RESPONSE OF GROWING RABBITS TO DIETARY ANTIOXIDANT VITAMINS E AND C. 1. EFFECT ON PERFORMANCE

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ABSTRACT

A seven-treatment experiment was carried out to study the response of seventy 6-week old New Zealand White (NZW) rabbits to dietary supplementation with the antioxidant vitamins, α-tocopheroyl acetate (vitamin E) and ascorbic acid (vitamin C), provided individually or in a combination on some performance traits. Rabbits were equally allocated to one of the following supra-nutritional levels of the two vitamins per kg diet up to the 13th week of age: 1, control with no extra vitamin supplement (40 ppm vitamin E provided through the vitamin A and mineral premix; 2, supplemented with 40 ppm vitamin E (E40); 3, supplemented with 80 ppm vitamin E (E80); 4, supplemented with 200 ppm vitamin C (C200); 5, supplemented with 400 ppm vitamin C (C400); 6, supplemented with 40 ppm vitamin E and 200 ppm vitamin C (E40C200); and 7, supplemented with 80 ppm vitamin E and 400 ppm vitamin C (E80C400).
The C200 group recorded the highest live weight gain and best feed conversion ratio (1312 g and 2.68 vs. 943 g and 3.68 in control group; $P<0.01$); E80 group had the highest dressing percentage (65.9% vs. 62.5 in control group; $P<0.05$) and C400 group showed the highest total antioxidant capacity values and lymphocytes percentage (2.4 mmol/l and 63% vs. 1.89 mmol/l and 58% in control group; $P<0.01$). In conclusion, the vitamin E and/or vitamin C successfully enhanced the growth performance, anti-oxidant status and immunity traits of growing rabbits.

Key words: Rabbit, Vitamins E and C, Growth performance, Antioxidant capacity, Immunity.

INTRODUCTION

Under normal conditions, the body has sufficient antioxidant reserves to cope with the production of free radicals (oxidants), which are produced continuously during metabolism and may increase as a result of pathological and other circumstances. When oxidants generation exceeds the body's antioxidant production capacity, oxidative stress develops (Roth, 2000). The formation of these oxidants is counteracted by natural anti-oxidants. 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 a.l*, 1994). Vitamin C can reduce the generation of oxidants and regenerates α-tocopherol from its oxidation form (Reed, 1992). Based on that anti-oxidant action, both vitamins have been used under stress or un-coming conditions to improve the performance *in vivo*. In rabbits, Meshreky and Shaheed (2003) and Corino *et al.* (2007) and others working on vitamin E, and Abdel-Hamid (1994) and Sedki *et al.* (2002) working on vitamin C, reported a growth promoting action for the two vitamins, Yet, other studies failed to prove such response (Castellini *et al.*, 1998, 2001; Oriani *et al.*, 2001; Dal Bosco *et al.*, 2004; Botsoglou *et al.*, 2004; Selim *et al.*, 2004).

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 the performance of growing rabbits.

MATERIALS AND METHODS

Animals and diets

Seventy NZW rabbits were evenly sexed, weighed and individually caged at 6 week of age to evaluate the response to supranutritional levels of α-tocopheryl (vitamin E) and vitamin C, provided individually or in a combination per kg diet as follows: C: control diet with no extra vitamin supplement (40 ppm vitamin E provided through the vitamin and min. premix; NRC 1977), E40: 40 ppm vitamin E, E80: 80 ppm vitamin E, C200: 200 ppm vitamin C, C400: 400 ppm vitamin C, E40C200: 40 ppm vitamin E +200 ppm vitamin C, and E80C400: 80 ppm vitamin E+ 400 ppm vitamin C. Basal diet was formulated to satisfy the NRC (1977) recommendations. 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 the water, and then sprayed over the pellets, in every other day interval. During the 49-day growth trial period, live body weight and feed intake were recorded in weekly intervals. Weight gain and feed conversion ratio were calculated.

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, dicalcium phosphate 0.50%, NaCl 0.30%, vitamin and mineral premix* 0.30%, Dl-Methionine 0.20%, anti-coccidial 0.10%, and anti-fungal 0.10 %
Chemical composition:	DM, 89%; CP, 17.06%; DE, 2605 kcal/kg; CF, 13.12%; Ca, 0.91%; P, 0.64%; Lysine, 0.87%; Methionine + cystene 0.69%

*Supplied per 1 kg. of diet: 12000 IU vitaminA; 2200 IU vitamin D3; 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

Carcass traits

Six rabbits of each treatment were fasted for 12 hours, and then slaughtered; fur was immediately loosened, and peeled. The hot carcass, head, liver, kidneys, and heart as the dressing percentage were considered. Dressing, liver and abdominal fat was proportioned to the live weight upon slaughtering.

Determination of vitamins E and C

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

Determination of plasma antioxidant status

Blood samples of three rabbits were withdrawn from the ear vein over Na_2ETDA (1-2 mg/ml blood) and centrifuged (10000 X g for 10 minutes at 4°C). The supernatant was used immediately for determining plasma antioxidant status (oxidants-antioxidant equilibrium) as total peroxyl radical-trapping antioxidant parameter (TRAP) as ascribed by Koracevic and Koracevic (2001).

Hemoglobin (Hb), red blood cells (RBCs) and white blood cells (WBCs) count and differential

As indicators to immune function status, fresh blood samples of three rabbits of each treatment were assigned for and Hb concentration (g/dl), RBCs (10^6/mm^3) and for WBCs (10^6/mm^3) count and differentiation; lymphocytes, neutophils, Monocytes, eosinophils, and basophils, according to the methods reported by Schalm *et al*. (1975).

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

Growth performance and carcass traits

Data on growth performance and carcass traits are summarized in Table 2. It is quite clear that rabbits had access to extra levels of both vitamins beyond recommendation level achieved ($P<0.01$) better performance in growth terms (live weight gain, feed intake and feed conversion ratio) compared to the control group. Massive additional increase in total weight gain, ranged between 21.0 and 39.1%, matched with substantial reduction in feed conversion ratio, estimated by 15.2 and 27.7% were recorded. The minimum values for both variables were belonged to the E40 group, while the superiority was always for the C100 group. These results partially run parallel with those reported by Sedki *et al.* (2002), Meshreky and Shaheed (2003) and Corino *et al.* (2007) in that live weight gain, but not feed intake or feed conversion was improved as growing rabbits allowed to extra doses of vitamin E, while other reports (Castellini *et al.*, 1998, 2001; Oriani *et al.*, 2001; Dal Bosco *et al.*, 2004; Botsoglou *et al.*, 2004) failed to detect a growth promoting action for vitamin E. Also, the current findings and those cited by Abdel-Hamid (1994) and Sedki *et al.* (2002) accord that vitamin C has a growth promoting action, which conflict with the results of Selim *et al.* (2004) reporting no further response to supplemental vitamin C on growth performance of rabbits. The improvement in growth traits could be attributed to the enhancement of the total antioxidant status as indicated in Table 3. Yet, the fluctuations on the response to vitamins E and C might be due to several factors. More important that most studies dealt with these two vitamins aimed at first to study the effect on meat quality (Bernardini *et al.*, 1996; Lopez-Bote *et al.*, 1997; Castellini *et al.*, 1998, 2001; Corino *et al.*, 1999, 2007; Oriani *et al.*, 2001; Botsoglou *et al.*, 2004; Lo Fiego *et al.*, 2004). Also, the initial weight and the length of the study may be involved. Giovannangelo *et al.* (2001) with 2.0 kg, Botsoglou *et al.* (2004) with 1.47 kg and Corino *et al.* (2007) with 1.8 kg, they started the growth trial part of the work. So, not surprisingly, that the study carried out by Giovannangelo *et al.* (2001) lasted for 29 days and that reported by Corino *et al.* (1999) lasted only for 15 days. Other factors can not be ignored; e.g. the experimental conditions under which the study was performed (environmental condition; summer or normal, dose of the vitamin studied, way of introduction; diet, water, oral or injection).

Table 2: Growth performance and carcass traits

	Growth performance (6-13 week of age)				Carcass traits		
	Initial weight (g)	live weight gain (g)	Feed intake (g)	Feed conversion ratio	Dressing %	Liver %	Abdominal fat %
Control (C) (40 ppm VE)	749±34	943^{c}±42	3422^{d}±42	3.68^{a}±0.18	62.5^{b}±1.67	5.14±0.34	1.60±0.23
Cont.+E40	715±42	1141^{b}±35	3540^{bc}±46	3.12^{b}±0.10	62.4^{b}±0.62	4.76±0.28	0.93±0.16
Cont.+E80	711±38	1277^{a}±33	3493^{cd}±36	2.75^{c}±0.07	65.9^{a}±0.36	4.65±0.39	1.44±0.44
Cont.+C200	742±41	1312^{a}±14	3523^{bc}±13	2.68^{c}±0.04	61.4^{b}±0.73	5.28±0.38	1.36±0.29
Cont.+C400	724±50	1266^{ab}±44	3545^{bc}±17	2.81^{bc}±0.09	61.5^{b}±0.73	5.36±0.34	1.26±0.23
Cont.+E40C200	715±39	1214^{ab}±21	3613^{ab}±16	2.98^{bc}±0.05	63.3^{ab}±0.99	4.91±0.35	1.35±0.27
Cont.+E80C400	719±39	1202^{ab}±63	3655^{a}±13	3.09^{b}±0.13	63.61^{ab}±0.65	4.86±0.31	1.61±0.16
significance	ns	**	**	**	*	ns	ns

E40=40 ppm vitamin E , E80=80 ppm vitamin E , C200=200 ppm vitamin C, C400=400 ppm vitamin C, E40C200=40 ppm vitamin E +200 ppm vitamin C, E80C400=80 ppm vitamin E+ 400 ppm vitamin C (/kg diet)
ns=not significant, *= ($P<0.05$), **= ($P<0.01$)

Of studied carcass traits (dressing, liver and abdominal fat), only dressing percentage was improved significantly ($P<0.05$) with the E80 group. Also, the observations made by Abdel-Hamid (1994) with vitamin C and Corino *et al.* (2007) with vitamin E indicated that dressing % was significantly

improved with such supplementation. Other studied, carried out by Sedki *et al.* (2002), Selim *et al.*(2004) and Castellini *et al.* (1998) reported no effect of vitamin C or E on carcass traits.

Total antioxidant capacity

Results in Table 3 indicate that both vitamins E and C, except the E80 and E80C400 treatments showed higher (P<0.01) TRAP values relative to the control. TRAP is a single measure used as good indicator to describe the dynamic equilibrium between pro-oxidants and anti-oxidants in plasma compartment (Ghiselli *et al.*, 2000). These data suggest that both vitamins can effectively control the excessive generation of oxidants in live rabbits and latter improve muscle lipostability (Giovannangelo *et al.*, 2001). Previous works (Bernardini *et al.*, 1996; Giovannangelo *et al.*, 2001; Dal Bosco *et al.*, 2004) reported that vitamin E enhanced the plasma TRAP, such response was attributed to the longer ingestion period of the vitamin (Bernardini *et al.*, 1996).

Hb concentration, RBC's count and WRC's count and differentiation

Results provided in Table 3 illustrate the Hb, RBCs count and WBCs (leukocytes) count and differentiation (lymphocytes, neutrophils, monocytes, eosinophils and basophils). Of these immunity related parameters, lymphocytes percentage by C400 and E80C400 groups, neutrophils percentage by E40 group and basophils percentage by E80 group were significantly (P<0.01) leveled up. Completely in agreement with findings of this study, the contentions reported by Sedki *et al.* (2002) working on vitamins E, C and their combination, and Meshreky and Shaheed (2003) working on vitamin E accord that these vitamins had only appreciable significant effect on lymphocytes % that consider a good indicator of increasing the immunity efficiency. Badwev and Karnovsky (1980) reported that vitamin E plays an important role in protecting leukocytes and macrophages during phagocytosis, it protects leukocytes from the toxic products that produced from ingested bacteria.

Table 3: Total antioxidant capacity (TAC), Hb, RBC's count and WBC's count and differentiation

	TAC (mmol/l)	Hb (g/dl)	RBC's (10^6/mm^3)	WBC's (10^3/mm^3)	Lymph. %	Neuto. %	Mono. %	Eosino. %	Baso. %
Control (C)	1.89^{c}±0.02	15.06±0.27	5.70±0.11	7.05±0.62	58.0^{bc}±1.50	36.60^{ab}±1.20	2.66±0.30	2.00±0.60	0.66^{ab}±0.30
(40 ppm VE)									
ont. + E40	1.97^{b}±0.03	14.16±0.14	5.37±0.09	7.75±0.55	55.66^{c}±1.20	39.00^{a}±1.15	3.00±0.57	1.66±0.33	0.66^{ab}±0.30
ont. + E80	1.91^{c}±0.03	13.50±0.41	5.11±0.14	6.80±0.10	60.60^{ab}±0.66	34.30^{b}±0.33	2.33±0.30	1.66±0.33	1.00^{a}±0.00
Cont.+ C200	1.99^{b}±0.01	13.63±0.32	5.08±0.06	6.72±0.64	61.30^{ab}±0.88	33.30^{bc}±0.82	2.00±0.57	2.66±0.33	0.33^{b}±0.30
Cont.+C400	2.40^{a}±0.06	13.63±0.60	5.19±0.20	6.30±0.71	63.00^{a}±1.53	30.33^{c}±1.30	3.00±0.58	3.00±0.57	0.33^{b}±0.30
Cont.+E40C200	2.37^{a}±0.10	12.86±0.77	4.84±0.24	6.75±0.66	60.66^{ab}±0.67	33.33^{bc}±1.30	3.00±0.57	2.33±0.33	0.33^{b}±0.30
Cont.+E80C400	1.92^{c}±0.04	13.10±1.06	4.94±0.37	7.58±0.70	63.00^{a}±1.00	30.00^{c}±1.00	3.33±0.66	2.33±0.33	0.33^{b}±0.30
Significance	**	ns	ns	ns	**	**	ns	ns	**

E40=40 ppm vitamin E , E80=80 ppm vitamin E , C200=200 ppm vitamin C, C400=400 ppm vitamin C, E40C200=40 ppm vitamin E +200 ppm vitamin C, E80C400=80 ppm vitamin E+ 400 ppm vitamin C (/kg diet). ns= not significant, **= (P<0.01)

CONCLUSIONS

From performance traits it can concluded that both vitamin E and C improves growth rate, feed efficiency but no dressing out percentage (only the highest dose of vit E); effects of vitamin E seem to be dose-dependent, whereas the highest vitamin C dose does not improve the results obtained with the lower vitamin C dose; the effects of vitamin E and vitamin C do not seem to be additive.

Accordingly the recommendation would be to use the higher dose of vitamin E. But if we are not interested in dressing out percentage the use of the low dose of vitamin C might be enough.

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