

INTERACTION BETWEEN SUPPLEMENTAL VITAMIN E AND ENDOGENOUS ANTIOXIDANT ENZYMES OF DIFFERENT RABBIT GENETIC RESOURCES: 2- EFFECT ON MEAT QUALITY

Abdel-Khalek A.M.^{1*}, Gihan Shaaban F.², El-Refaay W.H.³, Azoz A.A.¹,
Selim Nesslerin A.¹, El-Medany Sh.A.³, Abd El-Salam Amal B.¹

¹Animal Production Research Institute, ARC, Dokki, Giza, 12618, Egypt.

²Department of Poultry Science, Faculty of Agriculture, Fayoum University, Fayoum, 63514, Egypt.

¹Animal Production Research Institute, Dokki, ARC, Egypt.

³Regional Centre for Food and Feed, ARC, Egypt.

*Corresponding author: aabdelkhalek_apri@yahoo.com

ABSTRACT

Lipid oxidation is a limiting factor of meat quality. Integration between supplemental antioxidants and the activity of endogenous antioxidants' enzymes, as well as, introduction of proper rabbit breed could participate to improve the quality of the rabbit meat. 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 on the activities of endogenous antioxidants' enzymes (glutathione peroxidase; GSH-Px, superoxide dismutase; SOD, and catalase; CAT), lipid oxidative stability and α -tocopherol content of the hind legs' muscles (oxidative muscle fibers) kept refrigerated (up to 8 days at 4°C) or frozen (30 days at -20°C). 36 samples distributed among 6 experimental treatments were used in the study. Results indicate that V-line had higher lipid oxidative stability and α -tocopherol content, but lower GSH-Px and CAT activities compared to Gabali breed. The increase in dietary vitamin E was associated with a linear increase in the oxidative stability and α -tocopherol content of the muscle, without affecting antioxidants' enzymes activity. Prolonged storage (irrespective of storage method) decreased the activities of endogenous antioxidants' enzymes, lipid oxidative stability and α -tocopherol content of the hind legs' muscles. Conclusively, rabbit breed and supplemental vitamin E have a clear effect on meat quality as it increased the α -tocopherol content and lipid oxidative stability of the meat.

Key words: Rabbit meat, vitamin E, breed, antioxidants' enzymes, oxidative stability.

INTRODUCTION

Rabbit meat is interesting for its dietetic and nutritional characteristics and bio-security for the consumer. In addition, the consumer as a product of high quality already accepts it (Abdel-Khalek, 2007). 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). It begins with oxidation of the double bonds of the phospholipids present in the cell membranes, leading to the production of reactive oxygen species (ROS). This leads to discolouration, 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 and exogenous antioxidants work synergistically to neutralize the action of these ROS.

Endogenous antioxidants' enzymes, mainly, GSH-Px, SOD and CAT work together to convert superoxide anion radical, through H_2O_2 to H_2O , thereby minimizing the production of hydroxyl radical, the most potent oxidant encountered in biological systems. 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).

However, endogenous antioxidants' enzymes represent the principal antioxidants' defence 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. Hernández *et al.*, (2002) reported that during refrigerated storage (up to 5 days), the activity of CAT in rabbit meat was stable for hind leg (HL), while in the *longissimus dorsi* (LD), the activity decreased significantly between 0 and 2 days with no difference between 2 and 5 days. GSH-Px activity decreased significantly over storage days in HL and LD. Moreover, No changes were shown in lipid rancidity of the HL (measured as TBARS values) through days of refrigerated storage between the two breeds studied.

A plethora of studies has appreciated the influence of supplemental dietary vitamin E on rabbit meat quality. It participates in increasing the functionality (nutritional quality) of the meat as it increases the meat content of α -tocopherol (Botsoglou *et al.*, 2004; Lo Fiego *et al.*, 2004; Bielański and Kowalska, 2008; Selim *et al.*, 2008), and more interesting is its involvement in enhancing the oxidative stability of the meat muscles during refrigeration (Botsoglou *et al.*, 2004; Lo Fiego *et al.*, 2004), freezing (Selim *et al.*, 2008; Zsédely *et al.*, 2008) or even cooking (Botsoglou *et al.*, 2004).

Selection for fast growth might favour a glycolytic energy metabolism in muscle tissue and reduce the intramuscular lipids of the rabbit (Dalle Zotte, 2000) 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.

The current study was conducted to investigate the interaction between dietary vitamin E levels and rabbit breeds, and their respective effect on endogenous antioxidants' enzymes activities, α -tocopherol content and oxidative stability of the rabbit meat.

MATERIALS AND METHODS

Feeding protocol

One hundred-fifty–6 week rabbits were divided into six groups 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.

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: DM, 89%; CP, 16.0%; DE (kcal/kg) 2460; CF, 14.3%; Ca, 0.71%; P, 0.39%; Lysine, 0.60%; methionine + cystine 0.50%.

*Supplied per kg diet to meet NRC' (1977) vitamins & minerals recommendations for growing rabbits.

Breeds

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

Muscle sampling

At 16 weeks of age, 6 rabbits (three males and three females) per group, with a weight close to the average of the group ($\pm 10\%$) were selected, and slaughtered at local plant. After slaughter (d-0) the two hind legs of each rabbit carcasses were stored in the dark at $2.5 \pm 5^\circ\text{C}$ prior to processing. After 24 h (d-1) the hind legs' muscles were divided into two portions; the 1st was kept refrigerated at 4°C , and the 2nd was kept frozen at -20°C . Determination of the muscles α -tocopherol, activities of GSH-Px, SOD, and CAT, and oxidative stability of the muscle lipids (as TBARS) were carried out.

Analyses

Vitamin E (α -tocopherol): α -tocopherol content of the muscles was determined at days 4 and 8 of refrigeration, and 1 month of freezing, according to Buttriss and Diplock (1984).

Endogenous antioxidants' enzymes activities: Antioxidants enzymes' activity measurements were carried out at days 1, 4 and 8 of refrigeration, and 1 month of freezing. SOD was measured according to Marklund and Marklund (1974). CAT activity was measured according to Aebi (1974). GSH-Px activity was assayed according to Agergaard and Jensen (1982).

TBARS: Rate of lipid oxidation of refrigerated (4 and 8 days) and frozen (1 month) muscles, as thiobarbituric acid-reactive substance (TBARS) test was carried out according to AOAC (1990).

Statistical procedures

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

RESULTS AND DISCUSSION

α -tocopherol deposition in muscles

Results in Table 2 indicate that V-line rabbits were more efficient than Gabali in α -tocopherol deposition in the hind legs muscle ($p=0.0001$). This could be attributed to the increase in feed intake in V-line rabbits compared to the Gabali (data are not shown) which means more vitamin E ingestion in the V-line. Also, there was a linear significant ($p=0.0001$) increase in α -tocopherol deposition with the increase in supplemental vitamin E level. These results are supported by the findings of Botsoglou *et al.*, (2004), Lo Fiego *et al.*, (2004), Bielański and Kowalska (2008), and Selim *et al.*, (2008), 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. The muscle content of α -tocopherol was decreased ($p=0.0001$) with the increase in days in storage (irrespective of the method used; refrigerated *vs.* frozen). 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 -20°C . The decrease in α -tocopherol content in the muscles with prolonged storage may be due to that the vitamin was used by the muscle to withstand oxidation processes with prolonged storage.

Oxidative stability of the muscles' lipids

Data shown in Table 2 reveal that Gabali meat was ($p=0.0001$) more prone to lipid oxidation than the V-line meat. However, Hernández *et al.*, (2002), reported no breed effect on lipid oxidation of the hind legs. 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. The negative correlation ($p=0.0001$; Tables 2 and 3) 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.*, (2004), Botsoglou *et al.*, (2004), Botsoglou *et al.*, (2004), Lo Fiego *et al.*, (2004), Selim *et al.*, (2008), and Zsédely *et al.*, (2008), where supplemental 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 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). Oxidative stability of the muscle was negatively ($p=0.0001$) responded to storage period, irrespective of the method (Table 2). An increase in days in storage was followed by a corresponding increasing in TBARS values. Also, 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. All interactions studied significantly 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.

Endogenous antioxidants enzymes activities in muscles

Data presented in Table 2 indicate that Gabali meat significantly had higher GSH-Px and CAT activities than the V-line, but no effect on SOD activity was found. More interesting, as indicated in Tables 2 and 3 is that supplemental vitamin E levels or its deposited form (α -tocopherol) had no significant effect or correlation with the muscle antioxidants' enzymes activities. Again, days in storage significantly affected the enzymes' activities. Correlations data (Table 3) revealed positive correlation between GSH-Px and SOD activities ($r=0.247$; $p<0.05$) and SOD and CAT activities ($r=0.413$; $p<0.01$). The current findings completely disagree with those of Hernández *et al.*, (2002) who stated that genetic differences had no effect on antioxidants' enzymes activities of the rabbit meat, and that the antioxidants' enzymes in rabbit meat seems enough to control oxidation during refrigerated storage. However, Dal Bosco *et al.*, (2009) reported that genotype of the rabbit had a clear effect on antioxidant power and reactive oxygen species generation values.

Table 2: Main effects of genotype, dietary vitamin E level (mg/kg diet), and time in storage on α -tocopherol ($\mu\text{g/g}$), TBARS (ng/g) and antioxidants' enzymes activities (u/g) of hind-legs' muscles.

	α -tocopherol	TBARS	Endogenous antioxidants' enzymes		
			GSH-Px	SOD	CAT
Genotype					
V-line	2.03 ^A	29.75 ^B	5.09 ^B	1.116	0.923 ^B
Gabali	1.95 ^B	42.08 ^A	6.99 ^A	1.213	1.688 ^A
Pooled SE	0.009	0.36	0.37	0.094	0.141
Supplemental vitamin E level					
40 mg (Control)	1.44 ^C	54.71 ^A	5.79	1.176	1.088
80 mg	2.02 ^B	40.42 ^B	5.86	1.148	1.247
120 mg	2.51 ^A	12.62 ^C	6.49	1.169	1.581
Pooled SE	0.011	0.451	0.46	0.115	0.173
Time in storage					
1 days (4°C)	ND	ND	7.63 ^A	1.91 ^A	2.189 ^A
4 days (4°C)	2.75 ^A	27.78 ^C	6.59 ^B	1.18 ^B	1.589 ^B
8 days (4°C)	2.39 ^B	34.70 ^B	5.02 ^C	0.38 ^C	0.394 ^D
30 days (-20°C)	0.82 ^C	45.27 ^A	4.92 ^C	1.19 ^B	1.049 ^C
Pooled SE	0.011	0.450	0.52	0.133	0.200
<i>p</i> value					
Genotype	0.0001	0.0001	0.001	0.473	0.0001
Vitamin E level	0.0001	0.0001	0.490	0.983	0.132
Time in storage	0.0001	0.0001	0.001	0.0001	0.0001
Genotype X vitamin E	0.002	0.0001	0.067	0.025	0.0001
Genotype X time	0.04	0.001	0.114	0.454	0.079
Vitamin E X time	0.0001	0.0001	0.980	0.521	0.131
Genotype X vit. E X time	0.598	0.002	0.867	0.415	0.083

ND: not determined

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

	α -tocopherol	TBARS	GSH-Px	SOD	CAT
α -tocopherol	1.000	-0.684**	0.212	-0.257	0.028
TBARS	-0.684**	1.000	-0.054	0.052	-0.076
GSH-Px	0.212	-0.54	1.000	0.247*	0.140
SOD	-0.257	0.052	0.247*	1.000	0.413**
CAT	0.028	-0.076	0.140	0.413**	1.000

*correlation is significant at the 0.05 level (2-tailed).

**correlation is significant at the 0.01 level (2-tailed).

CONCLUSION

- No relationship between the exogenous dietary vitamin E level and endogenous antioxidants' enzymes is found.
- Lipid oxidative stability of the meat increases with increasing the supplemental vitamin E level, irrespective the endogenous antioxidants' enzymes profile.
- Indigenous (native) rabbit breed (Gabali) has higher endogenous antioxidants' enzymes (GSH-Px, SOD, and CAT) activities than the exotic breed (V-line). Nevertheless, meat of the

faster growth rabbit breeds (V-line) is less prone to lipid oxidation during meat storage, as genetically has lower intra-muscular lipid content compared to lower growth rate breeds.

REFERENCES

- Abdel-Khalek A.M. 2007. Safe alternative additives to antibiotics in rabbit nutrition: A review. *In Proc.:5th International Conference on Rabbit Production in Hot Climates, Hurghada, Egypt, 113 - 134.*
- Aebi H. 1974. Methods enzymology analysis. *In: Bergmeyer H.U. (Ed.), New York: Academic press, 673-682.*
- Agergaard N., Jensen P. 1982. Procedure for blood glutathione peroxidase determination in cattle and swine. *Acta Veterinaria Scandinavia, 23, 515–527.*
- AOAC Official Methods of Analysis 1990. Re-approved in 1997: 2-Thiobarbituric acid value-Direct method sampling and analysis of commercial fats and oils.
- Bianchi M., Petracchi M. Cavani, C. 2006. Effects of dietary inclusion of dehydrated lucerne and whole linseed on rabbit meat quality. *World Rabbit Sci., 14, 247-258.*
- Bielanski P., Kowalska, D. 2008. Use of linseed oil and antioxidant (vitamin E) in rabbit diets to improve dietetic traits of rabbit meat. *In Proc.: 9th World Rabbit Congress, Italy, 1319-1323.*
- Botsoglou N., Florou-Paneri P., Christaki E., Giannenas I., Spais A. 2004. Performance of rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with oregano essential oil. *Arch. Animal Nutr., 58, 209-218.*
- Buttriss J.L., Diplock, A.T. 1984. HPLC methods for vitamin E in tissues. *In: Parcker L., Glazer A.N. (Eds.), Methods in enzymology, New-York: Academic press, 131-138.*
- Castellini C., Dal Bosco A., Bernardini M. 2000. Improvement of lipid stability of rabbit meat by vitamin E and C administration. *J. Sci. Food and Agric., 81(1), 46-53.*
- Dal Bosco A., Castellini C., Bianchi L. Mugani C. 2004. Effect of dietary α -linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. *Meat Sci., 66, 407-413.*
- Dal Bosco A., Mugnai C., Mourvaki E., Cardinali R., Moscati L., Paci, G. Castellini C. 2009. Effect of genotype and rearing system on the native immunity and oxidative status of growing rabbits. *Italian J. Animal Sci., 8 (2), 781-783.*
- Dalle Zotte A. 2000. Main factors influencing the rabbit carcass and meat quality. *In Proc.: 7th World Rabbit Congress, Valencia, Spain.*
- Duncan D.B. 1955. Multiple range and multiple F tests. *Biometrics, 11, 1-42.*
- Gray J.I., Goma E., Buckley D. 1996. Oxidative quality and shelf life of meats. *Meat Sci., 43 (Suppl.), S111-S123.*
- Hernández P., López A., Marco M. and Blasco A. 2002. Influence of muscle type, refrigeration storage and genetic line on antioxidant enzyme activity in rabbit meat. *World Rabbit Sci., 10, 141-146.*
- Lo Fiego D., Santoro P., Macchioni P., Mazzoni D., Piattoni F., Tassone F., De Leonibus E. 2004. The effect of dietary supplementation of vitamin C and E on the α -tocopherol content of muscles, liver and kidney, on the stability of lipids, and on certain meat quality parameters. *Meat Sci., 67, 317-327.*
- Machlin L., Bendich A. 1987. Free radical damage: Protective role of antioxidant defense systems. *FASEB J. 1:441-445.*
- Marklund S.L., Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European J. Biochem., 47, 469–474.*
- Morrissey P., Sheehy P., Galvin K., Kerry J. and Buckley D. 1998. Lipid stability in meat and meat products. *Meat Sci., 49 (Suppl. 1), S73-S86.*
- NRC 1977. Nutrient requirements of domestic rabbits. *National Acad. Sci., Washington DC, USA.*
- Selim N., Abdel-Khalek A., Nada Sh., El-Medany Sh. 2008. Response of growing rabbits to dietary antioxidant vitamins E and C. 2. Effect on meat quality. *In Proc.: 9th World Rabbit Congress, Italy, 1437-1441.*
- SPSS 1999. User's Guide: Statistics. *Version 10, SPSS Inc. Chicago, IL, USA.*
- Ullrey, D. E. 1981. Vitamin E for swine. *J. Anim. Sci. 53: 1039-1056.*
- Zsédely E., Tóth T., Eiben Cs., Virág Gy., Fábán J., Schmidt J. 2008. Effect of dietary vegetable oil (sunflower, linseed) and vitamin E supplementation on the fatty acid composition, oxidative stability and quality of rabbit meat. *In Proc.: 9th World Rabbit Congress, Italy, 1473-1477.*