

**EFFECTS OF VITAMIN E, SWEET CHESTNUT WOOD EXTRACT AND HOPS  
SUPPLEMENTATION ON PIG MEAT QUALITY AND OXIDATIVE  
STABILITY**

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**Abstract**

The aim of the study was to investigate the effect of vitamin E and two natural additives with potential antioxidative activity (sweet chestnut wood extract – Farmatan, and hops) on growth performance, meat quality and meat oxidative stability of pigs. For this purpose, 41 crossbred pigs (20 gilts and 21 barrows) were assigned to four treatment groups. Control group (Control; N=11) was given a standard diet (13 MJ ME, 16% crude protein), while pigs in three treatment groups received the same diet supplemented with either 150 mg/kg  $\alpha$ -tocopherol acetate (Vit E, N=7), 3% of tannin rich extract Farmatan (Tannin, N=12) or 0.4% of hop cones (Hop, N=11). Meat quality was evaluated in muscle *longissimus dorsi* by measuring pH at 24 h post-mortem, color (lightness, redness, yellowness), drip loss, thawing and cooking losses, and hardness. Meat chemical composition was assessed using NIRS (near-infrared spectroscopy). Oxidative stability of meat was studied by measuring concentrations of vitamin E, by total plasma antioxidant capacity of water-soluble compounds (ACW), fat oxidation by malondialdehyde (MDA) concentration and fatty acid composition and protein oxidation by concentration of carbonyl groups. Data was analysed using the General Linear Models (GLM) procedure of the SAS/STAT module. No differences in growth performance and meat quality were observed among experimental groups. Feeding the supplements also showed no influence on fatty acid composition, MDA and carbonyl groups concentration and on ACW. Concentration of  $\alpha$ -tocopherol was higher in the group supplemented with vitamin E.

*Keywords:* nutrition, antioxidants, tannin, hops, vitamin E, oxidative stability, finishing pigs

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## Introduction

Lipid oxidation is one of the main factors limiting the quality and acceptability of meat and meat products and is reflected in adverse changes in flavor, color, texture and nutritive value, and by possible production of toxic compounds. It can be reduced or inhibited by the use of antioxidants, the product quality and shelf-life can thus be improved (Shah et al., 2014). Vitamin E occupies an important position in the overall antioxidant defense; its supplementation above the requirements has been found to be effective in reducing lipid oxidation in meat and meat products (Morrissey et al., 1998). Several studies investigating the effect of natural antioxidants on oxidative stability of meat and meat products have been published. Tannins exert anthelmintic, antimicrobial and antioxidant properties (Mueller-Harvey, 2006). One of the main sources of hydrolysable tannins is sweet chestnut (*Castanea sativa* Mill.) wood extract (SCW). Hops (*Humulus lupulus* L) is also reported to be a source of polyphenolic substances and can act as a potent antioxidant (Krofta et al., 2008). As hops and SCW (commercial name Farmatan) are the two natural supplements available and economically relevant in Slovenia, their effect on meat quality and oxidative stability in comparison to control and vitamin E supplemented feed mixture was evaluated in the present study.

## Material and Methods

At an average age of 140 days, 41 crossbred pigs (20 gilts and 21 barrows) were assigned to four treatment groups. Control group (Con; N=11) was given a standard corn based finishing diet (13.09 MJ ME, 16% crude protein; Table 1), while pigs in three treatment groups received the same diet supplemented with either 150 mg/kg *all-rac- $\alpha$ -tocopherol* acetate (VitE, N=7), 3% of Farmatan (Tannin, N=12) or 0.4% of hop cones (Hop, N=11). The diets were fed on the *semi ad libitum* basis. The number of pigs per group was different due to different size of pens and was adjusted to have the same space area per pig (1.2 m<sup>2</sup>). After 69 days, the pigs were slaughtered and samples of *longissimus dorsi* (LD) muscle were collected to determine meat quality and oxidative stability.

Table 1. Chemical and fatty acid composition, and oxidative stability of experimental diets

	Supplemented			
	Control	VitE	Tannin	Hop
Dry matter (g/kg)	873.0	869.0	867.1	873.3
Crude protein (g/kg)	155.1	152.9	149.6	153.1
Crude fat (g/kg)	25.3	25.3	24.4	25.2
Crude fiber (g/kg)	49.6	50.7	51.0	49.9
Crude ash (g/kg)	59.4	48.2	48.9	47.4
Nitrogen-free extract (g/kg)	583.4	592.1	593.2	597.7
Fatty acid composition (wt % of fat)				
SFA	17.4	17.5	17.3	17.4
MUFA	24.2	24.0	25.0	24.2
PUFA	58.5	58.6	57.8	58.4
n-3 PUFA	3.38	3.43	3.49	3.40
n-6 PUFA	55.1	55.1	54.3	55.0
n-6/n-3 PUFA	16.3	16.1	15.6	16.2
Vitamin E (mg/kg)				
$\alpha$ -tocopherol	62.1	143.6	41.0	37.1
$\beta$ + $\gamma$ -tocopherol	19.6	19.1	17.5	16.5
$\delta$ -tocopherol	18.8	18.5	14.9	16.8
Oxidative stability				
MDA ( $\mu$ mol/kg)	2.23	3.04	3.21	3.89
ACW (mmol/kg)	26.8	33.7	50.2	36.8

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; MDA = malondialdehyde; ACW = antioxidant capacity of the water soluble compounds

Color was assessed using Minolta Chromameter and CIE (1976) L\* (lightness), a\* (redness) and b\* (yellowness) color space. Muscle ultimate pH was determined in the central area of *LD*. Two 2.5 cm thick slices of *LD* were taken for the determination of drip loss (EZ method; Christensen, 2003), thawing and cooking loss, shear force and chemical composition. In order to determine thawing loss, the samples were thawed and reweighed. Cooking loss and shear force was determined in *LD* samples cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72°C. Shear force was measured using a TA Plus texture analyzer (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with a 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s. Chemical composition was determined according to Prevolnik et al. (2005) by near-infrared spectral analysis (NIR Systems 6500 Monochromator, Foss NIR System, Silver Spring, MD, USA).

Fatty acid (FA) composition of feed and *LD* samples was determined according to the method of Park and Goins (1994). Concentrations of vitamin E were analysed by Agilent 1260 Infinity HPLC system equipped with a 1260 Infinity Quaternary Pump (Agilent) according to the methodology of Abidi and Mounts (1997) and Rupérez et al. (2001). Concentrations of MDA in *LD* samples were analysed according to the methodology of

Vilà et al. (2002) with slight modifications. An Agilent 1260 Infinity HPLC system equipped with a 1260 Infinity quaternary pump (Agilent) was used. Protein oxidation in myofibril isolates was measured according to the method of Oliver et al. (1987) as modified by Mercier et al. (1998). The protein and carbonyl concentrations were measured spectrophotometrically in the supernatant. The antioxidant capacity of the water soluble compounds (ACW) in *LD* samples was measured by the photo-chemiluminescence method by PhotoChem® (Analytik Jena, Jena, Germany).

Data was analysed using the General Linear Models (GLM) procedure of the SAS/STAT module (SAS 8e, 2000; SAS Inc., Cary, NC, USA) with fixed effects of treatment and sex. Differences between groups were tested using Tukey-Kramer multiple comparison test with significance at  $P < 0.05$ . The results in the tables are presented as least square means (LS-means).

### Results and Discussion

Supplementing pig diets with vitamin E is a conventional way to improve meat quality and oxidative stability (Jensen et al., 1998). However, in response to demand for natural products and consumers' willingness to pay significant premiums for natural foods, meat industry is seeking for natural solutions to minimize oxidative rancidity and increase products' shelf-life (Karre et al., 2013). Lipid oxidation namely leads to the formation of several compounds which affect the quality of meat and meat products (Shah et al., 2014). Due to high protein content in muscle tissues, oxidation of proteins can also be implemented to evaluate the quality of meat (Falowo et al., 2014). In the present study, no differences in performance, carcass and meat quality traits were observed between control group and groups supplemented with vitamin E, SCW extract or hops (Tables 2 and 3). This corroborates with studies where supplementation of vitamin E (Cannon et al., 1996) and SCW extract (Prevolnik et al., 2012) showed no effect on performance, carcass and meat quality of pigs. On the other hand, some studies demonstrated improved performance or carcass and meat quality traits of  $\alpha$ -tocopherol ( $\alpha$ -toc) supplemented diet; i.e. increased dressing percentage (Corino et al., 1999), improved water holding capacity (Guo et al., 2006) and delayed color deterioration (Waylan et al., 2002). Regarding natural extracts, pigs fed a diet containing 1% hops showed an improved gain to feed ratio (Fiesel et al., 2014), while supplementation with 3% of SCW extract resulted in reduced feed intake and daily gain of entire male pigs (Čandek-Potokar et al., 2015).

Table 2. The effect of dietary supplementation on growth performance and carcass traits of fattening pigs

	Supplemented				P-value	RMSE
	Control	VitE	Tannin	Hop		
Initial body weight (kg)	75.2	75.1	75.5	74.2	0.989	9.7
Final body weight (kg)	133.9	131.6	130.4	128.4	0.839	14.5
Average daily gain (kg)	0.851	0.819	0.796	0.786	0.765	0.154
Hot carcass weight (kg)	109.4	106.2	103.9	101.9	0.509	12.02
Lean meat %	62.1	61.3	62.2	61.7	0.947	3.6
Fat thickness (mm)	11.8	12.6	12.1	13.1	0.932	4.9
Muscle thickness (mm)	82.0	79.1	82.9	81.9	0.488	5.2

Table 3. The effect of dietary supplementation on meat quality traits of fattening pigs

	Supplemented				P-value	RMSE
	Control	VitE	Tannin	Hop		
pH 24h	5.37	5.36	5.34	5.37	0.525	0.07
L*	53.5	53.5	52.1	53.6	0.421	2.4
a*	8.5	8.5	9.0	8.6	0.768	1.3
b*	2.1	2.2	2.2	2.3	0.950	0.8
Drip loss 24h (%)	4.1	4.8	3.9	3.3	0.182	1.4
Drip loss 72h (%)	5.9	6.8	6.1	5.7	0.544	1.6
Thawing loss (%)	12.0	12.3	11.8	9.8	0.453	3.7
Cooking loss (%)	31.7	28.7	29.1	29.6	0.221	3.4
Shear force (WBSF; N)	135	128	124	125	0.602	21
Chemical composition						
Water (%)	74.1	72.9	73.7	74.3	0.213	1.34
Protein (%)	22.1	22.0	22.1	22.1	0.581	0.1
Intramuscular fat (%)	3.0	4.3	3.3	2.9	0.278	1.6

WBSF = Warner-Bratzler shear force

Interest in meat FA composition stems mainly from the need to find ways to produce healthier meat, i.e. with a higher ratio of polyunsaturated (PUFA) to saturated FA and a more favorable balance between n-6 and n-3 PUFA. The FA composition of animal products depends on tissue FA biosynthesis and the FA composition of dietary lipids (Mourot and Hermier, 2001). Experimental diets in the present study differed only in terms of supplementation; consequently, no differences in *LD* FA composition and n-6/n-3 PUFA ratio were observed (Table 4). As the concentration of  $\alpha$ -toc was significantly higher in  $\alpha$ -toc supplemented group, changes in the studied markers of oxidative stability were expected, but no effects on the formation of MDA, carbonyl groups, and ACW of *LD* were observed.

Table 4. The effect of dietary supplementation on *LD* muscle fatty acid composition (wt % of fat)

	Control	VitE	Supplemented	
			Tannin	Hop
SFA	36.78	36.06	35.90	36.74
MUFA	43.56	43.82	43.42	43.21
PUFA	19.66	20.13	20.68	20.05
n-3 PUFA	0.99	1.04	1.06	0.99
n-6 PUFA	18.68	19.08	19.62	19.06
n-6/n-3 PUFA	18.93	18.29	18.58	19.30
LC PUFA	5.66	5.73	6.12	6.10
LC n-3 PUFA	0.61	0.68	0.68	0.64
LC n-6 PUFA	5.05	5.05	5.44	5.46
LC n-6/n-3 PUFA	8.23	7.42	7.96	8.48

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; LC = long chain

Table 5. Concentrations of  $\alpha$ -tocopherol, antioxidant capacity of water soluble compounds (ACW), malondialdehyde (MDA) and carbonyl groups in *LD* muscle

	Control	Vit E	Tannin	Hop	P-value	RMSE
$\alpha$ -tocopherol (mg/kg)	2.98 <sup>a</sup>	3.79 <sup>b</sup>	2.75 <sup>a</sup>	2.69 <sup>a</sup>	< 0.05	0.21
ACW ( $\mu$ mol/kg)	42.0	44.5	42.8	42.1	>0.95	0.49
MDA (nmol/g)	0.612	0.633	0.588	0.628	>0.95	0.014
Carbonyl groups ( $\mu$ mol/g protein)	0.82	0.70	0.85	0.83	0.20	0.15

### Conclusion

Supplementing pigs with vitamin E, tannins and hops showed no effect on growth performance and meat quality. Besides, no influence on fatty acid composition, MDA and carbonyl groups concentration and on ACW was found. Supplementing animal feeds with vitamin E increased the vitamin E level in muscle *LD*.

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