

THE EFFECT OF LINSEED OIL SUPPLEMENTATION ON PERFORMANCE, FATTY ACID COMPOSITION AND OXIDATIVE STATUS OF RABBITS

Tina TREBUŠAK¹, Alenka LEVART², Mojca VOLJČ³, Urška TOMAŽIN⁴, Tatjana PIRMAN⁵

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The effect of linseed oil supplementation on performance, fatty acid composition and oxidative status of rabbits

The objective of the present study was to determine the effect of linseed oil supplementation on performance, fatty acid composition and oxidative status of rabbits. Twelve male SIKA rabbits were divided into two groups. The control group (n = 4) received commercial diet and the linseed group (n = 8) received commercial diet containing 9% of linseed oil, which was sprayed onto the pellet. Rabbits were slaughtered at 115 days of age, 52 days after the start of the experiment. Live weight, weight gain, feed intake and feed efficiency were recorded. The fatty acid composition of muscle, adipose tissue and liver was determined by the in situ transesterification method and gas chromatography-flame ionisation detection (GC-FID). In order to evaluate the oxidative status of rabbits, the malondialdehyde (MDA) concentration in plasma, liver and muscle were measured. The results show that it is possible to enhance proportion of n-3 polyunsaturated fatty acids (PUFA) in rabbit's muscle, adipose tissue and liver by adding linseed oil in the diet without detrimental effect on productive performance. Linseed oil addition improved fatty acid composition in all tissues by increased PUFA proportion and decreased proportion of saturated and monounsaturated fatty acid. Linseed oil also reduced n-6/n-3 PUFA ratio in all tissues. However, linseed oil addition led to significantly higher MDA concentrations in plasma, liver and muscle. Since PUFA are highly susceptible to oxidation, further research is needed to focus on protecting animal and their products from lipid oxidation by adding various natural antioxidants to the diet.

Key words: rabbits / animal nutrition / feed additives / linseed oil / fatty acid composition / oxidative status / performance

Vpliv dodatka lanenega olja v krmo na proizvodne lastnosti, maščobnokislinsko sestavo in oksidacijski status kuncev

V poskusu smo preučevali vpliv dodatka lanenega olja v krmo na proizvodne lastnosti, maščobnokislinsko sestavo in oksidacijski status kuncev. Dvanajst kunccev slovenske mesne linije SIKA smo razdelili v dve skupini. Kontrolna skupina (n = 4) je uživala standardno krmo, poskusna skupina (n = 8) pa standardno krmo z dodatkom 9 % lanenega olja, ki smo ga nanесли na pelete. Kunce smo zaklali pri starosti 115 dni, 52 dni po začetku poskusa. V času poskusa smo zapisovali maso živali, prirast, zauživanje in izkoristek krme. Maščobnokislinsko sestavo mišic, maščobnega tkiva in jeter smo določili z in situ transesterifikacijo in kapilarno plinsko kromatografijo. Za določitev oksidacijskega statusa kuncev smo izmerili koncentracijo malondialdehida (MDA) v plazmi, jetrih in mišici. Rezultati so pokazali, da lahko z dodatkom lanenega olja v krmo kuncev povečamo delež n-3 večkrat nenasičenih maščobnih kislin (VNMK) v mišici, maščobnem tkivu in jetrih brez negativnih učinkov na proizvodne lastnosti. Dodatek lanenega olja je izboljšal maščobnokislinsko sestavo s povečanjem deleža VNMK in zmanjšanjem deleža nasičenih in enkrat nenasičenih maščobnih kislin, kar je znižalo tudi razmerje n-6/n-3 VNMK v vseh tkivih. Dodatek lanenega olja je značilno povečal koncentracijo MDA v plazmi, jetrih in mišici. Ker so VNMK zelo podvržene oksidaciji, so potrebne nadaljnje raziskave, v katerih se bo potrebno osredotočiti na zaščito živali in njihovih produktov pred lipidno oksidacijo z dodajanjem različnih naravnih antioksidantov v krmo.

Gljučne besede: kunci / prehrana živali / krmni dodatki / laneno olje / maščobnokislinska sestava / oksidacijski status / proizvodne lastnosti

1 Univ. of Ljubljana, Biotechnical Fac., Dept. of Animal Science, Groblje 3, SI-1230 Domžale, Slovenia, e-mail: tina.trebusak@bf.uni-lj.si

2 Same address as 1, Ph.D., e-mail: alenka.levart@bf.uni-lj.si

3 Same address as 1, e-mail: mojca.voljc@bf.uni-lj.si

4 Same address as 1, e-mail: urska.tomazin@bf.uni-lj.si

5 Same address as 1, Ass.Prof., Ph.D., e-mail: tatjana.pirman@bf.uni-lj.si

1 INTRODUCTION

In these days people are more and more aware of the importance of healthy diet. Polyunsaturated fatty acids (PUFA), especially n-3 PUFA, are known for their beneficial effects on human health. Western diets have a low intake of n-3 PUFA and a relatively higher consumption of n-6 PUFA and that leads to a very high n-6/n-3 PUFA ratio. According to this we can speculate that population in the developed world do not consume enough essential fatty acids with food. Recent recommendations for human diets suggest increased n-3 PUFA consumption and decreased the n-6/n-3 PUFA ratio. Deficiency of n-3 PUFA increases the risk of developing various diseases, especially cardiovascular diseases. Therefore, interest in obtaining animal products with high levels of n-3 PUFA has increased. The fatty acid composition of meat and other animal products (eggs, milk), intended for human consumption, can be improved by specific dietary strategies (Leskanick and Noble, 1997; Raes *et al.*, 2004; Wood *et al.*, 2003). Because of its high α -linolenic acid (C18:3 n-3) content, linseed or linseed oil is a suitable and frequently used plant source of n-3 PUFA. α -linolenic acid is the precursor fatty acid for the synthesis of eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3), which have beneficial cardiovascular and also anti-inflammatory properties (Connor, 2000).

The beneficial effects of PUFA on human health are well known and have been documented by numerous studies (review of Williams, 2000; review of Riediger *et al.*, 2009). Great numbers of experiments have been made in order to increase PUFA content in animal products (Leskanick and Noble, 1997; Bernardini *et al.*, 1999; Kouba *et al.*, 2003; Raes *et al.*, 2004; Wood *et al.*, 2003). Rabbit meat is often recommended by nutritionists because of its low lipid and cholesterol levels and high content of PUFA compared to other meat (Dalle Zotte, 2004). Fatty acid composition of rabbit meat is better compared to other meat, because rabbit's diet usually contains alfalfa with high n-3 PUFA content. With the addition of linseed oil in the diet the proportion of n-3 PUFA in meat increases and also affects the reduction of the n-6/n-3 PUFA ratio. Western diets are deficient in n-3 PUFA and have excessive amounts of n-6 PUFA. This leads to a very high n-6/n-3 PUFA ratio ranging from 15/1 to 20/1. The optimal ratio is thought to be from 5/1 to 10/1 in favour of the n-6 PUFA. According to Simopoulos (2002) the optimal ratio between n-6 and n-3 PUFA would be 4/1 or even lower. Dal Bosco *et al.* (2004) demonstrated that the addition of 8% linseed in rabbit's diet increased concentration of n-3 PUFA and decreased n-6/n-3 PUFA ratio in rabbit meat.

However, the higher content of PUFA could lead to

a higher susceptibility to lipid oxidation. This might alter tissue oxidation, which leads to the formation of free radicals, lipid peroxides, aldehydes (malondialdehyde (MDA) e.g.) and further oxidation products which have a negative effect on the dietetic value of fat and are harmful to the organism. Such enrichment of PUFA might reduce shelf-life of meat products and it can also negatively affect animals and their health. In meat and other animal products, oxidation might reduce its stability, nutritional and sensory quality (Gray *et al.*, 1996). It is therefore very important that we supplement animals with antioxidants in order to prevent lipid oxidation caused by feeding high levels of PUFA.

The aim of this study was to investigate the effect of linseed oil supplementation on performance, fatty acid composition and oxidative status in rabbits. In order to determine to what extent we can change the fatty acid composition of muscle, adipose tissues and liver of rabbits, we made an experiment in which we added high proportion of linseed oil to the commercial rabbit's diet. In order to evaluate the oxidative stress, the plasma, liver and muscle MDA concentrations were measured.

MATERIALS AND METHODS

All procedures were performed according to current legislation on animal experimentation in Slovenia. Animals used in this experiment were reared and slaughtered at the Department of Animal Science, Biotechnical faculty (Ljubljana, Slovenia).

2.1 ANIMALS AND DIETS

Twelve male SIKA rabbits (63 days old, 2284 g) were randomly divided into two groups and assigned into two different dietary treatments for 52 days: a control (n=4) and a linseed diet (n=8). The control diet was commercial diet and the linseed diet was commercial diet containing 9% of linseed oil, which was sprayed onto pellets. The experimental diet was prepared daily. Animals had free access to feed and water (nipple drinkers) and were housed individually in standard cages. Diet samples were taken during the experiment for the purpose of chemical analyses. The fatty acid composition of the diets was analysed using a gas chromatographic method after the *in situ* transesterification of lipids. The chemical and fatty acid composition of the diets are presented in Table 1.

2.2 EXPERIMENTAL PROCEDURE AND SAMPLE COLLECTION

Each day animals received weighed daily meal and the residue from the day before was weighed and discarded. Body weights were recorded each week during the experimental period and just before slaughter. After 52 days of treatment, rabbits were slaughtered at 115 days of age, by electric stunning and exsanguination. After sacrificing the animals, blood, liver, adipose tissue and leg muscle samples were collected. Blood samples, for the purpose of measuring MDA concentration in plasma, were collected into 6 ml evacuated tubes containing EDTAK2 anticoagulant (367864, BD-Plymouth, UK). Plasma was separated by centrifugation (1000g for 15 min at 4 °C), transferred into Eppendorf tubes and stored at -70 °C until analysis. The whole liver, part of the leg muscle and adipose tissue were taken, weighted and stored at -70 °C until analyses were performed.

2.3 DETERMINATION OF FATTY ACID COMPOSITION

The fatty acid composition of muscle and adipose tissues samples were analysed using a gas chromatographic method after the in situ transesterification of lipids. Each sample was analysed in duplicate. Methyl esters of fatty acids were prepared according to the procedure of Park and Goins (1994). A brief summary of the procedure is as follows: frozen muscle or adipose tissue samples were homogenized (Grindomix homogenizer, Retsch GmbH & Co, Haan, Germany) and approximately 0.5–0.7 g (muscle) or 0.1 g (adipose tissue) of the homogenized sample was weighed directly in a tube with stopper and mixed with 3 ml 0.5 M sodium hydroxide in methanol and 0.3 ml methylene chloride. In situ transesterification was performed by heating samples at 90 °C for 10 min in the closed tube. After cooling, 3 ml of 14% boron trifluoride in methanol was added and heating at 90 °C was continued for 10 min in the closed tube. Samples were cooled, and the fatty acid methyl esters (FAMES) were extracted into 1 ml hexane. Analysis of FAMES was performed by gas chromatography using an Agilent 6890 series gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with an Agilent 7683 Automatic Liquid Sampler, a split injector, a flame ionization detector and a fused silica capillary column Omegawax 320 (Supelco, USA). The injection volume was 1 µl. The chromatograms were evaluated by the Agilent GC Chem Station software. Separated FAMES were identified by retention time. Results are expressed as a percentage of the total fatty acids.

2.4 MALONDIALDEHYDE (MDA) DETERMINATION

The methodology of Wong *et al.* (1987) modified by Chirico (1994) and Fukunaga *et al.* (1995) was used to measure the concentration of MDA in blood plasma using HPLC. A brief summary of the procedure is as follows: 100 µl of the sample was mixed with 100 µl of 0.44 M phosphoric acid (H₃PO₄) and 10 µl of 0.2% ethanolic butylhydroxytoluene (BHT) in Eppendorf microcentrifuge tubes, left for 15 minutes, added 300 µl ethanol and then centrifuged (15000 g, for 15 minutes at 4 °C). The supernatant (350 µl) was mixed with 1.5 ml of 0.44 M H₃PO₄, 0.5 ml of 0.6% thiobarbituric acid (TBA) and 0.9 ml of Milli Q deionised water in a tube with stopper and heated at 90 °C for 60 minutes. After cooling, the samples were filtered through Millipore filters (pore size 0.22 µm) into autosampler vials. The MDA concentration in liver and muscle samples was determined following the method of Vila *et al.* (2002) with minor modifications. Briefly, frozen liver or muscle samples were homogenized (Grindomix homogenizer, Retsch GmbH & Co, Haan, Germany) and approximately 0.3 g of the homogenized sample was mixed with 1.5 ml of 2.5% trichloroacetic acid (TCA) in Eppendorf microcentrifuge tubes, left for 10 minutes and then centrifuged (15000 g, for 15 minutes at 4 °C). 1 ml of supernatant was mixed with 1.5 ml of 0.6% TBA and 1 ml of Milli Q deionised water in a tube with stopper and heated at 90 °C for 60 minutes. After the samples were cooled, they were filtered through Millipore filters (pore size 0.22 µm) into autosampler vials. A Waters Alliance 2690 (Waters, Milford, MA) equipped with Waters 474 scanning fluorescence detector was used to determine plasma, liver and muscle MDA. For the purpose of separation a reversed-phase HPLC chromatography column (HyperClone 5u ODS (C18) 120 A, 4.6 × 150 mm 5 micron; Phenomenex Inc., USA) and C18 ODS guard column (4 mm × 30 mm; Phenomenex Inc., USA) were used. The mobile phase consisted of 65% 50 mmol/l KH₂PO₄ buffer (pH 6.9) and 35% methanol. The mobile phase flow rate was 1.0 ml/min. The results of the analysis were evaluated using the Millennium32 Chromatography Manager Program.

2.5 STATISTICAL ANALYSIS

The data were analysed by the General Linear Models (GLM) procedure of the SAS/STAT module (SAS 8e, 2000; SAS Inc., Cary, NC, USA), taking into consideration the diet as the only main effect. Differences between groups were determined on the basis of Turkey's multiple comparisons test. The results in the tables are presented

as least square means (LSM) \pm standard deviation. If not stated otherwise, a least significant difference of 0.05 was used to separate treatment means.

3 RESULTS AND DISCUSSION

The animals adapted well to the experimental conditions. During the experiment, the animals had no health or other problems.

3.1 CHEMICAL AND FATTY ACID COMPOSITION OF THE DIETS

The chemical and fatty acid composition of the diets are presented in Table 1. As expected, the addition of linseed oil increased the proportion of crude fat from 4.2 to 12.2%. The fatty acid composition of the diets differed according to the ingredients. The linseed diet had higher proportion of n-3 PUFA and total PUFA, and lower proportion of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) compared to the control diet. The linseed diet had lower proportion of linoleic acid (C18:2 n-6) and higher proportion of α -linolenic acid (C18:3 n-3). Therefore, the linseed diet had lower n-6/n-3 ratio (0.63 vs. 6.30) compared to the control diet.

Table 1: Chemical and fatty acid composition of the diets
Preglednica 1: Kemijska in maščobnokislinska sestava krme

	Control diet	Linseed diet
Chemical analysis (%)		
Crude protein	16.4	14.9
Crude fat	4.2	12.2
Crude fibre	15.0	13.6
Crude ash	9.4	8.5
Main fatty acids (% of the total fatty acids)		
C16:0	14.31	7.85
C18:0	3.25	3.56
Σ C18:1	21.13	19.37
C18:2 n-6	50.26	25.85
C18:3 n-3	7.93	40.74
Σ SFA	19.64	12.60
Σ MUFA	22.05	20.81
Σ PUFA	58.32	66.59
Σ n-3 PUFA	7.99	40.74
Σ n-6 PUFA	50.33	25.85
n-6/n-3 PUFA	6.30	0.63

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

3.2 BODY WEIGHT GAIN AND DIET INTAKE

There were no significant differences in the initial and final body weight and also in the weight gain (Table 2). Some differences among treatments were observed in feed intake and feed efficiency. Animals in group that had diet enriched with linseed oil showed significantly lower feed intake (for about 41 g/day) and consecutively had significantly higher feed efficiency. That means that animals with supplemented diet consumed significantly ($P = 0.0006$) less feed (4.44 kg) than control animals (5.82 kg) for 1 kg weight gain. These differences were expected because of the much higher fat (energy) content in the linseed diet.

Table 2: Body weight (BW), weight gain and diet intake
Preglednica 2: Telesna masa živali, prirast in zauživanje krme

	Control group	Linseed group	P-value
Initial BW (g)	2343 \pm 129	2256 \pm 175	0.4026
Final BW (g)	3963 \pm 322	3903 \pm 202	0.6979
Weight gain (g/day)	31.8 \pm 4.0	32.3 \pm 3.7	0.8232
Feed intake (g/day)	183.4 \pm 13.0	142.4 \pm 9.5	<0.0001
Feed efficiency (%)	17.3 \pm 1.7	22.7 \pm 2.0	0.0010

Our results are in general agreement with other studies showing that diets high in n-3 PUFA did not significantly influence productive performances in rabbits (Bernardini *et al.*, 1999; Dal Bosco *et al.*, 2004; Kouba *et al.*, 2008; Bianchi *et al.*, 2009). Differences occurred in diet intake and diet efficiency between our and theirs results. These differences were expected because of the higher fat content in the diet in the linseed group in our experiment, while other had isoenergetic diets. On the contrary, Bianchi *et al.* (2006) found out that 8% of linseed supplementation determined a lower daily weight gain as well as a lower final live weight of rabbits compared to the group without linseed addition.

3.3 FATTY ACID COMPOSITION OF TISSUES AND OXIDATIVE STATUS OF RABBITS

Diet supplemented with linseed oil led to a higher proportion of total PUFA, due to a reduction of total SFA and MUFA levels in muscle, adipose tissue and liver. As expected, the linseed oil addition significantly increased ($P < 0.0001$) proportion of the α -linolenic acid and some other n-3 PUFA in all tissues (Table 3). The long chain n-3 PUFA content was at much higher level in muscle and liver compared to adipose tissue. The main differences between tissues were obtained in proportion of li-

Table 3: Fatty acid composition of muscle, adipose tissue and liver (% of total fatty acids)**Preglednica 3:** Maščobnokislinska sestava mišice, maščobnega tkiva in jeter (% od vseh maščobnih kislin)

	Muscle		Adipose tissue		Liver	
	Control	Linseed	Control	Linseed	Control	Linseed
C16:0	21.86 ^a	15.04 ^b	21.96 ^a	12.31 ^b	21.25 ^a	12.20 ^b
C16:1 n-7	3.46 ^a	1.53 ^b	2.68 ^a	0.93 ^b	1.40 ^a	0.22 ^b
C18:0	6.90 ^a	6.62 ^a	5.43 ^a	4.79 ^b	16.28 ^a	22.85 ^b
Σ C18:1	23.48 ^a	21.74 ^a	24.98 ^a	23.23 ^b	17.66 ^a	11.01 ^b
C18:2 n-6	28.98 ^a	25.77 ^b	34.47 ^a	27.41 ^b	29.16 ^a	32.88 ^b
C18:3 n-3	3.30 ^a	20.17 ^b	4.72 ^a	28.02 ^b	1.55 ^a	9.53 ^b
C20:4 n-6	3.49 ^a	2.45 ^a	0.15 ^a	0.09 ^b	4.96 ^a	5.37 ^a
C20:5 n-3	0.08 ^a	0.30 ^b	0.01 ^a	0.03 ^b	0.04 ^a	0.32 ^b
C22:5 n-3	0.57 ^a	1.37 ^b	0.05 ^a	0.11 ^b	0.29 ^a	1.27 ^b
C22:6 n-3	0.10 ^a	0.16 ^a	0.01 ^a	0.01 ^a	0.09 ^a	0.49 ^b
ΣSFA	33.64 ^a	24.86 ^b	31.35 ^a	19.34 ^b	40.22 ^a	36.66 ^b
ΣMUFA	27.83 ^a	23.77 ^b	28.65 ^a	24.72 ^b	20.03 ^a	11.55 ^b
ΣPUFA	38.53 ^a	51.37 ^b	40.00 ^a	55.93 ^b	39.75 ^a	51.78 ^b
n-3 PUFA	4.17 ^a	22.28 ^b	4.80 ^a	28.19 ^b	2.16 ^a	11.92 ^b
n-6 PUFA	34.32 ^a	29.06 ^b	35.14 ^a	27.70 ^b	37.54 ^a	39.85 ^a
n-6/n-3	8.22 ^a	1.31 ^b	7.31 ^a	0.98 ^b	17.43 ^a	3.41 ^b

^{a, b} values with different subscripts within each tissue are significantly different ($P < 0.05$); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

noleic acid (n-6 PUFA). The proportion of linoleic acid significantly decreased in muscle and adipose tissue but increased in liver. The differences also occurred in proportion of α -linolenic acid, which increased in all tissues but it was at much higher level in muscle and adipose tissue compared to the liver. Despite these differences the higher content of n-3 PUFA in linseed diet led to a significantly ($P < 0.0001$) lower n-6/n-3 PUFA ratio in all tissues.

Our results are in general agreement with other studies. Bernardini *et al.* (1999) found that addition of 160 g ground linseed/kg diet significantly increased the proportion of α -linolenic acid and decreased the n-6/n-3 PUFA ratio compared to the standard and fish oil diet (60 g fish oil/kg diet). Similar results were obtained when approximately 30 g of extruded linseed/kg diet was added. The linseed diet increased the level of α -linolenic acid in muscle, perirenal fat, and in raw and cooked meat. Linseed addition also increased the long chain n-3 PUFA contents in meat and consecutively caused a significant decrease in the n-6/n-3 PUFA ratio (Kouba *et al.*, 2008).

Decreased n-6/n-3 PUFA ratio in rabbit's muscle, adipose tissues and liver can provide the "functional food" for human consumption. Intake of such food contributes to a better ratio of n-6 to n-3 PUFA in the human diet and thus contributes to improved human health.

The MDA concentrations in plasma, liver and muscle were significantly influenced by the diet (Table 4). The linseed group, which was supplemented with linseed oil high in PUFA, showed significantly higher levels of MDA in plasma, liver and muscle compared to the control group. That showed that supplementing diet with linseed oil increased the oxidation. From the nutritional point of view these results are unwanted, because of reduced shelf-life of meat products and negative effect on animal health.

Table 4: MDA concentration in plasma, liver and muscle**Preglednica 4:** Koncentracija MDA v plazmi, jetrih in mišici

	Control	Linseed	P-value
Plasma MDA (nmol/ml)	0.26	0.36	0.0059
Liver MDA (nmol/g)	0.47	1.09	0.0319
Muscle MDA (nmol/g)	0.40	1.24	0.0015

Oxidative stress is defined as a persistent imbalance between antioxidants and pro-oxidants in favour of the latter. Namely, oxidation can be reduced if we provide enough antioxidants to prevent the formation of free radicals. Factors affecting the extent of oxidation are: PUFA content in the membranes, the amount of free

radicals and the levels of antioxidants which can be of endogenous or exogenous origin (Brenes *et al.*, 2008). We could prevent oxidation using different dietary strategies. Vitamin E is the most frequently used antioxidant. There are some studies dealing with feeding and nutritional value of rabbit's meat, but there is a lack of them dealing with rabbit plasma and other tissues more involved in the animal's metabolism, such as the liver, at the same time (Tres *et al.*, 2009).

Bianchi *et al.* (2006) indicated that the addition of 35% alfalfa with 200 mg vitamin E/kg feed increased the proportion of α -linolenic acid without having a negative impact on the oxidative stability of meat. Simultaneous addition of 8% linseed has a strong influence on the fatty acid composition of meat, with increasing proportion of PUFA, especially α -linolenic acid, which has a positive effect on the ratio of n-6 and n-3 PUFA. Similar results were also demonstrated in a recent study about the effects of different inclusion rates of whole linseed in the diet to the quality of meat, where the proportion of PUFA also increased at the expense of SFA (Bianchi *et al.*, 2009). They found that inclusion of 3% linseed in diets for growing rabbits provided favourable fatty acid composition and also meat quality. Dal Bosco *et al.* (2004) indicated that the addition of 8% linseed with 200 mg of vitamin E/kg feed increased the concentration of n-3 PUFA and narrowed the ratio of n-6 and n-3 PUFA in rabbits without negative effect on the oxidative stability of meat. It is of note that these authors used much higher quantities of vitamin E when linseed oil was added in the diet compared to the control diet (200 mg/kg vs. 50 mg/kg). Petracci *et al.* (2009) found that the dietary inclusion from 3 to 6% of linseed together with 200 mg of vitamin E/kg might be considered as a way to increase the proportion of α -linolenic acid in the rabbit meat and also ensure sufficient oxidative stability of the product.

The addition of 3% linseed oil in the diet had a beneficial effect on fatty acid composition of rabbit meat as it reduced the proportion of total SFA and the level of cholesterol and increased the proportion of n-3 PUFA. The addition of vitamin E prevented oxidation processes (Bielanski and Kowalska, 2008). Zsédely *et al.* (2008) studied the effect of sunflower oil and linseed oil in combination with various concentrations of vitamin E on fatty acid composition and oxidative stability of rabbit meat. It was expected that addition of oil would significantly increase the proportion of PUFA due to the reduction of SFA and MUFA and decrease the ratio between n-6 and n-3 PUFA. In addition, vitamin E improved oxidative stability compared to the groups without vitamin E addition. Plasma and liver fatty acids composition also reflected feed's fatty acid profile (Tres *et al.*, 2009).

4 CONCLUSIONS

Nowadays consumers are more concerned about their health and the nutritional value of their food. Therefore, interest in obtaining animal products with better nutritional value has increased. Our results represent to what extent we can improve fatty acid composition of rabbits tissues with linseed oil supplementation. To summarize, the overall fatty acid composition was influenced by dietary linseed oil inclusion without significant effect on productive performance. The linseed oil caused a lower content of total SFA and MUFA and a higher content of PUFA. Linseed oil also reduced n-6/n-3 PUFA ratio in all tissues. However, the higher content of PUFA in tissues led to higher susceptibility to lipid oxidation. Such enrichment might reduce the shelf-life of meat products and it can also negatively affect animals and their health. Therefore, further research is needed to protect animals and their products from lipid oxidation by adding various natural antioxidants to the diet.

5 REFERENCES

- Bernardini M., Dal Bosco A., Castellini C. 1999. Effect of dietary n-3/n-6 ratio on fatty acid composition of liver, meat and perirenal fat in rabbits. *Animal Science*, 68: 647–654
- Bianchi M., Petracci M., Cavani C. 2006. Effects of dietary inclusion of dehydrated lucerne and whole linseed on rabbit meat quality. *World Rabbit Science*, 14: 247–258
- Bianchi M., Petracci M., Cavani C. 2009. The influence of linseed on rabbit meat quality. *World Rabbit Science*, 17: 97–107
- Brenes A., Viveros A., Goñi I., Centeno C., Sáyago-Ayerdy S.G., Arija I., Saura-Calixto F. 2008. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Science*, 87: 307–316
- Chirico S. 1994. High-performance liquid chromatography-based thio-barbituric acid tests. *Methods in Enzymology*, 233: 314–318
- Connor W.E. 2000. Importance of n-3 fatty acids in the health and disease. *The American Journal of Clinical Nutrition*, 71: 171S–175S
- Dal Bosco A., Castellini C., Bianchi L., Mugnai C. 2004. Effect of dietary α -linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. *Meat Science*, 66: 407–413
- Dalle Zotte A. 2004. Perception of rabbit meat quality and major factor influencing the rabbit carcass and meat quality. *Livestock Production Science*, 75: 11–32
- Fukunaga K., Takama K., Suzuki T. 1995. High-performance liquid chromatography determination of plasma malondialdehyde level without a solvent extraction procedure. *Analytical Biochemistry*, 230: 20–23

- Gray J.I., Gomaa E.A., Buckley D.J. 1996. Oxidative quality and shelf life of meats. *Meat Science*, 43: S111–S123
- Kouba M., Benatmane F., Blochet J.E., Mourot J. 2008. Effect of linseed diet on lipid oxidation, fatty acid composition of muscle, perirenal fat, and raw and cooked rabbit meat. *Meat Science*, 80: 829–834
- Kouba M., Enser M., Whittington F.M., Nute G.R., Wood J.D. 2003. Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. *Journal of Animal Science*, 81: 1967–1979
- Leskanich C.O., Noble R.C. 1997. Manipulation of the n-3 polyunsaturated fatty acid composition of avian eggs and meat. *World's Poultry Science Journal*, 53: 155–181
- Park P.W., Goins R.E. 1994. In situ preparation on fatty acid methyl esters for analysis of fatty acid composition in foods. *Journal of Food Science*, 59: 1262–1266
- Petracci M., Bianchi M., Cavani C. 2009. Development of rabbit meat products fortified with n-3 polyunsaturated fatty acids. *Nutrients*, 1: 111–118
- Raes K., De Smet S., Demeyer D. 2004. Effect of dietary fatty acid on incorporation of long chain polyunsaturated fatty acid and conjugated linoleic acid in lamb, beef and pork meat. *Animal Feed Science and Technology*, 113: 199–221
- Riediger N.D., Othman R.A., Suh M., Moghadasian M.H. 2009. A systemic review of the roles of n-3 fatty acids in health and disease. *Journal of the American Dietetic Association*, 109: 668–679
- Simopoulos A. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy*, 56: 365–379
- Tres A., Bou R., Codony R., Guardiola F. 2009. Dietary n-6- or n-3-rich vegetable fats and α -tocopheryl acetate: effects on fatty acid composition and stability of rabbit plasma, liver and meat. *Animal*, 3: 1408–1419
- Vila B., Jaradat Z.V., Marquardt R.R., Frohlich A.A. 2002. Effect of T-2 toxin on in vivo lipid peroxidation and vitamin E status in mice. *Food and Chemical Toxicology*, 40: 479–486
- Williams C.M. 2000. Dietary fatty acids and human health. *Annales de Zootechnie*, 49: 165–180
- Wong S.H., Knight J.A., Hopfer S.M., Zaharia O., Leach C.N., Sunderman F.W. 1987. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clinical Chemistry*, 33: 214–220
- Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., Sheard P.R., Enser M. 2003. Effect of fatty acid on meat quality: a review. *Meat Science*, 66: 21–32