DIETARY VITAMIN E SUPPLEMENTATION IN RABBIT : ANTIOXIDANT CAPACITY AND MEAT QUALITY

BERNARDINI M.[1], DAL BOSCO A.[1], CASTELLINI C.[1], MIGGIANO G.[2]

[1] Istituto di Zootecnica Generale- Facoltà di Agraria, Università di Perugia - Italy
[2] Istituto di Chimica Biologica - Facoltà Medicina e Chirurgia, Università Cattolica Sacro Cuore, Roma - Italy

Abstract - The effect of dietary vitamin E supplementation in rabbit feeding was evaluated for the possibility of increasing the (n-3) PUFA content of the meat especially those with ≥ 20 C for their possible health benefits. Four groups of male hybrids (Provisal) were given, from 35 to 80 days of age, different diets: basal (50 mg/kg vit. E), basal supplemented (200 mg/kg vit. E), basal + 2% fish meal (50 mg/kg vit. E), and fish supplemented (200 mg/kg vit. E). Supplementation improved oxidative stability of diet lipids particularly of (n-3) fatty acids (≥ 20 C). It enhanced the plasma antioxidant capacity, measured as TRAP, mainly at 80 days of age (365 vs 317 µmol/l, basal ; 435 vs 356 µmol/l, fish). As a consequence peroxidation processes were lower (Conjugates dienes: 0.62 vs 0.86 a.u. basal ; 0.51 vs 0.74 fish - peroxide index: 2.82 vs 2.99 meqO_2/kg, basal ; 2.70 vs 3.33, fish) and the (n-3) fatty acid (≥ 20 C) levels in *longissimus dorsi* were higher (3.69 vs 3.18%, basal ; 6.42 vs 4.48%, fish).

INTRODUCTION

In developed countries the increased consumption of meat has resulted in an increase in saturated fatty acid assumption. Consequently results (in connection with low fiber, high energy diets) some chronic diseases such as obesity and cardiovascular problems have increased.
Many consumers, time aware of the close association dietary intake and health standards, have modified the consumption models, orienting their choices to products which satisfy their personal dietetic and nutritional requirements.
From this point of view, rabbit meat is certainly one of the best, given that it is a lean meat, easily digestible, not allergic and containing high levels of polyunsaturated fatty acids (PUFA).
Unfortunately, this last desirable characteristic could cause problems for meat storage, processing and cooking. In fact, polyunsaturated fatty acids are very susceptible to oxidation and hence flavour, colour, texture, nutritive value and the safety of the food can be affected.
To reduce oxidation, many substances can be used. Vitamin E is the primary antioxidant in biological systems and breaks the chain of lipid peroxidation in cell membranes, preventing the formation of hydroperoxides (HALLIWELL, 1987 ; DAVIES, 1988). It is usually incorporated in diets as α-tocopheryl acetate which functions as an antioxidant after de-esterification in the gastrointestinal tract.
Many studies have already shown the positive influence of this substance on the rate of lipid peroxidation, colour, water-holding capacity, and cholesterol oxidation in beef, pig and poultry meat (BRUNI, 1993 ; BUCKLEY *et al.*, 1995 ; LIU *et al.*, 1995 ; MORDENTI and SARDI, 1995). However, no references have been found on rabbit meat. Therefore the purpose of this study was to verify the real benefits of dietary vitamin E supplementation on the quality of rabbit meat obtained from animals fed or not on diets containing fish meal, rich in (n-3) fatty acids. High dietary intake of PUFA is recognized as an important factor influencing the vitamin E requirement. It has also been (MEYDANY *et al.*, 1987; ALEXANDER *et al.*, 1995) that increased consumption of (n-3) fatty acids leads to a greater reduction in vitamin E levels in the blood and tissues than does consumption of (n-6) PUFA.

MATERIALS AND METHODS

The study was carried out in the experimental rabbitry of the Istituto di Zootecnica. The environment was partially controlled; temperature was 18.5 ± 3.1 °C and photoperiod 16 hours light/day.
At weaning (35 days), sixty hybrid males (Provisal) were divided into four groups (15 rabbits per group), homogeneous according to weight and litter, and assigned to diet treatments consisting of : basal diet (50 mg/kg

vit. E); basal diet supplemented (200 mg/kg vit. E); basal diet + 2% fish meal (50 mg/kg vit. E); and fish diet supplemented (200 mg/kg vit. E). Vitamin E was incorporated as α-tocopheryl acetate.

At 40 and 80 days of age blood samples were drawn. The blood was collected over Na2ETDA (1-2 mg/ml blood) and immediately centrifuged (10,000 x g for 10 min at 4 °C). The supernatant was used immediately for evaluating plasma antioxidant status as total peroxyl radical - trapping antioxidant parameter (TRAP) (MILLER *et al.*, 1993).

Animals were slaughtered at 80 days of age; without fasting the carotid arteries and jugular veins were cut, the blood was drained and the skin, digestive tract, genital organs and bladder were removed. About an hour after slaughter, the carcasses were put in a ventilated cold room (+ 4 °C) and chilled for 24 hours. The perirenal fat and the *longissimus dorsi* were then dissected.

The peroxide index in the fat was measured according to AOAC methods (1995).

Conjugated dienes (CD) and fatty acid composition were determined in the *longissimus dorsi.*

CD were measured by using second derivative spectrophotometry according to the method of CORONGIU *et al.* (1986). The chloroform lipid solutions were dried under nitrogen at 40 °C. The lipids were then dissolved in 500 µl of cyclohexane and vortexed for 30 s. The lipid solutions were immediately scanned from 200 to 300 nm using a spectrophotometer managed by a computer. Operating conditions were a scanning speed of 100 nm/min and an interval of 0.25 nm. For the fatty acid analysis, the fat was extracted with a mixture of chloroform : methanol (2:1) as described by FOLCH *et al.* (1957) ; the fatty acids were determined as methyl esters with a VARIAN model 3700 Chromatograph. Statistical analyses were done using the GLM procedure (SAS/STAT, 1990) with a linear model, evaluating the diet effect for all variables ; the age effect was also considered for the TRAP variable.

RESULTS AND DISCUSSION

Protection of dietary fatty acids

Table 1 - Pufa composition of diets as % total fatty acids

Fatty acids	Basal	Basal + Vit E	Fish	Fish + Vit E
C18:2n6	47.08	50.19	44.19	45.86
C18:3n3	10.67	10.86	9.39	9.53
C18:4n3	-	0.15	0.41	0.15
C20:2n6	0.16	0.19	0.21	0.12
C20:3n6	-	-	0.14	0.11
C20:4n6	0.11	0.14	0.12	0.14
C20:3n3	-	0.07	0.12	0.38
C20:5n3	0.28	0.28	0.31	0.48
C21:5n3	-	0.17	0.27	0.75
C22:5n3	0.13	0.19	0.36	0.62
C22:6n3	0.11	0.18	0.55	0.89
Total PUFA	58.54	62.42	56.06	59.18
(n-3) ≥ 20 C	0.52	0.89	1.63	3.12
(n-6) ≥ 20 C	0.27	0.33	0.43	0.52

The data reported in table 1 show that vitamin E played a positive role on the protection of polyunsaturated fatty acids during mixing and pelletting of feedstuffs, consequently the PUFA levels were superior in the supplemented diets. This positive action is very important because the animal's natural defense system, namely the glutathione peroxidases, could be overwhelmed by high dietary peroxides, allowing their passage through the intestinal mucosa, and having a destabilizing effect on muscle microsomes, as BUCHLEY *et al.* (1989) found in pig.

Plasma antioxidant capacity

Table 2 : Plasma antioxidant capacity measured as TRAP (ls means)

Age		Basal	Basal + Vit. E	Fish	Fish + Vit E	SDE
40 days	µmol/l	149.4a	166.0b	141.7a	155.9b	30.8
80 days	µmol/l	317.5AB	365.0Ab	355.9 Ab	434.6B	67.8

a, b: P ≤ 0.05 ; A, B: P ≤ 0.01

The total antioxidant capacity of plasma (table 2) was significantly higher in animals fed on the supplemented diets than in those given non supplemented diets. The differences were much more remarkable at 80 days of age (365 vs 317 µmol/l, basal ; 435 vs 356 µmol/l, fish), because of longer ingestion

period. It is known that the greater the amount of vitamin E fed and/or the longer the supplementation, the higher the tissue concentration of α-tocopheryl acetate (ARNOLD *et al.*, 1993a), and those the plasma levels of it.

Lipid peroxidation in fat and longissimus dorsi muscle

The peroxide index (table 3) showed lower values in groups fed on supplemented diets (2.82 vs 2.99 meqO_2/kg, basal ; 2.70 vs 3.33, fish). The lipid stability was more evident in muscle where oxidative processes were quantified as conjugates dienes which gave significantly lower values either expressed as arbitrary units (0.62 vs 0.86, basal ; 0.51 vs 0.74, fish) or as a dienes/proteins ratio (0.81 vs 1.21, basal ; 0.75 vs 1.16, fish).

Table 3 : Oxidation levels in fat and muscle.

Parameters		Basal	Basal + Vit. E	Fish	Fish + Vit E	SDE
Peroxide index	meqO2/kg	2.99	2.82	3.33	2.70	0.48
Conjugates dienes (CD)	a.u..*	0.86B	0.62A	0.74B	0.51A	0.19
CD/ proteins		1.21b	0.81a	1.16b	0.75a	0.49

a, b: P ≤ 0.05 ; A, B,: P ≤ 0.01. *a.u.: arbitrary units.

Effect on the acidic composition of longissimus dorsi muscle

Table 4 - PUFA composition of *longissimus dorsi* muscle as % total fatty acids (lsmeans)

Fatty acids	Basal	Basal + Vit E	Fish	Fish + Vit E	SDE
C18:2n6	31.24	30.30	30.29	29.22	3.02
C18:3n6	5.24	5.71	5.29	5.03	0.96
C18:3n3	0.76	1.02	1.33	1.46	0.53
C18:4n3	0.76	1.02	1.03	1.42	0.59
C20:2n6	0.51	0.66	0.66	0.87	0.17
C20:3n6	0.35	0.41	0.48	0.65	0.46
C20:4n6	3.78	3.42	3.98	3.84	0.45
C20:3n3	0.52	0.65	0.99	1.50	1.07
C20:5n3	0.75	0.86	0.89	1.13	0.07
C21:5n3	0.64	0.78	0.49	1.24	0.98
C22:5n3	0.72b	0.85b	1.04a	1.26a	0.77
C22:6n3	0.55b	0.55b	1.07A	1.29B	0.89
Total PUFA	45.82	46.23	47.54	48.91	5.17
(n-3) ≥ 20 C	3.18a	3.69b	4.48A	6.42B	4.56
(n-6) ≥ 20 C	4.64	4.49	5.12	5.36	3.51

a, b: P ≤ 0.05 ; A, B : P ≤ 0.01

The data in table 4 show that vitamin E supplementation improved the oxidative stability of muscles, with *longissimus dorsi* being richer in PUFA in animals fed diets supplemented with this antioxidant. The stability concerned (n-3) fatty acids (≥ 20 C) particularly when the diet contained fish meal (3.69 vs 3.18, basal ; 6.42 vs 4.48, fish).

As a whole dietary vitamin E supplementation (200 mg/kg vs 50 mg/kg) from 35 to 80 days of age improved the meat quality resulting in an increase in PUFA level and particularly of (n-3) fatty acids (≥ 20 C). Vitamin E exerted its protective action on diets, by increasing the oxidative stability of lipids. The greater amount of ingested (n-3) fatty acids was also found in *longissimus dorsi* because the vitamin E enhanced the plasma antioxidant capacity.

The average value of the peroxide index was low (2.96) at least in reference to the one (6.69) obtained by CABANES-ROIRON *et al.* (1994) in perirenal fat after two days of storage at + 4 °C. It is not possible to comment on the dienes since reference data are lacking.

The pre-eminent protective action of vitamin E on the (n-3) fatty acids shows the necessity of using a higher amount of this antioxidant when diets are rich in (n-3) PUFA. The recent interest in the possible health benefits associated with increased consumption of (n-3) PUFA suggests the need to carry on research in this field.

Acknowledgements - Research supported by the National Research Council of Italy- Special Project RAISA. Sub-Project no. 3. Paper no
The Authors wish to thanks the Vitamin Institute of Milano that supplied a-tocopheryl acetate.

REFERENCES

ALEXANDER D.W., McGUIRE S.O., CASSITY N.A., FRITSCHE K.L., 1995. Fish oils lower rat plasma and hepatic, but not immune cell α-tocopherol concentration. *J. Nutr.*, **125**, 2640-2649.

AOAC, 1995. Official Methods of Analysis. 16th Ed. Vol. II. AOAC International. Arlington, VA, USA.

ARNOLD R.N., ARP S.C., SCHELLER K.K., WILLIAMS S.N., SHAEFER D.M., 1993. Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *J. Anim. Sci.*, **71**, 105-118.

BRUNI M., 1993. Vitamina E e qualità della carne. *Avicoltura* **62**, (7/8), 15-19.

BUCKLEY D.J., GRAY J.I., ASGHAR A., BOOREN A.M. CRACKEL R.L. PRICE J.F., BUCKLEY D.J, MORRISSEY P.A., GRAY J.I., 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *J. Anim. Sci.*, **73**, 3122-3130.

CABANES-ROIRON A., OUHAYOUN J., GILBERT S., 1994. Qualité de la viande de lapin. *VIèmes Journées de la Recherche Cunicole*, **2**, 393-402.

CORONGIU F.P., POLI G., DIANZIANI M.U., CHEESEMAN K.H., SLATER T.F., 1986. Lipid peroxidation and molecular damage to polyunsaturated fatty acids in rat liver. Recognition of two classes of hydroperoxides formed under conditions in vivo. *Chem. Biol. Interactions*, **59**, 147-155.

DAVIES K.J.A., 1988. Proteolytic systems as secondary antioxidant defences. *Cellular Antioxidant Defence Mechanisms*, 25-67, D.K. Chow (Ed.), CRC Press, Boca Raton, FL

FOLCH J., LEES M., SLOANE-STANLEY H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497-509.

HALLIWELL B., 1987. Oxidants and disease : Some new concepts. *FASEB J.*, **1**, 358.

LIU Q., LANARI M.C., SCHAEFER D.M., 1995. A review of dietary vitamin E supplementation for improvement of beef quality. *J. Anim. Sci.*, **73**, 3131-3140.

MEYDANI S., SHAPIRO A.C., MEYDANI M., MACAULEY J.B., BLUNBERG J.B., 1987. Effect of age in dietary fat (fish, corn and coconut oil) on tocopherol status of C57BL/6 Nia mice. *Lipids*, **22**, 345-350.

MILLER E.R., 1989. Effects of dietary antioxidants and oxidized oil on membranal lipid stability and pork product quality. *J. Food Sci.*, **54**, 1193-1197.

MILLER N.J., RICE-EVANS C., DAVIES M.J., GOPINATHAN V., MILNER A., 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*, **84**, 407-412.

MORDENTI A., SARDI L., 1995. Come ottenere buona carne bovina. *Informatore zootecnico*, **18**, 19-23.

SAS/STAT, 1990. User's guide, Version 6, Cary, NC, USA.

Effetto della vitamina E sul potere antiossidante *in vivo* e sulla qualità della carne di coniglio - La possibilità di aumentare il contenuto di polinsaturi n-3 della carne, soprattutto di quelli > 20 C, evidenziata in precedenti ricerche ci ha suggerito l'opportunità di valutare l'effetto della integrazione della dieta con vit. E. Allo scopo sono stati istituiti 4 gruppi di conigli maschi (ibrido Provisal) cui sono stati somministrati dal 35° all'80° giorno di età 4 diversi alimenti : dieta base (50 mg/kg vit. E) dieta base supplementata (200 mg/kg vit. E) dieta base + 2% farina di pesce (50 mg/kg vit. E) dieta pesce supplementata (200 mg/kg vit. E). L'aggiunta di vitamina E ha migliorato la stabilità ossidativa dei lipidi durante la pellettatura e segnatamente quella degli acidi grassi superiori ω3. Ha inoltre incrementato la capacità antossidante del plasma, misurata come TRAP, soprattutto a 80 giorni (365 vs 317 µmol/l dieta base ; 435 vs 356 µmol/l dieta con pesce). Sono pertanto risultati più ridotti i processi di perossidazione valutati come dieni coniugati (0,62 vs 0,86 dieta base ; 0,51 vs 0,74 dieta con pesce) e più elevate le quantità di polinsaturi (n-3, ≥ 20C) presenti nel *longissimus dorsi* (3,69 vs 3,18 % dieta base ; 6,42 vs 4,48 % dieta con pesce).