Response of growing rabbits to dietary antioxidant vitamins E and C: 2-effect on meat quality

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Summary

A seven-treatment experiment was carried out to study the response of seventy, each 6 weeks old New Zealand White rabbits to dietary supplementation with the antioxidant vitamins; α-tocopheryl acetate (vitamin E) and ascorbic acid (vitamin C), provided individually or in combinations on some meat quality traits following a 7 week growth trial. Rabbits were equally allocated to one of the following supra-nutritional levels of the two vitamins/kg diet up to the 13th week of age; 1: Control with no extra vitamin supplement (40 mg vit. E provided through the vitamin & mineral premix; NRC 1977' recommendation), 2: 40 mg vit. E (E40), 3: 80 mg vit. E (E80), 4: 200 mg vit. C (C200), 5: 400 mg vit. C (C400), 6: 40 mg vit. E+200 mg vit. C (E40C200), and 7: 80 mg vit. E+ 400 mg vit. C (E80C400). α-tocopherol and ascorbate contents of meat at days 10 and 20 of frozen storage were higher (*p*<0.01) in the vitamin-supplemented groups, especially the E80C200 treatment for α-tocopherol, and C400 group at day 10 and E40C200 group at day 20 for ascorbate content. TBA-RS levels of stored meat was significantly (*p*<0.01) lower in the vitamin-supplemented groups, substantially in the C200 fed group. Again, C400 and E40C200 groups significantly (*p*<0.01) maintained the higher desirable polyunsaturated fatty acids (PUFAs) content of the storage meat. It is concluded that vitamin E and/or vitamin C successfully ameliorated the quality traits of meat produced in terms of improved oxidative stability and the introduction of a highly nutritional food (rabbit meat) source rich in vitamins E and C for human.

Key words: Rabbit, Vitamins E and C, Meat quality.

Introduction

Oxidation of lipids in food has received considerable attention because of possible health effects related to consumption of oxidized lipids (Addis and Park, 1989). Rabbit meat contains high levels of PUFAs. Unfortunately, appreciable character could cause problems for meat storage, processing and cooking, since PUFAs are very susceptible to oxidation, resulting in reduced oxidative stability of muscles and hence nutritive value and the safety of the food can be affected (Bernardini *et al*., 1996), which means a deterioration in the self-life of the meat (Dal-Bosco *et al*., 2004). The oxidation of muscle tissue lipids can be reduced by antioxidants. Dietary supplementation has been proved to be a simple and convenient strategy to introduce a natural antioxidant that may effectively inhibit the oxidation reactions (Botsoglou *et al*., 2004). α-tocopherol is a highly effective natural antioxidant that protects cellular membranes against oxidative damage (Morrissey *et al*., 1994). Vitamin C can reduce the generation of oxidants and regenerates α-tocopherol from its oxidation form (Reed, 1992). Numerous studies approved the positive relationship between the deposited vitamin E and meat quality in protecting the PUFAs against oxidation (Botsoglou *et al*., 2004; Lo Fiego *et al*., 2004). Such relationship was not completely proved in the case of vitamin C. Only, Lo Fiego *et al*., (2004) reported that supplemental vitamin C increased the rabbit lipid stability in the low α-tocopherol diet fed rabbits, which disappeared with the large α-tocopherol doses. The aim of this study was to evaluate, under field conditions, the relationships between the antioxidant nutrients (vitamin E or C and their combination) and meat quality of growing rabbits.

Materials and Methods

Animals and diets

Seventy, 6 weeks old New Zealand White rabbits were evenly sexed, weighed and individually caged to evaluate the response to supra-nutritional levels of α-tocopheryl acetate (vitamin E) and vitamin C, provided individually or in a combination/kg diet as follows 1: control with no extra vitamin supplement (40 mg vit. E provided through the vit.& min. premix; NRC 1977), 2: 40 mg vit. E (E40), 3: 80 mg vit. E (E80), 4: 200 mg vit. C (C200), 5: 400 mg vit. C (C400), 6: 40 mg vit. E +200 mg vit. C (E40C200), and 7: 80 mg vit. E+ 400 mg vit. C (E80C400). Basal diet was formulated to satisfy the NRC (1977) recommendation. Ingredient and chemical composition of the basal diet are presented in Table 1. To avoid vitamin C oxidation during pelleting process, the vitamin was dissolved in about 20-30 ml water, and then sprayed over the pellets, in every other day intervals.

Table 1: Ingredients and calculated chemical composition of the experimental diet

Ingredients: Wheat bran 25.5%, barley 23.0%, soybean meal (44%) 21.5%, yellow corn, 7.5%, wheat straw 19.5%, limestone 1. 5%, di calcium phosphate 0.50 %, NaCl 0.30%, vitamins & minerals premix* 0.30%, Dl- Methionine 0.20%, anti-coccidial 0.10%, and anti-fungal 0.10 % ; Total 100.0%

Chemical composition: DM, 89%; CP, 17.06%; DE (MJl/kg) 10.88; CF, 13.12%; Ca, 0.91%; P, 0.64%; Lysine, 0.87%; methionine + cystene 0.69%

*Supplied per kg. of diet: 12000 IU vit.A; 2200 IU vit. D3; 13.4 mg vit. E (determined); 2.0 mg vit. K_3; 1.0 mg vit. B_1; 4.0 mg vit. B_2; 1.5 mg vit. B_6; 0.0010 mg vit. B_{12}; 6.7 mg vit. PP; 6.67 mg vit. B_5; 0.07 mg B_8; 1.67 mg B_9; 400 mg choline chloride; 133.4 mg Mg; 25.0 mg Fe; 22.3 mg Zn; 10.0 mg Mn;1 .67 mg Cu; 0.25 mg I and 0.033 mg Se.

Determination of vitamins E and C

Dl-α-tocopheryl acetate in the vitamin-mineral premix added to feed formula, also, in the pure supplement and α-tocopherol in the loin meat of vitamin E and E+C groups were assayed using HPLC, according to Leth and Sondergaro (1983). Vitamin C in the pure supplement and ascorbate in the loin meat of vitamin C and E+C groups was assayed using HPLC, according to Danish Official (1996).

Determination TBA-RS

For determining lipid peroxidation of frozen meat, the thio-barbituric acid-reactive substance (TBA-RS) test was carried out using three frozen loin meat samples (-20ºC) of each treatment at days 10 and 20 after slaughter, according to AOAC (1990). The TBA value is defined as the increase of absorbance measured at 530 nm due to the reaction of the equivalent of 1 mg of the sample per 1 ml volume with 2-thio-barbituric acid. Secondary oxidation products of oils and fats react with 2-thio-barbituric acid forming condensation products.

Fatty acid profile of the meat

Fatty acids profile determination of the loin meat was carried out in three samples of each treatment according to AOAC (2000) at days 10 and 20 of freezing storage (-20°C).

Statistical analysis

Data were subjected to a one-way analysis using SAS (1990). Variables having significant differences were compared using Duncan's Multiple Range Test (Steel and Torrie, 1960).

Results and discussion

Vitamins E and C content of the meat

Data provided in Table 2 indicate that α-tocopherol and ascorbate in the frozen loin meat after 10 or 20 days of frozen storage significantly ($p<0.01$) increased as the level of α-tocopheryl and ascorbic acid increased in the diet. More interesting is that vitamin C had an additive effect to maintain high level of vitamin E in the meat, also both vitamins worked synergistically ($p<0.01$) to minimize the loss %, especially in α-tocopherol, as compared to the loss % in ascorbate. These findings supported by the results of Lopez-Bote *et al*., (1997), Castellini *et al*., (1998), Botsoglou *et al*., (2004) and Lo Fiego *et al*., (2004), reporting that the increase in the α-tocopherol concentration of the muscles depends on the increase in the α-tocopheryl acetate level of the diet. Moreover, ascorbate restores the activity of α-tocopherol (Niki, 1984, Sies and Stahl, 1995). Lo Fiego *et al*., (2004) reported the same effect of vitamin C (500 mg/kg diet) in low (40 mg/kg diet) but not in high (300 or 500 mg kg^{-1}) vitamin E diets, and found significantly increased the α-tocopherol content of refrigerated meat.

Oxidative stability of the muscle lipids

Lipid oxidation of the rabbit muscle expressed as TBA-RS at days 10 and 20 of frozen storage, and the increase in deterioration rate values are illustrated in Table. It was found that TBA-RS values were lowered ($p<0.01$) by supplementation of the diets with the antioxidant vitamins, especially in the low-ascorbic acid fed groups. The negative correlation between the α-tocopherol content of the muscle and the rate of lipid oxidation as was found in the present study is supported by previous studies of Lopez-Bote *et al*., (1997), Castellini *et al*., (1998 and 2000), Corino *et al*., (1999 and 2007), Oriani *et al*., (2001), Botsoglou *et al.,* (2004), and Lo Fiego *et al*., (2004), where vitamin E forms increased the oxidative stability of muscular lipids, or in other terms, delayed lipid oxidation. The effect of vitamin E was probably because of quenching free radicals originating from lipid oxidation (Machlin and Bendich, 1987) or the reduction in lipid oxidation was due to the reduction in NADPH oxidase when rabbits were fed on supplemental vitamin E diet as earlier reported by Chan *et al*., (1983).

Table 2: Vitamins E and C content and TBA-RS values of meat at days 10 and 20 of frozen storage (-20ºC).

	Control	E40	E80	C200	C400	E40C200	E80C400	Sig.
	Vitamins in meat (µg/g)							
Vit. E (d 10)	$2.16^{d}\pm0.01$	$2.66^{c}\pm0.03$	$3.45^{b}\pm0.04$	ND	ND	$2.82^{c}\pm0.10$	$3.68^{a}\pm0.02$	**
Vit. E (d 20)	$0.46^{e}\pm0.04$	$0.91^{d}\pm0.03$	$1.17^{c}\pm0.05$	ND	ND	$1.90^{b}\pm0.04$	$2.14^{a}\pm0.01$	**
% loss	$78.8^{a}\pm2.04$	$65.9^{b}\pm0.93$	$66.1^{b}\pm1.03$	ND	ND	$32.5^{d}\pm2.76$	$41.9^{c}\pm0.07$	**
Vit.C (d 10)	$2.70^{d}\pm0.04$	ND	ND	$4.19^{c}\pm0.10$	$5.50^{a}\pm0.11$	$4.40^{c}\pm0.11$	$5.16^{b}\pm0.01$	**
Vit.C (d 20)	$0.34^{d}\pm0.01$	ND	ND	$0.69^{c}\pm0.05$	$1.05^{b}\pm0.01$	$1.45^{a}\pm0.04$	$1.41^{a}\pm0.01$	**
% loss	$87.2^{a}\pm0.35$	ND	ND	$83.5^{b}\pm0.81$	$80.8^{c}\pm0.10$	$67.1^{e}\pm0.05$	$72.6^{d}\pm0.21$	**
	TBA-RS (ng/g)							
Day 10	$46.6^{a}\pm1.70$	$35.4^{b}\pm0.94$	$26.1^{c}\pm1.19$	$21.7^{d}\pm1.15$	$22.6^{d}\pm1.24$	$26.6^{c}\pm1.24$	$44.0^{a}\pm0.17$	**
Day 20	$58.6^{a}\pm1.60$	$49.1^{b}\pm0.72$	$39.1^{d}\pm1.18$	$31.6^{e}\pm0.60$	$37.2^{d}\pm1.22$	$43.9^{c}\pm1.03$	$49.1^{b}\pm0.66$	**
% increase	$25.8^{bc}\pm2.4$	$38.6^{abc}\pm5.7$	$50.3^{ab}\pm11.4$	$45.9^{ab}\pm4.9$	$66.7^{a}\pm14.7$	$65.7^{a}\pm11.8$	$11.7^{c}\pm1.76$	**

ND= not determined, ** ($p<0.01$)

Fatty acid profile of intramuscular fat

Fatty acid profile of frozen loin meat (-20ºC) at days 10 and 20 *post mortem* is illustrated in Tables 3 and 4. In vitamin-enriched groups, the proportions of PUFAs, especially $C_{18:2}$ and $C_{18:3}$ was higher ($p<0.01$), while the proportion of mono-unsaturated fatty acids (MUFAs) was lower ($p<0.01$), as compared to the control, with the exception recorded for the E80 (days 10 and 20) and E80C200 (day 20) groups. This might be due to the high deposition of the antioxidant vitamins in the meat, protecting these fatty acids from oxidative damage during storage. These results are supported by results of Bernardini *et al*., (1996) and Dal Bosco *et al.,* (2004) whose studies clarified that vitamin E (200 mg kg^{-1} diet) resulted in an increase in the PUFAs ratio of rabbit meat. Dal Bosco *et al*., (2004) found that vitamin E inhibited the peroxidation of PUFAs, which are highly susceptible to oxidation, rather than of the more stable MUFAs or saturated fatty acids. On the other hand, Lopez-Bote *et al*., (1997) reported that 200 mg vitamin E kg^{-1} diet had no effect on the fatty acid profile of rabbit muscle.

In contrast to our findings, Corino *et al*., (2007) reported that oleic acid ($C_{18:1\ n\text{-}9}$) and total MUFAs were higher, while, PUFAs were lower in meat from rabbits fed on 240 mg vitamin E kg^{-1} diet as compared to the control (60 mg vitamin E kg^{-1} diet).

Table 3: Fatty acid profile (weight % of total fatty acids) of frozen meat (day 10).

	Control	E40	E80	C200	C400	E40C200	E80C400	Sig
$C_{10:0}$	Not detected						0.30±0.01	-
$C_{12:0}$	Not detected						0.30±0.01	-
$C_{14:0}$	3.0^{b}±0.01	2.75^{c}±0.05	3.05^{ab}±0.05	3.15^{a}±0.05	2.85^{c}±0.05	2.80^{c}±0.01	3.05^{ab}±0.1	**
$C_{15:0}$	0.55±0.05	0.50±0.01	0.55±0.05	0.50±0.01	0.50±0.01	0.55±0.05	0.60±0.01	ns
$C_{16:0}$	31.9^{a}±0.35	30.1^{b}±0.7	32.6^{a}±0.50	31.6^{a}±0.30	31.7^{a}±0.15	31.2^{ab}±0.1	32.4^{a}±0.2	*
$C_{16:1}$	4.3^{cd}±0.30	5.5^{ab}±0.20	5.0^{b}±0.01	6.1^{a}±0.30	4.1^{d}±0.20	3.9^{d}±0.01	4.9^{bc}±0.15	*
$C_{16:3}$	Not detected				0.30±0.01	0.15±0.15	0.30±0.01	-
$C_{17:0}$	0.60±0.01	0.80±0.20	0.50±0.01	0.50±0.01	0.60±0.01	0.60±0.05	0.50±0.01	ns
$C_{18:0}$	6.6±0.2	6.4±0.1	6.0±0.1	5.9±0.1	6.4±0.1	6.1±0.3	5.8±0.2	ns
$C_{18:1\ (n\text{-}9)}$	26.1^{a}±0.7	24.7^{bc}±0.2	24.7^{bc}±0.2	25.4^{ab}±0.2	23.5±0.1	24.2^{cd}±0.1	23.2^{d}±0.2	**
$C_{18:1\ (n\text{-}7)}$	2.25^{b}±0.1	1.90^{bcd}±0.2	2.65^{a}±0.2	1.65^{cd}±0.1	1.55^{d}±0.2	1.55^{d}±0.1	2.05^{bc}±0.1	**
$C_{18:2\ (n\text{-}6)}$	20.9^{c}±0.6	24.8^{a}±0.6	22.6^{b}±0.2	23.5^{b}±0.3	25.4^{a}±0.2	25.4^{a}±0.1	22.9^{b}±0.2	**
$C_{18:3\ (n\text{-}3)}$	1.20±0.10	1.75±0.40	1.30±0.10	1.25±0.15	1.30±0.01	1.55±0.05	1.30±0.01	ns
$C_{20:4\ (n\text{-}6)}$	Not detected				0.80±0.01	0.75±0.05	0.60±0.01	-
TFAs	97.35±2.3	99.15±0.7	98.90±0.1	99.60±0.1	98.85±0.2	98.60±0.2	98.15±0.5	-
SFAs	42.7^{a}±0.6	40.6^{b}±0.5	42.7^{a}±0.4	41.7^{ab}±0.2	41.9^{ab}±0.3	41.2^{b}±0.2	42.9^{a}±0.4	*
UFAs	54.7±1.8	58.7±1.3	56.3±0.4	57.9±0.2	56.9±0.1	57.5±0.1	55.3±0.2	ns
MUFAs	32.6^{a}±1.1	32.1^{a}±0.2	32.4^{a}±0.1	33.2^{a}±0.2	29.4^{b}±0.3	29.8^{b}±0.1	30.4^{b}±0.1	**
PUFAs	22.1^{d}±0.7	26.6^{ab}±1.0	23.9^{cd}±0.4	24.7^{bc}±0.4	27.5^{a}±0.2	27.7^{a}±0.1	24.9^{bc}±0.2	**

ns=not significant,* : (*p*<0.05) , ** (*p*<0.01)

TFAs: total fatty acids, SFAs: saturated fatty acids, UFAs: unsaturated fatty acids, MUFAs: mono-unsaturated fatty acids, PUFAs: poly-unsaturated fatty acids.

Table 4: Fatty acids profile (weight % of total fatty acids) of frozen meat (day 20).

	Control	E40	E80	C200	C400	E40C200	E80C400	Sig
$C_{10:0}$	0.35±0.05	0.30±0.01	0.30±0.01	0.30±0.01	0.30±0.01	0.30±0.01	0.30±0.01	ns
$C_{12:0}$	0.20±0.01	0.20±0.01	Not detected					-
$C_{14:0}$	2.95±0.05	2.90±0.01	3.10±0.10	2.95±0.05	2.95±0.15	2.70±0.01	3.10±0.1	ns
$C_{15:0}$	0.50^{b}±0.01	0.60^{a}±0.01	0.50^{b}±0.01	0.50^{b}±0.01	0.60^{a}±0.01	0.60^{a}±0.01	0.65^{a}±0.01	**
$C_{16:0}$	32.6^{ab}±0.2	31.6^{bc}±0.2	33.5^{a}±0.6	31.4^{bc}±0.2	31.2^{bc}±0.7	30.9^{c}±0.2	33.6^{a}±0.2	**
$C_{16:1}$	5.2^{a}±0.05	4.4^{bc}±0.05	4.6^{b}±0.05	5.1^{a}±0.20	4.1^{cd}±0.15	3.9^{d}±0.05	4.3^{bc}±0.10	**
$C_{16:3}$	0.35±0.05	0.30±0.01	0.30±0.01	0.30±0.01	0.30±0.01	0.30±0.01	0.30±0.01	ns
$C_{17:0}$	0.60^{a}±0.01	0.60^{a}±0.01	0.60^{a}±0.01	0.50^{b}±0.01	0.60^{a}±0.01	0.60^{a}±0.01	0.60^{a}±0.05	*
$C_{18:0}$	6.1^{bc}±0.10	6.7^{a}±0.01	5.9^{cd}±0.10	5.8^{d}±0.15	6.1^{bc}±0.01	6.1^{bc}±0.01	6.4^{b}±0.10	**
$C_{18:1\ (n-9)}$	25.5^{a}±0.1	23.3^{bc}±0.2	23.9^{b}±0.1	23.1^{d}±0.2	22.4^{bcd}±0.4	23.1^{cd}±0.1	22.8^{cd}±0.2	**
$C_{18:1\ (n-7)}$	2.00±0.01	1.80±0.10	2.25±0.05	2.00±0.01	1.75±0.20	1.80±0.01	2.00±0.01	ns
$C_{18:2\ (n-6)}$	20.6^{c}±0.40	24.0^{b}±0.01	21.1^{c}±0.20	24.0^{b}±0.01	26.2^{a}±0.70	26.1^{a}±0.05	21.5^{c}±1.00	**
$C_{18:3\ (n-3)}$	1.00^{d}±0.01	1.20^{bcd}±0.10	1.05^{cd}±0.05	1.30^{abc}±0.01	1.45^{ab}±0.10	1.55^{a}±0.05	1.10^{cd}±0.01	**
$C_{20:4\ (n-6)}$	0.40^{b}±0.10	0.85^{ab}±0.05	0.45^{b}±0.05	0.60^{ab}±0.01	1.00^{a}±0.30	1.00^{a}±0.01	0.40^{b}±0.10	*
TFAs	98.10±0.40	98.60±0.70	97.50±0.50	97.95±0.30	98.70±0.10	98.75±0.15	97.40±0.90	-
SFAs	43.1^{b}±0.20	42.8^{bc}±0.20	44.0^{ab}±0.60	41.5^{cd}±0.01	41.6^{cd}±0.50	41.2^{d}±0.10	45.0^{a}±0.50	**
UFAs	55.0^{bc}±0.2	55.9^{ab}±0.40	53.5^{cd}±0.10	56.5^{ab}±0.05	57.1^{a}±0.40	57.6^{a}±0.10	52.4^{d}±1.4	**
MUFAs	33.0^{a}±0.10	29.8^{bcd}±0.3	30.9^{b}±0.10	30.6^{bc}±0.05	28.5^{e}±0.80	29.0^{de}±0.01	29.4^{cde}±0.30	**
PUFAs	22.0^{c}±0.30	26.1^{b}±0.10	22.6^{c}±0.20	25.9^{b}±0.01	28.6^{a}±1.20	28.6^{a}±0.10	23.0^{c}±1.10	**

ns=not significant,* : ($p<0.05$) , ** ($p<0.01$)

TFAs: total fatty acids, SFAs: saturated fatty acids, UFAs: unsaturated fatty acids, MUFAs: mono-unsaturated fatty acids, PUFAs: poly-unsaturated fatty acids.

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