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EFFECT OF VITAMIN E IN SWINE DIET ON ANIMAL GROWTH PERFORMANCE AND MEAT QUALITY PARAMETERS

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Lipid oxidation is the primary cause of deterioration in the quality of frozen meat and meat products. Oxidative deterioration of meat lipids during frozen storage can directly affect the colour, flavour, texture, nutritive value, and safety of food. Natural antioxidants reduce lipid oxidation and as a consequence may improve meat quality. In the present study we investigate the effect of three levels of dietary vitamin E on animal growth performance and on meat oxidation. HPLC analyses were performed in order to assess α-tocopherol levels in blood serum and its deposition in muscles. The oxidative stability of muscle was examined over 7 days of refrigeration storage by means of thiobarbituric acid reactive substances (TBARS). We concluded that supplementation with vitamin E augmented α-tocopherol levels in blood serum and muscles from pig samples receiving 300 mg/kg feed. Moreover lipid oxidation in chilled meat was successfully reduced.

INTRODUCTION

The bright red colour in meat is one of the most important quality attributes influencing the consumer's decision to purchase. Meat colour and lipid stability are major factors limiting the quality and acceptability of meat and meat products [Gatellier *et al.,* 2001].

The oxidative stability of muscle depends upon the balance between anti-oxidants, such as α-tocopherol and pro-oxidants including the free iron in the muscle and concentrations of polyunsaturated fatty acids (PUFA) [Yang *et al.,* 2002].

Lipid oxidation in muscle foods is initiated by stressors arising from both internal and external sources. The most important stressors are the reactive oxygen species (ROS) including free radicals and peroxides [Descalzo *et al.,* 2005].

Vitamin E plays a fundamental role in the prevention of radical formation in biological systems like plasma, membranes and tissues. Vitamin E is the collective name for the eight naturally occurring forms α, β, γ and δ tocopherols and α, β, γ and δ tocotrienols. Historically, α-tocopherol was reported to have the highest biological activity [Hewavitharana *et al.,* 2004]. Antioxidant vitamins are essential in the animal diet for normal health [Fiego *et al.,* 2004]. Supplementation of the swine diet with vitamin E during the growing and finishing periods may improve pork quality. Vitamin E is a potent antioxidant and has been demonstrated to decrease lipid oxidation, decrease drip loss, and improve the colour of pork. Vitamin E supplementation also has been shown to improve average daily gain and feed efficiency of pigs [Cannon *et al.,* 1996].

This trial was carried out to investigate the effects of three different dietary levels of vitamin E on its deposition in the muscles and to determine whether these dietary supplementations affect the oxidative stability of muscular lipids and various meat quality characteristics.

MATERIALS AND METHODS

Animals and diets. Many factors affect pork quality, including genetics, preslaughter handling of the pig, and postslaughter handling of the carcass. An accumulating body of evidence shows that various nutritional factors including vitamin E affect pork quality [Morrissey *et al*., 1996; Monahan *et al*., 1992; Cannon *et al*., 1996; O'Sullivan *et al*., 1997; Asghar *et al*., 1991].

The levels of vitamin E in the feed formulation used until now were at the level of nutritional requirements. Nowadays the level of vitamin E is not related only to the aimed productivity performances but also to the quality parameters intended to be attained.

Composition of nutrient levels. The biological material was represented by a number of 86 pigs of Large White breed of average weight 52 kg divided into three groups: two of 29 and one of 28 pigs. The groups were randomly assigned a growing-finishing diet (Table 1) supplemented with the following levels of all-*rac*-α-tocopheryl acetate: 11 (C = control), 100 (E1), 300 (E2) mg/kg of diet. Feed formulation was achieved by means of BRILL computerized program, taking into account the chemical composition of the raw materials, which was determined by laboratory tests. Mixed feed quality parameters represent the mean values of the three samples from three batch formulation processes; they were similar

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TABLE 1. Experimental design.

Specification	Group		
	C	E1	E2
Number of tested animals	29	28	29
Period (days) of feeding	61	61	61
Vitamin E in feed (mg/kg)	11.0	100.0	300.0

TABLE 2. Chemical composition of the mixed feed.

Ingredients	Content (%)
Maize	68.79
Full fat soy	9.00
Sunflower meal	6.00
Soybean meal (44%)	10.00
Choline mix	0.10
Lysine	0.25
Bone meal	4.46
Salt	0.40
Trace mineral mix and vitamin mix*	1.00
Quality Parameters – calculated value	
Crude protein (%)	16.96
Net Energy (EM) (Kcal /kg)	3 227.0
Lysine (%)	0.91
Methionine + Cysteine (%)	0.58
Lipids (%)	5.0
Crude fiber (%)	4.31
Calcium (%)	1.03
Phosphorus (%)	0.85
Quality parameters – as analyzed **	
Crude protein (%)	16.50
Crude fat (%)	4.91
Crude fiber (%)	4.48
Calcium (%)	1.21
Phosphorus (%)	0.74

* Trace mineral mix and vitamin mix had content in vitamin E as follows: 1100 UI group C; 10 000 UI group E1 and 30 000 UI; **The results represent the mean values of the laboratory analysis of the samples from three batch formulation processes for the 3 experimental groups.

with one exception, the concentration of vitamin E. The gross and chemical composition of the diet is provided in Table 2.

The main fat source in the mixed feed is represented by maize and full fat soy. In the feed formulation 5 g/100 g feed represents the crude fat. Out of the crude fat 2.85 g/100 g feed is the amount of fat brought by corn and 1.67 g/100 g feed comes from full fat soy.

During the 61 days of experiment the feed daily intake was monitored and animals were individually weighed at the beginning of the biological testing, after 30 days, respectively after 61 days from the day 1 of the experiment.

Sample collections. Analyses were performed at the beginning of the experiment on the animals selected from the C, E1 and E2 groups, for plasma α-tocopherol, fatty acids and cholesterol. Blood samples were taken from eighteen randomly selected animals for the three groups (six from each group) at the beginning of the study, at 30 days, and at slaughter.

Blood samples were withdrawn into vacuum tubes and analyzed for α-tocopherol, cholesterol, and fatty acid profile.

At the end of the first experimental period three animals from each group were slaughtered and samples of liver, loin and thigh, *Longissimus dorsi* and fat were taken.

Sub-samples were used to measure TBARS content at days 1, 3, and 7 *post mortem* (*p.m.*). All LD samples were subjected to triplicate analyses and were kept wrapped with PVC film and stored in the dark at 4°C until 7 days *p.m*.

Lipid oxidation measurement. Lipid oxidation was assessed according to Fiego *et al.* [2004]. A sample of 10 g of muscle was homogenized, 30 s at high speed, with 25 mL of 20% trichloroacetic acid (TCA) and 20 mL of distilled water. After centrifugation of the homogenate (1000 g for 20 min at 4°C), the supernatant was filtered through Whatman #1 filter paper. Two milliliters of filtrate were combined with 2 mL of 0.02 mol/L aqueous 2-thiobarbituric acid solution (TBA), heated in boiling water bath for 20 min together with a blank containing 2 mL of a TCA/water mix (1/1) and 2 mL TBA reagent and subsequently cooled in running tap water. The absorbance of the resulting solution was measured at 532 nm with a Helios Alpha spectrophotometer (Unicam - Thermo Electron) and the results were expressed as absorbance values.

pH. Measurements of pH were carried out with an Inolab 720 pH-meter, equipped with a Sen Tix Sp Spear type pH electrode.

HPLC determination of vitamin E in serum. The determination of liposoluble vitamins in foods usually requires saponification of the fatty material and extraction of the vitamins from the unsaponified purified residue prior to chromatographic separation and determination [Delgado--Zamarreno *et al*., 2001]

The method used in this experiment is based on the saponification with potassium hydroxide in ethanol and extraction of vitamin E in petrol ether by evaporation followed by dissolving of the residue in methanol. The extract was then injected in the HPLC (Perkin-Elmer detector UV/VIS Series 200, Pomp 200 Series and Degasser Series 200, chromatographic column Perkin Elmer – SPHERI-5 RP--18 5 μm, 250 × 4.6 mm). The mobile phase consisted of a mixture of 95:5 methanol-water. A sample is weighed with a precision of 0.0001 g in the saponification flask, and then the KOH solution is added. The saponification takes place on a water bath for 30 min, after that the solution is slowly cooled down, distilled water is added, and is quantitatively placed on a separation funnel. Sample extract (20 μL) is injected into the HPLC and the peak area of DL-α-tocopherol is measured. The average of the three successive injections is calculated and then related to the average area of the DL--α-tocopherol standard peak.

HPLC determination of vitamin E in LD muscle. α-Tocopherol concentration was determined using the fol-

lowing procedure: 1-g sample was mixed with 0.25 g of ascorbic acid and 7.3 mL of saturated KOH. The mix was then heated at 80°C for 15 min and saponified in a water bath. The mixture was cooled on ice, then 4 mL isooctane was added and vortexed for 2 min. The separation took place for 1 min, then 2 mL of the isooctane phase is taken and evaporated in a rotary evaporator with vacuum pomp. Methanol (0.50 mL) was added to the evaporated mixture. High performance liquid chromatography was used to quantify α-tocopherol concentration using a C8 column. The mobile phase was 45% methanol 45% acetonitrile and 10% water. Peaks were detected by UV. Samples were compared with vitamin E standards.

Longissimus muscle samples for proximate analysis were prepared by homogenizing closely trimmed muscle samples in a blender.

Fatty acids profile analysis with GS. The fatty acids are transformed in methyl esters of fatty acids, followed by the separation of the components on the chromatographic column, identification by comparison with the standards and their quantitative analysis.

The gas chromatograph consisted of a FID detector using a separation column filled with 10% FFAP on Chromosorb W/HP.

After a fat sample is weighed into a conical glass, 2% H_2SO_4 in methanol is added and is boiled in a reflux water bath for 25–30 min. After cooling down, the sample is transferred into a separation funnel, distilled water is added to wash the saponification glassware and hexane, then it is thoroughly mixed.

The aqueous layer is placed into another separation funnel and then again hexane is added, vigorously agitated and the organic layer (with hexane) is transferred again in the first separation funnel. Subsequently the hexane layer is washed with distilled water. The organic phase is dried on anhydrous Na_2SO_4 into a rotary evaporator and is concentrated, and after the residue is recovered with hexane.

The methylated esters solution of fatty acids (1–2 μL) is injected into the gas chromatograph. The fatty acid content in the sample is calculated and the sample area is related to the standard area taking into account the dilution factor.

RESULTS AND DISCUSSION

Growth performance

The priority at the beginning of the experiment was to create homogeneous groups as far as weight was concerned. Therefore the average initial weight was 52 kg (Table 3). At the end of the first period, after 30 days, a significant performance in weight gain was observed in group E2 (feed 300 UI vitamin E). The average weight in this group was 78.66 kg compared with 75.17 kg average weight of the control group.

TABLE 3. Body weight evolution and the daily growth rate (DGR).

Parameter	Group		
	C	E1	E2
Initial body weight (Kg)	52.10	52.70	52.69
Body weight at the end of the first experimental period (Kg)	75.17	76.90	78.66
Final body weight (Kg)	97.00	98.14	100.86
DGR period I (Kg)	0.769	0.807	0.866
DGR period II (Kg)	0.704	0.685	0.716
DGR per total (Kg)	0.736	0.745	0.790

The final average weight, at day 61, was not significantly different in the three groups: 97.0 kg control group, 98.14 kg E1 and 100, 86 kg E2 group ($p>0.05$).

As far as the daily growth rate is concerned, it was higher in the first period for group E2 compared with the control group, but not significantly different from group E1.

In the second period, the daily growth rates were smaller compared with the first period, but within the genetic potential of the breed, without significant differences among the three groups ($p>0.05$).

Throughout the duration of the experiment an increase was observed in the daily growth rate that was proportional to vitamin E content of the feed.

α-Tocopherol content of blood serum

Mean serum α-tocopherol concentration did not change significantly in the control group, but increased significantly in each of the other groups during the first 4 weeks ($p<0.05$) (Table 4).

Serum from swine supplemented with 300 UI vitamin E (E2) had the highest mean concentration of α-tocopherol throughout the first 4 weeks of the experiment, followed by the 200 UI fed swine (E1).

Blood levels of α-tocopherol were 2.7 to 3.5 times higher in pigs fed on the supplemented diet than in those on the basal diet. Similar results were reported by Asghar *et al*. [1991] and Buckley *et al*. [1995].

α-Tocopherol concentrations of muscle

Table 5 presents the α-tocopherol concentrations (μg/g) in fresh LD samples during refrigeration storage. Prolonged dietary supplementation seems necessary to allow α-tocopherol incorporation into muscle subcellular compartments [Buckley *et al*., 1995]. Jensen *et al*. [1988], Lanari *et al*. [1995], and Hoppe *et al*. [1993] reported muscle vitamin E levels in the range of 7.5 to 8.24 mg/kg in growing pigs sup-

TABLE 4. α-Tocopherol content of blood serum.

	C	E1	E2	Significance	
	α-Tocopherol content of blood serum (μg/μL)			M/ E1	M/ E2
Initial	0.66±0.164	0.66±0.164	0.66±0.164		
First period	0.091±0.012	0.164±0.064	0.184±0.064	p=0.01	p=0.05
Second period	0.072±0.028	0.259±0.134	0.2±0.115	p=0.05	p=0.05

TABLE 5. α-Tocopherol concentrations in LD muscle.

Group	α-Tocopherol concentrations (μg/g) 1st day post slaughter	α-Tocopherol concentrations (μg/g) 3rd day post slaughter	α-Tocopherol concentrations (μg/g) 7th day post slaughter
C	3.254	3.1664	2.338
E1	5.164	4.1738	3.618
E2	6.096	5.527	4.125

plemented with vitamin E (160 to 405 mg/kg feed) for 105 to 137 days. In the present study, the level of vitamin E in the LD muscle of pigs supplemented with 300 mg/kg of all-*rac*-a-tocopheryl acetate after slaughter reached 6.09 mg/kg (pigs were supplemented with vitamin E for 61 days). The mean ɑ-tocopherol level in muscle was 1.87-fold higher at group E2 compared with the control group in the first day post slaughter.

Blood cholesterol level

The level of blood cholesterol in different growing periods was assessed and it was observed that the cholesterol values were significantly lower in group E2 (animals fed with 300 mg vitamin E / kg feed). This fact can be explained by the antioxidant properties of α-tocopherol exerted on the polyunsaturated fatty acids from the feed formulation. Thus the potential to reduce blood cholesterol concentrations in animals involves the intake of unsaturated fatty acids matched by a higher dietary intake of vitamin E (Figure 1).

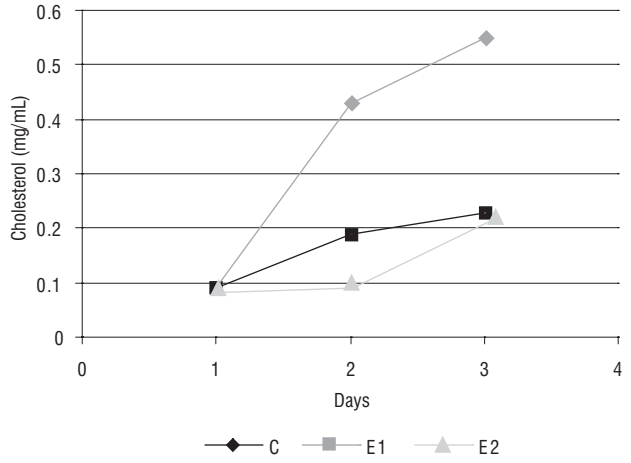

FIGURE 1. Blood cholesterol levels.

Fatty acid profile

Major changes in individual fatty acids were found for linolenic (18:3) acid (Table 6) for LD muscles with differences among the three groups. In the present study the level of linolenic acid and oleic acid in skeletal muscle was higher in group E2 compared to the other two groups C and E1. Highly unsaturated fatty acids represent only a small percentage of the total lipid but small differences in their contents can account for quite large effects in terms of oxidation.

Monahan *et al*. [1992] observed that skeletal muscle from pigs fed a soybean oil diet along with vitamin E had significantly higher C18:2/C18:1 ratios in the neutral and polar lipid fractions of muscle tissue and the total fraction of adipose tissue.

TABLE 6. Fatty acid (FA) profile (g in 100 g total fat).

	C (FA g in 100 g total fat)	E1 (FA g in 100 g total fat)	E2 (FA g in 100 g total fat)
		Thigh	
Miristic acid	1.23	1.43	1.09
Miristoleic acid	0.12	-	0.11
Palmitic acid	23.64	24.72	19.76
Palmitoleic acid	1.13	1.98	1.25
Stearic acid	46.85	45.54	55.80
Oleic acid	18.53	24.09	15.17
Linoleic acid	2.84	0.85	4.47
Linolenic acid	5.66	1.39	2.35
		Loin	
Miristic acid	1.46	1.68	1.79
Miristoleic acid	0.14	0.15	-
Palmitic acid	26.68	24.46	23.06
Palmitoleic acid	0.70	2.23	2.26
Stearic acid	50.14	44.31	45.32
Oleic acid	18.02	24.91	24.21
Linoleic acid	1.83	0.94	2.04
Linolenic acid	1.03	1.32	1.32
		Muscle LD	
Miristic acid	1.48	1.52	0.24
Miristoleic acid	0.11	-	-
Palmitic acid	26.68	25.37	13.11
Palmitoleic acid	2.66	0.33	1.03
Stearic acid	52.61	49.80	44.61
Oleic acid	14.44	14.74	23.79
Linoleic acid	0.29	2.62	0.39
Linolenic acid	1.73	5.62	16.83
		Liver	
Miristic acid	0.30	0.26	1.73
Miristoleic acid	-	-	0.16
Palmitic acid	16.31	17.30	26.66
Palmitoleic acid	1.38	1.51	1.56
Stearic acid	39.22	35.49	52.74
Oleic acid	20.14	29.26	13.39
Linoleic acid	2.40	0.57	2.08
Linolenic acid	20.25	15.61	1.68

Oxidation stability

The absorbance of the resulting solution, prepared as described in the material and methods section, was measured at 532 nm with a Helios Alpha spectrophotometer and the results were expressed as absorbance values (Figure 2).

The oxidative stability of LD lipids during storage was favourably affected by dietary vitamin E supplementation. Thus, TBARS development was reduced in the muscle of the

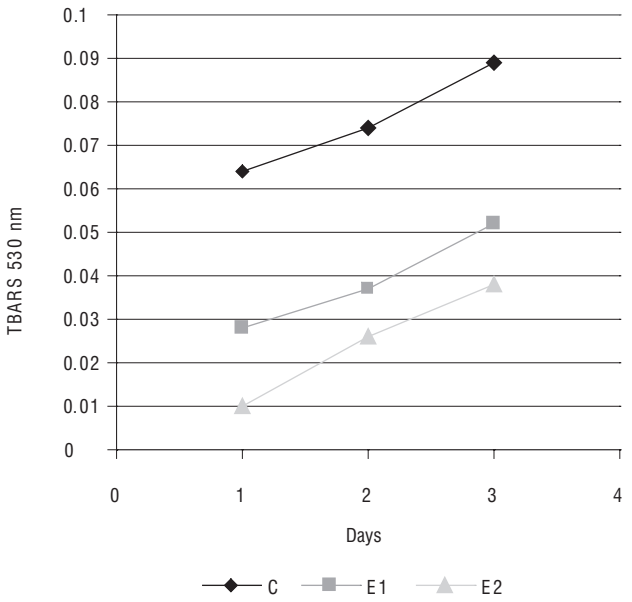

FIGURE 2: Lipid oxidation in *Longissimus dorsi* (mean TBARS values expressed as absorbance at 532 nm).

E2 group compared to C; at day 5 the antioxidant effect was considerable for muscle samples of both E1 and E2 groups.

Lipid oxidation in refrigerated meat was successfully reduced in meat from pigs fed a diet supplemented with 300 mg of α-tocopherol per kilogram of feed.

The extent of lipid oxidation in meat was dependent on the α-tocopherol concentration in the tissue, this findings being in accordance with other experiments [Buckley *et al*., 1995; Monahan *et al*., 1992] which showed that dietary supplementation (up to 200 mg of α-tocopheryl acetate/kg of feed) significantly improved the oxidative stability of pork muscle during refrigeration storage.

CONCLUSIONS

High levels of vitamin E supplementation (300 mg/kg) in the last 60 days of heavy pig finishing increased α-tocopherol levels in tissues and reduced the production of thiobarbituric acid reactive substances (TBARS). This may improve the organoleptic qualities of the meat and subsequently of the meat products. The TBARS reduction is attributable to increased vitamin E levels in muscle.

The administration of feed with high levels of vitamin E in the swine diet had direct implications on the fatty acids profile with regard to the polyunsaturated fatty acids distribution in different anatomic parts, being significantly higher in muscles of groups E1 and E2 compared with the control group.

An interesting fact noticed was the decrease in the level of blood cholesterol in different growing periods. The cholesterol values were significantly lower in group E2 (animals fed with 300 mg vitamin E / kg feed), which was attributed to the level of vitamin E protecting the unsaturated fatty acids against oxidation.

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