RESPONSE OF GROWING RABBITS TO DIETARY ANTIOXIDANT VITAMINS E AND C. 2. EFFECT ON MEAT QUALITY

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ABSTRACT

A seven-treatments experiment was carried out to study the response of seventy, 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 a combination, on some meat quality traits at the end of 7-weeks experimental 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 additional vitamin supplementation (40 mg vitamin E provided through the vitamin and mineral premix); (2) 40 mg vitamin E (E40); (3) 80 mg vitamin E (E80); (4) 200 mg vitamin C (C200); (5) 400 mg vitamin C (C400); (6) 40 mg vitamin E+200 mg vitamin C (E40C200); (7) 80 mg vitamin E+400 mg vitamin C (E80C400). Meat α-tocopherol and ascorbate contents of the 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, also C400 group at day 10 and E40C200 group at day 20 for ascorbate content. TBARS 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 polyunsatured fatty acids (PUFAs) content of the stored 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 PUFA's. Unfortunately, appreciable character could cause problems for meat storage, processing and cooking, since PUFA's are highly 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). Also, the shelf-life of the meat deteriorates (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 oxidised form (Reed, 1992). Numerous studies (*e.g.* Botsoglou *et al*., 2004; Lo Fiego *et al*., 2004) approved the positive relationship between the deposited vitamin E and meat quality in protecting the PUFA's against oxidation. 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 lipid stability of meat in low α-tocopherol diet fed rabbits, which disappeared by 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 NZW rabbits were 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^{-1} diet as follows 1: control with no additional vitamin supplement (40 mg vitamin E provided through the vitamin and mineral premix; NRC 1977), 2: 40 mg vitamin E (E40), 3: 80 mg vitamin E (E80), 4: 200 mg vitamin C (C200), 5: 400 mg vitamin C (C400), 6: 40 mg vitamin E + 200 mg vitamin C (E40C200), and 7: 80 mg vitamin E + 400 mg vitamin C (E80C400). Basal diet was formulated according to the NRC (1977) recommendation. Ingredients and nutrient content 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 the water, and then sprayed over the pellets, in every consecutive day interval.

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

Ingredients:	Wheat bran 25.5%, barley 23.0%, soybean meal (44%) 21.5%, wheat straw 19.5%, limestone 1. 5%, di calcium phosphate 0.50 %, NaCl 0.30%, vitamin & mineral premix* 0.30%, DL-methionine 0.20%, coccidiostat 0.10%, and fungostat 0.10 %; Total 100.0%
Nutrient content:	DM, 89%; CP, 17.06%; DE (MJl/kg) 10.88; CF, 13.12%; Ca, 0.91%; P, 0.64%; Lysine, 0.87%; methionine + cysteine 0.69%

**Supplied per kg of diet: 12000 IU vitamin A; 2200 IU vitamin D_3; 13.4 mg vitamin E (determined); 2.0 mg vitamin K_3; 1.0 mg vitamin B_1; 4.0 mg vitamin B_2; 1.5 mg vitamin B_6; 0.0010 mg vitamin B_{12}; 6.7 mg vitamin PP; 6.67 mg vitamin 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 method (1996).

Determination TBARS

For determining the rate of lipid peroxidation of frozen meat, the thiobarbituric acid-reactive substance (TBARS) test was carried out using three loin meat samples of each treatment at days 10 and 20 after slaughtering, according to AOAC (1990). The TBARS 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-thiobarbituric acid. Secondary oxidation products of oils and fats react with 2-thiobarbituric 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 incorporated α-tocopherol and ascorbate in the frozen loin after 10 or 20 days of storage significantly (P<0.01) increased as the level of α-tocopherol 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, which means that those vitamins act synergistically (P<0.01) to minimize the loss of α-tocopherol as compared to the loss of 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 of α-tocopherol concentration of the muscles depends on the increase in the α-tocopheryl acetate level of the diet. Moreover, ascorbate supplementation 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^{-1}) in low (40 mg kg^{-1}) but not in high (300 or 500 mg kg^{-1}) vitamin E content diets, and found significantly increased α-tocopherol content of refrigerated meat.

Oxidative stability of the muscle lipids (TBARS value)

Lipid oxidation of the rabbit muscle (TBARS) at days 10 and 20 of frozen storage, and the increase in deterioration rate values is illustrated in Table 2. It was found that TBARS values were lowered (P<0.01) by the supplementation of the diets with 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 (TBARS value) as was found in 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 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 by 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: Contents of vitamins E and C and TBARS values of meat at days 10 and 20 of frozen storage

	Control	E40	E80	C200	C400	E40C200	E80C400	Sig.
				Vitamins in meat (µg/g)				
Vit. E (d 10)	2.16^{d}±0.01	2.66^{c}±0.03	3.45^{b}±0.04	ND	ND	2.82^{c}±0.10	3.68^{a}±0.02	**
Vit. E (d 20)	0.46^{e}±0.04	0.91^{d}±0.03	1.17^{c}±0.05	ND	ND	1.90^{b}±0.04	2.14^{a}±0.01	**
% loss	78.8^{a}±2.04	65.9^{b}±0.93	66.1^{b}±1.03	ND	ND	32.5^{d}±2.76	41.9^{c}±0.07	**
Vit.C (d 10)	2.70^{d}±0.04	ND	ND	4.19^{c}±0.10	5.50^{a}±0.11	4.40^{c}±0.11	5.16^{b}±0.01	**
Vit.C (d 20)	0.34^{d}±0.01	ND	ND	0.69^{c}±0.05	1.05^{b}±0.01	1.45^{a}±0.04	1.41^{a}±0.01	**
% loss	87.2^{a}±0.35	ND	ND	83.5^{b}±0.81	80.8^{c}±0.10	67.1^{e}±0.05	72.6^{d}±0.21	**
				TBA-RS (ng/g)				
Day 10	46.6^{a}±1.70	35.4^{b}±0.94	26.1^{c}±1.19	21.7^{d}±1.15	22.6^{d}±1.24	26.6^{c}±1.24	44.0^{a}±0.17	**
Day 20	58.6^{a}±1.60	49.1^{b}±0.72	39.1^{d}±1.18	31.6^{e}±0.60	37.2^{d}±1.22	43.9^{c}±1.03	49.1^{b}±0.66	**
% increase	25.8^{bc}±2.4	38.6^{abc}±5.7	50.3^{ab}±11.4	45.9^{ab}±4.9	66.7^{a}±14.7	65.7^{a}±11.8	11.7^{c}±1.76	**

ND= not determined; **(P<0.01)

Fatty acid profile of intramuscular fat

Fatty acid profile of frozen loin meat at days 10 and 20 *post mortem* is illustrated in Tables 3 and 4. The proportions of PUFA, especially C18:2 and C18:3 was higher (P<0.01), while the proportion of monounsaturated fatty acids (MUFAs) was lower (P<0.01) as compared to the control, except in 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 supported by the results of Bernardini *et al*. (1996) and Dal Bosco *et al*. (2004) whose studies clarified that vitamin E (200 mg kg^{-1} diet) resulted increase of PUFAs ratio of rabbit meat. Dal Bosco *et al*. (2004) found that vitamin E inhibits 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} rabbit 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 C18:1 n-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}).

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

	Control	E40	E80	C200	C400	E40C200	E80C400	Sign.
C10:0				Not detected			0.30±0.01	-
C12:0				Not detected			0.30±0.01	-
C14:0	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	**
C15:0	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
C16:0	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	*
C16:1	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	*
C16:3		Not detected			0.30±0.01	0.15±0.15	0.30±0.01	-
C17:0	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
C18:0	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
C18:1(n-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	**
C18:1(n-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	**
C18:2(n-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	**
C18:3(n-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
C20:4(n-6)		Not detected			0.80±0.01	0.75±0.05	0.60±0.01	-
TFA	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	-
SFA	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	*
UFA	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
MUFA	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	**
PUFA	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	**

Table 4: Fatty acids (FA) profile of frozen meat (day 20)

	Control	E40	E80	C200	C400	E40C200	E80C400	Sign.
C10:0	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
C12:0	0.20±0.01	0.20±0.01			Not detected			-
C14:0	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
C15:0	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	**
C16:0	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	**
C16:1	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	**
C16:3	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
C17:0	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	*
C18:0	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	**
C18:1 (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	**
C18:1 (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
C18:2 (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	**
C18:3 (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	**
C20:4 (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	*
TFA	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	-
SFA	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	**
UFA	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	**
MUFA	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	**
PUFA	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)

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