6.53 g/100gFA on the average, whereas samples from the C group contained 7.51 g/100gFA. As compared with the results of studies investigating the effects of supplementing rabbit diets with fresh alfalfa or alfalfa flax sprouts, seasonal diets in our experiment resulted in a lower content of stearic FA [Capra *et al*. 2013, Dal Bosco *et al*. 2014, Dal Bosco *et al*. 2015].

As compared with other reports on slow-growing rabbits, the SFA content in this study was lower than that reported by Schiavone *et al*. [2013], while it was somewhat higher than that obtained by D'Agata *et al*. [2009], who studied the FA profile of "grey-coloured" rabbits fed an organic diet. Those differences may have also resulted from the age at slaughter. However, the diet is considered the main factor affecting the FA profile of rabbit meat [Dalle Zotte and Szendrö 2011].

In our study, meat samples differed significantly also in the content of total short chain SFA (SCSFA), the lowest amount being recorded in the S group. Samples from the W-group contained significantly ($P<0.05$) more capric (C10:0) and lauric (C12:0) acids compared with the S and C groups (Tab. 3 and 4).

Table 4. Mean contents (±SD) of fatty acid categories in meat (g/100 g FA) and selected fatty acid indices in seasonally fed (S and W) and in control (CS, CW) rabbits

Fatty acid	Summer		Winter		Significant differences		
	summer diet (S) n = 14	control diet (CS) n = 8	winter diet (W) n = 14	control diet (CW) n = 8	S/CS	W/CW	S/W
SCSFA	0.28±0.02	0.58±0.03	0.45±0.03	0.58±0.02	***	***	***
LCSFA	40.61±0.63	41.49±0.17	45.77±0.32	41.26±0.40	**	***	***
OBCFA	4.84±0.16	2.41±0.05	5.03±0.50	2.43±0.04	***	***	*
MUFA	29.51±1.19	31.14±0.63	28.40±0.99	31.14±0.63	***	***	***
PUFA	25.59±0.56	24.50±0.23	16.28±0.59	24.63±0.14	***	***	***
n-6	20.60±0.60	22.05±0.19	12.42±0.29	22.17±0.15	***	***	***
n-3	5.23±0.28	2.45±0.10	3.78±0.12	2.46±0.08	***	***	***
AI	0.80±0.03	0.84±0.01	1.23±0.02	0.83±0.01			
TI	1.01±0.03	1.32±0.02	1.50±0.02	1.31±0.02	***	**	***
H/H	1.49±0.04	1.41±0.02	1.00±0.01	1.43±0.02		***	***
n-6/n-3	3.95±0.26	9.01±0.36	3.29±0.16	9.02±0.32	**	***	***
Lipid (%)	2.96±0.08	3.24±0.06	2.02±0.10	3.24±0.06	***	***	***

*p<0.05; **p<0.01; ***p<0.001.
SCSFA – short-chain saturated fatty acids; LCSFA – long-chain saturated fatty acids; OBCFA – odd-numbered and branched-chain fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; AI – atherogenic index; TI – thrombogenic index; H/H – hypocholesterolemic/hypercholesterolemic ratio.

When the two groups of control rabbits (CS – summer and CW – winter) were compared with respect to the FA composition of meat lipids, no significant differences in any FA was found except for the palmitic acid (C16:0) content, which was slightly higher (by 1.3%; $P<0.05$) in the summer control group. The control rabbits from both groups were fed the same pelleted diet throughout the year (as described in the Materials and Methods).

On the other hand, summer forages administered to rabbits in the summer season (S group) were rich in PUFA compared with the W and C groups, while the grass×alfalfa mixture was rich in α-linolenic acid (*9c12c15c C18:3*; Tab. 1). This resulted in a higher content of PUFA in meat of group S (25.59 g/100 g FA) than in group W (16.28 g/100 g FA), while the same applied to the principal n-6 and n-3 acids (linoleic and α-linolenic; Tab. 3). Similar findings were reported by Capra *et al*. [2013] and by Forrester-Anderson *et al*. [2006]. Meat derived from the S group contained two-fold greater amounts of PUFA belonging to the n-3 family of FA, than that from the C group, probably because of a much greater intake of α-linolenic acid with the summer diet as compared to both other groups (W and C; see Tab. 2 and 3). Capra *et al*. [2013] reported that the feeding strategy including fresh alfalfa significantly improved the composition of fat, mainly by increasing the content of α-linolenic acid (n-3 FA). Also Dal Bosco *et al*. [2014] confirmed that alfalfa added to the diet of rabbits resulted in a 2-fold increase of n-3 FA content in meat; in the case of α-linolenic acid, the increase was 3-fold when compared with the C group. Regarding the content of linoleic acid, our results (for the S-group) are in agreement with those given by Schiavone *et al*. [2013], who studied FA in meat of slow-growing rabbits.

Among the FAs from the n-3 group, the long-chained PUFA, which are products of desaturation and elongation of α-linolenic acid, are considered the most important [Poławska *et al*. 2011, Poławska *et al*. 2013]. As demonstrated in this study, as well as by Dalle Zotte and Szendrö [2011], rabbit meat contained several valuable long-chain FAs: C20:3, C20:5 – EPA, C22:5 – DPA and C22:6 – DHA. Their total content was higher in meat from the S than W group. When compared with the C group, much higher contents of C20:5 – EPA, C22:5 – DPA and C22:6 – DHA were recorded in meat from seasonally fed rabbits, especially in samples derived from the S diet. It needs to be emphasised that EPA and DHA are the most bioactive forms of n-3 FA, while α-linolenic acid exhibits low bioactivity, its conversion to EPA being very low in human beings. As reported by Forrester-Anderson *et al*. [2006], the contents of EPA, DPA and DHA increased in animals fed green forages. Also other reports confirmed that pasture-rearing and organic production may provide alternative (natural) methods of enhancing the content of n-3 FA in rabbit meat, thus offering consumers a healthier choice [Capra *et al*. 2013, Dal Bosco *et al*. 2014, Pla 2008, Dalle Zotte and Szendrö, 2011]. Also Leiber *et al*. [2008] and Strzałkowska *et al*. [2009] indicated a positive effect of alfalfa on the endogenous chain elongation in the n-3 family FA.

It should be emphasized that meat derived from seasonally fed rabbits (S and W) had a favorable n-6/n-3 ratio: 3.95 and 3.29, respectively (Tab. 4). The discussed

ratio is below 4, as recommended for the human diet [Simopoulos 2002]; a similar ratio was found in rabbits fed linseed diet [Kouba *et al*. 2008]. A more favourable, lower ratio (1.9) was found in rabbits fed a diet supplemented with fresh alfalfa [Dal Bosco *et al*. 2014]. Because of an excessive intake of PUFA (n-6 FA) in the human diet, consumption of food containing n-3 FA should be increased [Simopoulos 2009]. According to López-Farré and Macaya [2006], three main mechanisms are involved in the cardiovascular protective effect of n-3 fatty acids: their anti-inflammatory effect, antithrombotic effect and anti-arrhythmic action. By increasing the ratio of n-3/n-6 PUFA in the Western diet, the incidence of these chronic inflammatory diseases might be reduced [Patterson *et al*. 2012]. Due to the favourable FA composition (high levels of unsaturated and especially long-chain n-3 PUFA), the meat of seasonally fed rabbits (the summer season) may constitute a valuable component of human diet.

A relatively high content of MUFA in rabbit meat may have been expected irrespectively of the adopted feeding strategy. Among MUFA, oleic acid *c9*C18:1 predominated, the S group having slightly higher values than the W group (24.89 and 23.02 g/100gFA, respectively). The results obtained in this study were higher than those reported by Dal Bosco *et al*. [2014] for the meat of rabbits supplemented with fresh alfalfa, and by Cavani *et al*. [2004] for rabbits reared outdoors, but similar when compared with those reared indoors (24%). The level of oleic acid reported by Preziuso *et al*. [2008] in meat of slow-growing rabbits (*Grigia Rustica*) kept outdoors or by Paci *et al*. [2014] in slow-growing "grey" rabbits was similar to that reported in this study. An opposite trend was observed for palmitoleic FA (C16:1 n-7), whose content in meat derived from the W diet was about 30% higher compared with samples of rabbits fed the S diet. However, it should be emphasised that the content of C16:1 n-7 FA in meat of our Popielno White rabbits was greater irrespectively of the diet (S, W or C), and exceeded the levels reported by other researchers [D'Agata *et a*l. 2009, Paci *et al*. 2013, Dal Bosco *et al*. 2014, Forrester-Anderson *et al*. 2006]. Thus the main factor determining the content of that FA may have been the rabbit breed. Studies with animal models revealed a beneficial effect of palmitoleic acid on reducing muscle insulin resistance and preventing beta-cell apoptosis [Yang *et al.* 2011].

Lower contents of SFA and higher of MUFA and PUFA in the S and C group resulted in a more favourable atherogenic (AI) value (0.80) than in the W group (1.23) (Table 4). A lower AI value was recorded in rabbits fed a diet supplemented with fresh alfalfa (0.60; Capra *et al*. 2013; Dal Bosco *et al*. 2014). In our study the S diet resulted in a better thrombogenic index (TI) value (1.01) when compared with other regimens (W and C) and slightly lower than reported by Dal Bosco *et al*. [2014]. Moreover, the S diet rich in green forage, similarly as in the C diet, resulted in a favourable hypocholesterolemic/hypercholesterolemic ratio (H/H) (Tab. 4), as confirmed by Dal Bosco *et al*. [2014].

Regarding the FA composition of rabbit meat the significance of microbial lipid digestion with significant microbial fermentation in the hindgut (*caecum*) is of importance. The microbial fermentation of ingested feed is associated with

biohydrogenation and isomerisation of unsaturated FA. This results in generating considerable amounts of several *trans*-FA, conjugated linoleic (CLA) and stearic acids, as well as branched-chain FA (BCFA) in *de novo* synthesis [Leiber *et al*. 2008]. In this study, meat samples from the S group contained appreciable amounts of *trans* C18:1 FA, its content being undetected either in the W or in C samples. This suggests a less intense lipid metabolism in the rabbits of the W group, in which diet carrots, potatoes and fodder beets predominated. However, substantial amounts of OBCFA (in which 15 compounds were identified), including BCFA, were found in analysed meat samples from seasonally fed rabbits (S and W) irrespectively of the season (two-fold greater than in the C group), those amounts being favourably higher than those reported by other researchers [Papadomichelakis *et al*. 2010b]. The anticancer activity of BCFA attracted great attention; *iso* and *anteiso* C15:0 inhibited various cancer lines both *in vitro* and *in vivo*, by inducing apoptosis without toxic side-effects [Yang *et al*. 2000]. The highest content (0.4g/100 g FA) of those acids was assayed in samples derived from the W group, the contents of C17:0 and C15:0 being higher in the S than in the W rabbits. Seasonal feeding (S and W) resulted also in the synthesis of the main CLA isomer – *c9t11*C18:2, which content (0.085 g/100 g FA, on average) was similar to that found by Leiber *et al*. [2008] in New Zealand breed rabbits fed various diets (forage – ryegrass meal or alfalfa meal – vs. forages + oats), slaughtered after a 25-week fattening period. Our studies confirmed the statement of Papadomichelakis *et al*. [2010a] that increasing BCFA in rabbit meat by dietary intervention may prove beneficial in human nutrition.

In conclusion, the presented results showed that the meat of Popielno White rabbits fed the summer diet had remarkable nutritional attributes: high levels of PUFA, especially the long-chain n-3 (C20:5 – EPA, C22:5 - DPA and C22:6 – DHA), that improved the thrombogenic index. Seasonal diet, irrespectively of the season, improved the n-6/n-3 ratio and the content of OBCFA when compared to the control (commercial) diet. Irrespectively of the diet, the high content of palmitooleic acid was distinctive for the meat of Popielno White rabbits. This local population seems to be well adapted to the feeding strategies used with outdoor rearing, practiced in small farms. Thus, initiatives including promotion of native, slow-growing rabbit breeds as a source of valuable and functional nutrients, deserve to be supported.

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