INTERACTION BETWEEN SUPPLEMENTAL VITAMIN E AND ENDOGENOUS ANTIOXIDANT ENZYMES OF DIFFERENT RABBIT GENETIC RESOURCES: 1- EFFECT ON PERFORMANCE DURING SUMMER SEASON

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ABSTRACT

Supplemental antioxidants, in association with the action of endogenous antioxidants' enzymes could bring back the rabbit performance as high as possible during stress episode. The current study was initiated to explore the effect of the relationship between rabbit breed (exotic; V-line *vs.* native; Gabali) and dietary vitamin E level; 40 (control), 80, or 120 mg/kg diet, and their respective effect on growth performance, carcass traits, and blood plasma levels of α-tocopherol and endogenous antioxidants' enzymes glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT). One hundred-fifty-six week old rabbits of both genotypes (25 rabbit allocated for each vitamin E level), were distributed among the 6 experimental treatments. Results indicate that breed had no effect on growth performance and plasma antioxidants' enzymes activities. However, V-line rabbits had significantly higher hot carcass and total edible carcass quarters, but lower plasma α-tocopherol level compared to Gabali rabbits. Rabbits fed the highest dietary vitamin E level (120 mg/kg) significantly showed the highest live weight gain, best feed conversion ratio, highest plasma α-tocopherol level, and lowest GSH-Px activity compared to 40 or 80 mg/kg diet level. The interaction between rabbit breed and dietary vitamin E level significantly affected total weight gain and plasma α-tocopherol level. Other studied traits (feed conversion ratio, carcass traits' percentages, or plasma antioxidants' enzymes or α-tocopherol levels) were not significantly affected by the interaction.

It could be recommended adding a high level of vitamin E (120 mg/kg diet) to alleviate some of the impact of heat stress on rabbits, irrespective the breed used, and without expected contribution of the endogenous antioxidant enzymes activities.

Key words: Growing rabbit, vitamin E, rabbit genotype, antioxidant enzymes, heat stress.

INTRODUCTION

During stress episode, reactive oxygen species (ROS) generation exceeds the body's antioxidant production capacity, and oxidative stress develops (Roth, 2000). These active metabolites could result in drastic damage to the cell structures; protein, lipids and DNA, and further induce physiological and pathological changes, resulting in poor performance, reduced welfare of the live animal and worsen its meat quality (Abdel-Kalek, 2010). In the rabbit, stress associated with exposure to high ambient temperatures decreases growth performance, possibly because of excessive production of ROS that oxidize and destroy cellular biological molecules (Liu *et al.,* 2011).

Endogenous antioxidants' enzymes are the major cell defense against oxidative stress. Glutathione peroxidase (GSH-Px) and catalase (CAT) are considered the major peroxide-removing enzymes located in the cytosol. superoxide dismutase (SOD) plays an important role in protecting against damage by the superoxide anion radical (Chan and Decker, 1994). GSH-Px in the rabbit liver is about 6-12 folds that in cattle, sheep and cat (Tapel *et al.,* 1982), indicating its importance in scavenging excessive ROS produced by the tissues. On the other hand, dietary supplementation has been proved to be a simple and convenient strategy to introduce a natural antioxidant that may effectively inhibit the ROS reactions (Botsoglou *et al.,* 2004). Alpha-tocopherol is a highly effective natural antioxidant that protects cellular membranes against oxidative damage (Morrissey *et al.,* 1994). Most studies on supplemental antioxidants to growing rabbits have been carried out without due stress factor (Castellini *et al.,* 1998, 2000, and Oriani *et al.,* 2001). While, Liu *et al.,* (2011) in 21-day growth trail, fed rabbits reared under constant heat stress conditions (35ºC) diets supplemented with an extract of chestnut wood, rich in the antioxidant poly-phenolic compounds. They reported an increase in plasma SOD, and GSH-Px in liver.

In the rabbit, no studies are available that explicitly show the relationship between exogenous (supplemental) and endogenous antioxidants on rabbit performance, especially under stress conditions. Moreover, how proper rabbit genotypes respond well to such relationship.

The current study was performed to explore the relationships between supplemental vitamin E level [40 (control), 80, or 120 mg/kg diet] and the rabbit genotype [exogenous; V-line *vs.* indigenous; Gabali] on growth performance, carcass traits, and blood plasma levels of α-tocopherol and antioxidants' enzymes under summer heat stress conditions.

MATERIALS AND METHODS

Feeding, management, and slaughtering protocol

One hundred-fifty–6 week rabbits were divided into six groups (2 breeds*3 dietary vitamin E level) and fed the same basal diet for 10 experimental weeks with different levels of vitamin E; 40 (provided by the vitamin-mineral premix; control), 80 or 120 mg/kg diet of *all* rac-α-tocopheryl acetate. The basal diet was formulated to satisfy the NRC' (1977) recommendation. Ingredient and chemical composition of the basal diet are presented in Table 1. Rabbits were kept under the same managerial routine during July-September months. Rabbitry air temperature and relative humidity were recorded daily. Six rabbits (three males and three females) were assigned for hot carcass and total edible parts (including the head) determination (as percentages of live weight of slaughtering).

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

Ingredients: clover hay 31.81%, wheat bran 22.35%, barley 30.5%, soybean meal (44%) 11.0%, molasses 3.0%, limestone 0.35%, NaCl 0.30%, vitamins & minerals premix* 0.30%, di calcium phosphate 0.10 %, Dl- methionine 0.09%, anti-coccidial 0.10%, and anti-fungal 0.10 %; **Total** 100.0%.
Chemical composition: Dry matter, 89%; crude protein, 16.0%; digestible energy (kcal/kg) 2460; crude fiber, 14.3%; Ca, 0.71%; P, 0.39%; Lysine, 0.60%; methionine + cysteine 0.50%.

*Supplied per kg diet: 6000 IU vit. A; 2200 IU vit. D_3; 11.9 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.

Breeds

Two rabbit breeds were (25 rabbit/treatment) used in the study. V line, a maternal line selected for litter size at weaning, and characterized by its high growth rate. It has been developed by Animal Science Dept., Valencia, Spain, where the climate is not widely different from the weather of Delta of Nile in Egypt. Gabali, an Egyptian breed that has shown acceptable breeding ability.

Blood plasma analyses

Rabbits used in slaughtering test were assigned for blood plasma antioxidants determination. α-tocopherol was determined according to Buttriss and Diplock (1984). GSH-Px activity, according to Agergaard and Jensen (1982), SOD, according to Marklund and Marklund (1974), and CAT activity was measured according to Aebi (1974).

Statistical procedures

Data were subjected to two-way analysis of variance with genotype and vitamin E treatment as the main effects, using SPSS (1999). Significant means were compared according to Duncan's (1955).

RESULTS AND DISCUSSION

Throughout the ten-week growth trial, the recorded daily air temperatures averaged 32.4°C, while relative humidity values averaged 46.3%.

Growth performance and carcass traits

Results provided in Table 2 indicate that the rabbit genotype (V-line *vs.* Gabali breed) had no significant effect on rabbit growth performance (live weight gain and feed conversion ratio). Hot carcass and total edible parts percentages were significantly higher in the V-line compared to the Gabali. The highest dietary vitamin E level (120 mg/kg) recorded the highest total live weight gain and feed conversion ratio (*P*=0.0001) compared to the other dietary vitamin E levels (40 or 80 mg vitamin E/kg). Both hot carcass and total edible parts percentages tended to increase with increasing dietary vitamin E level. The interaction between the genotype and dietary vitamin E level significantly affected live weight gain, but not feed conversion ratio or carcass traits. Neither carcass percentage nor total edible parts percentages were significantly affected by the interaction studied.

The lack of breed effect on growth performance reported on the current study could be account for the selection of V-line rabbits for high environment temperatures. In most cases, they did not differ from the environmental conditions that the Gabali has been adapted for. So, continuous genetic improvements within each genotype, may minimize the differences in growth performance. Supplemental vitamin E improved (13%) growth of heat stressed rabbits which agrees with previous works reported by Abdel-Samee and El-Masry (1997) using a supplementation with 0.354 mg Se plus 40 mg vitamin E/kg diet for rabbits grown under sub-tropical conditions. Also, Meshreky *et al.,* (2002) reported that injection with vitamin E (75 IU/kg weight/ week) improved daily gain, and dressing percentage of rabbits subjected to heat stress. Recently, in a 21-day growth trail, Liu *et al.,* (2011) reported that diets supplemented with polyphenols, significantly, improved live weight gain and feed conversion ratio of the rabbits reared under heat stress conditions. They stated that chestnut tannins might be used in compensating the decline in the activities of antioxidant enzymes by means of reacting directly with free radicals..

Plasma α-tocopherol and antioxidant enzymes' levels

Results provided in Tables 2 & 3 indicate that studied factors significantly affected (*P*=0.0001) the plasma α-tocopherol content. Gabali rabbit had a higher ability to deposit α-tocopherol than the V-line. Increasing dietary vitamin E level was associated with a parallel increase in plasma α-tocopherol level. Also, it is quite clear that the response to dietary vitamin E level was rather high in the Gabali than in the V-line (interaction). Studies on the rabbit have shown that an increase in supplemental dl-α-tocopheryl acetate level produced an increase in the α-tocopherol level of the blood plasma (Castellini *et al.,* 1998 and 2000, and Oriani *et al.,* 2001).

Results illustrated in Tables 2 & 3 indicate that except the significant (*P*=0.05) negative relationship between dietary vitamin E and GSH-Px, no significant relationship was found between the studied factors (rabbit genotype, dietary vitamin E level, and their interaction) and plasma antioxidants' enzymes activities (GSH-Px, SOD, and CAT). However, genetic variation in GSH-Px activity has previously been reported in the rabbit (Mézes *et al.,* 1994). In the current study, there was a tendency for breed effect on GSH-Px.

Table 2: Main effects of genotype, dietary vitamin E level (mg/kg diet) and their interaction on growth performance, carcass traits (% slaughtering weight), and blood plasma α-tocopherol (μg/g),and antioxidants' enzymes activities (u/g)

	Growth performance			Carcass traits		Blood plasma			
	Initial live weight (g)	Live weight gain (g)	Feed conversion Ratio	Hot carcass (%)	Total edible parts (%)	α-tocopherol	GSH-Px	SOD	CAT
Genotype									
V-line	801	1313	3.32	53.5A	63.5A	1.62B	1.18	0.338	563.6
Gabali	801	1280	3.33	51.9B	61.3B	2.14A	0.80	0.372	604.0
Pooled SE	18.7	21.8	0.07	0.004	0.004	0.014	0.157	0.015	53.44
Supplemental vitamin E level									
40 mg (Control)	800	1226B	3.53B	51.8	61.4	1.68C	1.38A	0.346	560.8
80 mg	801	1271B	3.43B	52.9	62.7	1.90B	0.88AB	0.333	551.2
120 mg	801	1392A	3.03A	53.4	62.4	2.06A	0.71B	0.386	639.5
Pooled SE	22.9	26.8	0.08	0.005	0.005	0.018	0.192	0.019	65.4
Interaction									
V-line- 40 mg vitamin E	800	1282bc	3.47	52.8	62.7	1.48c	1.86	0.330	537.5
V-line- 80 mg vitamin E	801	1306bc	3.37	53.8	63.5	1.52c	1.01	0.318	467.3
V-line-120 mg vitamin E	801	1352ab	3.13	54.0	63.1	1.86b	0.69	0.367	686.0
Gabali - 40 mg vitamin E	800	1169d	3.58	50.9	60.1	1.88b	0.90	0.362	584.0
Gabali - 80 mg vitamin E	801	1237cd	3.48	52.0	62.0	2.28a	0.75	0.348	635.0
Gabali-120 mg vitamin E	801	1433a	2.93	52.8	61.8	2.27a	0.73	0.405	593.0
Pooled SE	32.3	37.9	0.11	0.007	0.007	0.025	0.27	0.026	92.4
P value									
Genotype	0.996	0.276	0.915	0.005	0.003	0.0001	0.09	0.130	0.597
Vitamin E level	0.999	0.0001	0.0001	0.07	0.154	0.0001	0.05	0.129	0.583
Genotype X vitamin E level	0.999	0.029	0.289	0.867	0.586	0.0001	0.18	0.986	0.382

There is a lack on studies investigating the relationship between supplemental antioxidants and their counterparts of endogenous body origin (Tables 2 & 3). Guo *et al.,* (2003) stated that 100 mg vitamin E/kg broiler diet did not influence activities of hepatic CAT and SOD of the liver. In the rabbit, Liu *et al.*, (2011) reported that feeding growing rabbits a diet rich on polyphenols increased SOD level in plasma and GSH-Px in liver.

Table 3: Pearsons's correlation coefficients (*r*) for variables studied

	α-tocopherol	GSH-Px	SOD	CAT	Carcass %	Total edible parts %
α-tocopherol	1.00	-0.404	0.246	0.231	0.384	0.247
GSH-Px	-0.404	1.00	-0.265	0.107	-0.150	0.111
SOD	0.246	-0.256	1.00	0.229	-0.121	-0.121
CAT	0.231	0.107	0.229	1.00	0.205	0.230
Carcass %	0.384	-0.150	-0.121	0.205	1.00	0.943
Total edible parts %	0.247	-0.111	-0.121	0.230	0.943	1.00

CONCLUSION

In the current work, it could be suggested that the supplemental antioxidant (Vitamin E) had no positive relationship with endogenous antioxidants' enzymes activities. Also, rabbit breeds of different genetic background, but has close stress response, show close endogenous antioxidant profile.

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