Acta vet. scand. 1983, *24*, 259—268.

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EFFECT OF DIETARY AND INJECTABLE VITAMIN E ON THE ANTIOXIDANT STATUS OF PIGS

By

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JENSEN, P. THODE, V. DANIELSEN, H. E. NIELSEN and T. LETH: *Effect of dietary and injectable vitamin E on the antioxidant status of pigs.* Acta vet. scand. 1983, *24*, 259—268. — An experiment concerning 6 different vitamin E treatments was conducted with 30 young pigs. From 4 to 15 weeks of age the pigs were kept in individual pens and fed a selenium supplemented basal diet consisting mostly of propionic acid treated barley and soybean meal, and containing 4.4 mg vitamin E per kg. The treatments were periods with or without vitamin E supplement (20 mg/kg) or a vitamin E injection (200 mg). Blood samples collected during the period of investigation were examined for vitamin E and for resistance against erythrocyte lipid peroxidation (ELP) in order to evaluate the antioxidant status.

Analysis of variance showed a litter effect on ELP values at all the weekly investigations and a treatment effect from two weeks after the experiment had started. Also the blood vitamin E level was litter dependent and influenced by treatment. Paired comparisons by Student's t-test showed a delay of 1 to 2 weeks in the effect on ELP of a dietary vitamin E supplement. In contrast, both ELP and vitamin E changed very rapidly (hours) after vitamin E injections.

Independently of the vitamin E treatments there was a rise in ELP within the first 2 or 3 weeks after weaning; this was taken as an index of a reduced antioxidant status during that period.

Vitamin E; antioxidant status; erythrocyte lipid peroxidation; pigs.

In Denmark supplementation of pig feed with 0.1 mg inorganic selenium per kg seems to prevent deaths from dietetic hepatosis, while deaths from mulberry heart disease (MHD) still occur (*Pedersen & Simesen* 1977). This and other observations have provided evidence to suggest that MHD is chiefly associated with vitamin E deficiency (*Van Vleet et al.* 1975, *Hakkarainen et al.* 1978, *Moir & Master* 1979, *Simesen et al.* 1982). Deaths

occur principally during the first few weeks after weaning, involving the largest pigs in the litter.

Increasing the feed supplement of vitamin E may improve the status of the young pigs. Intramuscular injection of vitamin E may perhaps be more efficient, since most of the deaths occur in the period after weaning when the feed intake is relatively low. The present experiment was designed to evaluate the efficacy of, respectively, oral and parenteral vitamin E supplementation to vitamin E deficient pigs. At the same time the antioxidant status of pigs during a period of vitamin E deprivation was investigated.

MATERIALS AND METHODS

The experimental animals were piglets born to sows fed a diet based on barley and soybean meal with vitamins and minerals, and with a supplemental content of Se (0.1 mg per kg as sodium selenite) and vitamin E (20 mg per kg as dl-α-tocopheryl acetate).

The piglets were weaned at 4 weeks of age, 3 days after castration of the males. At weaning, 6 equally sized piglets from each of 5 litters (blocks) were randomly assigned to 6 different vitamin E treatments (I—VI). The pigs were kept in single pens and fed ad libitum during the experimental period of 11 weeks. The pigs were provided access to a basal diet formulated to contain 23 % protein, and comprised of barley (51.1 %), soybean meal (20 %), dried skim milk (6 %), fish meal (6 %), linseed (3 %), dried yeast (3 %), sugar (3 %), animal fat (5 %), mineral-trace-mineral-vitamin mixture without vitamin E (2.9 %). The barley used had a moisture content of approximately 20 %. After treatment with 1 % propionic acid it was stored for at least 8 weeks before it was used. The purpose of this treatment was to reduce the vitamin E content. The diet was supplemented with 0.1 mg Se per kg (as sodium selenite) and had a total vitamin E content of 4.4 mg per kg.

Pigs in treatment groups I and II received the basal diet without any supplements; 11 weeks old the pigs in treatment group II were injected intramuscularly (IM) with 200 mg α-tocopheryl acetate in a micellar aqueous dispersion (IDO-E®vet., Ferrosan, Denmark). The pigs in treatment groups III to VI received the same diet, supplemented with 20 mg α-tocopherol per kg in weeks Nos. 12 and 13 (Treatment III), weeks Nos. 5, 6, 13, 14 and 15 (Treatment IV), weeks Nos. 5, 6, 7, 8, 13, 14 and 15

Table 1. Experimental design. O: Basal diet; E: Dietary vitamin E supplement; E-inj.: Vitamin E injection.

Treatment No.	Age in weeks 4	5	6	7	8	9	10	11	12	13	14	15
I	O	O	O	O	O	O	O	O	O	O	O	
II	O	O	O	O	O	O	O	E-inj. ↓ O	O	O	O	
III	O	O	O	O	O	O	O	E	E	O	O	
IV	E	E	O	O	O	O	O	O	E	E	E	
V	E	E	E	E	O	O	O	O	E	E	E	
VI	E	E	E	E	E	E	E	E	E	E	E	

(Treatment V), and throughout the experimental period (Treatment VI). The experimental design is outlined in Table 1.

The vitamin E supplemented diet was found on analysis to contain 27.3 mg vitamin E per kg. Heparinized blood samples were collected from the anterior vena cava once a week and as required. The blood cells were tested for resistance against lipid peroxidation (ELP) as described previously (*Jensen et al.* 1979). The method used is based on the reaction of 2-thiobarbituric acid (TBA) with malonylaldehyde produced in vitro by peroxidation with hydrogen peroxide. Blood vitamin E was determined on plasma samples when the pigs were 11 or 12 weeks old and at 15 weeks, and, for treatment groups IV and V, 2 days after the termination of the first supplemental period. Furthermore, at the beginning of the experiment plasma vitamin E was determined on pools each including plasma from 3 litter mates. Vitamin E determination was made by high pressure liquid chromatography of the unsaponifiable matter on a column of kieselgel, and measuring of the fluorescense of the eluate at 327 nm upon excitation at 290 nm. Plasma for vitamin E determination was kept at —20°C until analysed.

Statistical analyses were made in accordance with the Statistical Analysis System described by *Helwig & Council* (1979). The analyses included t-test on least squares means and analysis of variance, both performed by the general linear model (GLM) procedure with block (litter) and treatment as independent variables.

RESULTS

No deaths occurred among the experimental animals. The analysis of variance showed a significant litter (block) effect on ELP values at all of the weekly investigations ($P < 0.05$) and a treatment effect from week No. 7 until week No. 15 ($P < 0.05$). Also the blood vitamin E level seemed to be litter dependent as is seen from Table 2 which shows the plasma vitamin E level for each litter at the beginning of the experiment.

Table 2. Tocopherol levels in plasma pools, each including three 4-week-old pigs (μg α-tocopherol/g plasma).

Item	Block (litter)				
	1	2	3	4	5
Pool A	0.89	1.11	2.26	1.13	1.09
Pool B	0.37	0.96	2.19	1.28	2.23

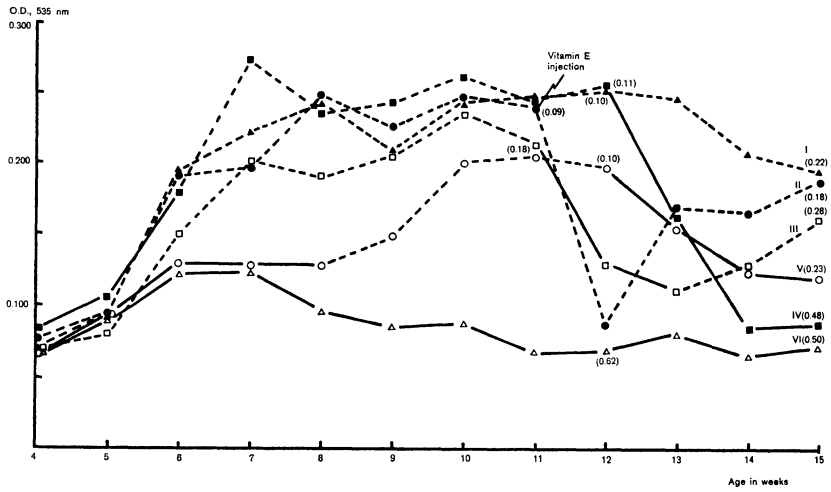

Figure 1. Weekly ELP mean values for the different treatment groups (I—VI). Figures represent mean plasma vitamin E levels (μg/g). - - - - - - - Basal diet without vitamin E; ———— Vitamin E supplemented diet.

Table 3. Mean values of measured variables (s.e.m. in brackets) for the different treatments. For details see: Materials and Methods.

Measured variables	Treatments					
	I	II	III	IV	V	VI
ELP, O.D. 535 nm, 4 weeks	0.070 (0.005)	0.077 (0.006)	0.070 (0.003)	0.082 (0.014)	0.067 (0.007)	0.065 (0.006)
„ „ „ , 5 weeks	0.091^{ab} (0.024)	0.092^{ab} (0.025)	0.079^{a} (0.022)	0.106^{b} (0.032)	0.094^{ab} (0.030)	0.087^{ab} (0.014)
„ „ „ , 6 weeks	0.192^{a} (0.045)	0.190^{a} (0.045)	0.148^{ab} (0.051)	0.179^{ab} (0.040)	0.130^{b} (0.045)	0.121^{b} (0.016)
„ „ „ , 7 weeks	0.220^{a} (0.055)	0.196^{ab} (0.040)	0.198^{ab} (0.046)	0.272^{a} (0.041)	0.127^{b} (0.036)	0.123^{b} (0.025)
„ „ „ , 8 weeks	0.240^{a} (0.035)	0.246^{a} (0.033)	0.189^{ab} (0.043)	0.234^{a} (0.026)	0.126^{b} (0.050)	0.095^{bc} (0.011)
„ „ „ , 9 weeks	0.208^{a} (0.032)	0.224^{a} (0.026)	0.205^{a} (0.022)	0.241^{a} (0.029)	0.147^{b} (0.028)	0.084^{c} (0.007)
„ „ „ , 10 weeks	0.242 (0.036)	0.246 (0.041)	0.234 (0.061)	0.260 (0.028)	0.199 (0.044)	0.086^{a} (0.007)
„ „ „ , 11 weeks	0.244 (0.029)	0.239 (0.024)	0.212 (0.053)	0.243 (0.037)	0.204 (0.035)	0.067^{a} (0.005)
„ „ „ , 12 weeks	0.250^{a} (0.015)	0.086^{bd} (0.007)	0.129^{b} (0.038)	0.252^{a} (0.013)	0.197^{c} (0.025)	0.067^{d} (0.004)
„ „ „ , 13 weeks	0.244^{a} (0.041)	0.168^{ab} (0.062)	0.109^{b} (0.047)	0.160^{ab} (0.035)	0.153^{b} (0.051)	0.079^{b} (0.004)
„ „ „ , 14 weeks	0.206^{a} (0.042)	0.163^{ac} (0.041)	0.127^{bc} (0.038)	0.083^{b} (0.012)	0.121^{bc} (0.043)	0.064^{b} (0.004)
„ „ „ , 15 weeks	0.192^{a} (0.024)	0.186^{ac} (0.042)	0.159^{ac} (0.048)	0.086^{b} (0.015)	0.119^{bc} (0.035)	0.070^{b} (0.006)
Blood vit. E, μg/ml, 11 weeks		0.09 (0.02)	0.18 (0.03)			
„ „ „ , 12 weeks				0.10 (0.03)	0.11 (0.05)	0.62 (0.09)
„ „ „ , 15 weeks	0.22 (0.08)	0.18 (0.07)	0.28 (0.06)	0.23 (0.08)	0.49 (0.18)	0.50 (0.13)

^{a,b,c,d} Means with different superscript within row differ significantly ($P < 0.05$).

The weekly ELP mean values, as related to age and kind of treatment, are shown in Fig. 1; and in Table 3 means and standard errors of both ELP and plasma vitamin E values are given with reference to the different treatments. Results of comparisons between mean values are also given in this table (t-test on least squares means).

During the first 2 weeks the ELP values increased (meaning reduced resistance against peroxidation) and there was no real difference in mean values between the treatment groups. From the pigs were 7 weeks old there was a significant difference in ELP values between treatments (Table 3) with a high level for the unsupplemented groups and a low level for Group VI, which was given vitamin E supplement through the whole experimental period. From the 11th to the 15th week the ELP values changed for treatment groups II to V owing to the vitamin E injection (II) and the vitamin E supplements introduced in this period (III—V). It appears that with an oral vitamin E supplement it took about 2 weeks for the total effect on ELP values to materialize (Treatments III, IV and V). The results of vitamin E injection (Treatment II) as compared to oral vitamin E treatment with the same total amount of vitamin E (III) are shown in details in Fig. 2. As seen from the figure, ELP and plasma vitamin E changed much more rapidly after the injection than after the oral treatment, though with a reduction of the effect already 1 or 2 weeks after the injection, i.e., before the appearance of the total effect of the oral treatment.

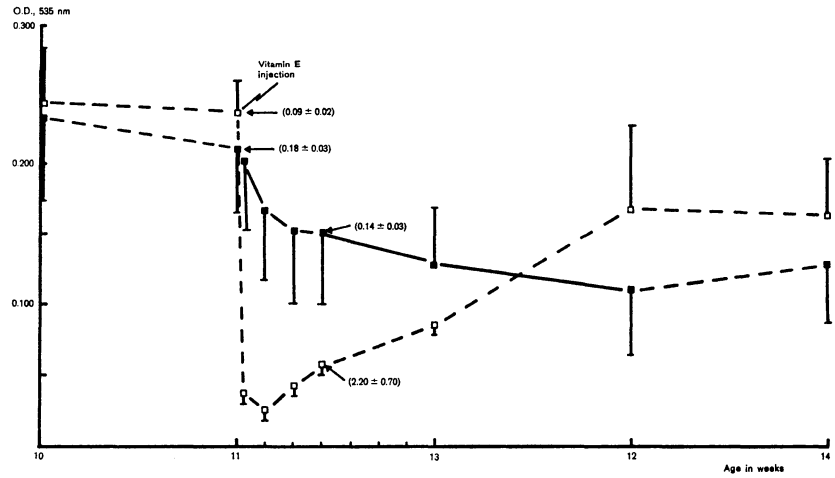

Figure 2. Vitamin E injection (II) compared to oral vitamin E treatment (III). ELP mean values with s.e.m. indicated by vertical bars. Figures represent mean plasma vitamin E values (μg/g) ± s.e.m. ------- Basal diet without vitamin E; ——— Vitamin E supplemented diet.

The plasma vitamin E levels found at 11 or 12 weeks and at 15 weeks are consistent with the ELP values, considering the variations recorded.

DISCUSSION

The resistance of the blood cells against peroxidation (ELP) and the blood vitamin E level has previously been found highly correlated in young pigs, and both variables seem to be reliable indices of the antioxidant status of the pigs (*Fontaine & Valli* 1977, *Simesen et al.* 1982, *Jensen et al.* 1983). Also during this investigation there was a remarkable coincidence between ELP and blood vitamin E both of which were found mainly to be determined by the supply of vitamin E to the pigs. Except within the first 2 weeks after weaning, there seems to be a delay of 1 or 2 weeks in the change in ELP, and presumably also in blood vitamin E, which follows a changed vitamin E content in the feed. When using injection therapy instead of a dietary supplement of vitamin E to deficient pigs, this delay is very short, i.e., a few hours (Fig. 2). Injection therapy is therefore recommendable as first aid in cases of sudden deaths from mulberry heart disease among young pigs. At the same time it will be advisable to give a dietary supplement of vitamin E, since the effect of the vitamin E injection on the ELP and on the blood vitamin E level will be sustained for only a few days or, at most for 1 or 2 weeks (Fig. 2). This is the same as found by *Mahan & Moxon* (1980) and by *Van Vleet* (1982).

The exchange of vitamin E between blood and tissue is relatively unexplored in pigs, but in other mammals and in chickens the exchange rate is known to depend on the character of the vitamin preparation and on the organs, generally with a slow vitamin E uptake in muscle and heart tissue (cf. *Gallo-Torres* 1980). Assuming a similar variation in pigs, the antioxidant status measured on blood samples will not reflect the antioxidant status of all tissues till after a period with a constant vitamin E supply.

The blood levels of vitamin E and ELP recorded at the beginning of this experiment were lower, respectively higher, than in comparable groups of pigs in a previous experiment (*Jensen et al.* 1983). A main cause of this may be different feed qualities, in that the quality of the dietary fat was found in the previous

investigation to influence the antioxidant status of the pigs significantly.

As in the previous experiment (*Jensen et al.* 1983) there was, during the first 3 weeks after weaning, an increase in the mean ELP values in all treatment groups, independently of the vitamin E supply. The rise in the ELP values corresponds to the post weaning decline in blood vitamin E found by *Simesen et al.* (1979) and by *Mahan & Moxon* (1980) when using, respectively, a barley and soybean based diet containing 45 mg α-tocopherol per kg, and a corn-soybean-oat based diet containing 39 mg α-tocopherol per kg. The initial plasma vitamin E levels were the same (1—3 μg/ml) in all these investigations. In an experimental study, using a semisynthetic diet for pigs with a low initial mean plasma α-tocopherol level (0.5 μg per ml). *Van Vleet* (1982) found a persistently low plasma α-tocopherol level during the first 4 weeks post weaning, even in pigs fed 33 mg α-tocopherol per kg feed.

The decline in blood vitamin E and the rise in ELP values even in well nursed, vitamin E supplied pigs may help to explain the occurrence of vitamin E-Se deficiency during the post weaning period. The change in feed composition caused by weaning, together with a relatively low post-weaning feed intake, may possibly explain the low post-weaning antioxidant status in pigs.

A high vitamin E content in the weaning diet and/or intramuscular vitamin E injection shortly after weaning may perhaps by ways to avoid deficiency problems in this high-risk period.

ACKNOWLEDGEMENTS

The experiment was carried out at the "Sjælland III" Experimental Station, Roskilde, belonging to the Danish bacon Factories.

Thanks are due to Mr. N. Kjeldsen and Mr. A Rask, National Institute of Animal Science, Copenhagen, for valuable help during the experimental work.

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SAMMENDRAG

Virkningen af vitamin E som fodertilskud, henholdsvis injektion, på den biologiske antioxydantstatus hos svin.

Ialt 30 grise blev i perioden fra de blev fravænnet 4 uger gamle og til de var 15 uger gamle holdt i enkeltstier og fodret med et selenberiget grundfoder hovedsagelig bestående af propionsyrebehandlet byg og soyaskrå og indeholdende 4,4 mg α-tocopherol pr. kg. Der blev anvendt 6 forsøgsbehandlinger i form af vitamin-E injektion eller forskellige perioder med et vitamin-E tilskud på 20 mg α-tocopherol-

acetat pr. kg foder. Blodprøver udtaget 2 gange i forsøgsperioden blev undersøgt for vitamin-E indhold i plasma, ligesom blodlegemernes resistens mod peroksydering (ELP) blev bestemt ugentlig for herved at vurdere grisenes antioxydantstatus.

Ved variansanalyse fandtes en konstant kuldeffekt på ELP-værdierne, ligesom disse var afhængige af forsøgsbehandlingerne fra 2 uger efter forsøgets start og forsøgsperioden ud. Også blodets vitamin-E indhold var influeret af kuld og forsøgsbehandling. Ved parrede sammenligninger (t-test) fandtes ELP først at ændre sig signifikant 1—2 uger efter at et vitamin-E-tilskud til foderet var påbegyndt. Når vitamin-E blev givet som injektionsbehandling, skete ELP-ændringerne i løbet af få timer.

Uafhængig af om grisene fik vitamin-E-tilskud eller ej fandtes der en nedsat resistens mod peroksydering (forhøjede ELP værdier) i de første 2 til 3 uger efter forsøgets start (fravænningsperioden) som tegn på en nedsat antioxydantstatus i denne periode.

(Received July 25, 1983).

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