# COLOUR AND pH OF RABBIT MEAT AND FAT DEPOSITS AS AFFECTED BY THE SOURCE AND DOSE OF DIETARY VITAMIN E SUPPLEMENTATION

# Virág Gy.<sup>1</sup>\*, Eiben Cs.<sup>1</sup>, Tóth T.<sup>2</sup>, Schmidt J.<sup>2</sup>

<sup>1</sup>Research Institute for Animal Breeding and Nutrition, Isaszegi ut 200, 2100, Gödöllő, Hungary
<sup>2</sup>University of West-Hungary, Faculty of Agricultural and Food Sciences, H-9200 Mosonmagyaróvár, Vár 2., Hungary
\*Corresponding author: virag@katki.hu

#### ABSTRACT

A trial was carried out to determine the effect of level and source of vitamin E supplementation on the pH and colour of rabbit thigh and loin meat as well as on perirenal and scapular fat (N=15 per treatment). The animals received a compound feed containing 2% sunflower oil and 2% linseed oil. Natural vitamin E (a by-product of the oil industry, i.e. fatty acid distillate) or synthetic dl- $\alpha$ -tocopheryl-acetate was used as vitamin E source. The feed was supplemented with 150 mg/kg and 300 mg/kg synthetic dl- $\alpha$ -tocopherylacetate, or 60+90 mg/kg and 60+240 mg/kg synthetic plus natural (d-alfa-tocopherol) vitamin E. Higher level of vitamin E compared to the lower level significantly increased loin pH (5.96 and 5.90; P<0.05). Vitamin E level had not further significant effect on the examined parameters. L\* (53.12 vs. 50.74; P<0.005) of loin; a\* of loin (0.07 vs. -0.39; P<0.05) and fat deposits (4.44 vs. 3.38; P<0.01) furthermore thigh b\* (7.10 vs. 6.37; P<0.01) were significantly higher at natural vitamin E supplementation. Fat deposit L\* (76.44 vs. 78.27; P<0.03) was lower in this group. Overall colour difference was well visible for thigh meat and perceptible for fat deposit what could influence consumer's choice and decision for shopping.

Key words: Vitamin E, Meat, Fat, pH, Colour.

#### INTRODUCTION

High PUFA, particularly high oleic or linolenic acid concentration in rabbit pellet was found to change rabbit meat FA composition, increase P:S, decrease n6/n3 ratio (Bianchi *et al.*, 2006; Corino *et al.*, 2007; Dal Bosco *et al.*, 2004) and increase unsaturation index (Lopez-Bote *et al.*, 1997) what further improves its wholesomeness as human food. Oxidative instability of unsaturated fatty acids however could influence meat quality just after slaughter and during chilling, processing, display and long term storage (Bianchi *et al.*, 2006). Lipid oxidation occurs in the muscles ante mortem and continues post mortem immediately after slaughter in the meat coupled with the oxymyoglobin (oxyMb) oxidation. The balance between oxyMb oxidation to metmyoglobin (metMb) and its reverse process is the main route by which diet could affect meat colour (Gray *et al.*, 1996).

Vitamin E is an efficient antioxidant which protects feed (Bernardini *et al.*, 1996) and muscle PUFAs against peroxidation. Dietary  $\alpha$ -tocopherol increases muscle  $\alpha$ -tocopherol concentration and improves oxidative stability (Corino *et al.*, 2007; Castellini *et al.*, 1998; Dal Bosco *et al.*, 2004; Lopez-Bote *et al.*, 1997; Oriani *et al.*, 2001) of muscles, fresh and processed meat. It is available and generally used in the form of synthetic dl- $\alpha$ -tocopheryl acetate which consist a racemic mixture of eight possible stereoisomers. Less common in its natural form as RRR-  $\alpha$  tocopherol isolated from plant sources, which contains mainly the biologically most available d- $\alpha$  tocopherol (Jensen and Lauridsen, 2007; Jensen *et al.*, 1995).

Linch *et al.* (1998) proposed potential mechanisms by which  $\alpha$ -tocopherol is involved in the metMb reduction to oxyMb and Faustman *et al.* (1998) summarized how these can affect beef colour. Oxidative and glycolytic muscles or adipose tissue are different in their sensitivity towards

peroxidation. The effect of these reactions was studied and was found to be important on rabbit loin meat surface colour stored for a long interval (Corino *et al.*, 1999).

The aim of the present study was to investigate the effect of source and level of dietary vitamin E supplementation on pH and colour of fresh rabbit thigh and loin meat and on fats deposited in the rabbit carcass.

## MATERIALS AND METHODS

### Animals and experimental design

Sixty New Zealand White rabbits were divided into four groups (N=60, mixed sex). The rabbits were fed ad libitum from 21 days of age until slaughter at 80 days of age. The diet was composed to cover nutritional requirements of fattening rabbits (Table 1). The included vegetable oils (2% linseed plus 2% sunflower oil) provided its energy content as well as high amounts of PUFAs.

Ingredients	Proportion	Chemical composition	
Vegetable oil (sunflower oil and linseed oil) (%):	2 and 2	Dry matter (%)	90.48
Alfalfa meal (16.5% crude protein)	333	DE rabbit (MJ/kg)	11.36
Wheat	100	Crude protein (%)	15.46
Extracted soybean meal (46% crude protein)	28	Ether extract (%)	5.60
Extracted sunflower meal	120	Crude fibre (%)	15.53
Whole corn meal	50	NDF (%)	32.43
Straw pellet	75	ADF (%)	21.52
Sugar beet pulp	90	ADL (Lignin) (%)	4.09
Dried apple pulp	50	Vitamin A (NE/kg)	10000
BIOLIZIN (a lysine supplement)	4	Vitamin D3 (NE/kg)	1000
KNP-843-Ro/Ti+Otc. Premix <sup>1</sup>	40	Vitamin E (mg/kg)*	300
WAFOLIN-S pellet binder	10		
Linseed oil <sup>2</sup>	50		
Perfett sunflower oil <sup>3</sup>	50		
Total:	1000		

<sup>1</sup>KNP-843-Ro/Ti+OTC (Bábolna Feed Ltd.) robenidin: 50 mg/kg, oxitetraciklin: 500 mg/kg, tiamulin: 50 mg/kg <sup>2</sup>60% steam flaking maize+40% linseed oil

<sup>3</sup>60% steam flaking maize+40% supplement containing sunflower oil (ABOMIX Co.)

\*The same base diet had been supplemented with synthetic or with synthetic+natural Vitamin E in low (150 or 60+90 mg/kg respectively) or in high (300 or 60+240 mg/kg) dose. Natural Vitamin E was provided as plant oil distillation product mixed with the wheat

Diets differed only in Vitamin E supplementation level and source: 150 mg/kg and 300 mg/kg synthetic dl-alfa-tocopherylacetate, or 60+90 mg/kg and 60+240 mg/kg synthetic plus natural (d-alfa-tocopherol) vitamin E. At  $80\pm3$  days of age rabbits weighing  $2680\pm33.3$  g were slaughtered at a commercial slaughterhouse and carcasses were chilled at 4°C for 24 h. After chilling the loin and the thigh were removed from the left side of each carcass and used for pH and colour measurements. Perirenal and interscapular adipose tissue depots were collected, pooled, mixed with hand to have the sufficient size of sample for colour measurement. Corino *et al.* (2007) found that perirenal and interscapular fats were fully developed showing similar hystology to the age of 9 weeks and for this field error from inhomogeneity of pooled, mixed samples was not presumed.

#### pH and colour measurement

Meat pH was determined using a pH/M201 portable pH meter (RadioMeter Analytical, France) equipped with pHC3031-9 spear shaped electrode. Colour was measured with Minolta Chromameter 300 (Minolta Co., Ltd., Osaka, Japan) using light source D65 and 8 mm  $\emptyset$  measuring area, diffuse illumination and 0° viewing angle. The equipment was calibrated to white plate before each session of

measuremets. The CIELAB L\* (lightness) a\* and b\* (green-red and blue-yellow chromaticity coordinates, resp.) colour space (CIE, 1976) was used to determine the colour; H $^{\circ}$  (metric hue) and C\* (chroma) were calculated using the equations given in the equipment's Instructional Manual (Minolta Co., Ltd., 1991). Total colour difference between treatments was calculated as follows:

$$\Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}$$

Loin and thigh colour was measured on the cut surface at the level of the 7<sup>th</sup> lumbar vertebra, and on the periosteal epimysial surface, respectively. pH was determined at the same locations with the electrode inserted 1 cm deep into the muscle.

#### Statistical analysis

ANOVA was performed using GenStat.8 (Lawes Agricultural Trust, 2004) software. Variability of data is expressed as standard errors (s.e.) and significance of differences between treatments was detected by calculated least significant difference.

#### **RESULTS AND DISCUSSION**

Results of the pH and colour measurements with calculated colour indicators are summarized in Table 2. Interaction between treatments was not found in any case, results are not shown. Dietary vitamin E level had no effect on the investigated parameters except the pH of the loin with the highest value detected at high dose supplementation, and lowest at low dose (5.96 and 5.90, P<0.05). In parallel with higher pH there was a tendency for darker and duller meat. Corino *et al.* (2007) found the same tendency for *longissimus lumborum* when used 60 mg/kg or 240 mg/kg dietary vitamin E supplementation of control diet containing 0.5% sunflower oil, but not for the diet containing added conjugated linolenic acid (CLA). Similarly, higher vitamin E supplementation resulted higher pH connected with decreased drip loss of pork loin in the experiment of Lauridsen *et al.* (1999). In contrast other studies (Castellini *et al.*, 1998) did not found pH difference of rabbit meat caused by the level of vitamin E supplementation and there is not obvious explanation for this effect.

-	Source of V	Source of Vit E suppl.		Dose		F prob	
	Synthetic	+Natural	Low	High	s.e.	Туре	Dose
Thigh							
pH	6.25	6.27	6.23	6.28	0.04	n.s.	n.s.
L*	51.62	52.60	52.11	52.11	0.44	n.s.	n.s.
a*	4.47	4.48	4.52	4.42	0.29	n.s.	n.s.
b*	6.37 <sup>a</sup>	$7.10^{b}$	6.53	6.94	0.19	< 0.01	n.s.
${ m H_{ab}}^{\circ}$	56.30	58.20	56.00	58.50	1.68	n.s.	n.s.
C*	7.87	8.47	8.04	8.31	0.27	n.s.	n.s.
Loin							
pH	5.94	5.92	5.90 <sup>a</sup>	5.96 <sup>b</sup>	0.02	n.s.	< 0.05
Ĺ*	50.74 <sup>a</sup>	53.12 <sup>b</sup>	52.53	51.33	0.56	< 0.005	n.s.
a*	-0.39 <sup>a</sup>	$0.07^{b}$	-0.09	-0.23	0.18	< 0.05	n.s.
b*	3.70	4.41	4.16	3.95	0.39	n.s.	n.s.
${ m H_{ab}}^{\circ}$	97.60	90.40	92.40	95.60	2.86	< 0.1	n.s.
C*	3.88	4.50	4.27	4.10	0.38	n.s.	n.s.
Fat deposits							
L*	78.27 <sup>b</sup>	76.44 <sup>a</sup>	77.94	76.77	0.58	< 0.03	n.s.
a*	3.38 <sup>a</sup>	4.44 <sup>b</sup>	3.63	4.19	0.61	< 0.01	n.s.
b*	12.17	12.64	12.43	12.38	0.35	n.s.	n.s.
${ m H_{ab}}^{\circ}$	76.9	72.1	75.5	73.5	2.14	n.s.	n.s.
C*	12.97	13.59	13.17	13.39	0.50	n.s.	n.s.

**Table 2**: Effect of source and dose of Vitamine E supplementation on colour parameters of rabbit thigh, loin meat and fat deposits after 24 hours ageing at 4°C temperature

Mean and standard error (s.e.). Differences are significant (P<0.05) between means with different letters in the same row within the same trait

Source of vitamin E supplementation had significant effect on the thigh b\* (7.10 vs. 6.37; P<0.01), resulting more yellowish color for natural compared to the synthetic vitamin E. The same tendency was observed for b\* in the loin and the deposited fats, but that were not significant. Loin L\* (53.12 vs. 50.74; P<0.005) and a\* values (0.07 vs. -0.39; P<0.05) were higher in case of natural vitamin E supplementation compared to the synthetic one, and H° showed a tendency of lower figure (90.4 vs. 97.6; P<0.10). Chromacity measured at the red-green axis (a\*) was however very weak, what means pale grayish coloration as the consequence of the very low concentration of oxyMb in this meat, in accordance with its low Mb content. The negative sign means few greenish colour caused by the presence of deoxyMb, because the loin has smaller surface what can be exposed to the air during chilling. C\* furthermore is very low at both treatments, what means that color intensity is very weak, the sample colour is dull. Very high H° is the reflection of the low a\* and means somewhat yellowish true colour, although this is not unique in the case of rabbit loin (Lambertini *et al.*, 2004; Bovera *et al.*, 2004).

Though considerable proportion of rabbit meat is sold as whole carcass containing abdominal and scapular fat deposits there are only very few reports about the stored fat colour. Ouhayoun *et al.* (1987), Pla and Cervera (1996) and Oliver *et al.* (1997) found that the origin of the dietary fat substantially affected perirenal fat colour. However studies on the effect of Vitamin E supplementation of vegetable oil containing diet on the deposit fat colour has not been published earlier. In our experiment deposit fat colour was influenced by the source of Vitamin E supplement. Pooled samples of perirenal and scapular fat were darker (L\* 76.44 vs. 78.27, P<0.03) and more red (a\* 4.44 vs. 3.38, P<0.01) for rabbits consuming feed supplemented with natural Vitamin E compared to those with synthetic one. If natural vitamin E was more effective to protect PUFAs against oxidation in the adipose tissue and preventing metHb formation in the erythrocytes present in the vessels that could account for these results.

Total colour difference resulted by the source of vitamin E supplementation was not detectable by naked eye in the case of thigh meat ( $\Delta$ E 2.17), just perceptible for fat ( $\Delta$ E 3.84) however it was well visible for loin meat ( $\Delta$ E 4.3) according to the scale proposed by Abril *et al.* (2001).

#### CONCLUSIONS

In agreement with other results the level and source of vitamin E supplementation has only few effects on the single colour parameters of the 24 hours chilled rabbit meat. In the case of deposited fats and thigh meat however overall colour difference caused by the vitamin E supplement's source is perceptible or apparent by naked eye. The observed overall colour deviation can influence consumer's acceptance and their decision for shopping. Further studies are necessary to know better the changes of rabbit meat colour and the preferences of the consumers.

#### ACKNOWLEDGEMENTS

Acknowledgements: The research was supported by GAK-OMFB-01336/ALAP1-00121/2004 project. The technical assistance of Barbara Végi is gratefully appreciated.

#### REFERENCES

Abril M., Campo M.M., Onenc A., Sanudo C., Alberti P., Negueruela A.L. 2001. Beef color evolution as a function of ultimate pH. *Meat Sci.*, 58, 69-78.

Bianchi M., Petracci M., Cavani C. 2006. Effects of dietary inclusion of dehydrated lucerne and whole linseed on rabbit meat quality. *World Rabbit Sci.*, 14, 247-258

Bernardini M., Dal Bosco A., Castellini C., Miggiano G. 1996. Dietary vitamin E supplementation in rabbit: antioxidant capacity and meat quality. *In: Proc.* 6<sup>th</sup> World Rabbit Congress, 1996 July, Toulouse, France, Vol. 3, 137-140.

- Bovera F., Di Meo C., Barone C., Gazaneo M.P., Taranto S., Nizza A. 2004. A survey on carcass and meat characteristics of ischia rabbits raised in pits. *In: Proc.* 8<sup>th</sup> World Rabbit Congress, 2004 September, Puebla, Mexico, 1366-1371
- Castellini C., Dal Bosco A., Bernardini M., Cyril H.W. 1998. Effect of dietary vitamin E on the oxidative stability of raw and cooked rabbit meat. *Meat Sci.*, 50, 153-161.
- CHROMA METER CR-300/CR-310/cr-321/CR-331/CR-331C Instruction Manual. 1991. Minolta Co., Ltd., Osaka, Japan. pp. 78-79.
- CIELAB 1976. Official recommendations on uniform colour spaces, colour differences equations and metric colour terms. Suppl. 2 to CIE Publications n. 15. Commission Internationale de Eclairage, Colorimetry. Paris, France.
- Corino C., Lo Fiego, D.P., Macchioni, P., Pastorelli, G., Di Giancamillo, A., Domeneghini, C., Rossi, R. 2007. Influence of dietary conjugated linolenic acids and vitamin E on meat quality, and adipose tissue in rabbits. *Meat Sci.*, 76, 19-28.
- Corino C., Pastorelli G., Pantaleo L., Oriani G., Salvatori G. 1999. Improvement of color and lipid stability of rabbit meat by dietary supplementation with vitamin E. *Meat Sci.*, 52, 285-289.
- Dal Bosco A., Castellini C., Bianchi L., Mugnai 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.
- Faustman C., Chan W.K.M., Schaefer D.M., Havens A. 1998. Beef color update: the role for vitamin E. J. Anim. Sci., 76, 1019-1026.
- Gray J.I., Gomaa E.A., Buckley D.J. 1996. Oxidative quality and shelf life of meats. Meat Sci., 43 (Suppl.), S111.
- Jensen C., Skibsted L.H., Jakobsen K., Bertelsen G. 1995. Supplementation of broiler diets with all-rac-alpha- or a mixture of natural source RRR-alpha-,gamma-,delta-tocopheryl acetate. 2. Effect on the oxidative stability of raw and precooked broiler meat products. *Poultry Sci.*, 74(12), 2048-56.
- Jensen S.K., Lauridsen C. 2007. Alpha-tocopherol stereoisomers. Vitam. Horm., 76, 281-308.
- Lambertini L., Vignola G., Beone G.M., Zaghini G., Formigoni A. 2004. Effects of chromium yeast supplementation on growth performances and meat quality in rabbit. *World Rabbit Sci.*, 12, 33-47.
- Lauridsen C., Nielsen J.H., Henckel P., Sorensen M.T. 1999. Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E and copper. J. Anim. Sci., 77, 105–115.
- Lawes Agricultural Trust 2004. GENSTAT A general Statistical program. Numerical Algorithms Group, Oxford, England.
- Linch M.P., Faustman C., Chan W.K.M., Kerry J.P., Buckley D.J. 1998. A potential mechanism by which α-tocopherol maintains oxymyoglobin pigment through cytochrome b<sub>5</sub> mediated reduction. *Meat Sci.*, 50, 333-342.
- Lopez-Bote C.J., Rey A., Sanz M., Gray J.I., Buckley D.J. 1997. Dietary vegetable oils and alpha tocopherol reduce lipid oxidation in rabbit muscle. J. Nutr., 127, 1176-1182.
- Oliver M.A., Guerrero L., Diaz I., Gispert M., Pla M., Blasco A. 1997. The effect of fat-enriched diets on the perirenal fat quality and sensory characteristics of meat from rabbits. *Meat Sci.*, 47, 95-103.
- Oriani G., Corino C., Pastorelli G., Pantaleo L., Ritieni A., Salvatori G. 2001. Oxidative status of plasma and muscle in rabbits supplemented with dietary vitamin E. J. Nutr. Biochem., 12, 138-143.
- Ouhayoun J., Kopp J., Bonnet M., Demarne Y., Delmas D. 1987. Influence of dietary fat composition on rabbit perirenal fat properties and meat quality. *Sci. Aliments*, *7*, *521-534*.
- Pla M., Cervera C. 1996. The effect of diet fat type on carcass composition and meat quality in rabbits. 1996. In: Proc. 6<sup>th</sup> World Rabbit Congress, 1996 July, Toulouse, France, Vol. 3, 233-237.