EFFECT OF DIETARY VEGETABLE OIL (SUNFLOWER, LINSEED) AND VITAMIN E SUPPLEMENTATION ON THE FATTY ACID COMPOSITION, OXIDATIVE STABILITY AND QUALITY OF RABBIT MEAT

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ABSTRACT

This experiment was conducted to investigate the effect of dietary vegetable oil (sunflower and linseed) and different vitamin E supplementation on the fatty acid (FA) profile, vitamin E content and oxidative stability of rabbit meat. In addition, the FA composition of cooked and roasted meat samples was analyzed. A total of 200 New Zealand White rabbits were randomly assigned to one of four dietary treatments (50 rabbits per treatment) and fed the experimental diets between 35 and 80 days of age. All diets were supplemented with 60 mg/kg dl-α-tocopherol-acetate. A negative control (NC) diet with no added oil (energy content 10.61 MJ/kg) and three diets supplemented with 2% sunflower and 2% linseed oil (energy content 11.36 MJ/kg) were used. In addition to the 60 mg/kg dl-α-tocopherol-acetate content, no more vitamin E (PC), 240 mg/kg synthetic vitamin E (dl-α-tocopherol-acetate) (SE) or 240 mg/kg natural vitamin E (fatty acid distillate, d-α-tocopherol, a by-product of the oil industry) (NE) were added to the oil supplemented diets. The oil addition to the diets (PC, SE and NE groups) significantly increased the UFA, PUFA and decreased the SFA and MUFA content in limb muscle compared to the NC rabbits. The meat samples of the PC, SE and NE rabbits had a lower n-6/n-3 PUFA ratio (2.39, 2.14, 2.76 vs. 4.26) and a significantly ($P<0.05$) higher PUFA/SFA ratio (1.92, 1.86, 2.01 vs. 0.66) in comparison to the NC group, respectively. It was found that supplementing the diets with vitamin E (SE and NE groups) significantly increased the α-tocopherol content of limb meat in rabbits and improved its oxidative stability compared to the NC and PC groups. The utilization of the synthetic form of vitamin E (SE group) was higher compared to the natural form (NE group), but the natural source of vitamin E was more efficient in improving the oxidative stability of the rabbit meat samples as indicated by a decreased MDA value. Our results proved that the fatty acid composition of rabbit meat cannot be altered by cooking or roasting.

Key words: Fatty acid, Vitamin E, Meat, Storage stability.

INTRODUCTION

It is well known that n-3 fatty acids have several beneficial effects on human health. A great number of scientific studies have been conducted in order to increase the PUFA content in animal products. In monogastric animals, including rabbits, it has been demonstrated that the quantity and chemical composition of the fatty acids can be altered by dietary manipulations (Cobos *et al*., 1994; Oliver *et al.*, 1997; Maertens, 1998; Xiccato, 1999). It has been demonstrated that supplementing the diet with vegetable oils can decrease PUFA/SFA ratio and increase n-3 concentration in rabbit meat. Flaxseed (or linseed) oil is one of the richest source of linolenic acid (C18:3 n-3). Dal Bosco *et al.* (2004) demonstrated that supplementing the rabbit feed with 8% linseed increased the C18:3 n-3 concentration and decreased the n-6/n-3 ratio in rabbit meat compared to the control group. On the other hand, high PUFA concentration in meat can have a negative effect on its storage stability due to the higher susceptibility to peroxidation (Xiccato, 1999). The rate of lipid oxidation in tissue is influenced by several factors. These include the fat content, fatty acid profile and antioxidant capacity

of the muscle. In most studies, vitamin E was used as the antioxidant. Different levels (10-500 mg/kg feed) of dietary vitamin E supplementations were applied in fat supplemented rabbit diets (Lopez-Bote *et al*., 1997; Castellini *et al*., 1998; Oriani *et al*., 2001). The results of those studies indicated that the dietary addition of α-tocopherol can be an efficient way to improve the storage stability of rabbit meat. However, the sufficient rate of vitamin E supplementation has not been fully assessed.

The objective of this study was to investigate the effect of adding fat rich in polyunsaturated fatty acids to the diets on the fatty acid composition of thigh and loin in rabbits, and to assess the effectiveness of α-tocopherol supplementation from different sources (synthetic and natural form) on the vitamin E concentration and the oxidative stability of meat. In addition, the research assessed whether cooking or roasting can modify the FA profiles of the meat.

MATERIALS AND METHODS

Animals and experimental design

This study was conducted using 200 New Zealand White rabbits (mixed sex). Animals were randomly assigned to one of the four treatment groups (50 rabbits/treatment) and fed the experimental diets between 35 and 80 days of age. A negative control (NC) diet with no added oil (energy content 10.61 MJ/kg, crude protein 155.5 g/kg feed, ether extract 19.3 g/kg feed, crude fiber 157.4 g/kg feed) and three diets supplemented with 2% sunflower and 2% linseed oil (energy content 11.36 MJ/kg, crude protein 154.6 g/kg feed, ether extract 56.0 g/kg feed, crude fiber 155.3 g/kg feed) were used (Table 1). In addition to the 60 mg/kg dl-α-tocopherol-acetate content, no more vitamin E (PC), 240 mg/kg synthetic vitamin E (dl- α-tocopherol-acetate) (SE) or 240 mg/kg natural vitamin E (NE) were added to the oil supplemented diets. Therefore, the diets supplemented with a higher level of vitamin E (SE and NE) contained a total of 300 mg/kg vitamin E supplementation. The natural vitamin E source was fatty acid distillate, a by-product of the oil industry. At 80 days of age all the rabbits were slaughtered.

Chemical analysis

After 24 hours chilling at 4°C following slaughter, 10 rabbits from each treatment were randomly selected and meat samples were collected to determine fatty acid profile, vitamin E content and oxidative stability of thigh and loin. The fatty acids from meat were extracted and measured as methyl esters with a gas chromatograph (HP Agilent Technologies 6890N) using Supelco SPTM 2560 Fused Silica Capillary Column (0.25 mm ∅, 100 m length). The α-tocopherol concentration in meat was determined using high performance liquid chromatography (HPLC) according to 44/2003 (IV.26.) MARD order 10. appendix. To evaluate the oxidative stability of the rabbit meat, samples were kept refrigerated -16°C and oxidation (TBARS) was measured (Ramanathan *et al*., 1992) after one and two months of storage. In addition, meat samples from each treatment group were cooked or roasted. The samples were cooked in a pot for 1.5 hour. The roasting lasted for two hours in an oven. We did not use spice or salt. After the cooking or roasting, the fatty acid profile of the meat was analyzed.

Statistical analysis

The effect of diet on fatty acid composition, tocopherol concentration and lipid oxidation was analyzed using ANOVA in STATISTICA 6.0 program. T-test was used to test the differences between treatment means.

RESULTS AND DISCUSSION

The results of the chemical analysis indicated that the fatty acid composition of the lipids in limb meat and *longissimus dorsi* muscle was influenced by the dietary oil supplementation (Table 1). As expected, the vegetable oil (rich in PUFA) addition significantly increased the linoleic (C18:2 n-6) and

α-linolenic acid (C18:3 n-3) and decreased the SFA and MUFA proportion, mainly due to the reduction of the C16:0 and C18:1 concentration, in the limb muscle and longissimus dorsi compared to the NC rabbits. These results are in agreement with other reports (Cobos *et al*., 1994; Oliver *et al*., 1997) and our previous experiment (Zsédely *et al*., 2006). Vitamin E supplementation (SE and NE) did not affect the composition of fatty acids in meat.

As a result of the 2% linseed oil supplementation, the meat samples of the PC, SE and NE rabbits had a lower n-6/n-3 PUFA ratio (2.39, 2.14, 2.76 vs. 4.26) and a significantly ($P<0.05$) higher PUFA/SFA ratio (1.92, 1.86, 2.01 vs. 0.66) in comparison to the NC group, respectively. This can be a beneficial effect in terms of human nutrition.

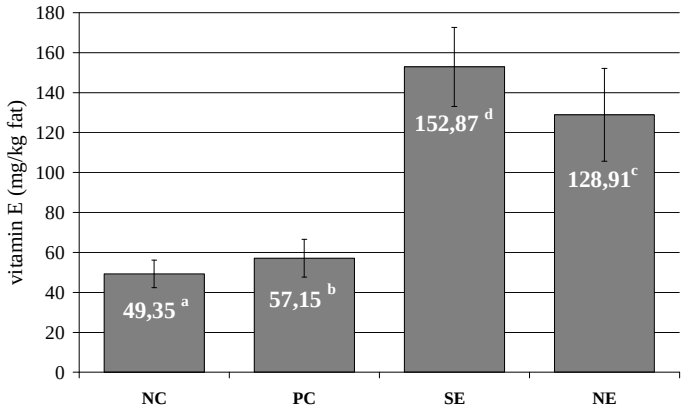
Table 1: Fatty acid composition of muscles and diets (% of total fatty acids)

Fatty acid	Thigh meat				Loin meat				Diets	
	NC[1]	PC[2]	SE[3]	NE[4]	NC	PC	SE	NE	NC	PC, SE, NE
C14:0	3.05^{b}	1.49^{a}	1.58^{a}	1.50^{a}	3.02^{b}	1.54^{a}	1.71^{a}	1.62^{a}	0.50	0.26
C16:0	28.86^{b}	17.09^{a}	17.82^{a}	16.72^{a}	29.13^{c}	18.54^{ab}	19.16^{b}	17.79^{a}	14.73	10.09
C16:1 n-7	4.26^{b}	1.29^{a}	1.32^{a}	1.35^{a}	4.85^{b}	1.49^{a}	1.70^{a}	1.71^{a}	0.22	0.14
C18:0	6.46^{b}	5.99^{a}	5.77^{a}	5.75^{a}	5.97^{a}	5.95^{a}	5.64^{a}	5.63^{a}	3.07	3.53
C18:1 n-9	24.59^{b}	20.05^{a}	20.35^{a}	20.39^{a}	24.38^{b}	20.08^{a}	20.58^{a}	20.68^{a}	15.49	19.22
C18:2 n-6	20.82^{a}	34.36^{b}	32.82^{b}	36.42^{c}	18.73^{a}	31.59^{b}	30.47^{b}	34.36^{c}	40.72	36.20
C18:3 n-3	4.74^{a}	14.25^{c}	15.20^{d}	13.08^{b}	3.98^{a}	13.17^{c}	14.25^{d}	12.47^{b}	18.24	27.21
C20:4 n-6	0.49^{a}	0.65^{b}	0.57^{ab}	0.56^{ab}	1.54^{b}	1.52^{b}	1.13^{a}	0.97^{a}	0.00	0.00
C20:5 n-3	0.04^{a}	0.07^{b}	0.07^{b}	0.06^{c}	0.11^{a}	0.16^{b}	0.14^{b}	0.10^{a}	0.05	0.00
C22:5n-3	0.24^{a}	0.39^{b}	0.37^{b}	0.29^{a}	0.49^{a}	0.76^{b}	0.61^{b}	0.43^{a}	0.00	0.00
C22:6 n-3	0.05^{a}	0.06^{a}	0.06^{a}	0.05^{a}	0.11^{b}	0.13^{b}	0.10^{b}	0.07^{a}	0.00	0.00
SFA	40.83^{b}	26.29^{a}	26.75^{a}	25.44^{a}	39.92^{b}	27.44^{a}	27.82^{a}	26.31^{a}	19.48	14.60
MUFA	30.56^{b}	22.37^{a}	22.71^{a}	22.69^{a}	31.33^{b}	22.78^{a}	23.46^{a}	23.42^{a}	15.83	19.45
PUFA	27.09^{a}	50.43^{b}	49.70^{b}	51.03^{b}	25.98^{a}	48.19^{b}	47.44^{b}	49.11^{b}	59.09	63.46
n-6/n-3	4.26^{d}	2.39^{b}	2.14^{a}	2.76^{c}	4.47^{d}	2.36^{b}	2.12^{a}	2.73^{c}	2.27	1.33
PUFA/SFA	0.66^{a}	1.92^{b}	1.86^{b}	2.01^{b}	0.65^{a}	1.76^{b}	1.71^{b}	1.87^{b}	3.03	4.35

a, b, c, d: different superscripts within a row indicate significant differences ($P<0.05$); [1]NC (negative control), no added fat, low energy content, 60mg/kg dl-α-tocopherol-acetate; [2]PC (positive control) 2% linseed oil + 2% sunflower oil, 60mg/kg dl-α-tocopherol-acetate; [3]SE 2% linseed oil + 2% sunflower oil, 60 + 240 mg/kg dl-α-tocopherol-acetate ; [4]NE 2% linseed oil + 2% sunflower oil, 60 mg/kg dl-α-tocopherol-acetate + 240 mg/kg d- α-tocopherol

There was a close relationship between the vitamin E content of limb meat and that of the diets (Figure 1). Extra dietary supplementation of vitamin E in the amount of 240 mg/kg feed significantly increased the vitamin E content of thigh (49.35 and 57.15 vs. 152.87 and 128.91 mg/kg fat). It was found that the synthetic form of vitamin E (SE) was utilized significantly better than the natural d-α-tocopherol (NE). It is in conflict with our previous results in broiler chicken (not publicated), where we found that natural form had a better utilization and reported that tocopherol transfer protein prefers natural 'd' to 'dl' racemic form. Further research is necessary to determine which form of vitamin E is utilized better.

After 1 and 2 months of deep frozen storage, the oxidative stability of limb meat and *longissimus* samples were measured by thiobarbituric acid (TBARS) method and was expressed in nmol/g tissue. The results of the current study indicated that supplementing the diets with 2% sunflower and linseed oil increased the oxidation of the rabbit meat compared to that of the NC group. Similarly, previous studies found that feeding vegetable oils with high PUFA concentration reduces the storage stability of meat (Dal Bosco *et al*., 2004). In contrast, Lopez-Bote *et al*. (1997) reported that meat samples from rabbits fed fat-enriched diet (supplemented with 30 g/kg olive or sunflower oil) decreased the level of oxidation in comparison to those rabbits that were fed non fat-supplemented diets. Dietary antioxidant supplementation seems to be an effective way to increase shelf-life, because in the present study we found that after one month of storage MDA value decreased to 23.4 in the SE and 20.3 in the NE group compared to 32.2 nmol/g meat in the PC group, which had only 60 mg dl- α-tocopherol-acetate in their diet.



a, b, c, d Different letters mean significant differences (P<0.05)

Figure 1: Vitamin E concentration of limb meat

In the current study, we compared efficiency of the natural and synthetic form of α-tocopherol against oxidation in refrigerated rabbit meat samples. After two months of storage it was found (Figure 2) that the natural form (NE) was more effective as it led to a lower MDA value compared to the SE samples. A higher MDA value was recorded from the negative control (NC) samples with no oil and extra vitamin E supplementation.

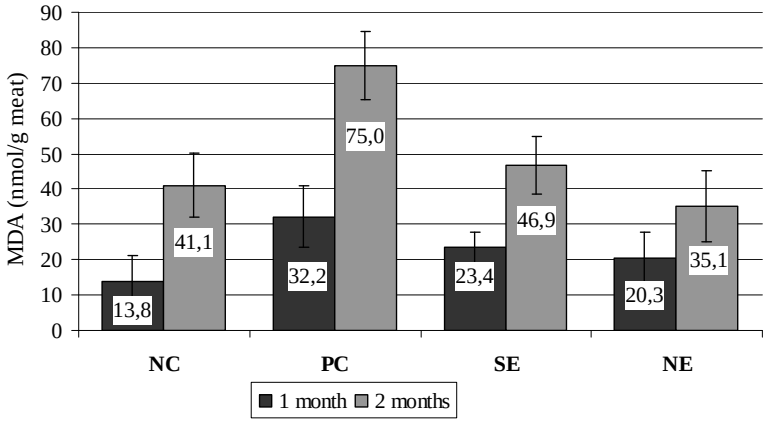


Figure 2: Effect of vitamin E (from different sources) supplementation on the oxidative stability of loin meat

The present study assessed whether cooking or roasting can modify the FA profile of the meat samples. There is a lack of studies in this subject. The results indicated that the C18:2 n-6 and C18:3 n-3 proportion of meat changed, as compared to the NC group (Table 2).

Table 2: The effect of cooking and roasting on the C18:2 n-6 and C18:3 n-3 concentration (% of ether extract) of meat samples

		Longissimus dorsi		Limb meat	
		$C_{18:2}$ (n-6)	$C_{18:3}$ (n-3)	$C_{18:2}$ (n-6)	$C_{18:3}$ (n-3)
Cooked in pot	NC	20.22	3.31	18.57	4.11
	PC	30.64	11.77	35.82	14.50
	SE	30.11	12.73	32.47	14.50
	NE	35.79	11.18	38.03	12.92
Roasted in oven	NC	18.31	3.33	18.72	4.47
	PC	31.15	11.29	35.83	14.23
	SE	28.52	12.60	31.02	14.06
	NE	33.97	11.24	37.95	12.62

Our results proved that cooking and roasting did not have major effect on C18:2 n-6 and C18:3 n-3 fatty acid proportion as in the oil supplemented groups (PC, SE and NE) linolenic acid was about three times higher in comparison with the NC group. The n-6/n-3 ratio of meat samples slightly increased as a result of meat preparation. This result confirms that dietary sunflower and linseed oil supplementation (2-2%) is an effective way to increase the C18:3 n-3 level in rabbit meat, which can have a positive effect on human nutrition.

CONCLUSIONS

Similarly to the findings of other studies, the results of the current experiment demonstrated that linseed and sunflower oil addition to rabbit diet is an effective way to increase the long chain n-3 fatty acid and decrease SFA content, and therefore, to decrease the n-6/n-3 ratio in the thigh and loin meat of rabbit. These changes can enhance the nutritional value of the rabbit meat.

Vitamin E addition did not have an effect on the FA profile of the meat samples. As a result of the 240 mg/kg extra vitamin E supplementation in the SE and NE groups, the amount of α-tocopherol in limb meat was about 3-times higher compared to the negative or positive control groups (NC and PC) fed only 60 mg/kg vitamin E in their diet. The natural form (dl-α-tocopherol) of vitamin E was more efficient to decrease the MDA value, and to increase the storage stability of rabbit meat compared to its synthetic form. Our results confirmed that the fatty acid composition of rabbit meat cannot be altered by cooking or roasting. Therefore, supplementing the rabbit feed with vegetable oils can improve the nutritional value of the rabbit meat.

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