



## EFFECTS OF DIETARY VITAMIN E AND VITAMIN C SUPPLEMENTATION ON LEVEL OF ALPHA –TOCOPHEROL AND L-ASCORBIC ACID IN MUSCLE AND ON THE ANTIOXIDATIVE STATUS AND MEAT QUALITY OF PIGS

R. Lahučký, I. Bahelka, K. Novotná, K. Vašíčková  
Research Institute of Animal Production, 94992 Nitra, Slovak Republic

### Background

Vitamin E and C are primary antioxidants in biological systems and break the chain of lipid peroxidation. Many studies suggest that vitamin C and vitamin E act synergistically (Gay 1998). Previous studies evaluating the efficiency of relatively high levels of vitamin have been inconsistent in producing a growth or feed efficiency response. By Mahan et al. (1994) was used a stable source of vitamin C (magnesium-L-ascorbyl-2-phosphate) in pig feeding experiment. Another stable source of vitamin C (L-ascorbyl-2-polyphosphate, Rovimix<sup>®</sup> Stay-C<sup>®</sup> 25, Roche) was used by de Rodas et al. (1998) and Sahin et al. (2002). Data from pig experiment introduced by Kremer et al. (1999) suggest that vitamin C supplementation before slaughter can improve parameters of meat quality.

### Objectives

The objective of this study was to evaluate further the effects of vitamins E and C supplementation on level of  $\alpha$ -tocopherol and ascorbic acid and lipid peroxidation status in fresh and chill-stored meat and on some quality parameters

### Materials and methods

Thirty Slovak White Meaty pigs were used in this experiment. Control group (n = 10) and two experimental groups were homozygotes, negative on malignant hyperthermia (defined by DNA based test). Control group was fed with a diet supplemented with basal level  $\alpha$ -tocopherol (Table 1). Experimental groups received a supplemental (Table 1) level  $\alpha$ -tocopherol (500 mg/kg), (group E, n = 10) and a supplemental level  $\alpha$ -tocopherol (500 mg/kg) and ascorbic acid (200 mg/kg), (group EC, n = 10) for 30 days before slaughter. Vitamin E (ROVIMIX<sup>®</sup> E-50 SD, stable source of vitamin E in feed) and vitamin C (ROVIMIX<sup>®</sup> STAY-C<sup>®</sup> 35) were provided by a commercial company (Roche, Germany). Animals were slaughtered at average live weight of 110 kg. The sample of longissimus dorsi (part lumborum, LD) muscle was used immediately (24 h) and the remaining samples were wrapped in aluminium film and stored in a refrigerator at 4°C for 5 days. The concentration of vitamin E ( $\alpha$ -tocopherol) of the samples (fresh, cooked, frozen) was measured by HPLC and vitamin C (ascorbic acid) with 2,4-dinitrophenylhydrazin as a color reagent was estimated. Lipid oxidation in samples (fresh and 5 days chill-stored) and the stability of the skeletal muscle (fresh samples) lipids against stimulated (by Fe<sup>2+</sup>/ascorbate) lipid peroxidation were assessed by the 2-thiobarbituric acid method (TBARS) and expressed in terms of malondyaldehyde (MDA, mg/kg tissue) as described earlier (Lahucky et al., 2001). The pH value of the carcass (m. longissimus – between 13<sup>th</sup> and 14<sup>th</sup> rib) 45 min post mortem, electrical conductivity 3 h, color by Miniscan 24 h, total water, protein and intramuscular fat were also measured (Lahucky et al., 2001). Drip loss analyses were made according to Honikel (1998). Shear force was determined in cooked samples (internal temperature 80°C, used also for further analyses) with Warner-Bratzler (W-B) apparatus. Statistical analyses were calculated as mean values and standard deviations and differences were evaluated by t-test.

### Results and discussion

The supplementation of vitamin E ( $\alpha$ -tocopheryl acetate) to pigs increased about 2 folds the  $\alpha$ -tocopherol levels (Fig 1) of fresh (24 h) and 5 days chill-stored meat. The levels of  $\alpha$ -tocopherol in longissimus dorsi muscle are higher or comparable with previously reported results (Honikel et al., 1998, Lahucky et al., 2000, 2001). Dietary supplementation of vitamin C increased fresh meat vitamin C concentrations (Fig 2) and in some extent in stored meat (P<0.05). The values of the moisture, crude protein and intramuscular fat (Table 2) were not influenced by dietary treatments. Improvement in pH value (P=0.06) after vitamin C and vitamin E supplementation (group E + C, Table 3) can support data from one experiment (Kremer et al.,



1999). They suggest that adding sodium oxalate or vitamin C to final meal given to pigs before slaughter resulted in higher early post-mortem pH, but further studies on glycolysis and glycogen metabolism would be useful as vitamin C is known as precursor of oxalic acid and sodium oxalate inhibits a key glycolytic enzyme, pyruvate kinase.

A tendency of improving drip loss (lower value) in longissimus dorsi in 24 h post mortem of normal on malignant hyperthermia pigs supplemented with vitamin E (Table 3) and significant lower ( $P<0.05$ ) value in pigs supplemented with vitamin E and vitamin C (group E + C) were received. It seems that adding a high level of vitamin E and/or vitamin C to the diet will reduce drip loss in some situations, but perhaps not in all situations and genetic background (occurrence of mutation on ryanodine receptor gene, malignant hyperthermia status) of experimental pigs could influence the results as was also discussed earlier (Lahucky et al., 2000). Dietary levels of vitamin E (group E) and vitamin E and vitamin C (group E + C) did not substantially affect the development of lipid oxidation (Fig 3) in the fresh meat (24 h), but significant differences ( $P<0.05$ ) we mainly received in chill-stored (5 days) between control vs. supplemented pigs (group E and group EC). Whereas the MDA of the control were increasing during 30 min of incubation, the increase was significantly ( $P<0.05$ ) lower in supplemented groups. Significant differences ( $P<0.05$ ) we also received between vitamin E vs. vitamin E + C group (Fig 4).

## Conclusions

Dietary supplementation of vitamin E (500 mg  $\alpha$ -tocopheryl acetate/kg feed) and vitamin C (200 mg/kg feed) to grow-finishing pigs increases the concentrations of  $\alpha$ -tocopherol and ascorbic acid in meat (longissimus dorsi). Supplementation vitamin E and vitamin C improves meat quality parameters (drip loss, pH), but results can be influenced by genetic background of animals (occurrence of mutation on ryanodine receptor gene, malignant hyperthermia status). Lipid oxidation measured as TBARS (MDA) and antioxidative capacity ( $\text{Fe}^{2+}$ /ascorbate induced) of meat can be positively influenced by supplementation of vitamin E to grow-finishing pigs.

## References

- Buckley D. J., Morrissey P.A., Gray J. I. (1995) *J. Anim. Sci.*, 73, 3122-3131.
- Gey K. F. (1998) *BioFactors* 7, 113-174.
- Honikel K. O., Rosenbauer H., Fischer K., Muller W. D. Przytule J. (1998) *Fleischwirtschaft*, 78, 1205-1207.
- Kremer B. T., Stahly T. S., Ewan R. C. (1999). *J. Anim. Sci.* 77 (Suppl. 1), 46 (Abstr.)
- Lahučký R., Krška P., Kuchenmeister U., Nurnberg K., Bahelka I., Demo P., Kuhn G., Ender K. (2001) *Czech J. Anim. Sci.*, 46, 7, 327-332.
- Mahan D.C.; Lepine A.J.; Dabrowski K. (1994) *J. Anim. Sci.* 72, 2354-2361.
- McDowell L.R. (1989): *Vitamin in Animal Nutrition: Comparative Aspects to Human Nutrition*. Academic Press, San Diego, CA.



Table 1. Composition and nutritive value of diet

Item	%	Item	Control	Vitamin E+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	Vitamin A (m.j.)	5 400.00	5 500.00
Fodder salt	0.4	α-tocopherol – added (mg)	-	500.00
Biofactor supplement	0.6	- analysed (mg)	33.60	515.00
		Vitamin C – added (mg)	-	200.00
		- analysed (mg)	90.30	189.20

Table 2. Chemical composition of muscle *longissimus dorsi*

Item	Control		Vitamin E		Vitamin E+C		Significance
	mean	S.D.	mean	S.D.	mean	S.D.	
Total water, %	74.23	0.70	73.82	0.84	73.93	0.75	-
Total proteins, %	22.41	0.41	22.53	0.50	22.46	0.89	-
Intramuscular fat, %	2.76	0.76	2.81	0.94	2.81	0.81	-

Table 3. Pork quality (*m. longissimus dorsi*)

Trait	Time	Control		Vitamin E		Vitamin E+C		Significance
		mean	S.D.	mean	S.D.	mean	S.D.	
pH	45 min	6.27	0.22	6.38	0.19	6.45	0.26	-
El. conductivity, μS	3 h	4.06	1.01	3.67	1.22	3.93	1.12	-
Colour (L)	24 h	48.67	3.64	48.58	2.36	48.60	2.14	-
Free water, %	24 h	37.84	2.95	36.73	3.33	36.41	3.25	-
Drip loss, %	24 h	4.86	1.03	4.12	1.05	4.05	0.88	*
Colour (L)	5 day	51.63	2.82	51.69	3.75	50.84	2.66	-
Free water, %	5 day	35.87	2.90	34.26	2.94	33.75	2.86	-
Shear force, kg	5 day	4.09	1.15	4.82	0.71	4.66	0.66	-

\*P&lt;0.05

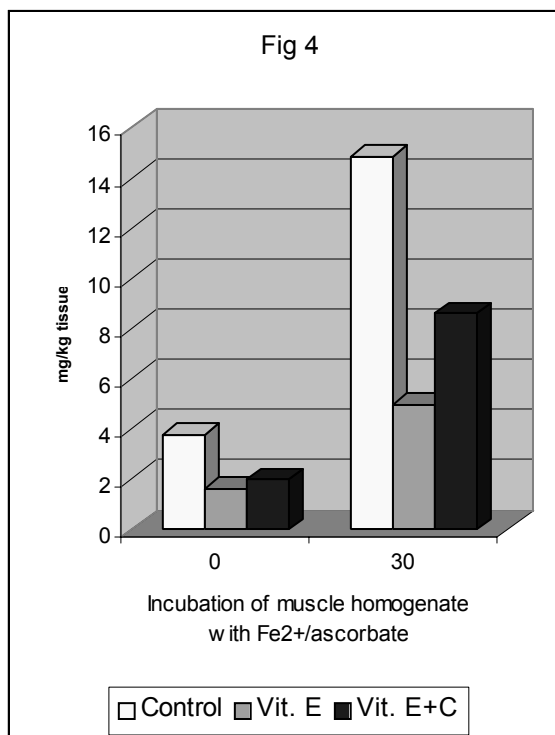
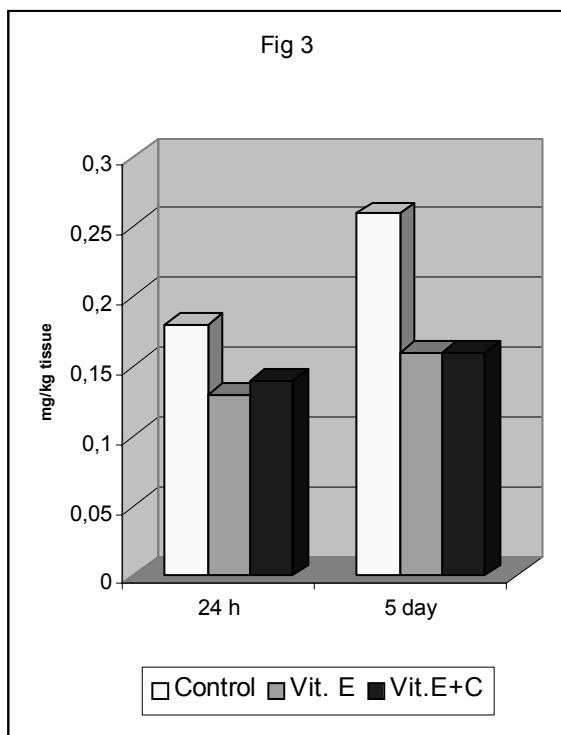
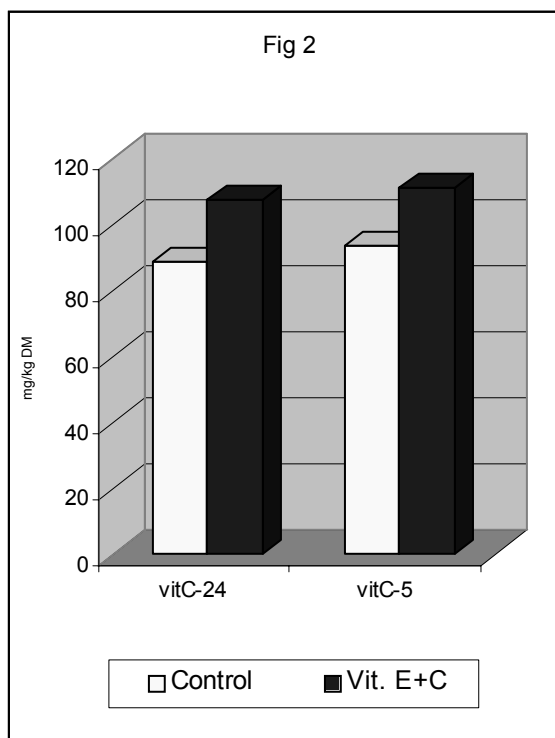
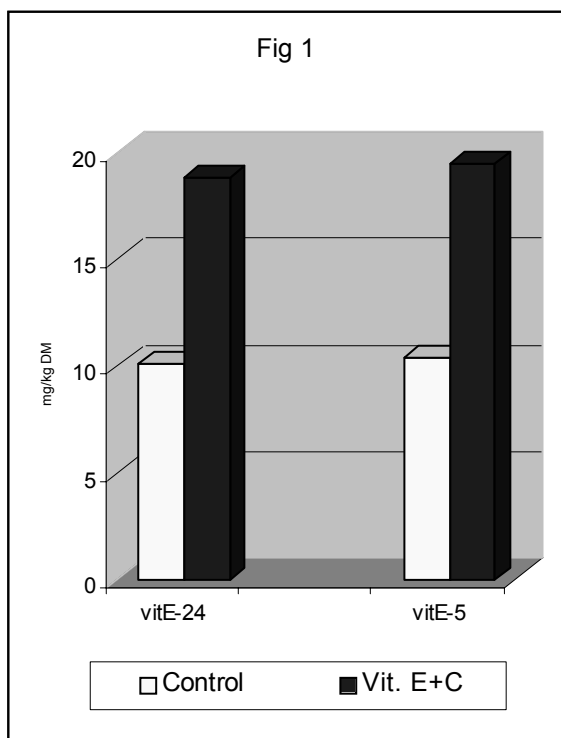


Figure 1. Content of  $\alpha$ -tocopherol in muscle  
vitE-24 = fresh meat 24 h  
vitE-5 = chill-stored meat 5 days

Figure 2. Content of ascorbic acid in muscle  
vitC-24, 5,

Figure 3. Level of thiobarbituric acid  
reactive substances (TBARS, MDA) in muscle

Figure 4. Antioxidant stability of muscle  
(incubation of muscle homogenate with  
 $\text{Fe}^{2+}$ /ascorbate)