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INTERACTION EFFECTS BETWEEN DIETARY VITAMIN E SUPPLEMENTATION AND ENDOGENOUS ANTIOXIDANT ENZYMES OF DIFFERENT RABBIT GENETIC RESOURCES ON SOME GROWTH PERFORMANCE, VITAMIN E CONTENTS AND OXIDATIVE STABILITY DURING SUMMER SEASON

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ABSTRACT

The current study was initiated to explore the effect of the relationship between dietary vitamin E at levels; 40 (control), 80, or 120 mg/kg diet, and male and female of two rabbit breeds (exotic; V-line vs. native; Gabali) and their respective effect on some growth performance, carcass traits, and α-tocopherol contents and the activities of endogenous antioxidant enzymes (glutathione peroxidase; GSH-Px, superoxide dismutase; SOD, and catalase; CAT), lipid oxidative stability (TBARS) of the hind legs' muscle kept frozen (30 or 60 days at -20ºC). One hundred-fifty-six week old rabbits of both genotypes (allocated for each vitamin E level and sex (13 male or 12 female rabbits)), were distributed among the 12 experimental treatments. At the end of experiment, 36 samples from hind legs' muscle (3 animals/ dietary vitamin E levels/ breed/ sex) were used in the study of meat quality traits.

***Results indicate that** the estimated THI values were 30.66, 29.32 and 30.21 during the experimental period (June, July and August) with average 30.063, these values indicating exposure of rabbits to severe heat stress. Body weight at 16 weeks of age, daily weight gain, the best feed conversion ratio, slaughter weight and hot carcass weight tended to increase significantly (P<0.001) with increasing dietary vitamin E levels. Increase in dietary vitamin E was associated with a linear increase in α-tocopherol content of the muscle, delayed lipid oxidation (19.36 or 43.17 vs.57.43±0.468 ng/g in 120 or 80 vs. 40 mg vitamin E/kg diet supplemental groups) and increase in antioxidants' enzymes activity. Positive correlations between α-tocopherol deposited in muscle and GSH-Px and CAT activities (r= 0.229 and 0.278; P<0.05) and SOD activities (r= 0.186) were found. However, negative correlations were observed between TBARS and GSH-Px, SOD (r= -0.199, -0.129) and CAT activities (r= -0.232; P<0.05).*

The rabbits genotype (V-line vs. Gabali breed) had no significant effect on some growth performance, however, V-line rabbits had significantly higher slaughter weight, hot carcass and dressing percentage, but lower plasma α-tocopherol levels compared to the Gabali rabbits. V-line breed had higher significantly (P<0.0179) α-tocopherol content (1.667 vs. 1.537±0.046 µg/g) in muscle and lipid oxidative stability (39.52 vs. 40.64±0.382; P<0.0681), however, lower GSH-Px and CAT activities as compared to Gabali breed.

Male rabbits had significantly higher daily weight gain, better feed conversion ratio value and insignificantly highest in dressing percentage than female rabbits. Significant difference between the male and female rabbit in against oxidation in frozen meat samples (38.48 vs. 41.5±0.382 ng/g). Male rabbits have lower endogenous antioxidant enzyme activities than the female. Prolonged storage decreased (P<0.0001) α-tocopherol content of the hind legs' muscles, the activities of endogenous antioxidants' enzymes and lipid oxidative stability.

***Conclusively**, adding a high level of vitamin E (120 mg/kg diet) to alleviate some of the impact of heat stress on different genotype rabbits, extra supplemental vitamin E, rabbit genotype and sex have a clear effect on meat quality as it increased the α-tocopherol content and lipid oxidative stability of the meat, during summer season.*

Key words: Growing rabbit, vitamin E, genotype, antioxidant enzymes, oxidative stability, summer months heat stress.

INTRODUCTION

Supplemental antioxidants, in association with the action of endogenous antioxidants' enzymes could bring back the rabbit performance as high as possible during stress episode. 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. 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). No studies are available that explicitly show the relationship between exogenous (supplemental) and endogenous antioxidants on rabbit performance, especially under different stress conditions. Moreover, how proper rabbit genotypes respond well to such relationship.

Food safety is an important and essential aspect for consumers, especially on the meat sector since numerous crises have recently affected meat production. Rabbit meat is highly valued for its nutritional and dietary properties; it is a lean meat with a low-fat content and less saturated fatty acids and cholesterol than other meats (Hernández and Gondret, 2006). Lipid oxidation is one of the main factors limiting the quality and acceptability of meats (Morrissey *et al.*, 1998). It could influence meat quality *ante mortem* and continues *post mortem* and during chilling, processing, display and long term storage (Bianchi *et al.*, 2006). Lipids of rabbit meat can suffer alterations during refrigerated storage, owing to lipolysis and oxidation, resulting in quality deterioration (Alasnier *et al*., 2000) characterised by sensory degradation and losses of nutritional value (Campo *et al*., 2006). It begins with oxidation of the double bonds of the phospholipids present in the cell membranes, leading to the production of free radicals hydro-peroxides (reactive oxygen species; oxidants). This leads to discoloration, drip losses, off- odour and off-flavour development, production of potentially toxic compounds (Gray *et al.*, 1996), and deterioration in the self-life of the meat (Dal Bosco *et al.*, 2004). In this case, endogenous (mainly: glutathione peroxidase; GSH-Px, superoxide dismutase; SOD, and catalase; CAT) and exogenous antioxidants enzymes work synergistically to neutralize the action of these oxidants. However, endogenous antioxidants' enzymes represent the principal antioxidants' defense line against oxidative damage, the subject did not receive an equivalent interest from rabbit scientists, and limited information is available concerning their role against oxidative damage. There has been an increasing interest in the use of antioxidants in rabbit feed formulas because the dietary manipulation of tissue lipid composition to produce meat with a high PUFA content could decrease meat oxidative stability (Hernández *et al*., 2008).

Recently, human resources have been expended to widely study the effects of various biological and zootechnical factors on rabbit carcass and meat quality (Dalle Zotte, 2002). Dietary vitamin E increases muscle Vit E concentrations, protects muscle polyunsaturated fatty acids against oxidative deterioration and improves meat quality (Zhang *et al*., 2012).Vitamin E is commonly used in animal feeds as an indispensable component of biological membranes with stabilizing properties and a high antioxidant activity. Vitamin E is the generic term used to describe at least eight naturally occurring compounds that exhibit the biological activity of α-tocopherol (Morrissey *et al*., 2000). Increasing the functionality (dietetic) of the meat as it increases the meat content of α-tocopherol (Lo Fiego *et al.*, 2004; Kowalska and Bielański, 2009), and more interesting is its involvement in enhancing the oxidative stability of the meat muscles during refrigeration, freezing (Zsédely *et al.*, 2008) or even cooking. Also, a high α-tocopherol level improves some

physical traits of meat, reducing shear values and increasing water holding capacity (Castellini *et al*., 1998).

Differences between rabbit lines were found in the fatty acid composition of the hind leg meat (Hernández *et al.*, 2008). Genetic rabbit line has an effect on intramuscular fat deposition and related characteristics that could lead to differences in meat quality (Zomeño *et al.*, 2010). Several reports have shown that rabbit genetic origin influence muscle lipid content (Gasperlin *et al*., 2006). However, there are no studies about the influence of genetic line on lipogenic activity in rabbit muscle.

The objective of this study was conducted to assess the effectiveness of α-tocopherol supplementation levels on some growth performance traits, carcass traits, α-tocopherol contents, endogenous antioxidants' enzymes activities and the oxidative stability of male and female in each of V-line as exogenous breed and Gabali as indigenous breed rabbits meat, under summer heat stress conditions.

MATERIALS AND METHODS

The present study was conducted at El-Gemeza Research Station, Garbia Governorate, Agricultural Research Centre, Ministry of Agriculture, Egypt from June to September 2011.

One hundred-fifty, 6 week rabbits (average 801±15 gm live body weight) were divided into twelve groups (3 dietary vitamin E levels x 2 breeds x 2 sex, n=12 female or 13 male/group), fed the same basal diet for 10 experimental weeks with different levels of vitamin E; 40 mg/kg diet (provided by the vitamin-mineral premix as recommended in NRC (1977) as control diet, 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 experiment period.

Breeds: Two rabbit breeds were used in the study: V line, a maternal line selected for litter size at weaning, and is characterised by 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, and Gabali as Egyptian breed, has shown acceptable breeding ability and very promising results.

Environmental condition: Air temperature (^{o}C) and relative humidity (%) inside the semi-closed rabbitry building were measured weekly throughout

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

Ingredients	**%**
Clover hay	31.81
Wheat bran	22.35
Barley	30.50
Soybean meal (44%)	11.00
Molasses	3.00
Limestone	0.35
NaCl	0.30
Vitamins & minerals premix*	0.30
Di calcium phosphate	0.10
Dl- methionine	0.09
anti-coccidial	0.10
anti-fungal	0.10
Total	**100.00**
Chemical composition	
DM	89.00
CP	16.00
DE (kcal/kg)	2460
CF	14.3
Ca	0.71
P	0.39
Lysine,	0.60
methionine + cystene	0.50

***Supplied per kg diet:** 6000 IU Vit. A; 2200 IU Vit D3; 11.9 mg Vit. E (determined); 2.0 mg Vit. K3; 1.0 mg Vit. B1; 4.0 mg Vit. B2; 1.5 mg Vit. B6; 0.0010 mg Vit. B12; 6.7 mg Vit. PP; 6.67 mg Vit. B5; 0.07 mg B8; 1.67 mg B9; 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.

the experimental periods between 12.00 to 14.00h using automatic thermo-hygrometer (Table 2). The temperature-humidity index (THI) was calculated using the equation
proposed by Marai *et al.* (2001) as follows: THI = db^{o}C – [(0.31 - 0.31 RH) (db^{o}C - 14.4)], where db^{o}C = Dry bulb temperature in Celsius and RH= Relative humidity percentage/100. The THI values obtained were then classified as follows: <27.8= Absence of heat stress, 27.8 - < 28.9= Moderate heat stress, 28.9- <30.0 = Severe heat stress and 30.0 and more = Very severe heat stress.

Some growth performance and Carcass quality traits: Body weight and feed intake were recorded only. At the end of the experimental diet (16 weeks of age), 36 rabbits (3 animals in each of vitamin E supplemental

Table 2. Maximum, minimum and average air temperature ºC, percentages of relative humidity(%) and temperature humidity index (THI) during the experimental periods.

Months	Air temperature (ºC)			Relative humidity (%)			THI
	Maximum	Minimum	Mean	Maximum	Minimum	Mean	
June	40.8	26.1	33.7	72.4	29.7	49.0	30.66
July	37.9	25.2	31.4	84.2	40.4	60.5	29.32
August	37.8	27.6	32.4	84.4	37,1	60.7	30.21
Overall means							

group / breed / sex), with a weight close to the average of the group (±10%) were selected, and slaughtered at local plant and assigned to study carcass traits (determination as percentages of live weight of slaughtering).

***Blood plasma analyses*:** Rabbits used in slaughtering test were assigned for blood plasma α-tocopherol according to Buttriss and Diplock (1984).

Muscle sampling and analysis after Storage duration: After slaughter, the two hind legs of each rabbit carcasses were stored in the dark at 4ºC prior to processing. After 24 hours chilling following slaughter, part of the hind legs' muscles were kept frozen at -20ºC for determination of the muscles α-tocopherol, activities of GSH-PX, SOD, and CAT, and oxidative stability of the muscle lipids (measured as TBARS) after one and two months of storage. α-tocopherol content of the muscles was determined according to Buttriss and Diplock (1984). After saponification and hexane extraction, samples were analyzed by normal phase HPLC fitted with fluorimetric detection. Endogenous antioxidants enzymes' activity measurements were carried out at days 1 of refrigeration (4ºC), and at one and two months of freezing. Total superoxide dismutase activity (Cu-Zn SOD and Mn SOD) was measured according to Marklund and Marklund (1974) using inhibition of pyrogallol autoxidation in a basic medium. Catalase activity was measured by the rate of disappearance of H_2O_2 (Aebi, 1974). GSH-Px activity was assayed with a GSH reduction coupled to a NADPH oxidation by glutathione reductase (Agergaard and Jensen, 1982). For determining the rate of lipid oxidation of muscles (oxidative stability), the thiobarbituric acid-reactive substance (TBARS) test was carried out 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.

Statistical analysis: Data of growth performance and carcass traits were statistically analyzed using Least Squares Analysis of Variance using the

General Linear Model Program of SAS® (1998) according to the following fixed model: $Y_{ijkl} = \mu + A_i + B_j + S_k + AB_{ij} + AS_{ik} + BS_{jk} + e_{ijkl}$,

Where; Y_{ijkl}=The observed value of a given dependent variable, μ=Overall adjusted mean, A_j=Fixed effect of i^{th} treatments (vitamin E supplementation), i = 40, 80 and 120 mg/kg diet of *all* rac-α-tocopheryl acetate, B_i=Fixed effect of j^{th} breed, j =V-line and Gabali, S_k = Fixed effect of k^{th} sex, k= Male and Female, AB_{ij}, AS_{ik} and BS_{jk} =Interactions, interaction among vitamin E levels, breed and sex was discard from the model, since the variable was not significant statistically; e_{ijkl}=Error of the model.

Data of the muscles α-tocopherol, activities of GSH-PX, SOD, and CAT, and oxidative stability of the muscle lipids (measured as TBARS) were statistically analyzed using the following fixed model:

$Y_{ijklm} = \mu + A_i + B_j + S_k + D_l$ + all double interactions available + e_{ijklm},

Where; where, Y_{ijklm}, μ, A_i, B_j, S_k were as defined in the previous model and D_l=Fixed effect of l^{th} storage duration, j = After 24 hours chilling following slaughter, kept frozen at -20ºC after one and two months of storage. The third and fourth interactions were discard from the model, since the variables were not significant statistically.

Data of α-tocopherol content of the blood plasma were statistically analyzed using the following fixed model:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk},$$

Where, Y_{ijk}, μ, A_i, B_j, were as defined in the previous model. Significance of the differences was tested by Duncan's New Multiple Range Test (Duncan, 1955). Differences between groups at $P<0.05$ were considered as significant.

RESULTS AND DISCUSSION

The estimated THI values were 30.66, 29.32 and 30.21 during the experimental period (Table 2) with average 30.063, these values indicating exposure of rabbits to severe heat stress. Marai *et al*. (2002) mention that when exposed to THI 30 or more, rabbits can no longer regulate internal temperature and heat prostration sets in.

Growth performance and carcass traits

Data in Table (3) showed that dietary 120 mg vitamin E /kg diet recorded the highest final body weight at 16 weeks of age, daily weight gain and the best feed conversion ratio ($P<0.0001$) as compared to the other dietary vitamin E levels (40 or 80 mg vitamin E/kg). Slaughter weight and hot carcass weight tended to increase significantly with increasing dietary vitamin E level. Also, vitamin E supplementation in the diet (80 or 120 mg vitamin E/kg) improved dressing weight (%). These results were agreed 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 weight gain, and dressing weight percentage of rabbits subjected to heat stress. Recently, in a 21-day growth trail, Liu *et al.,* (2011) reported that diets supplemented with polyphenols improved significantly 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.

The rabbit genotype (V-line *vs.* Gabali breed) had no significant effect on some growth performance (live body weight at 16 weeks of age and daily weight gain), whereas, slaughter weight, hot carcass and dressing (%) were significantly higher in the V-line compared to the Gabali rabbits (Table 3). 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. So, continuous genetic improvements within each genotype, may minimize the differences in growth performance.

Male rabbits had significantly higher daily weight gain, better feed conversion ratio value and insignificantly highest in dressing percentage than female rabbits (Table 3).

Interaction between dietary vitamin E levels and genotype was significantly affected body weight at 16 weeks of age ($P<0.0686$), daily feed intake ($P<0.0001$), slaughter weight ($P<0.0451$), hot carcass weight ($P<0.0452$) and dressing weight percentage ($P<0.0465$). Interaction between dietary vitamin E levels and sex was significantly affected daily feed intake ($P<0.0557$) and dressing weight percentage ($P<0.0395$). Interaction between sex and genotype was significantly affected only daily feed intake ($P<0.0002$) and Abdominal fat percentage ($P<0.0237$). Indicating that the factors studied had great influence on some growth performance and carcass traits of the rabbit.

α-tocopherol contents in plasma & hind legs muscle

Increasing dietary vitamin E level was associated with a parallel increase ($P<0.0001$) in plasma α-tocopherol level (Tables 3). 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.,* 2000 and Oriani *et al.,* 2001). There was a linear significant ($P<0.0001$) increase in α-tocopherol deposition in the hind legs muscle with the increase in supplemental vitamin E level (Table 3). These results are supported by the findings of Bielański and Kowalska (2008) and Kowalska and Bielański (2009), reporting that the increase in the α-tocopherol concentration of the

muscles depends on the increase in the α-tocopherol acetate level of the diet. In other studies the vitamin E supplementation induced, at least, a 2-fold increase in rabbit meats (Zsédely *et al*., 2008 and Tres *et al*., 2009). Also, Castellini *et al*. (2000) found that extra supplementation of Vitamin E in the diet (200 mg/kg) improve the oxidative stability of the meat and led to an increase of almost 50% of Vitamin E in rabbit meat. Zhang *et al.* (2012) also found that α-tocopherol contents in the serum, liver and meat increased when the dietary vitamin E levels increased ($P<0.0001$), the liver exhibited the greatest concentration of α-tocopherol, followed by the meat, and the serum.

Results shown in Table 3 indicate that Gabali rabbit had a higher plasma α-tocopherol than the V-line. However, V-line rabbits were more efficient ($P<0.0179$) than Gabali in α-tocopherol deposition in hind leg muscles (1.667 *vs.* 1.537±0.046 µg/g). This could be attributed to the increase in feed intake in V-line rabbits compared to the Gabali (Table 3) which means more vitamin E ingestion in the V-line.

Irrespective of the genotype effect, significant ($P<0.0001$) difference was observed between male and female rabbits in α-tocopherol deposition. With the increase in days of storage, the muscle content of α-tocopherol was decreased ($P<0.0001$). In this regard, Selim *et al.*, (2008) reported a loss in α-tocopherol of the loin meat after 20 days of storage relative to 10 days of storage at -20ºC. The decrease in α-tocopherol content in the muscles with prolonged storage may be due to that the vitamin was used by the muscle (oxidative muscle) to withstand oxidation processes.

In the present study interactions between vitamin E and storage durations, genotype and storage durations, sex and storage durations were significantly ($P<0.001$ or 0.05) affected α-tocopherol deposition in muscle, indicating that the factors studied had great influence on the oxidative stability of the rabbit meat.

Oxidative stability of the muscles' lipids

After 1 and 2 months of deep frozen storage, the oxidative stability of hind leg samples were measured by thiobarbituric acid (TBARS) method and was expressed in ng/g tissue (Table 4). The results of the current study indicated that extra vitamin E supplementation in the rabbit diets increased ($P<0.0001$) the oxidative stability of muscular lipids, or in other terms, delayed lipid oxidation (19.36 or 43.17 *vs*.57.43±0.468 ng / g in 120 or 80 *vs.* 40 mg vitamin E/kg diet supplemental groups). 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). Different levels (10-500 mg/kg feed) of dietary vitamin E supplementations were applied in fat supplemented rabbit

Table 4: α-tocopherol (µg/g) in blood plasma and muscle of hind-legs, TBARS (ng/g) and antioxidants enzymes activity (u/g) of hind-legs muscle of rabbits at 16 weeks of ages as affected by vitamin E supplementation (mg/kg diet), genotype, sex, various storage durations and their interactions.

Factors	α-tocopherol		TBARS	Endogenous antioxidants enzymes		
	plasma	muscle		GSH-Px	SOD	CAT
Vitamin E supplementations:						
40 mg (Control)	1.682^{c}	1.117^{c}	57.43^{a}	5.042^{b}	1.051^{b}	0.833^{b}
80 mg	1.905^{b}	1.737^{b}	43.17^{b}	5.595^{b}	1.239^{a}	1.003^{b}
120 mg	2.061^{a}	2.001^{a}	19.36^{c}	6.545^{a}	1.152^{ab}	1.576^{a}
Pooled S.E.	±0.018	±0.051	±0.468	±0.326	±0.069	±0.095
Genotype:						
V-line	1.624^{b}	1.689^{a}	39.52	4.548^{b}	1.129	0.740^{b}
Gabali	2.145^{a}	1.547^{b}	40.64	6.907^{a}	1.166	1.535^{a}
Pooled S.E.	±0.014	±0.040	±0.382	±0.266	±0.053	±0.077
Sex:						
Male	--	1.497	38.48^{b}	5.025^{b}	1.071	1.066
Female	--	1.740	41.50^{a}	6.431^{a}	1.223	1.209
Pooled S.E.	--	±0.040	±0.382	±0.266	±0.053	±0.077
Storage durations:						
One day (4ºC)	--	3.301^{a}	ND	7.661^{a}	1.908^{a}	2.188^{a}
One month (-20ºC)	--	0.825^{b}	38.60^{b}	4.917^{b}	1.233^{b}	1.049^{b}
Two months (-20ºC)	--	0.729^{b}	41.38^{a}	4.605^{b}	0.301^{c}	0.175^{c}
Pooled S.E.	--	±0.051	±0.382	±0.326	±0.069	±0.095
		P values				
Vit. E supplementation	0.0001	<.0001	<.0001	0.0060	0.0524	<.0001
Genotype (G)	0.0001	0.0179	0.0681	<.0001	0.7175	<.0001
Sex	--	0.0001	0.0575	0.0003	0.1433	0.1929
Storage duration (SD)	--	<.0001	<.0001	<.0001	<.0001	<.0001
Vit. E x G	0.0001	0.2079	<.0001	0.1697	0.0001	<.0001
Vit. E x Sex	--	0.4816	0.0005	0.3001	0.0022	0.7429
Vit. E x SD	--	<.0001	0.0900	0.0446	0.3350	0.0002
G x Sex	--	0.3312	0.0210	0.0716	0.0208	0.2882
G x SD	--	0.0600	0.8220	0.0043	0.0179	<.0001
Sex x SD	--	<.0001	0.0522	0.1044	0.9981	0.7024

ND: Not determined

diets (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.

Results shown in Table 4 reveal that Gabali meat was more prone (P<0.0681) to lipid oxidation than the V-line meat. Hernández *et al.,* (2002) reported no breed effect on lipid oxidation of the hind legs. Thus, part of the

genetic variation of intramuscular fat (IMF) is independent of the genetic variation in overall lipid content of the carcass. Different lines with different genetic composition can lead to different genetic relationships between IMF and carcass fat. In the current study, the decrease in the rate of lipid oxidation in the V-line *vs.* the Gabali could be due to the higher growth rate of the V-line compared to the Gabali (data are not shown), as fast growth is accompanied by low lipid deposition in the muscle (Dalle Zotte, 2000) that might affect the oxidative rancidity of the meat. In addition, selection for fast growth might favor a glycolytic energy metabolism in muscle tissue and reduce the intramuscular lipids of the rabbit that might affect the oxidative rancidity of the meat, and this in turn may affect the need for a given level of endogenous/exogenous antioxidants.

Irrespective of the breed, data shown in Table 4 reveal significant (P<0.0575) difference between the male and female rabbit in against oxidation in frozen meat samples (38.48 *vs.* 41.5±0.382 ng/g). However, Ghosh and Mandal (2008) reported that the breed (Soviet Chinchilla and Grey Giant) and sex had no significant effects on various meat quality traits.

Oxidative stability of the muscle was negatively (P<0.0001) responded to storage period (Table 4). An increase in days in storage was followed by a corresponding increasing in TBARS values (38.6 and 41.38±0.382 ng/g post one and two months of storage in -20 °C). Lo Fiego *et al.,* (2004) and Selim *et al.,* (2008) reported an increase in TBARS values either in refrigerated or frozen rabbit muscles with the increase in storage days. Most interactions studied (Vit. E with genotype, Vit. E with sex, Vit. E with storage durations, genotype with sex, sex with storage durations) significantly (P<0.01 or 0.05) affected TBARS, indicating that the factors studied had great influence on TBARS values of the rabbit meat while exploring the factors to improve the oxidative stability of the rabbit carcass (Table 4).

Endogenous antioxidants enzymes activities in muscles

Meat contains several natural antioxidants such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Studies on meat of several species (Pradhan *et al*., 2000) indicate that endogenous antioxidant enzymes could potentially delay the onset of oxidative rancidity in refrigerated stored meat. Indeed, GSH-Px could have an important role controlling lipid oxidation due to its high activity in rabbit meat when compared to other species (Hernández *et al*., 2002). Data presented in Table 4 indicate that supplemental vitamin E levels had significant (P<0.006 or 0.0524 or 0.0001) effect with the muscle antioxidants enzymes activities (GSH-Px, SOD and CAT). GSH-Px and CAT enzymes were increased with increase in level of vitamin E supplementation in the rabbit diet. Several authors (Castellini *et al*., 1999) have shown that the deposition of α-

tocopherol in rabbit muscle is very efficient and has a strong relationship with the supplementation level used in the diet. Dietary α-tocopheryl acetate supplementation has been found to stabilize color of raw meat (Corino *et al*., 1999), even after refrigerated storage (Dalle Zotte, 2000). Vitamin E (α-tocopherol) leads the second class of protective exogenous antioxidants. They are capable of scavenging reactive oxygen species and spare GSH-Px activity (Ullrey, 1981), and by this, together with the action of endogenous preventive antioxidants, prevent or delay the onset of lipid oxidation process (Morrissey *et al.*, 1998).

Gabali meat significantly ($P<0.001$) had higher GSH-Px and CAT enzymes activities than the V-line, but no effect on SOD activity was found (Tables 4). The current findings completely agree with those reported by Dal Bosco *et al.*, (2009) who stated that genotype of the rabbit had a clear effect on antioxidant power and reactive oxygen species generation values. However, Hernández *et al.*, (2006) reporting no differences between different rabbit lines for muscle glycolytic and oxidative enzyme activity. Recently, Zomeño *et al*. (2010) showed an influence of genetic rabbit line on lipogenic, lipolytic, and oxidative enzyme activities.

Irrespective of the genotype, meat samples taken from female rabbits had significantly ($P<0.003$) higher GSH-Px enzyme activities in comparison to male rabbits (Table 4).

Storage duration significantly ($P<0.001$) affected the antioxidants enzymes activities (Table 4).

Most interactions studied significantly ($P<0.01$ or 0.05) affected the muscle antioxidants enzymes activities, indicating that the factors studied had great influence on GSH-Px, SOD and CAT values of the rabbit meat while exploring the factors to improve the oxidative stability of the rabbit carcass and seems to be an effective way to increase shelf-life during storage (Table 4). The same observation were obtained by Lo Fiego *et al*. (2004) who found that vitamin E has also been effective in reducing lipid oxidation during refrigerated and frozen storage of meat. In addition, Vitamin E supplementation increases the oxidative stability of cooked rabbit meat (Castellini *et al*., 1999), whatever the different cooking methods studied (Dal Bosco *et al*., 2001). In recent years, different studies have examined the effects of dietary extra supplementation with vitamin E on the deposition of α-tocopherol in tissues, on meat quality characteristics, and on oxidative stability and the shelf life of rabbit meat. Zsédely *et al.,* (2008) who state that dietary extra dl- α-tocopherol-acetate in the diet as a antioxidant supplementation increased shelf-life, because he found that after one month of storage MDA value decreased to 20.3 in the 240 mg/kg extra

vitamin E supplementation group compared to 32.2 nmol/g meat in the which had only 60 mg/kg extra vitamin E supplementation in their diet.

Correlation coefficients for variables studied in hind-legs muscle of rabbits:

The negative correlation ($P<0.01$; Table 5) 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 Castellini *et al.,* (2000) and Zsédely *et al.,* (2008). Also, correlations data (Table 5) revealed positive correlation between α-tocopherol deposited and GSH-Px and CAT activities ($r= 0.229$ and 0.278; $P<0.05$) and SOD activities ($r= 0.186$). Hernández and Gondret (2006) reviewed the use of vitamin E in the diet as the antioxidant.

Table 5: Pearsons's correlation coefficients (*r*) for variables studied in hind-legs muscle of rabbits at 16 weeks of ages as

Items	α-tocopherol	TBARS	GSH-Px	SOD	CAT
α-tocopherol	1.000				
TBARS	-0.883**	1.000			
GSH-Px	0.229*	-0.199	1.000		
SOD	0.186	-0.129	0.269*	1.000	
CAT	0.278*	-0.232*	0.304*	0.427**	1.000

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

Correlations data (Table 5) revealed also negative correlation between TBARS and GSH-Px, SOD ($r= -0.199, -0.129$) and CAT activities ($r= -0.232$; $P<0.05$). Positive correlations between GSH-Px and SOD activities ($r= 0.269$; $P<0.05$), GSH-Px and CAT activities ($r= 0.304$; $P<0.05$) and SOD and CAT activities ($r= 0.427$; $P<0.01$) were found.

CONCLUSION

In the current work, it could be suggested that adding a high level of vitamin E (120 mg/kg diet) to alleviate some of the impact of heat stress on rabbits. The endogenous antioxidants' enzymes profile and lipid oxidative stability increase with increasing supplemental vitamin E. The native rabbit breed (Gabali) has higher endogenous antioxidant enzyme (GSH-Px, SOD, and CAT) activities than the exotic synthetic line (V-line), nevertheless the meat of fast growth rabbit breeds, as genetically has lower intra-mascular lipid content compared to low growth rate breeds, are less prone to lipid oxidation during meat storage. Male rabbits have lower endogenous antioxidant enzyme (GSH-Px, SOD, and CAT) activities than the female, has lower intra-mascular lipid content, which led to a decrease in lipid oxidation during meat storage.

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التفاعل بين إضافة فيتامين هـ للغذاء و الإنزيمات المضادة للأكسدة الذاتية لأرانب من موارد وراثية مختلفة: تأثير على بعض أداء النمو و محتويات فيتامين هـ والاستقرار التأكسدي للحوم الأرانب خلال موسم الصيف

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تم عمل هذه الدراسة لاستكشاف تأثير العلاقة بين مستوى التغذية على فيتامين هـ : ٤٠ (الضابطة)، ٨٠، أو ١٢٠ مج / كج غذاء و ذكور و إناث سلالتين من الأرانب (المستوردة؛ الفيلاين مقابل المحلية؛ الجبلي) و تأثير كل منها على بعض أداء النمو، صفات الذبيحة، ومحتويات α توكوفيرول وأنشطة الإنزيمات المضادة للأكسدة الذاتية (الجلوتاثيون بيروكسيديز؛ GSH-PX، ديسموتاز الفائق؛ SOD، والكاتلاز؛ CAT)، والاستقرار الدهون المؤكسدة (TBARS) من عضلات الرجلين الخلفيتين التي أبقيت متجمدة (٣٠ أو ٦٠ يوما في -٢٠درجة مئوية). مائة وخمسون

أرانب عمر٦ أسبوع من كلا الأنماط الجينية (وزعت لكل مستوى فيتامين هـ و جنس (١٣ ذكور أو ١٢ أنثى أرنب)، وزعت بين أثنى عشر معاملة تجريبية. في نهاية التجربة، تم استخدام ٣٦ عينة من عضلات الأرجل الخلفيتين (٣ حيوانات / مستوي فيتامين هـ / سلالة / جنس) في دراسة جودة اللحم.

تشير النتائج إلى أن دليل الجرارة و الرطوبة ٣٠.٦٦، ٢٩.٣٢ و ٣٠.٢١ خلال فترة التجربة (يونيو، يوليه و أغسطس) بمتوسط ٣٠.٠٦٣، هذه القيم تدل على تعرض الأرانب لإجهاد حراري شديد. وزن الجسم عند ١٦ أسبوع من العمر، زيادة اليومية في وزن الجسم، وأفضل نسبة تحويل غذائي، وزن الذبح ووزن الذبيحة الساخن تميل إلى زيادة معنوية ($P<0.001$) مع زيادة مستوى فيتامين هـ فى الغذاء. زيادة التغذية على فيتامين هـ مرتبطا مع زيادة خطية ($P<0.0001$) في محتوى العضلات من الالفاتوكوفيرول، و تأخير أكسدة الدهون (١٩.٣٦ أو ٤٣.١٧ مقابل ٠.٤٦٨±57.43 نانوجرام/جرام في المجموعات المضاف إليها ١٢٠ أو ٨٠ مقابل ٤٠ ملجم فيتامين E/ كجم غذاء) وزيادة في نشاط إنزيمات مضادات الأكسدة. وجدت علاقة إيجابية بين تركيز الالفا توكوفيرول في العضلات و نشاط أنزيمات GSH-Px و CAT (r= 0.229 and 0.278; $P<0.05$)، و نشاط أنزيم SOD (r= 0.186). كشفت البيانات أن هناك ارتباطات سلبية بين TBARS وأنزيم GSH-Px و- ,r= -0.199) SOD (0.129 و نشاط أنزيم CAT (r= -0.232; $P<0.05$).

ليس هناك تأثير معنوي لسلالة الأرانب (الفيلاين مقابل الجبلي) على بعض أداء النمو، بينما كانت أرانب الفيلاين أعلى في وزن الذبح و الذبيحة الساخنة و نسبة التصافي، لكن أقل في مستوى الالفاتوكوفيرول في البلازما مقارنة بالأرانب الجبلي. كان أرانب الفيلاين أعلى في محتواة من الالفاتوكوفيرول فى العضلات (1.667 vs. 1.537±0.046) واستقرار أكسدة الدهون ($P<0.0681$; 0.382±40.64 vs. 39.52)، ولكن أقل في أنشطة أنزيم GSH-Px وCAT مقارنة بسلالة الجبلي.

كانت ذكور الأرنب أعلى معنويا في زيادة وزن الجسم اليومية، أفضل قيمة معدل تحويل غذائي و أعلى غير معنويا نسبة تصافى مقارنة بإناث الأرانب. كان هناك فرق معنوي بين الأرانب الذكور والإناث ضد الأكسدة في عينات اللحوم المجمدة (38.48 *vs.* 41.5±0.382 ng/g). ذكور الأرانب أقل في نشاط الإنزيمات المضادة للأكسدة الذاتية عن الإناث. أدى التخزين لفترات طويلة إلى انخفاض معنوي ($P<0.0001$) فى المحتوى من الالفاتوكفيرول في عضلات الأرجل الخلفية، وأنشطة أنزيمات مضادات الأكسدة الذاتية' واستقرار أكسدة الدهون.

التوصية: إضافة مستوى مرتفع من فيتامين هـ (١٢٠ ملجم/كجم غذاء) لتخفيف بعض آثار الإجهاد الحراري على الأرانب من مصادر وراثية مختلفة، إضافة فيتامين هـ بزيادة، وسلالة الأرانب والجنس له تأثير واضح على جودة اللحوم حيث ارتفعت محتوى الالفاتوكوفيرول واستقرار أكسدة الدهون فى اللحوم خلال موسم الصيف.