EFFECT OF SUPPLEMENTATION OF LINSEED OIL, VITAMIN E AND SELENIUM IN DIET ON MEAT QUALITY OF GROWING RABBITS

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ABSTRACT

The objective of our study was to examine the fatty acid (FA) profile and the selenium (Se) and vitamin E contents of meat from rabbits fed on a diet enriched with linseed oil, vitamin E and Se. Supplemented diets were enriched with 180 mg vitamin E/kg, whereas Se content was increased from 0.1 to 0.46 mg/kg and 3% linseed oil replaced the same amount of sunflower oil. Rabbits were weaned at the age of 5 weeks, and the supplementation (S) was fed for 1 (S1), 2 (S2), 3 (S3) or 4 weeks (S4) in the pre-slaughter period. The control group (C) was fed a non-supplemented diet throughout the study. Rabbits were slaughtered at 11 weeks of age and *Longissimus dorsi* (MLD) meat and thigh (HL) were sampled. The ∑n-6 FA content of HL of S4 was significantly lower than the other groups ($P<0.01$); this decrease was continuous and statistically significant ($P<0.001$) in the MLD for all groups. The n-3 FA content increased, compared to the C group, of 2.5, 4.8, 4.8 and 3.6-fold in the HL, and of 2.0, 2.8, 3.1 and 3.7-fold in the MLD, for groups S1, S2, S3 and S4, respectively. The larger increment was found between groups C and S4 for C20:5 n-3 (increased by 5.9 and 5.7-fold), which was followed by C18 n-3 (increased by 4.0 and 4.7-fold), by C22:5 n-3 (increased by 2.8 and 3.1-fold), and by C22:6 n-3 (increased by 2.0 and 2.5-fold) in the HL and MLD, respectively. The n-6/n-3 ratio decreased after 2 weeks of S feeding from 14.8 and 13.6 to 4.5 and 4.2, and in the S4 group to 2.3 and 2.4, for HL and MLD respectively. The vitamin E content increased significantly from C and S3 and S4 only in the MLD, whereas in the HL its concentration was lower in the S3 group compared to C and S1. In the meat of the S diet-fed-rabbits the Se concentration increased parallelly with the feeding time interval. Compared to the C group, S1, S2, S3 and S4 groups the Se increment was of 1.4, 1.6, 1.8 and 2.0-fold in the HL, and of 1.4, 1.4, 1.6 and 2.0-fold in the MLD. Results have proven that 2-3 weeks of dietary supplementation of linseed oil, vitamin E and Se increases significantly their content in the rabbit meat, improving the functional value of the rabbit meat.

Key words: Rabbits, meat quality, fatty acids, Vitamin E, selenium.

INTRODUCTION

Since the industrial revolution, particularly the last 150 years a rapid change in human diet could be observed. Today, the Western diets are characterized by an increase in energy, saturated fat, omega-6 fatty acids (FA) and trans FA, cereal grain, and a decrease in omega-3 FA, complex carbohydrates and fibre, fruits and vegetables, protein, antioxidants, vitamins especially C, E and D, trace elements and calcium intake (Simopoulos, 2008). The optimal ratio of omega-6/omega-3 varies from 1/1 to 4/1 but in the Western diets is 15/1 to 25/1 or higher (Simopoulos, 2010). The beneficial health effect of omega-3 FA, C20:5 n-3 (EPA) and C22:6 n-3 (DHA), relates to low rate of coronary heart disease, asthma, hypertension, type 1 diabetes, osteoporosis, arthritis and other inflammatory and autoimmune diseases, obesity, dry eye disease, age-related macular degeneration, mental health, multiple sclerosis, many cancers, especially cancer of breast, colon and prostate (Simopoulos, 2008). DHA is essential for normal functional development of brain and retina, particularly in premature infants (Simopoulos, 2011). Vitamin E (αtocopherol) is the most effective chain-breaking lipid-soluble antioxidant (Valk and Hornsta, 2000). In humans, because its antioxidative properties, vitamin E is believed to help prevent diseases associated with oxidative stress, such as cardiovascular disease, cancer, chronic inflammation and neurologic disorders. However, recent clinical trials undertaken to prove this hypothesis failed to verify a consistent benefit (Brigelius-Flohé *et al*., 2002). According to Zhang *et al*. (2010) in humans, selenium (Se) deficiency is associated with decreased immune function resulting in increased susceptibility to cancer, cardiovascular diseases, muscular dystrophy, diabetes, arthritis, stroke cataracts and other diseases. Reilly (1998) gives a more detailed list of diseases induced in compromised human Se supply. Whereas major changes have taken place in human diet our genes have not changed. Humans today live in a nutritional environment that differs from that for which our genetic constitution was selected (Simopoulos, 2010). The functional foods aim to decrease the gap between the Western diet and the genetically determined demands. Rabbit meat itself is very healthy, but with targeted specific feeding its nutritive value can be further improved (Dalle Zotte and Szendrő, 2011).

The objective of our study was to examine the health protective effect of meat from rabbits fed on a diet with linseed oil, vitamin E and Se supplementation. In particular the ∑n-3 FA content, the n-6/n-3 FA ratio and the vitamin E and Se contents was considered.

MATERIALS AND METHODS

Animals and experimental design

The experiment was carried out at the Kaposvár University using the maternal line of the Pannon Breeding Programme. The rabbits (n=360) were weaned at the age of 5 weeks and housed in pairs. The daily lighting period was 16 hours, while the temperature ranged between 16-19 °C.

Rabbits were randomly allocated into 5 experimental groups; one group was fed control diet (C) during the entire experiment (until 11 weeks of age), while in the other 4 groups the rabbits were fed diet C after the weaning until the finishing phase. In the finishing phase rabbits were fed for 1, 2, 3 or 4 weeks with an experimental diet (S) containing linseed oil, vitamin E and selenium.

The sunflower oil, Se and vitamin E content of the C diet (crude protein: 15.7%; crude fibre: 16.8%) were 3%, 0.1 mg/kg and 60 mg/kg, respectively, and as medication it contained 0.5% Robenidin. The sunflower oil content of the S diet was substituted with 3% linseed oil, whereas its vitamin E and Se content was 0.46 mg/kg and 260 mg/kg. Organic Se was used as Se supplement (Sel-Plex, Alltech). During the last week of the experiment all groups were deprived of coccidiostatic. Dietary FA, vitamin E and Se contents are shown in Table 1.

Rabbits were slaughtered at the end of the trial (at 11 weeks of age) and the carcass was dissected according to the recommendations of the WRSA (Blasco and Ouhayoun, 1996). Meat samples of m. *Longissimus dorsi* (MLD) and hind leg (HL) were taken, and stored at -80 °C until chemical analysis.

Laboratory analysis

MLD and HL samples were extracted according to Folch *et al.* (1957) method and lipids were converted to methyl esters according to Christie (2003) after addition of C19:0 internal standard. Gas chromatography was performed as described earlier (Szabó *et al*., 2005). FA results were given as mg FA/kg of meat. Vitamin E content was determined with a HPLC method (Qingping *et al.,* 1996), whereas Se with a fluorimetric method (Rodriguez *et al.,* 1999).

Statistical Analysis

The meat quality traits were evaluated with one-way ANOVA, using SPSS10.0 software package.

Table 1: Fatty acids, vitamin E and selenium contents of diets (mg/kg)

	Diets	
	Control (C)	Supplemented (S)
C18:3 n-3	1.52	15.11
C20:3 n-3	0.014	0.017
C18:2 n-6	23.5	17.3
C18:3 n-6	0.134	0.143
C20:2 n-6	0.018	0.024
C20:3 n-6	0.012	0.026
∑ SFA	6.93	6.63
∑ MUFA	1.27	9.40
∑ PUFA	25.5	32.6
∑ n-6	23.7	17.5
∑ n-3	1.53	15.1
n-6/n-3	15.5	1.16
Vitamin E	60	240
Selenium	0.1	0.46

RESULTS AND DISCUSSION

Highly Unsaturated FA (HUFA) content, FA classes, vitamin E and Se content of meat samples are summarized in Table 2. The HUFA profile of meat is primarily determined by the diet. In our study the n-6 FA provided by sunflower oil inclusion was substituted with the n-3 FA of the linseed oil. The saturated FA (SFA) content was significantly lower in the HL of groups S4 compared to S2 (7.88 *vs* 13.85 mg/kg; $P<0.05$), whereas in the MLD a decreasing trend was found from S1 to S4 ($P=0.051$). The MUFA content remained unchanged in both meat portions, notwithstanding the large difference between diets (Table 1). The polyunsaturated FA (PUFA) content of HL was significantly lower in the S4 group, compared to the S2 and S3 groups, while in the MLD only a trend was found between groups C and S4 ($P=0.092$). The Σ n-6 content in HL meat was the lowest in S4, whereas in the MLD the decrease from C to S4 was continuous and statistically significant ($P<0.001$). The Σ n-3 FA content of HL significantly ($P<0.001$) increased of 2.5, 4.8, 4.8 and 3.6-fold in S1, S2, S3 and S4 when compared to the C, whereas in the MLD the increase was of 2.0, 2.8, 3.1 and a 3.7-fold, respectively ($P<0.001$). The largest increment was found between groups C and S4 for C20:5 n-3 (x 5.9 and 5.7-fold), followed by C18 n-3 (x 4.0 and 4.7-fold), C22:5 n-3 (x 2.8 and 3.1-fold), and C22:6 n-3 (x 2.0 and 2.5-fold) in the HL and MLD, respectively. The n-6/n-3 ratio decreased significantly after 1 week of S feeding in both HL and MLD meat.

The increase of C18:3 n-3 of rabbit meat can be achieved most effectively with the feeding of whole linseed or linseed oil supplementation. Close correlations between the content of whole linseed in the diet and the content of C18:3 n-3 in meat were found (Bianchi *et al.,* 2009). In numerous studies the above-mentioned supplementation was applied since weaning (Dal Bosco *et al.,* 2004; Kouba *et al*., 2008; Bianchi et al., 2009; Tres *et al*., 2009) thus increasing C:18 n-3 in the meat and decreasing its n-6/n-3 ratio. Szabó *et al.* (2001) concluded that after a 4-week feeding the FA profile of rabbit meat can be effectively modified when animals are fed diets with different FA supplementations. Our results proved that a single week on the S diet before slaughter can increase C18:3 n-3 by two-fold and beat down the n-6/n-3 ratio by 50%. With two and three weeks of S-diet feeding these differences were three and four-fold lower. Literature data report a supplementation effectiveness for 2-3 weeks feeding before slaughter (Bianchi *et al*., 2006; Maertens *et al.,* 2008; Gigaud and Combes, 2008). The shorter feeding period is reasoned mostly by the increased cost of the supplemented diet.

The vitamin E content increased significantly ($P<0.001$) in the MLD between groups C vs S3 and S4, but in the HL its content was lower in S3 than C and S1 groups. In other studies the vitamin E supplementation induced, at least, a 2-fold increase in rabbit meats (Castellini *et al*., 1998; Zsédely *et al*., 2008; Tres *et al*., 2009).

Table 2: Highly unsaturated fatty acid (HUFA), selenium and vitamin E content (mg/kg meat) in meat samples.

	Dietary groups					SE	Prob.
	C	S1	S2	S3	S4		
			HL meat				
n	10	10	10	10	10	-	-
C18:3 n-3	0.931^{a}	2.597^{ab}	4.038^{bc}	5.212^{c}	3.711^{bc}	0.292	<0.001
C20:3 n-3	0.101^{b}	0.102^{b}	0.095^{ab}	0.098^{b}	0.081^{a}	0.002	0.005
C20:5 n-3	0.011^{a}	0.034^{b}	0.042^{b}	0.058^{c}	0.065^{c}	0.003	<0.001
C22:5 n-3	0.093^{a}	0.133^{b}	0.163^{c}	0.217^{d}	0.257^{e}	0.009	<0.001
C22:6 n-3	0.021^{a}	0.024^{ab}	0.027^{b}	0.034^{c}	0.042^{d}	0.001	<0.001
C18:2 n-6	15.76^{b}	16.90^{b}	17.43^{b}	16.16^{b}	8.19^{a}	0.916	0.004
C18:3 n-6	0.077^{b}	0.078^{b}	0.080^{b}	0.077^{b}	0.044^{a}	0.004	0.009
C20:2 n-6	0.176^{b}	0.180^{b}	0.164^{b}	0.153^{ab}	0.085^{a}	0.009	0.002
C20:3 n-6	0.018^{a}	0.045^{ab}	0.061^{bc}	0.082^{c}	0.054^{bc}	0.004	<0.001
C20:4 n-6	0.913^{b}	0.892^{b}	0.865^{b}	0.850^{ab}	0.749^{a}	0.014	<0.001
∑ SFA	11.36^{ab}	12.35^{ab}	13.85^{b}	13.39^{ab}	7.88^{a}	0.692	0.044
∑ MUFA	8.65	9.80	10.99	10.23	5.91	0.600	0.061
∑ PUFA	18.10^{ab}	20.99^{ab}	22.96^{b}	22.94^{b}	13.28^{a}	1.101	0.020
∑ n-6	16.94^{b}	18.10^{b}	18.60^{b}	17.32^{b}	9.13^{a}	0.936	0.004
∑ n-3	1.158a	2.891^{ab}	4.365^{bc}	5.618^{c}	4.155^{bc}	0.300	<0.001
n-6/n-3	14.79^{d}	6.37^{c}	4.49^{b}	3.20^{a}	2.27^{a}	0.654	<0.001
Vitamin E	28.81^{b}	28.12^{b}	27.24^{ab}	18.72^{a}	24.70^{ab}	1.06	0.012
Selenium	64.5^{a}	89.1^{b}	101.9^{bc}	115.2^{cd}	126^{d}	3.47	<0.001
			m. *Longissimus dorsi*				
n	10	10	10	10	10	-	-
C18:3 n-3	0.198^{a}	0.501^{b}	0.723^{bc}	0.805^{c}	0.934	0.048	<0.001
C20:3 n-3	0.064^{c}	0.060^{bc}	0.054^{ab}	0.048^{a}	0.049^{a}	0.001	<0.001
C20:5 n-3	0.009^{a}	0.023^{b}	0.033^{c}	0.043^{d}	0.051^{e}	0.002	<0.001
C22:5 n-3	0.054^{a}	0.082^{b}	0.101^{c}	0.132^{d}	0.167^{e}	0.006	<0.001
C22:6 n-3	0.011^{a}	0.015^{b}	0.017^{b}	0.022^{c}	0.028^{d}	0.001	<0.001
C18:2 n-6	3.813^{c}	3.364^{c}	3.122^{bc}	2.403^{ab}	2.224^{a}	0.128	<0.001
C18:3 n-6	0.022^{c}	0.020^{bc}	0.019^{abc}	0.016^{ab}	0.015^{a}	0.001	<0.001
C20:2 n-6	0.049^{c}	0.043^{bc}	0.034^{ab}	0.032^{a}	0.026^{a}	0.002	<0.001
C20:3 n-6	0.005^{a}	0.013^{b}	0.015^{bc}	0.021^{cd}	0.023^{d}	0.001	<0.001
C20:4 n-6	0.681^{d}	0.655^{cd}	0.609^{bc}	0.554^{ab}	0.527^{a}	0.011	<0.001
∑ SFA	3.47	3.36	3.33	2.74	2.70	0.095	0.051
∑ MUFA	2.36	2.32	2.41	1.89	1.91	0.084	0.115
∑ PUFA	4.91	4.78	4.73	4.08	4.04	0.132	0.092
∑ n-6	4.569^{c}	4.095^{c}	3.801^{bc}	3.026^{ab}	2.814^{a}	0.136	<0.001
∑ n-3	0.336^{a}	0.681^{b}	0.928^{bc}	1.050^{cd}	1.229^{d}	0.055	<0.001
n-6/n-3	13.59^{e}	6.01^{d}	4.16^{c}	2.94^{b}	2.37^{a}	0.587	<0.001
Vitamin E	19.69^{b}	16.47^{a}	23.30^{bc}	25.30^{c}	25.85^{c}	0.76	<0.001
Selenium	73.1^{a}	99.6^{b}	105.1^{b}	113.4^{b}	147.4^{c}	4.01	<0.001

$^{a, b, c, d}$ Means in a row with different subscripts were significantly different (P< 0.05)

In the meat of the S-diet-fed rabbits the Se concentration increased significantly with the feeding time interval (P<0.001). Compared to the C group, in the HL the Se increment was of 1.4, 1.6, 1.8 and 2.0-fold whereas in the MLD it was of 1.4, 1.4, 1.6 and 2.0-fold, for S1, S2, S3 and S4 groups, respectively. Dokoupilová *et al.* (2007) applying for 5 weeks the same organic Se supplementation used in our study at level of 0.50 mg/kg (instead of 0.12 in C diet) realized a 4-fold Se increase in both loin and HL meat. Large Se enrichments were found on meat of other species (Zhang *et al.*, 2010). Se is a part of the body's antioxidant defence system and with dietary Se supplementation the Se concentration in the meat can be effectively increased, improving the functional value of the rabbit meat (Dalle Zotte and Szendrő, 2011).

CONCLUSIONS

Results have proven that selenium, vitamin E and linseed oil supplementation improves the functional value of the rabbit meat. To reach the demanded effect a 2-3 week feeding period is recommended.

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