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GLUTATHIONE PEROXIDASE ACTIVITY AND ERYTHROCYTE LIPID PEROXIDATION AS INDICES OF SELENIUM AND VITAMIN E STATUS IN YOUNG PIGS*

By

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THODE JENSEN, P., V. DANIELSEN and H. E. NIELSEN: *Glutathione peroxidase activity and erythrocyte lipid peroxidation as indices of selenium and vitamin E status in young pigs.* Acta vet. scand. 1979, *20*, 92—101. — A randomized, blocked 2^3 factorial experiment was conducted with 48 pigs from sows fed a diet low in selenium and vitamin E. From 3 to 12 weeks of age the piglets were kept in single pens and fed a basic diet consisting mostly of barley, dried skim milk, soybean meal and dried yeast, and containing 55 μg selenium and 3 mg vitamin E per kg. The treatment factors — i.e. feed supplements — were 2 levels of Se (nil, 60 μg/kg), 2 levels of vitamin E (nil, 50 mg/kg), and 2 levels of the feed antioxidant ethoxyquin (nil, 150 mg/kg). Blood samples, collected at termination of the experiment, were examined for glutathione peroxidase activity (GSH-Px) and resistance against erythrocyte lipid peroxidation (ELP) to evaluate Se and vitamin E status, respectively. Analysis of variance showed the GSH-Px activity to be litter-dependent ($P < 0.001$) and influenced by selenium supplementation ($P < 0.001$) but not by the other supplements or by interactions between supplements. Resistance against ELP was influenced only by vitamin E supplementation ($P < 0.001$). GSH-Px and ELP thus seem to be valuable and simple methods for evaluating, respectively, Se status and vitamin E status in growing pigs.

glutathione peroxidase; erythrocyte lipid peroxidation; selenium; vitamin E; mulberry heart disease; dietetic hepatosis; nutritional muscular dystrophy; pigs.

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Selenium-vitamin E deficiency is the cause of several specific pathological conditions in domestic animals, viz. different myopathic conditions in calves, pigs, lambs and foals, hepatosis in pigs, and exudative diathesis and encephalomalacia in chickens. Also non-specific selenium or vitamin E responsive conditions have been described.

The main manifestations among growing pigs are 1) dietetic hepatosis (HD), 2) dietetic microangiopathy or mulberry heart disease (MHD), and 3) nutritional muscular dystrophy (NMD). In practice these lesions may occur separately or in any combination. The deficiency is usually characterized by sudden deaths among young pigs in herds with a relatively low overall mortality.

Because of the interrelatedness of selenium and vitamin E as nutrients, the development of simple methods to determine the "selenium status" and/or the "vitamin E status" of the animals in a herd is a matter of considerable importance.

Direct quantitative measurements of selenium and vitamin E levels in blood samples are laborious and expensive, and a specialized laboratory is required. Instead, serum enzyme determinations, such as ASAT, ALAT or CPK, have been proposed for clinical diagnostic purposes (*Hyldgaard-Jensen* 1973, *Lannek & Lindberg* 1975, *Fontaine et al.* 1977), but these tests, which reflect tissue damage, are non-specific and give no information about individual nutritional factors. Recently, determination of the enzyme glutathione peroxidase (GSH-Px, E.C.1.11.1.9) in blood samples has been described as a simple and specific method for indirect determination of the selenium status of pigs (*Bengtsson et al.* 1976, *Jensen* 1977, *Sivertsen et al.* 1977, *Hakkarrainen et al.* 1978). At the same time measurement of the resistance of erythrocytes against in vitro lipid peroxidation (ELP) was reported to be useable as a method for evaluating vitamin E status in pigs (*Fontaine & Valli* 1977).

This paper describes the use of GSH-Px and ELP determinations for demonstration of experimentally induced subclinical Se and/or vitamin E deficiency in young pigs.

MATERIAL AND METHODS

The experimental animals were piglets borne by sows fed a diet low in Se and vitamin E. The piglets were weaned at 3 weeks of age, after the males had been castrated 3 days before. On

weaning, 8 piglets from each of 6 litters were randomly allocated to 8 different treatments, i.e. feed supplements, in a blocked 2^3 factorial experiment (*Snedecor & Cochran* 1967). The treatment factors were: 2 levels of selenium, 2 levels of vitamin E and 2 levels of the feed antioxydant ethoxyquin (Table 1). All pigs were kept in single pens and fed ad lib. during the experimental period of 9 weeks. At the end of this period, the pigs were sacrificed for intensive examination (*Nielsen et al.* and *Basse et al.* to be published).

Table 1. Experimental design.

Se suppl. µg/kg (as sodium selenite)	0				60			
Vit. E suppl. mg/kg (dl-α-tocopheryl acetate, gelatin-coated)	0		50		0		50	
Ethoxyquin suppl. mg/kg	0	150	0	150	0	150	0	150
Number of pigs	6	6	6	6	6	6	6	6

Basic diet: 60.3 % barley; 5.0 % soybean meal; 12.0 % dried skim milk; 10.0 % dried yeast; 3.0 % sugar; 4.0 % lard; 3.3 % mineral-tracemineral mixture; 0.6 % vitamin mixture; 1.8 % lysine-methionine mixture. Native contents: 55 µg Se/kg, 3 mg vitamin E/kg.

Heparinized blood samples were collected from the anterior vena cava immediately before the pigs were killed. The GSH-Px activity was determined on whole blood by the simple "spot test" described by *Board & Peter* (1976). This test is based on defluorescence of NADPH by GSH-Px activity in the samples. Erythrocyte lipid peroxidation was estimated by the method of *Mengel et al.* (1964) as used by *Fontaine & Valli* (1977) but with small modifications. The method is based on the reaction of 2-thiobarbituric acid (TBA) with malonylaldehyde produced by lipid peroxidation. The pink chromogen produced has its maximum absorbance at 535 nm.

A volume of 0.5 ml heparinized blood was washed twice with 5 ml 0.9 % saline and centrifuged at 1200 × g for 10 min. at 20°C. The cells were resuspended in 2 ml 0.9 % saline and incubated, under rotation, at 37°C for 2 hrs. with 2 ml 1 % H_2O_2 in saline. The mixture was then precipitated with 2 ml 10 % trichloroacetic acid and filtered through a Whatman No. 1 paper. Two ml filtrate was thoroughly mixed with 2.5 ml 0.67 % 2-thio-

barbituric acid and heated in a boiling water bath for 15 min.; after cooling, the absorbance of the mixture at 535 nm was measured with H_2O_2 in saline as a blank.

Statistical procedure

Analysis of variance was performed by the general linear model procedure of Barr, Goodnight, Sall and Helwig, SAS Institute Inc., Raleigh, N.C., USA. The effect of each treatment as well as of the interactions between treatments were analyzed within blocks, i.e. litters.

RESULTS

The results of the analysis of variance are shown in Table 2, and in Table 3 the mean values and ranges of GSH-Px and ELP are given for each of the main treatments (—Se, +Se; —E, +E; —ethoxyquin, +ethoxyquin). F-values and corresponding P-values are all calculated after correction for influence, if any,

Table 2. Analysis of variance for GSH-Px and ELP.

Source	d.f.	GSH-Px			ELP		
		Sums of squares	F-values	P ≤	Sums of squares	F-values	P ≤
Block (litter)	5	3030	5.6	0.0007	9389	2.0	0.11
Se	1	21051	195.8	0.0001	1010	1.1	0.31
Vit. E	1	60	0.6	0.46	143671	149.4	0.0001
Ethoxyquin (Ethox.)	1	3	0.0	0.85	1718	1.8	0.19
Se × Vit. E	1	144	1.4	0.25	1140	1.2	0.28
Se × Ethox.	1	33	0.3	0.58	338	0.4	0.56
Vit. E × Ethox.	1	14	0.1	0.72	1742	1.8	0.19
Se × Vit. E × Ethox.	1	21	0.2	0.66	250	0.3	0.61
Error	34	3654			32706		

The model explained 87 % of the total variation for GSH-Px and 83 % of the total variation for ELP.

of the other factors, including litter effect. One of the pigs from the group fed basic diet without any supplements died the day before the experimental period was finished. Typical lesions of "mulberry heart disease" were found at necropsy. Statistically, whole-blood GSH-Px activity was found to be significantly influenced by Se supplement ($P < 0.001$). No effect of the other

Table 3. The effect on GSH-Px activity (reaction time, min.) and ELP (optical density at 535 nm) of selenium, vitamin E and ethoxyquin supplements to a pig diet low in selenium and vitamin E. Mean values together with minimum and maximum values in blood from 12 weeks old pigs after 9 weeks of experimentation.

Treatment	Number	GSH-Px			ELP		
		mean	min.	max.	mean	min.	max.
— selenium	23	62	90	35	0.075	0.012	0.218
+ selenium	24	19	25	9	0.069	0.013	0.169
— vitamin E	23	38	90	9	0.128	0.025	0.218
+ vitamin E	24	41	90	11	0.018	0.012	0.023
— ethoxyquin	23	40	90	11	0.076	0.013	0.218
+ ethoxyquin	24	40	90	9	0.068	0.012	0.169

supplements or of interactions between supplements was seen. However, besides being influenced by Se supplementation, the GSH-Px activity was litter-dependent ($P < 0.001$). ELP was influenced only by the vitamin E supplement ($P < 0.001$).

DISCUSSION

The discovery of selenium as an integral part of the enzyme glutathione peroxidase gives a possible biochemical explanation of the well-known nutritional interrelatedness of selenium and vitamin E (*Rotruck et al.* 1973, *Chow & Tappel* 1974). According to these authors and to *Hoekstra* (1975) vitamin E, as an antioxidant, inhibits fatty acid hydroperoxide formation, and selenium, as part of GSH-Px, reduces peroxides already formed.

The value of blood GSH-Px determination as a measure of "selenium status" is ascribable to the existence in various animal species, including sheep, cattle, pig and man, of a close positive correlation between blood GSH-Px activity and blood selenium content, and between these blood levels and the selenium uptake (*Oh et al.* 1974, *Allen et al.* 1975, *Peter & Board* 1975, *Bengtsson et al.* 1976, *Wilson & Judson* 1976, *Chauvaux et al.* 1977, *Perona et al.* 1977, *Sivertsen et al.* 1977, *Anderson et al.* 1978, *Hakkarainen et al.* 1978). *Ewan* (1976) found the serum GSH-Px activity low in selenium-deprived pigs and high in selenium-injected pigs. In a clinical investigation *Jensen* (1977) found a higher whole-blood GSH-Px activity among pigs fed a selenium-supplemented feed than among pigs fed rations low

in selenium or among pigs from litters fed rations with unknown selenium content and with at least 1 pig having died from mulberry heart disease or dietetic hepatosis.

Thompson et al. (1976) found only a low correlation between blood levels of GSH-Px and selenium in pigs, but unlike *Bengtsson et al., Sivertsen et al.* and *Hakkarainen et al.* their study did not include animals with low blood selenium levels. A possible genetic difference in blood GSH-Px activity between individual pigs or between breeds, as suggested by *Jørgensen et al.* (1977), could be another reason for the discrepancy, as could also technical differences.

A selenium-independent GSH-Px activity has been found in livers of different animal species, including sheep and pigs (*Lawrence & Burk* 1977). This enzyme could possibly influence the serum GSH-Px activity level. In the rat, which is the animal most thoroughly examined in this respect, selenium-independent GSH-Px activity has been found in the liver, but not in the erythrocytes, heart or skeletal muscles. If the tissue distribution of selenium-independent and selenium-dependent GSH-Px activity is the same in other animals as in the rat, the liver will generally have a higher total GSH-Px capacity than other organs, independent of the selenium supply. This might possibly explain why feed supplementation with selenium apparently protects more effectively against dietetic hepatosis than against mulberry heart disease (*Pedersen & Simesen* 1977).

The finding, in the present investigation, that the GSH-Px activity was influenced by feed Se supplementation but not by vitamin E or antioxidant supplementation or by interaction between supplements, corroborates the value of using GSH-Px determinations for evaluation of the selenium status of pigs. Genetic variation in pig erythrocyte GSH-Px activity, as assumed by *Jørgensen et al.* could be one explanation of the statistically significant variation between litters found in this experiment.

In rats, the influence of vitamin E on GSH-Px activity has been reported on differently. *Yang et al.* (1976) found a depressed GSH-Px activity in liver and plasma with both excess and deficiency of vitamin E, and *Chow et al.* (1973) found a higher GSH-Px activity in adipose tissue and muscles, but not in liver and kidney, in rats fed a diet low in vitamin E than in rats fed a vitamin E supplemented diet. A possible substrate-induced regulation of enzyme synthesis may to some extent

explain the influence. *Combs* (1978) has reported an increase in plasma GSH-Px activity in chickens fed ethoxyquin-supplemented rations.

The best known biochemical role of vitamin E is its function as a lipid antioxidant which protects biological membranes against non-enzymatic peroxidation of polyunsaturated fatty acids (*Glavind* 1973, *Anon.* 1978). The common method used to measure lipid peroxides is the reaction of TBA with malonylaldehyde, which is a breakdown product of lipid peroxides derived primarily from polyunsaturated fatty acids, such as linolenate (*Dahle et al.* 1962). In different investigations the TBA method has been found to be a valuable means for evaluating in vitro the lipid antioxidant capacity of an animal, which possibly reflects the vitamin E status (*Chow & Tappel* 1972, *Mengel* 1972).

The strong influence of vitamin E supplementation on ELP found in this investigation agrees with the results of *Fontaine & Valli* (1977). These results, together with the apparently high specificity of the test (ELP was not influenced by antioxydant or Se supplementation) corroborate the potential usefulness of this test for determination of the vitamin E status of pigs. For evaluation of its clinical usefulness, field studies will be necessary.

The breakdown of lipoperoxides by GSH-Px which is assumed to take place in vivo is probably inhibited in the in vitro ELP test, since the conditions are not optimized for the enzymic reaction.

Although no significant interaction between Se and vitamin E was reflected in the GSH-Px and ELP reactions, protection of the pigs is most likely to be achieved by a combined effect of the 2 feed supplements in vivo. Also ethoxyquin, though it was found without influence on the 2 parameters investigated, could possibly have a biological effect.

The results of the post-mortem examinations, which will throw light on other biological effects, will be published separately (*Nielsen et al.* and *Basse et al.* to be published).

In conclusion it seems justified to recommend GSH-Px and ELP as valuable and simple methods for evaluating selenium status and vitamin E status independent of one another in growing pigs.

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SAMMENDRAG

Glutation-peroxydase-aktivitet og erytrocyt-lipid-peroxydering som mål for selen- og vitamin E-status hos unge grise.

I et 2^3 faktorielt fodringsforsøg med tilfældigt sammensatte blokke blev anvendt grise fra søer, der var fodret med et foder fattigt på selen og vitamin E. Fra grisene var tre uger og til de blev tolv uger gamle, blev de fodret i enkeltdyrsstier med et grundfoder, der hovedsagelig bestod af byg, tørret skummetmælk, soyaskrå og tørgær, og som indeholdt 55 µg selen og 3 mg vitamin E pr. kg. Behandlingsfaktorerne var forskellige fodertilskud, hver i to niveauer: selen (0 og 60 µg/kg), vitamin E (0 og 50 mg/kg) og foder-antioxydanten ethoxyquin (0 og 150 mg/kg). Blodprøver, der blev udtaget ved forsøgets afslutning, blev undersøgt for glutation-peroxydase-aktivitet (GSH-Px) og resistens mod erytrocyt-lipid-peroxydering (ELP) med henblik på en vurdering af henholdsvis selen og vitamin E status.

Ved en variansanalyse fandtes GSH-Px at være afhængig af selentilskuddet ($P < 0.001$) og af kuld ($P < 0.001$), men ikke af de andre fodertilskud eller af samspil mellem forskellige fodertilskud. Resistens mod ELP var kun afhængig af vitamin E tilskuddet ($P < 0.001$). Vurderet ud fra denne undersøgelse synes GSH-Px og ELP at kunne være værdifulde simple metoder til en uafhængig vurdering af selen status og vitamin E status hos unge grise.

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