



## EFFECTS OF VITAMIN D<sub>3</sub> AND VITAMIN E ON QUALITY CHARACTERISTICS OF PIGS BY FEEDING

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### ABSTRACT

The effects of addition of vitamin D<sub>3</sub> and vitamin E to pig diet on blood plasma calcium concentration and meat quality (longissimus muscle) were investigated. The treatment consisted of supplementation with vitamin E (500 mg  $\alpha$ -tocopheryl acetate/kg diet) for 30 days and vitamin D<sub>3</sub> (500,000 IU/d) for 5 days to growing pigs before slaughtering these fullgrown ones. Animals fed with vitamin D<sub>3</sub> exhibited higher ( $P < 0.01$ ) plasma calcium concentration compared with control pigs. The dietary supplementation of vitamin E significantly ( $P < 0.05$ ) increased the concentration of  $\alpha$ -tocopherol in meat (longissimus muscle). Lipid oxidation measured as MDA (malondialdehyde) was positively influenced by the supplementation with vitamin E. Vitamin D<sub>3</sub> supplementation resulted in higher ( $P < 0.05$ )  $a^*$  values of loin chops at 5 d of shelf storage. Vitamin D<sub>3</sub> and vitamin E supplementation did not affect other meat quality characteristics or tenderness (quantified via Warner-Bratzler shear force).

**Key words:** pig, feeding, vitamin D<sub>3</sub>, vitamin E, meat quality

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### INTRODUCTION

Improving and stabilizing meat quality parameters is an area attracting increasing attention from all segments of the pork chain. Results from a number of studies showed that feeding of supra-nutritional levels of some nutrients may improve the water holding capacity, color and antioxidant capacity of pork. These nutrients include magnesium, selenium, vitamin E, vitamin C, tryptophan, creatine, conjugated linoleic acid (Buckley et al., 1995; Krska et al., 2001; Nürnberg et al., 2002; Swigert et al., 2004).

A major factor in determining consumer satisfaction with meat is tenderness. There has been development with the move towards higher lean growth genetics (Meisinger, Miler, 1998). The association of calcium with meat tenderness is well defined (Koochmaraie, 1992). It has been reported that in situ tenderization of meat and meat products involves the activities of "free" activated calpain, which is controlled by calcium ion concentration. Increasing

muscle calcium increases the activity of calpains, which are intracellular proteases responsible for postmortem meat tenderness (Koochmaraie, 1992). Montgomery et al. (2000) have reported that feeding very higher concentrations of vitamin D<sub>3</sub> to cattle results in increased levels of plasma and muscle calcium. But vitamin D<sub>3</sub> supra-nutritional supplementation was not an effective means of improving the tenderness characteristics of lamb muscles (Boleman et al., 2004). Similarly vitamin D<sub>3</sub> supplementation did not affect quality characteristics (Swigert et al., 2004) or tenderness (quantified via Warner-Bratzler shear force) of pork but changes in L\* and a\* values were positively influenced (Wiegand et al., 2002). Results of the further study (Wilborn et al., 2004) indicated that L\* values can be reduced, and subjective colour and firmness scores increased.

The objective of this study was to characterize the effects of dietary supplementation with vitamin D<sub>3</sub> individually, and in combination with vitamin E on meat quality characteristics of pork.

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## MATERIAL AND METHODS

### Animal and sample preparations

A total of 36 pigs were chosen for 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.

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

The 36 pigs were divided into the control groups and the experimental groups (12 pigs each: 7 MH normal = 3 barrows and 4 gilts, 5 MH heterozygote = 3 barrows and 2 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 D) received a supplemental level of vitamin D<sub>3</sub> (500,000 IU/kg of feed intake for 5 d) and a supplemental level of vitamin E (ROVOMIX® E-50 SD, 500 mg  $\alpha$ -tocopherol/kg of feed for minimum 30 days before slaughter. The levels of  $\alpha$ -tocopherol in the diets are shown in Table 1 (Protocol 1222-1223/2005, Central Control Institute of Agriculture, Bratislava).

The animals were stunned, slaughtered and exsanguinated in the slaughter house of RIAP, Nitra (transportation of about 200 m) with an average live weight of 110 kg. Blood was collected in heparinised tubes for calcium estimation. After 24 h of chilling (3-4°C), the longissimus muscle (LD) was removed from the carcass (right side) and then sliced into chops (2.5 cm thick). Once wrapped sample was stored in the refrigerator for 5 days at 4°C.

### Chemical analysis

The concentration of vitamin E ( $\alpha$ -tocopherol) in muscle was measured by HPLC (Berlin et al., 1994), (in cooperation with State Veterinary and Food Institute, Bratislava). Lipid oxidation was assessed by the 2-thiobarbituric acid (TBARS – thiobarbituric acid related substances) method of Salih et al. (1987) and was expressed as mg of malondialdehyde (MDA) produced per kg of sample. Calcium was determined by atomic absorption spectrometry 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 13th and 14th rib) was determined using a combined pH electrode (Ingold). Instrumental colour measurements were recorded for L\* (lightness; 0: black, 100: white), a\* (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 (1998). Shear force was determined on cooked samples (core temperature of 80°C) using a Warner-Bratzler apparatus.

### Statistical analysis

Statistical analyses were calculated as mean values (of three repeated measurements) and standard deviations and differences were evaluated by t-test.

**Table 1: Composition and nutritional value of the diet**

Item	%	Item	Group C	Group E
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			

**Table 2: Calcium level (mmol/l) in blood plasma of pigs**

Trait	Control ± s	Group D <sub>3</sub> ± s	Group D <sub>3</sub> + E ± s
Ca (mmol/l)	2.71±0,16a	3.90±0,28b	3.74±0,24b

a, b (P&lt;0.05)

**RESULTS AND DISCUSSION**

Feeding with supplemental vitamin D<sub>3</sub> at the 500.000 IU/kg level for 5 days before slaughter increased (P<0.01) plasma calcium concentration as is shown in Table 2 in groups with vitamin D<sub>3</sub> and vitamin D<sub>3</sub> in combination with vitamin E as well. The vitamin D<sub>3</sub> fed pigs exhibited greater plasma calcium concentration on the day of slaughter (5 d) as was shown also by Wiegand et al. (2002). Chemical compositions in total water, total

protein and intramuscular fat were also not influenced and significant differences (P>0.05) between control and experimental groups were not found (Table 3). Feeding with supplemental vitamin E at the 500 mg/kg level for 30 days before slaughter increased (P<0.05) the level of α-tocopherol in muscle (Table 3, group D<sub>3</sub> + E). The results are in agreement with those of Lauridsen et al. (1999) and Lahucky et al. (2001). Meat quality characteristics from the study are summarized in Table 4. Feeding supplemental vitamin D<sub>3</sub> and vitamin E did not (P>0.05) affect pH (1 and 24 h) and conductivity (24 h). Percentage drip loss after 24 h were lower in muscle from pigs that had been fed with supplemental vitamin D<sub>3</sub> and vitamin E (group D<sub>3</sub> + E) but differences were not significant (P>0.05). Colour data were not different (P>0.05) for L\*, a\* and b\* values as estimated 24 h after postmortem. At only 5 d postmortem the a\* value was higher (P<0.05) for LD muscle (higher redness) from vitamin D<sub>3</sub> and vitamin D<sub>3</sub> and vitamin E treated pigs. Similar results were reported also by Wiegand et al. (2002). Thiobarbituric acid reactive substance evaluated

**Table 3: Chemical composition and level of vitamin E of longissimus dorsi muscle**

Trait	Control ± s	Group D <sub>3</sub> ± s	Group D <sub>3</sub> + E ± s
Total water, %	73.86 ± 0,55	73.43 ± 0,62	73.62 ± 0,58
Total protein, %	22.63 ± 0,48	22.53 ± 0,32	22.83 ± 0,36
Intramuscular fat, %	2.44 ± 0,77	2.9 ± 0,70	2.56 ± 0,54
Vitamin E, mg/kg	2.015±0,65 <sup>a</sup>	n.d.	3.208±0,94 <sup>b</sup>

a, b (P&lt;0.05)

**Table 4: Pork quality of longissimus dorsi muscle**

Trait	Control ± s	Group D <sub>3</sub> ± s	Group D <sub>3</sub> + E ± s
pH <sub>1</sub>	6.24 ± 0,33	6.33 ± 0,16	6.32 ± 0,28
pH <sub>24</sub>	5.40 ± 0,05	5.37 ± 0,10	5.50 ± 0,27
Conductivity – 24 h, mS	6.52 ± 1,27	5.74 ± 3,00	5.55 ± 2,47
Drip loss %	3.42 ± 1,25	3.20 ± 1,06	3,04 ± 1,00
Colour L*	48.85 ± 3,12	46.72 ± 2,32	46.59 ± 4,15
a*	2.19 ± 2,09	2.44 ± 1,21	2.51 ± 0,44
b*	8.42 ± 0,63	7.74 ± 0,97	8.18 ± 0,66
TBA, mg/kg	0.173 ± 0,03	0.186 ± 0,06	0.154 ± 0,05
Colour L* - 5 d	49.20 ± 2,05	49.16 ± 2,15	48.30 ± 4,47
a* - 5 d	2.73a ± 0,76	3.74b ± 1,15	3.63b ± 0,81
b* - 5 d	8.56 ± 0,72	8.88 ± 0,84	9.32 ± 1,37
Shear force (W-B) kg	4.86 ± 1,04	4.77 ± 0,83	5.00 ± 0,72
TBA, mg/kg – 5 d	0.22 ± 0,03	0.21 ± 0,06	0.18 ± 0,02

a, b (P&lt;0.05)

as level of malondyaldehyde (MDA) was lower in muscle of pigs supplemented with vitamin E but only its value 5 d after postmortem was significant ( $P < 0.05$ ), which is in accordance with earlier findings (Lauridsen et al., 1999; Lahučký et al., 2001). The effect of supplemented dietary vitamin D<sub>3</sub> and the combination of vitamin D<sub>3</sub> and vitamin E on tenderness of LD muscle was determined by the Warner-Bratzler shear force method. Results from measures of tenderness indicated that feeding 500.000 IU of vitamin D<sub>3</sub> to pigs for 5 d did not improve shear force values for LD muscle ( $P > 0.05$ ). Results of received of our study are supported by others (Wiegand et al., 2002; Wilborn et al., 2004; Swigert et al., 2004), which stated feeding high concentrations of vitamin D<sub>3</sub> to pigs improves colour, but the mechanism responsible for this improvement is not clear. As reported by Wilborn et al. (2004) longer feeding time may be required to increase muscle calcium concentrations sufficiently to improve pork tenderness.

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## REFERENCES

- AOAC. 1995. Determination of calcium, magnesium and phosphorus in cheese. In: *Official Methods of analysis* (16th ed.). Arlington, VA : Association of Official Analytical Chemists, 1995.
- BOLEMAN, C. T. - MCKENNA, D. R. - RAMSEY, W. S. - PEEL, R. K. - SAVELL, J. W. 2004. Influence of feeding vitamin D<sub>3</sub> and aging on tenderness of four lamb muscles. In: *Meat Sci.*, vol. 67, 2004, s. 185-190.
- BUCKLEY, D. J. - MORRISSEY, P. A. - GRAY, J. I. 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. In: *J. Anim. Sci.*, 73, 1995, s. 3122-3131.
- FUJII, J. - OTSU, K. - ZORZATO, F. - DE LEON, S. - KHANA, V. K. - WEILER, J. - Ó BRIEN, P. - MAC LENNAN, D. H. 1991. identification of a mutation in the porcine ryanodine receptor that is associated with malignant hyperthermia. In: *Science*, Washington 253, 1991, s. 448-451.
- HONIKEL, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. In: *Meat Sci.*, vol. 49, 1998, p. 447-457.
- KOOHMARAIE, M. 1992. The role of Ca<sup>2+</sup> dependent proteases (calpains) in postmortem proteolysis and meat tenderness. In: *Biochemie*. vol. 74, 1992, p. 239-245.
- KRSKA, P. - LAHUCKY, R. - KÜCHENMEISTER, U. - NÜRNBERG, K. - PALANSKA, O. - BAHNELKA, I. - KUHN, G. - ENDER, K. 2001. Effects of dietary organic selenium and vitamin E supplementation on post mortem oxidative deterioration in muscles of pigs. In: *Arch. Tierz.*, Dummerstorf, vol. 44, 2001, p. 193-201.
- LAHUCKY, R. - KRSKA, P. - KÜCHENMEISTER, U. - NÜRNBERG, K. - BAHNELKA, I. - DEMO, P. - KUHN, G. - ENDER, K. 2001. Influence of vitamin E supplementation on antioxidative status in muscle and meat quality of pig. In: *Arch. Tierz.*, Dummerstorf, vol. 43, 2001, no. 5, p. 487-497.
- LAURIDSEN, CH. - NIELSEN, J. H. - HENCKEL, P. - SORENSEN M. T. 1999. Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E, and copper. In: *J. Anim. Sci.*, vol. 77, 1999, p.105-115.
- MEISINGER, D. J. - MILLER, R. K. 1998. Pork quality issues. In: *Proc. Pork Quality Summit*, Des Moines, IA, 1998, p. 29-32.
- MONTGOMERY, J. L. - PARISH, F. C. JR. - BEITZ D. C. - HORST, R. L. - HUFF-LONERGAN, E. J. - TRENKLE A. H. 2000. The use vitamin D<sub>3</sub> to improve beef tenderness. In: *J. Anim. Sci.*, vol. 78, 2000, p. 2615-2621.
- NÜRNBERG, K. - KÜCHENMEISTER, U. - KUHN, G. - NÜRNBERG, G. - WINNEFELD, K. - ENDER, K. - COGAN, D. - MOKADY, S. 2002. Influence of dietary vitamin E and selenium on muscle fatty acid composition in pigs. In: *Food Research International*, vol. 35, 2002, p. 505-510.
- SALIH, A. M. - SMITH, D. M. - PRICE, J.F. - DAWSON, L. E. 1987. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. In: *Poultry Sci.*, vol. 66, 1987, p. 1483-1488.
- SWIGERT, K. S. - MCKEITH, F. K. - CARR, T. C. - BREWER, M. S. - CULBERTSON, M. 2004. Effects of dietary vitamin D<sub>3</sub>, vitamin E, and magnesium supplementation on pork quality. In: *Meat Sci.*, vol. 67, 2004, p. 81-86.
- WIEGAN, B. R. - SPARKS J. C. - BEITZ D. C. - PARRISH F. C., jr. - HORST R. L. - TRENKLE A. H. - EWAN R. C. 2002. Short-term of vitamin D<sub>3</sub> improves color but does not change tenderness of pork loin chops. In: *J. Anim. Sci.*, vol. 80, 2002, p. 2116-2112.
- WILBORN, B. S. - KERTH, C. R. - OWSLEY, W. F. - JONES, W. R. - FROBISHL.T. 2004. Improving pork quality by feeding supranutritional concentrations of vitamin D<sub>3</sub>. In: *J. Anim. Sci.*, vol. 82, 2004, p. 218-224.

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