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SERUM GLUTATHIONE PEROXIDASE ACTIVITY AND BLOOD SELENIUM IN PIGS*

By

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HAKKARAINEN, J., P. LINDBERG, G. BENGTSSON and L. JÖNSSON: *Serum glutathione peroxidase activity and blood selenium in pigs.* Acta vet. scand. 1978, *19*, 269—284. — Blood serum glutathione peroxidase activity and blood selenium concentration were measured in blood samples from pigs subjected to experimentally induced selenium deficiency and dietary selenium supplementation on graded levels. A highly significant correlation between blood selenium and serum GSH-Px activity in pigs, especially in selenium deficient pigs, was demonstrated. There was also a strong relationship between blood selenium concentration and serum GSH-Px activity in pigs receiving dietary selenium at graded levels. Serum GSH-Px activity exhibited an excellent close-response relationship to dietary selenium. Linear regression analysis showed that the increased serum GSH-Px activity was a function of the dietary selenium concentration. The fitness of serum in monitoring slight changes of the selenium status of pigs with help of the estimation of GSH-Px activity was discussed. The measurement of serum GSH-Px activity seems to provide a useful and rapid means for defining selenium requirements and for identifying selenium deficiency in growing pigs.

serum glutathione peroxidase; blood selenium; dietary selenium; pigs

The prevention of oxidative damage to the membrane and hemoglobin of erythrocytes by selenium involves the utilization of glutathione (*Rotruck et al.* 1972). It was consequently assumed that selenium might function as a constituent of glutathione peroxidase. It has later been shown that glutathione per-

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oxidase (GSH-Px, EC 1.11.1.9.) is a selenium containing enzyme and that selenium through this enzyme exerts protection against peroxidative damage in tissues, as does vitamin E (*Rotruck et al.* 1973, *Flohé et al.* 1973). *Rotruck et al.* (1973) suggested also that measuring GSH-Px may provide a useful means of defining requirements of selenium and of identifying selenium deficiency in animals and humans.

Studies on the rat (*Chow & Tappel* 1974, *Hafeman et al.* 1974, *Smith et al.* 1974), chicken (*Noguchi et al.* 1973, *Omaye & Tappel* 1974) and sheep (*Oh et al.* 1974, 1976a,b, *Peter & Board* 1975) have shown that the activity of glutathione peroxidase in blood and tissues is a function of dietary selenium. The GSH-Px activity in whole blood, erythrocytes and/or plasma has also been shown to be positively related to the blood selenium concentration in rats (*Chow & Tappel, Hafeman et al., Smith et al.*), chicken (*Noguchi et al.*), sheep (*Oh et al.* 1974, 1976b, *Thompson et al.* 1976, *Wilson & Judson* 1976) and cattle (*Allen et al.* 1975, *Thompson et al., Wilson & Judson*).

In a preliminary study, levels of blood selenium and serum GSH-Px activity were found to be closely correlated in pigs (*Bengtsson et al.* 1976). The same condition was also valid concerning dietary selenium intake and GSH-Px. On the contrary, *Thompson et al.* did not find a similar correlation between the selenium content and GSH-Px activity in porcine blood.

Vitamin E and selenium deficiencies (VESD) in domestic animals, and especially in pigs, cause a group of disorders resulting in serious economic losses. However, the determination of selenium in blood and tissues is a rather complicated and time consuming procedure of analysis which furthermore demands great laboratory resources as well as high precision and accuracy. Therefore, it should be a fundamental task for the benefit of both future research and diagnostic work to try to establish the fitness of using GSH-Px activity in evaluating the selenium status in pigs.

Preliminary data (*Bengtsson et al.* 1976) proposed that there is a close correlation between blood selenium and glutathione peroxidase in pigs. Consequently, the present work comprises a systematic study to establish the correlation between blood selenium as well as dietary selenium on the one side and serum glutathione peroxidase activity on the other side. In this study, experimentally induced selenium deficiency and dietary selenium

supplementation on graded levels were the means applied to elucidate this problem.

MATERIAL AND METHODS

Selenium-deficient cows' milk casein was used as the protein source in a vitamin E deficient diet to pigs. The casein was prepared from the milk of cows which were fed selenium-deficient hay, oats and barley (*Bengtsson et al.* 1974). Batches holding 5—8 μg selenium/kg were selected for feeding cows, who were further supplemented with urea and a mineral mixture. The supply of selenium in excess of that present in the hay and cereals was found to be negligible. The selenium content of the casein gradually fell from 210 μg/kg DM to a more or less stable level of 30—50 μg/kg DM. Blood selenium of the cows fell at the same time from 40—46 ng/ml to 7—8 ng/ml.

The pigs' basic diet was composed of this low-selenium casein 17 %, wheat starch 53 %, sugar 20 %, cellulose 3 %, molecular distilled cotton-seed oil 3 %, and a mineral and vitamin mixture. The diet contained 8.0 ± 0.9 μg selenium/kg DM (mean $\pm$ 1s) and 2.5 mg tocopherol (1.4 mg α-tocopherol and 1.1 mg γ-tocopherol)/kg DM as determined according to *Lindberg* (1966, 1968). The composition of the basal diet in detail has been reported (*Bengtsson et al.* 1978a). This diet, supplemented with selenium and vitamin E, was able to support a growth rate of 450 g/day for 3 months following weaning. The final diet mixture was prepared twice a week and stored in a dark and cool place.

Four pregnant gilts (Swedish Landrace $\times$ Large English White) were fed potatoes, groats and skimmed milk (*Obel* 1953) for the last 3 weeks preceding delivery and during lactation. Thirty weaned pigs used for the experiment were obtained from the 4 sows. Pigs Nos. 7 to 16 (Sow 830) were born on August 31, pigs Nos. 17 to 25 (Sow 823) on September 1, pigs Nos. 26 to 36 (Sow 86) on September 2, and pigs Nos. 37 to 47 (Sow 816) on September 5, 1975). The pigs were kept in pens with concrete floors covered with a thin layer of pine shavings. The selenium content of the pine shavings was found to be 13 μg/kg DM. The pigs were given the basic diet as creep feed during the last 2 of the 7 weeks' sucking period in order to make the transition to this experimental diet as safe as possible.

The pigs were weaned on October 21, 1975. At weaning the pigs were divided into 6 groups with 5 pigs in each group and

transferred to the experimental diet on the same day. The general outlines of the experiment is presented in Table 1. The pigs in Group 1 composed the control group without supplementation of the basic diet with selenium and tocopherol. Groups 2, 3, 4, 5 and 6 were supplemented with 5 mg DL-α-tocopheryl acetate/kg food and with graded levels of selenium (0, 5, 15, 45 and 135 μg selenium, respectively, as sodium selenite/kg food). In Groups 4 and 5, vitamin E and/or selenium supplementations were changed on day 49 and on day 71, respectively (cf. Table 1). The new levels of supplementation were 15 mg DL-α-tocopheryl acetate plus 135 μg selenium/kg food for Group 4 on day 49 and 15 mg DL-α-tocopheryl acetate plus unchanged 45 μg selenium/kg food for Group 5 on day 71.

Blood selenium determination was carried out as described by *Lindberg* (1968).

Glutathione peroxidase (GSH-Px, EC 1.11.1.9.) activity was determined in blood serum at pH 7.4 and 25°C with a coupled test system according to *Paglia & Valentine* (1967), cumene hydroperoxide being used as the substrate (*Little et al.* 1970). The method is a linked reaction in which the oxidized glutathione is regenerated to reduced form enzymatically with glutathione reductase (EC 1.6.4.2.) and NADPH. Reaction rates are followed by continuously measuring the decrease of NADPH concentration spectrophotometrically at 340 nm. The "blank" oxidation rate of NADPH is registered by using water instead of serum. The error of the method was calculated to be 0.60 μkat/l, giving a coefficient of variation of 3.8 % ($n = 20$).

The blood samples were allowed to clot at 4°C. Serum was removed and immediately frozen and kept at —20°C until analyzed unless this was done the same day. In all cases the serum glutathione peroxidase determinations were carried out within 2 weeks. Storing for 2 weeks was shown not to have any influence on the level of serum GSH-Px activity.

RESULTS AND DISCUSSION

Composing experimental diets for groups of large animals for studies of deficiency conditions involves practical difficulties, mainly due to the high cost of pure ingredients. This is especially so when the critical factor is ubiquitously present in general foodstuffs. Studies on selenium deficiency in domestic animals are one example. The conditions are favorable in the case of herbi-

Table 1. Experimental plan and body growth of pigs. The basic diet contained 8.0 ± 0.9 μg Se/kg DM and 2.5 mg tocopherol (1.4 mg α-tocopherol)/kg DM. Each group was composed of 5 pigs.

Group	Added to diet		Body wt day 0, kg (mean ± 1 s)	Died or euthanized on day (mean ± 1 s)	Body wt on day of death, kg (mean ± 1 s)	Av. daily gain, g (mean ± 1 s)
	DL-α-tocopheryl acetate, mg/kg[a]	Se (as sodium selenite) μg/kg				
1	0	0	11.1 ± 1.3	22.4 ± 9.7	17.4 ± 7.7	290.6 ± 145.7
2	5	0	11.0 ± 1.1	26.4 ± 13.3	20.0 ± 6.4	307.0 ± 119.0
3	5	5	11.0 ± 1.1	32.2 ± 16.9	23.7 ± 8.1	360.2 ± 64.2
4	Days 0—48: 5 Days 49—75: 15	Days 0—48: 15 Days 49—75: 135	10.5 ± 1.5	66.0 ± 20.1	38.5 ± 12.4	407.0 ± 83.6
5	Days 0—70: 5 Days 71—89: 15	45	11.0 ± 1.3	89	51.0 ± 8.1	444.4 ± 87.0
6	5	135	10.9 ± 1.4	89	49.8 ± 3.3	432.2 ± 26.3

[a] Vitamin E dry powder 25 % (Merck Art. 501618).

vores, which can be fed naturally occurring low-selenium food plants. The diets are more difficult to compose for pigs, however, as they require a supply of animal protein for adequate body growth and development. Animal protein is consistently rich in selenium. The selenium-deficient experimental diets for pigs, which have been described in previous literature, seem to have been rather exclusively based on vegetable proteins.

We have made use of the naturally occurring selenium-deficient hay and cereals in Sweden. These were fed to cows which produced a selenium-deficient milk casein. This casein was used as the protein source in a vitamin E-deficient diet for pigs in studying the relationships between blood or dietary selenium and serum GSH-Px activity.

The results concerning the prophylactic effect of a combined addition of dietary selenium and tocopherol to this vitamin E- and selenium-deficient basic diet on the development of the vitamin E selenium deficiency (VESD) syndrome in weaned pigs are reported elsewhere (*Hakkarainen et al.* 1978a).

The relationship between blood selenium and serum GSH-Px activity is shown in Fig. 1. Levels of blood selenium and serum GSH-Px activity were found to be closely correlated within the

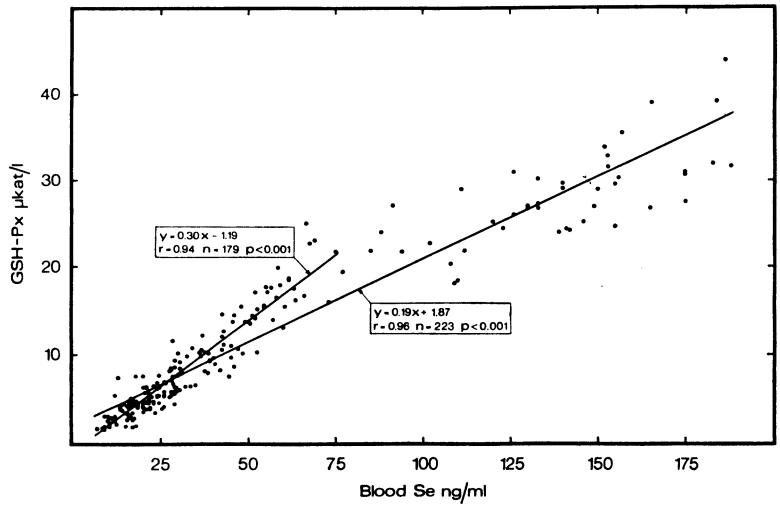

Figure 1. Relationship between serum glutathione peroxidase activity and blood selenium concentration in pigs. The enzyme activity (y) was positively ($P < 0.001$) related to blood selenium concentration (x) within the range observed (r = regression coefficient and n = the number of samples). For further details, see the text.

range observed ($y = 0.19x + 1.87$; $r = 0.956$; $n = 223$; $P < 0.001$). However, it should be pointed out that the slope of the regression line was greater within the range of the pronounced selenium deficiency, when the blood selenium concentration was less than 75 ng/ml, ($y = 0.30x - 1.19$; $r = 0.943$; $n = 179$; $P < 0.001$) than within the range of selenium concentrations between 75 and 200 ng/ml blood ($y = 0.14x + 8.44$; $r = 0.740$; $n = 44$; $P < 0.001$). Thus determination of serum GSH-Px activity should be a reliable way to evaluate the selenium status in pigs, and selenium deficiency in particular.

Contrary to our experiences, *Thompson et al.* (1976) found that the correlation between blood selenium and GSH-Px activity in the pig was poor. However, our preliminary (*Benglsson et al.* 1976) and present findings gained support by the observations of *Ewan* (1976). In addition to high correlations between the selenium content and GSH-Px activity in the liver, kidney and spleen, *Ewan* was able to find a low serum GSH-Px activity in selenium-deficient pigs and a high serum GSH-Px in pigs given selenium. Even the observations of *Jensen* (1977) and *Jørgensen et al.* (1977) support our conclusions concerning the usefulness of the estimation of GSH-Px activity in evaluating the selenium status in pigs. However, neither *Jensen* nor *Jørgensen et al.* have correlated their measurements of GSH-Px activity in swine blood to the levels of blood selenium.

Quite recently, *Sivertsen et al.* (1977) reported a close correlation between erythrocyte glutathione peroxidase and blood selenium in swine.

Figs. 2, 3, 4 and 5 demonstrate the strong and positive relationship between blood selenium concentration and serum GSH-Px activity in pigs receiving dietary selenium at graded levels. At the same time, the response of pig serum GSH-Px activity to alterations in dietary selenium intake confirms the essentiality of selenium for this enzyme. The mean value ± 1s on day 0 was for blood selenium 26.7 ± 3.4 ng/ml and for GSH-Px 6.6 ± 1.8 μkat/l ($n = 30$). The animal material was apparently rather homogenous at the onset of the experiment. When selenium was not added to the diet there was a continuous decrease of blood selenium level to 10 ng/ml or less accompanied by a decrease of the GSH-Px activity at the same time to 3—1.5 μkat/l (Fig. 2, Groups 1 and 2). The addition of 5 μg selenium/kg diet slightly delayed the decrease of the 2 constituents (Fig. 2, Group 3). The

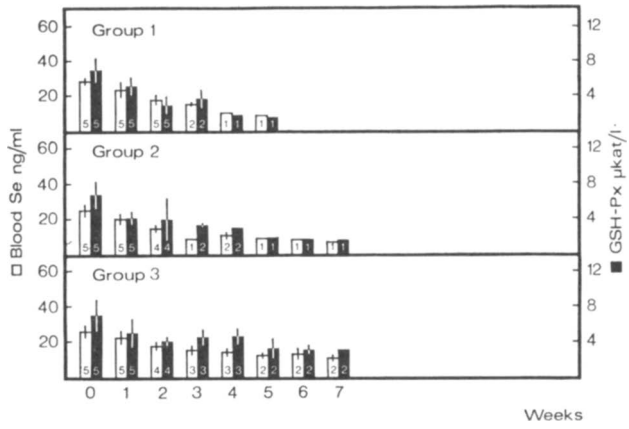

Figure 2

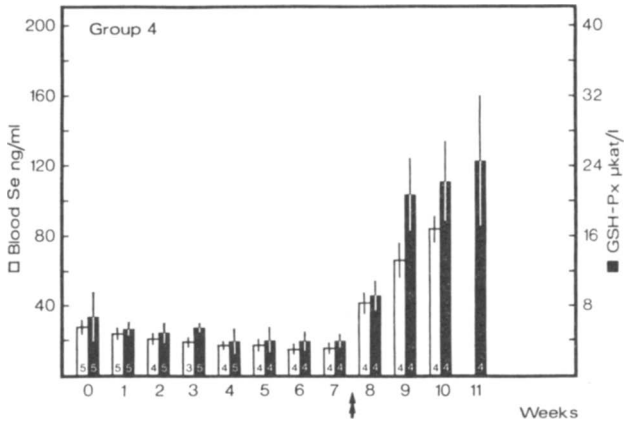

Figure 3

Figures 2 — 5. Effect of dietary selenium on blood selenium concentration and serum glutathione peroxidase activity in pigs. The experimental diets were introduced on day 0. The pigs in control Group 1 received the basic diet, which contained only traces of selenium and α-tocopherol. Groups 2, 3, 4, 5 and 6 were supplemented with 5 mg DL-α-tocopheryl acetate/kg diet and with graded levels of selenium (0, 5, 15, 45 and 135 μg selenium, respectively, as sodium selenite/kg diet). In Group 4 the α-tocopherol supplementation was

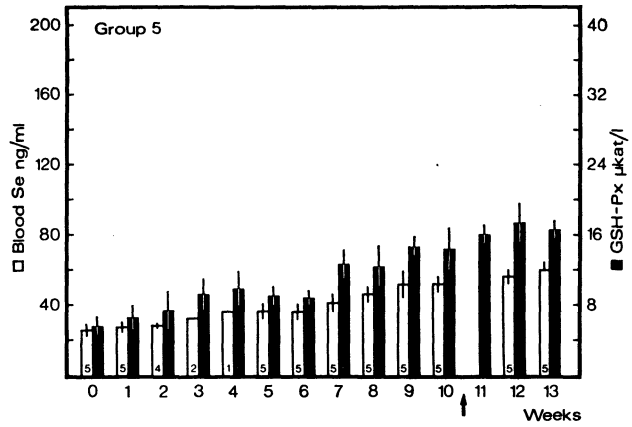

Figure 4

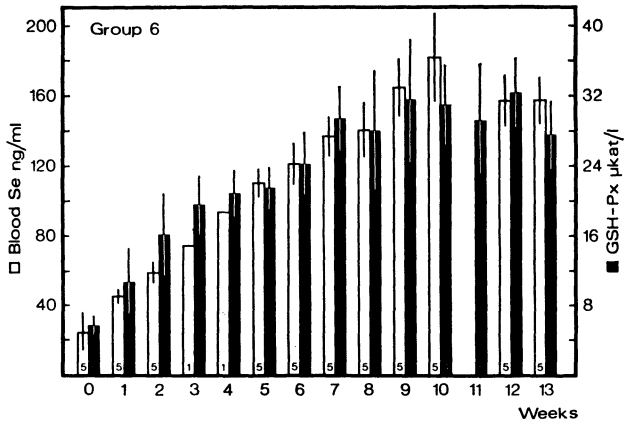

Figure 5

increased from 5 mg to 15 mg/kg diet and the selenium supplementation from 15 to 135 µg/kg diet on day 49 (double arrow). In Group 5 the α-tocopherol supplementation alone was increased from 5 mg to 15 mg/kg diet on day 71 (single arrow). The results for both blood selenium and serum glutathione peroxidase activity are given as the mean ± 1 s of the mean at each time of observation. Figures within the bars represent the number of pigs observed.

addition of 15 μg selenium/kg (Fig. 3, Group 4) was insufficient to retain the initial values for both blood selenium concentration and GSH-Px activity. The supply of 45 μg selenium/kg diet (Fig. 4, Group 5) gave rise to a slow increase of the blood selenium and GSH-Px levels, i.e. both parameters showed the same tendency of slight improvement of the selenium status of the pigs in this group. Blood selenium concentrations rose to about 60 ng/ml and GSH-Px activity to 16—17 μkat/l during 90 days. Finally, in Group 6 (Fig. 5), the addition of 135 μg selenium/kg food resulted in a comparatively fast rise of blood selenium to a level of 155—180 ng/ml followed by a similar rise of serum GSH-Px activity to a level of 30 μkat/l or slightly more within 7—9 weeks. The planing off of both blood selenium concentration and serum GSH-Px activity in this group (6) to a rather even level after the dietary supplementation of 135 μg selenium/kg food during 7—8 weeks may reflect just that situation when the selenium binding capacity of the blood and the serum of the pigs at the actual age is near to become saturated. In Groups 4 and 5, vitamin E and/or selenium supplementation were increased on day 49 and on day 71, respectively, when it was obvious that the original levels were insufficient to protect the pigs from the clinical signs of the VESD syndrome. The addition of more tocopherol alone on day 71, from 5 to 15 mg/kg diet, did not affect the curve of slight increase of blood selenium or that of GSH-Px activity. The slight increases were the results of the supplementation with 45 μg selenium/kg diet from the beginning of the experiment (Fig. 4, Group 5; cf. *Hakkarainen et al.* 1978a). On the contrary, the decreasing trend of both blood selenium concentration and serum GSH-Px activity seen in Group 4 (Fig. 3) receiving insufficient amounts of selenium and vitamin E was dramatically changed to an increasing one when the selenium supplementation was increased from 15 to 135 μg selenium/kg diet on day 49.

When the number of experimental animals in the respective groups allowed statistical calculations (Groups 4, 5 and 6), the difference in serum GSH-Px activity among the different dietary groups with graded selenium levels was highly significant ($P < 0.001$) at 7 weeks (Figs. 3, 4 and 5). An increment of enzyme activity was associated with each increment of dietary selenium supplementation from 0 to 135 μg selenium/kg diet. There was also a highly significant difference ($P < 0.001$) in serum GSH-Px

activity at 10 and 13 weeks between the 2 surviving groups supplemented with 45 μg selenium/kg and 135 μg selenium/kg (Figs. 4 and 5).

Thus, serum GSH-Px activity exhibited an excellent close-response relationship to dietary selenium. Linear regression analysis showed that the increased serum GSH-Px activity was a function of the dietary selenium concentration at observations 2, 3, 4, 5, 6 and 7 weeks after the experimental diets with 0, 5, 15, 45 and 135 μg selenium/kg food were introduced (Table 2). After 7 weeks, the only pigs surviving on the original diets (with unchanged selenium levels) were those in Group 5 (45 μg selenium/kg) and Group 6 (135 μg selenium/kg); therefore the linear regression analysis would not be fruitful for the observations between 8 and 13 weeks.

The separate linear regressions are shown in Table 2.

Table 2. The linear relationships between dietary selenium (x) and serum GSH-Px activity (y) at observations 2, 3, 4, 5, 6 and 7 weeks after the experimental diets with 0, 5, 15, 45 and 135 μg selenium/kg food were introduced. For further details, see the text and Figs. 2, 3, 4 and 5.

Observation after	$y = bx + a$	r	P	n
2 weeks	$y = 0.09x + 3.41$	0.899	< 0.001	28
3 weeks	$y = 0.12x + 3.72$	0.964	< 0.001	22
4 weeks	$y = 0.13x + 2.93$	0.967	< 0.001	21
5 weeks	$y = 0.14x + 2.18$	0.982	< 0.001	18
6 weeks	$y = 0.17x + 1.58$	0.975	< 0.001	17
7 weeks	$y = 0.21x + 1.89$	0.980	< 0.001	17

This close relationship between dietary selenium and GSH-Px activity emphasizes the fitness of the estimation of serum GSH-Px enzyme as a rapid monitoring method for the selenium status of an animal. The studies of *Omaye & Tappel* (1974) and *Smith et al.* (1974) also showed a close linear relationship between GSH-Px activity and dietary selenium. However, in their data the plasma GSH-Px activity increased linearly as a function of the logarithm of dietary selenium concentration. An explanation to this disagreement would seem to be the fact that the dominating part of their study covers that range of supplementation where selenium was added in excess of physiological amounts.

Our study mainly covered the range of selenium deficiency and the range of the amounts of supplementation according to suggestions for dietary selenium allowances in Scandinavia and the USA. A requirement of 0.1 p.p.m. has been suggested for pigs by *Trapp et al.* (1970), and has been recommended by the *N.R.C.* (1968). The studies of *Bengtsson et al.* (1976, 1978b) and *Hakkarainen et al.* (1978a,b) support the validity of this recommendation in Scandinavian circumstances.

Determination of GSH-Px activity in blood serum was used in the present study as a measure of selenium status. It was shown that the changes in blood selenium concentration were intimately followed by quite identical changes in serum GSH-Px activity on several levels of graded dietary supplementation of selenium.

In disagreement with our results, *Thompson et al.* found only negligible traces of the enzyme activity in pig plasma giving them the reason to use whole blood and to interpret the results to correspond closely to erythrocyte GSH-Px activity. Later on, *Jørgensen et al.* in comparing plasma and erythrocyte GSH-Px activity, noticed a relatively high enzyme activity in the plasma, although, not particularly well related to the activity in the red cells. In any case, it should be emphasized in this connection that the contribution of erythrocytes to total blood GSH-Px activity is superiorly dominating. (The average contribution of plasma and erythrocytes to total blood GSH-Px activity amounted to 14.2 % and 85.8 %, respectively, according to *Jørgensen et al.*). It should not be denied that in the majority of studies hitherto with rats, chickens, sheep, cattle and pigs, determination of GSH-Px activity in erythrocytes or in whole blood has been preferred. However, our observations of the usefulness of serum GSH-Px activity in estimating the selenium status of pigs are supported by the results of *Smith et al.* and *Omaye & Tappel.* In rats as well as chickens, the most drastic changes in GSH-Px activity due to dietary selenium supplementation occurred specifically in the plasma. Similarly, of all the tissues studied by *Omaye & Tappel,* including erythrocytes and plasma, the best correlation of GSH-Px activity with selenium levels was found in the blood plasma. The observations of *Hafeman et al.* (1974) suggested that selenium is incorporated into erythrocyte GSH-Px only during erythropoiesis. Thus, the rather long life span of the erythrocytes combined with the dominating role of erythro-

cytes in regard to GSH-Px activity in the blood could be a too powerful stabilizing factor and therefore be able to prohibit the use of whole blood and/or erythrocytes in monitoring slight changes or abnormal levels of enzyme activity related to improper nutrition and acute selenium deficiency. In addition, according to our observations during the experimental work, hemoglobin constitutes a methodologically disturbing factor in measuring GSH-Px activity by a direct spectrophotometric procedure such as the method of *Paglia & Valentine* (1967).

Blood serum rather than blood plasma was preferred in the present study for the determination of enzyme activity since heparin has been reported to inhibit glutathione peroxidase activity (*Günzler et al.* 1974).

The close correlation between blood selenium and the serum GSH-Px activity in pigs was demonstrated in this study by applying experimentally induced selenium deficiency and dietary selenium supplementation on graded levels. It was concluded that estimation of glutathione peroxidase activity is reliable in evaluating the selenium status in pigs, and that the level of this enzyme well reflects the changes of selenium in blood. Determination of GSH-Px activity in blood serum has been successfully applied by us later on to evaluate the selenium status in pigs (*Hakkarainen et al.* 1978b). Thus, the measurement of serum GSH-Px activity seems to provide a useful and rapid means for defining selenium requirements and for identifying selenium deficiency in growing pigs.

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SAMMANFATTNING

Serumglutationperoxidas-aktivitet och blodselen hos grisar.

Blodserumglutationperoxidas-aktivitet och blodselenkoncentration mättes i blodprov från grisar vilka var utsatta för experimentellt inducerad selenbrist och dietär supplementering med selen på olika nivåer. Höggradigt signifikant korrelation mellan blodselen och serum GSH-Px aktivitet hos grisar, speciellt hos grisar utsatta för selenbrist, påvisades. Ett starkt samband konstaterades vidare föreligga mellan

blodselenkoncentration och serum GSH-Px aktivitet hos grisar som fick selentillskott på olika nivåer i kosten. Höggradigt signifikant korrelation förelåg mellan serum GSH-Px aktivitet och dietärt intag av selen. Valet av serum vid glutationperoxidas-analyser för att avläsa grisens selenstatus diskuteras. Det fastslås att bestämning av serum-glutationperoxidas-aktivitet är en väl användbar och snabb metod för att fastställa selenbehov och att diagnostisera tillstånd av selenbrist hos växande grisar.

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