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# Effects of Vitamin E by dietary supplementation and of Calcium Ascorbate by post mortem Injection in Muscle on the Antioxidative status and on Meat quality of Pigs

#### Abstract

The effects of addition of vitamin E to pig diet and of calcium ascorbate injection in meat on the antioxidative status and meat quality (*longissimus* muscle) of pigs were investigated. The treatment consisted of supplementation with vitamin E (500 mg  $\alpha$ -tocopheryl acetate/kg diet) for a minimum of 30 days to growing-finishing pigs before slaughter. Furthermore, meat samples (*longissimus* muscle) were injected with 10% by weight of a 1.5% calcium ascorbate solution. The dietary supplementation of fat soluble antioxidant vitamin E significantly (P<0.05) increased the concentration of  $\alpha$ -tocopherol in meat (*longissimus* muscle). Lipid oxidation measured as TBARS, and antioxidative capacity (Fe<sup>2+</sup>/ascorbate induced) of meat were significantly (P<0.05) influenced by the supplementation with vitamin E, and the pH<sub>1</sub> and driploss was improved. Water soluble antioxidant calcium ascorbate, injected in meat, increased the concentration of calcium (P<0.05) and ascorbic acid (P<0.05), and stabilized the colour ("a" value; P<0.05) in chill-stored meat and improved (P<0.05) the antioxidative capacity (Fe<sup>2+</sup>/ascorbate induced). Further research is needed to optimise the dosage.

Key Words: pig, vitamin E, calcium ascorbate, lipid oxidation, meat quality

#### Zusammenfassung

# Titel der Arbeit: Auswirkungen einer Futtersupplementierung mit Vitamin E sowie einer Injektion von Calciumascorbat in den Muskel post mortem auf den antioxidativen Status und die Fleischqualität beim Schwein

In einem Experiment mit Schweinen wurde der Effekt einer Futtersupplementierung mit Vitamin E sowie die Injektion von Calciumascorbat in das Fleisch (*M. longissimus*) auf den antioxidativen Status und auf die Fleischqualität untersucht. Die Vitamin E Supplementierung (500 mg  $\alpha$ -tocopherylacetat/kg Futter) wurde in einem Zeitraum von mindestens 30 Tagen vor dem Schlachten vorgenommen. Die Injektion in das Fleisch erfolgte mit einer 10% igen Calciumascorbat-Lösung (10% Gewichtsanteil zum Fleisch). Die Futtersupplementierung mit Vitamin E erhöhte die  $\alpha$ -tocopherol Konzentration im Fleisch signifikant. Die Lipidoxidation, gemessen als TBARS und die antioxidative Kapazität (induziert durch Fe<sup>2+</sup>/Ascorbat) wurden durch die Supplementierung signifikant beeinflusst und der pH<sub>1</sub> sowie der Dripverlust wurden signifikant verbessert. Das in das Fleisch injizierte Calciumascorbat erhöhte sowohl die Calciumkonzentration als auch die Konzentration von Ascorbinsäure. Weiterhin wurde der Farbwert "a" des Fleisches bei einer Lagerung stabilisiert. Für eine optimale Dosierung sind weitere Untersuchungen notwendig.

Schlüsselwörter: Schwein, Vitamin E, Calciumascorbat, Lipidoxidation, Fleischqualität

## Introduction

Vitamin E is considered as the principal antioxidant defence agent against lipid oxidation in cell membranes in mammals. The advantages of vitamin E supplementation (commonly used as  $\alpha$ -tocopheryl acetate) at supranutritional levels in the diet of pigs to increase the oxidative stability of phospholipids in the subcellular muscle membranes have been shown (ASGHAR et al., 1991; MONAHAN et al, 1994;

FLACHOWSKY, 2000). Dietary supplementation of vitamin E increases the concentration of  $\alpha$ -tocopherol in muscle and reduces the susceptibility of the muscle to lipid peroxidation (BUCKLEY et al., 1995). The recommended level of dietary  $\alpha$ -tocopheryl acetate supplementation in growing pigs is 15 to 40 mg/kg of feed (ALBERS et al, 1984). However, some meat quality parameters and the oxidative stability improve when dietary  $\alpha$ -tocopheryl acetate levels are higher (200 to 500 mg/kg diet) as was shown by several researches (ASGHAR et al., 1991; MONAHAN et al., 1994; BUCKLEY et al., 1995; CHEAH et al., 1995; LAHUCKY et al., 2000, 2001). The studies showed the beneficial effects of vitamin E on lipid peroxidation in meat (NUERNBERG et al., 2002), usually evaluated as TBARS (thiobarbituric reactive substances) concentration. Skeletal muscle is particularly susceptible to oxidative reactions since it contains high concentrations of prooxidants (transition metals, haem-containing proteins, i.e. myoglobin, haemoglobin) and membrane lipids which contain high percentages of polyunsaturated fatty acids (KANNER, 1994).

Vitamins E and C are primary antioxidants in biological systems and break the chain of lipid peroxidation. Some studies suggest that vitamin C and vitamin E act synergistically (GEY, 1998; EICHENBERGER et al., 2001). In the body, vitamin E (mainly as  $\alpha$ -tocopherol) is found in the lipid, while vitamin C (ascorbic acid) is an outstanding antioxidant in plasma and aqueous compartments (ROCK et al, 1996). Lascorbic acid is unstable when exposed to either oxygen or certain minerals, and this can result in a rapid oxidation to the dehydroascorbic acid derivative. Ascorbate contributes to the development of a more stable colour of cured products and acts as a synergist to antioxidants (SOFOS and BUSTA, 1980). There is a consistent epidemiological evidence about the protective effect of vitamin C against heart disease (GEY, 1998). Calcium ascorbate (E-302) is employed as antioxidant and preservative in concentrated milk products, in cooked, cured or pulverized meat products, and in pickles in which pork and beef products are cured and packed (RUTH, 1989).

The objective of this study was to evaluate further the effects of dietary vitamin E supplementation and calcium ascorbate injected into the muscle postmortem on levels of  $\alpha$ -tocopherol, ascorbic acid and calcium in muscle, the antioxidative capacity, and parameters of meat quality.

### Material and Methods

### Animals and sample preparations

In total 20 pigs were used in this experiment. They originated from lines of Slovak White Meaty pigs. The RYR-1 genotype (FUJII et al., 1991) of these animals was determined by a DNA based test described previously (BAUEROVA et al., 1999).

The experiments were in accordance with the institutional guidelines for animal care (Research Institute of Animal Production, Nitra, 1999).

The 20 pigs were equally divided in the control group and the experimental group (10 pigs each: 5 MH normal = 2 barrows and 3 gilts, 5 MH heterozygote = 2 barrows and 3 gilts). The pigs were penned in double boxes at the institute (RIAP) facilities.

The control group (group C) received standard diet. The experimental group (group E) received a supplemental level of  $\alpha$ -tocopherol (500 mg/kg diet) as source of vitamin E (ROVIMIX<sup>®</sup> E-50 SD) for approximately 40 days (minimum 30 days) before slaughter. The levels of  $\alpha$ -tocopherol and ascorbic acid in the diets are shown in Table 1 (Protocol 1222-1223/2004, Central Control Institute of Agriculture, Bratislava).

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Item	% (Weight)	Item	Group C	
Wheat	24.0	Organic matter, %	82.15	82.15
Barley	40.0	Crude protein, %	17.42	17.42
Oat	10.0	Crude fat, %	2.79	2.79
Soybean meal	12.0	Crude fibre, %	4.51	4.51
Wheat meal	4.0	N-free extract, %	57.43	57.43
Lucerne meal	3.0	Ash, %	5.63	5.63
Meat and bone meal	2.0	Metabolisable energy, MJ	12.38	12.38
Fish meal	1.0	Lysine, %	0.91	0.91
Mineral supplement	3.0	$\alpha$ -tocopherol-added, mg/kg	-	500.00
Fodder salt	0.4	- analysed, mg/kg	35.50	524.00
Biofactor supplement	0.6	Vitamin C-added, mg/kg	-	-
		-analysed, mg/kg	92.40	90.80

 Table 1

 Composition and nutrition value of the diet (Zusammensetzung und N\u00e4hrwert des Futters)

The animals were stunned, slaughtered and exsanguinated in the slaughter house of RIAP Nitra (transportation about 200 m) with an average live weight of 110 kg. After 24 h chilling (3-4°C), the *longissimus* muscle was removed from the carcass (right side) and then sliced into chops (three pieces 2.5 cm thick). One piece of loin from each pig was used as a control (no injection, group C, group E). Two pieces were treated with calcium ascorbate (group C+Asc, group E+Asc). Treatments consisted of injecting (needle injector, MEDART, Slovakia) the muscle with 10% by weight of solution of 1.5 % Ca ascorbate (Sigma-Aldrich, 1.5 g/100 g water) with approximately 2.5 cm distances. Following injection, the muscle sections were covered with aluminium film to prevent evaporative losses and to allow equilibration (for 5 min), and were than wrapped in aluminium film.

One wrapped sample was stored in a refrigerator for 5 days at  $4^{\circ}$ C, and a second wrapped sample was frozen at -  $25^{\circ}$ C until analysing.

# Chemical analysis

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The concentration of vitamin E ( $\alpha$ -tocopherol) in muscle was measured by HPLC (BERLIN et al., 1994). For vitamin C (ascorbic acid) the methodology of DAVIDEK (1981) and OMAYE et al. (1979) with 2,4-dinitrophenylhydrazin as a colour reagent was used (in cooperation with State Veterinary and Food Institute, Bratislava). Lipid oxidation was assessed by the 2-thiobarbituric acid method of SALIH et al. (1987) and was expressed as mg of malondialdehyde produced per kg of sample. For evaluating the peroxidative stability of *longissimus* homogenates the determination of thiobarbituric acid reactive substances (TBARS) was used (KUECHENMEISTER et al., 1999). TBARS were expressed in equivalents of malondialdehyde (nM/mg homogenate protein), a breakdown product formed during peroxidation stimulated by Fe<sup>2+</sup>/ascorbate. The protein content of homogenates was estimated by a modified method of MARKWELL et al. (1978). Calcium was determined by atomic absorption spectrophotometry according to the AOAC method (AOAC, 1995). Total protein and intramuscular fat were measured by the Infratec-Analyser.

# Meat quality measurements

The pH value of the carcass (*longissimus* muscle between  $13^{th}$  and  $14^{th}$  rib) was determined using a combined pH electrode (Ingold). Instrumental colour measurements were recorded for L<sup>\*</sup> (lightness; 0: black, 100: white), a<sup>\*</sup>

(redness/greenness; positive values: red, negative values: green), and  $b^{*}$  (yellowness/blueness; positive values: yellow, negative values: blue) using a Spectrophotometer (Hunter Lab MiniScan). Drip loss analysis was made according to HONIKEL et al. (1998). Shear force was determined on cooked samples (core temperature of 80°C) using a Warner-Bratzler apparatus.

#### Statistical analysis

The data were analysed with the procedure GLM of the Statistical Analysis Package SAS. The statistical model included the fixed effects gender, genotype, and experimental group. The ANOVA showed no interactions between these effects. The gender was of no influence, however, the genotype was shown to be of influence on  $pH_1$ , colour L\*, and on driploss. These effects were taken care of by calculating the least-squares means (LSM) and the standard errors (SE) including these effects in the calculations. The tables and figures contain the LSM and the SE.

### Results

The results of the crude protein, moisture and intramuscular fat of meat (*longissimus* muscle) 24 h after slaughter (Table 2) show no differences between dietary treatments with different levels of vitamin E. Also, the difference in the concentration of vitamin C was not significant (P>0.05).

Table 2

Chemical composition and level of vitamin E and C of *longissimus* muscle (Chemische Zusammensetzung und Gehalt an Vitamin E und C im Longissimusmuskel)

Trait	Group C	Group E	SE	P-value
Total water, %	73.46	73.49	0.337	0.950
Total protein, %	22.10	22.44	0.132	0.085
Intramuscular fat, %	3.31	2.88	0.339	0.378
Vitamin E, mg/kg	2.54	4.89	0.159	< 0.0001
Vitamin C, mg/kg	25.07	25.72	1.278	0.721

The supplementation with vitamin E (500 mg  $\alpha$ -tocopheryl acetate/kg diet) increased (P<0.05) the  $\alpha$ -tocopherol level (Table 2) of fresh (24 h) meat compared to that observed in pigs fed a basal level of  $\alpha$ -tocopheryl acetate (35.5 mg/kg diet, Table 1) The pH<sub>1</sub> in *longissimus* muscle was higher (P<0.05) and drip loss was lower by about 1% in *longissimus* muscle of pigs supplemented with vitamin E (Table 3). The pH<sub>24</sub>, conductivity, colour, and TBARS (24 h) in *longissimus* muscle were not significantly (P>0.05) influenced by supplementation with vitamin E.

Table 3

Meat quality of *longissimus* muscle (Fleischqualität des Longissimusmuskels)

Trait	Group C	Group E	SE	P-value
pH <sub>1</sub>	6.03	6.24	0.20	0.033
pH <sub>24</sub>	5.43	5.35	0.04	0.134
Conductivity – 3 h, mS	3.71	3.40	0.38	0.570
Conductivity – 24 h, mS	6.77	5.83	0.62	0.294
Drip loss, %	4.53	3.48	0.20	0.002
Colour L*	50.9	49.0	1.09	0.213
TBARS, mg/kg	0.24	0.21	0.01	0.108

Supplementation of Ca ascorbate (1.5 %) by injection into the meat chops (*longissimus* muscle) 24 h post mortem increased vitamin C (ascorbate) concentrations, determined

following a 5 day storage (Table 4). The concentration of calcium (*longissimus* muscle) showed a significant increase (P<0.05) in the modified meat (supplemented with Ca ascorbate). The colour parameters "a" value improved (P<0.05) in groups injected with Ca ascorbate compared to control group. The TBARS values were higher in groups without supplementation of vitamin E. No significant differences (P>0.05) were received in shear force values.

Table 4

Effect of calcium ascorbate injection on Ca and ascorbate (Vit. C) level and meat quality values after storage (5 days) of longissimus muscle (see Material and Methods) (Einfluss einer Injektion mit Calciumascorbat auf den Calcium- und Ascorbatgehalt sowie die Fleischqualität des Longissimusmuskels nach einer Lagerung von 5 Tagen)

Trait	Group C	Group C+Asc	Group E	Group E+Asc	SE
Ca, mg/kg	0.168 <sup>a</sup>	0.233 <sup>b</sup>	0.156 <sup>a</sup>	0.214 <sup>b</sup>	0.011
Vit. C, mg/kg	n.d.	274.8	n.d.	277.9	12.0
Colour L*	51.34	50.56	50.61	50.42	0.837
а	$2.29^{a}$	3.05 <sup>b</sup>	$2.42^{a,b}$	$3.00^{a,b}$	0.262
b	9.46	9.63	8.99	9.30	0.346
TBARS, mg/kg	$0.290^{a}$	$0.287^{a}$	0.235 <sup>b</sup>	0.259	0.015
Shear force, kp	5.18	4.98	4.94	4.75	0.209

within a row, means with different superscript letter differ (P<0.05) n d not determined

n.d. not determined

The Figure shows the development of TBARS (expressed as nM MDA/mg homogenate protein) after incubation of homogenates of frozen stored muscle. Whereas the TBARS of the control was steeply increasing during the incubation, the increase was significantly (P<0.05) lower in the vitamin E group and also in groups with Ca ascorbate injected meat.

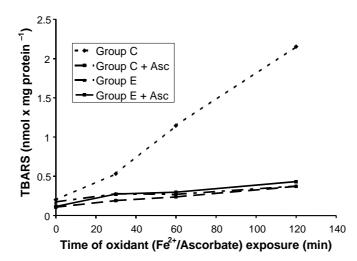


Figure: Peroxidative stability of *longissimus* muscle homogenate. The muscle homogenate was incubated for different time intervals with  $Fe^{2+}$ /ascorbate. The TBARS values of the Control group are significantly different from that of the other groups (Peroxidative Stabilität der Homogenate des Longissimusmuskels. Die TBARS-Werte der Kontrollgruppe (Group C) unterscheiden sich signifikant von denen der anderen Gruppen)

# Discussion

The levels of  $\alpha$ -tocopherol in *longissimus* muscle in the present study are comparable with previously reported results (BUCKLEY et al., 1995; LAHUCKY et al., 2001; NUERNBERG et al., 2002).

It is known (Den HERTOG-MEISCHKE et al., 1997; KRSKA et al., 2001) that the effect of vitamin E supplementation on meat quality values depends on the muscle. As was shown earlier (CHEAH et al., 1995) using a higher level of vitamin E ( $\alpha$ -tocopherol) supplementation (500 mg and or 1000 mg/kg diet) administered for 46 days positively influenced the drip loss and pH in unfrozen *longissimus thoracis* muscle in MH-heterozygotes and in MH-normal pigs. KERTH et al.(2001) showed, that the supplementation of at least 600 IU of vitamin E in the finishing diet of MH-normal pigs drastically reduced quality defects of the muscle. Some results (HONIKEL et al., 1998) did not show positive effects of vitamin E supplementation on pH and drip loss, pH 45) could also be a consequence of different levels and time of vitamin E administration and/or unknown (and different number) halothane (malignant hyperthermia) gene status of pigs as was discussed earlier (LAHUCKY et al. 2001). Our results, showing no differences in colour (Table 3), are in agreement with others (HONIKEL et al., 1998, LAHUCKY et al., 2001).

A higher "a" value in Ca ascorbate injected muscle after a five days chill storage (Table 4) could be connected to a reducing activity of ascorbic acid on metmyoglobin. Ascorbic acid could play a role in maximizing muscle colour stability by metmyoglobin reduction as was discussed by LEE et al. (1999). Recently, MANCINI et al. (2004) showed that application of ascorbic acid (2.5%) prevented lumbar vertebrae discoloration during a five day display at 1° C. Using higher concentrations of Ca ascorbate (29.17 g/kg) in fermented sausages, significant higher "a" values and lower "L\*" values were received (GIMENO et al., 2001). As was introduced by CONOLLY and DECKER (2004), addition of exogenous antioxidants with strong reducing activity (ascorbic acid) to postmortem tissue can partially convert peroxinitrite oxidized metmyoglobin back to oxymyoglobin and could help preserve colour.

There are only few results regarding shear force (tenderness) in pigs supplemented with vitamin E (LAHUCKY et al., 2001; WAYLAN et al., 2002). Ca<sup>2+</sup> transport as well as increased intracellular Ca<sup>2+</sup> concentration should be of influence on tenderness (KUECHENMEISTER and KUHN, 2003). In our experiment, there was no improvement in tenderness by diet supplementation of vitamin E and/or by injection of Ca ascorbate into the meat (Table 4). As was shown by SHEARD and TALI (2004) marination with different salt solutions (sodium tripolyphosphate and/or sodium bicarbonate) could reduce the shear force. Also, results reported by MONTGOMERY et al. (2000) that dietary vitamin D<sub>3</sub> given 9 days before slaughter improved tenderness of 14 days aged beef (interaction vitamin E and D<sub>3</sub>) were shown to be promising.

It has been proposed that a high level of vitamin E in the diet might reduce the damaging oxidation of meat, and this area has been reviewed extensively (MORRISSEY et al., 1994; BUCKLEY et al., 1995). The dietary level of vitamin E (group E and group E+Asc) positively influenced the lipid oxidation. It has been stated that additional vitamin C can either exhibit antioxidative or prooxidative effects (WONG et al., 2005). Different mechanisms of fat soluble (vitamin E) and water soluble (vitamin C) antioxidants can be expected (ROCK et al., 1996). For further studies different levels of Ca ascorbate (or acid ascorbate) injection in meat and using TEAC (Trolox equivalent antioxidant capacity) to evaluate water soluble (or total) antioxidants (ARTS et al., 2004) would be useful.

The possibility to slow the formation of peroxidative degradation products (MDA) in such systems is an indication of its antioxidative capacity (NUERNBERG et al., 2002; LAHUCKY et al., 2004). The illustrated differences (Figure) substantiate the protective action of feed supplemented with vitamin E and/or vitamin C incorporated into the muscle tissue against peroxidation. Such a beneficial effect of dietary vitamin E on the oxidative stability of pork chops and ground meat after storage has been also shown by others (ASGHAR et al., 1991; LAHUCKY et al., 2001; NUERNBERG et al., 2002).

From the data it is clear that a higher level of vitamin E in the pig's diet improves the oxidative stability of pork. To take the greatest advantage of cooperative effects with other antioxidants (ascorbic acid), it is necessary to clearly define the dosages to be administered, bearing in mind the results to be achieved.

In agreement with others (ISABEL et al., 2003) administration strategies for improving meat quality and antioxidative stability characteristics are not commonly used due to extra feeding costs, which are difficult to be recovered in the commercial setting. However, the relative higher added value of processed meats compared to fresh meat could provide an economic advantage by the use of administration (antemortem and/or postmortem) strategies for reducing deterioration during storage, or for improving quality characteristics. This provides an interesting area of research to define strategies to optimise quality, and their possible interactions with technological processes.

Further research is needed to optimise the dosage.

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