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ABSTRACT

THE INFLUENCE OF SUPPLEMENTAL VITAMIN E AND ARSANILIC ACID ON DIETARY SELENIUM UTILIZATION BY SWINE

By

John Paul Hitchcock

Two areas of investigation were undertaken in this research. The first area of research was designed to investigate the effects of supplemental arsanilic acid, selenium (Se) and vitamin E on performance, deficiency signs, hematology, tissue selenium and selenium balance in growing-finishing pigs fed diets low in vitamin E and selenium. One feeding trial with growing-finishing pigs and two selenium balance experiments with young pigs were conducted to evaluate these effects. Yorkshire, Hampshire and crossbred pigs were maintained in a modern confinement management system in the feeding trial. Young pigs (5 weeks of age) for the balance experiments were housed, shortly after weaning, in stainless steel metabolism cages and received a constant, near *ad libitum* intake of experimental diet in three meal feedings daily.

In the feeding trial, classic Se and/or vitamin E deficiency lesions were observed in four animals fed diets not supplemented with Se or vitamin E. Selenium supplementation (0.1 ppm Se, as sodium selenite) increased ($P<.01$)

liver, kidney and diaphragm muscle selenium concentrations. Arsanilic acid supplementation (99 ppm) increased ($P<.05$) diaphragm muscle selenium concentrations. Vitamin E (22 IU/kg) supplements had no effect on tissue selenium levels.

The two balance studies were concerned with Se absorption and retention on diets supplemented with Se as sodium selenite or seleniferous corn and the effects of arsanilic acid (99 ppm) and vitamin E (22 IU/kg) on Se balance. Supplemental selenium (0.1 ppm) from seleniferous corn increased percent fecal Se excretion (as a percent of intake) and decreased percent urinary Se excretion as compared to Se from sodium selenite. Vitamin E supplementation increased Se retention from seleniferous corn by decreasing urinary and fecal Se excretion. Arsanilic acid decreased Se excretion and increased Se retention in these experiments.

The second area of research was designed to study the effects of 0.1 ppm supplemental selenium as sodium selenite and four dietary levels of vitamin E (d-α-tocopheryl acetate) supplementation on performance, hematology, tissue selenium and selenium balance in growing-finishing pigs fed diets low in vitamin E and selenium. Pigs were housed and maintained during one feeding trial and one selenium balance study as described for the previous experiments. There were no lesions

indicative of vitamin E-selenium deficiency and no deaths due to the deficiency were observed in the feeding trial. Dietary supplements of Se or vitamin E had no significant effects on overall performance of pigs during the feeding trial. Selenium supplementation increased ($P<.01$) serum selenium concentrations, while vitamin E supplementation had no effect on serum selenium concentrations. Selenium supplementation increased ($P<.01$) *rectus abdominus* muscle, liver and kidney Se concentrations on both a wet and dry basis. There was a significant ($P<.05$) interaction between selenium and vitamin E on muscle selenium concentrations (wet basis). The addition of increasing levels of vitamin E to unsupplemented Se diets decreased muscle Se concentrations while on Se supplemented diets, muscle Se concentrations increased with increasing levels of vitamin E. Dietary level of vitamin E supplementation had no significant effect on tissue Se concentrations.

The balance study was conducted to study Se absorption and retention on a selenium supplemented basal diet (0.172 ppm Se) with dietary vitamin E added at levels of 0, 5, 10 or 15 IU E/kg of diet. Supplemental levels of vitamin E had no significant effects on selenium excretion or retention, serum selenium or tissue selenium concentrations on either a wet or dry basis in this short-term selenium balance study.

THE INFLUENCE OF SUPPLEMENTAL VITAMIN E AND ARSANILIC ACID ON DIETARY SELENIUM UTILIZATION BY SWINE

By

John Paul Hitchcock

A DISSERTATION

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INTRODUCTION

The role of selenium in nutrition during the past 150 years has changed dramatically. Selenium was first recognized for its toxicity characteristics and was identified as the toxic principle in "alkali disease in horses." The concept of selenium as a toxic element was generally accepted as its only role in nutrition until, in 1957, Schwarz and Foltz (1957) showed that sodium selenite would prevent liver necrosis in rats fed torula yeast diets. Eggert *et al.* (1957) and Grant and Thafvelin (1958) demonstrated a relationship between selenium deficiency and hepatosis dietetica in swine.

Thompson and Scott (1970) demonstrated that chicks receiving crystalline amino acid diets containing high levels of vitamin E but less than 0.02 ppm selenium required selenium supplementation for proper pancreatic function. This research established that selenium is indeed an essential trace element. The discovery of a specific function of selenium as a component of glutathione peroxidase in animals has been described by Rotruck *et al.* (1973) and Hoekstra (1974). These researchers indicated that decreases in glutathione peroxidase appear

to explain partially the degenerative diseases induced by selenium deficiency.

Naturally occurring selenium and/or vitamin E deficiency in ruminant farm animals has been recognized in certain areas of the United States for many years. Field cases of selenium and/or vitamin E deficiency were first observed in swine in this country in the late 1960's. Michel *et al.* (1969) were the first to describe the occurrence of the disease in commercial swine herds in Michigan on practical corn-soybean meal type diets. Trapp *et al.* (1970) suggested that the condition probably existed prior to the first observed cases in 1967. They observed that most herds in which selenium-vitamin E deficiency occurred were being fed corn-soybean meal diets supplemented with arsanilic acid as a feed additive. Arsanilic acid has been demonstrated to counteract selenium toxicity in swine (Wahlstrom *et al.*, 1955). Therefore, Trapp *et al.* (1970) suggested that arsanilic acid may have been antagonistic to selenium and thus enhanced the occurrence of a natural selenium-vitamin E deficiency.

Preliminary estimates of the dietary selenium requirement for the pig have been reported by Groce *et al.* (1973b). These studies indicated that the dietary selenium requirement for the pig when supplemental vitamin E does not exceed 11 IU/kg is 0.15 ppm. These studies also indicated a need for further research on

the interrelationship of Se with vitamin E. The National Research Council (1973) suggests that 11 IU of vitamin E be added per kg of diet until more specific information is obtained on vitamin E and selenium requirements of swine.

The studies presented in this dissertation were undertaken to define more clearly the effects of arsanilic acid, vitamin E and selenium supplementation in corn-soybean meal diets for swine. The studies on tissue selenium levels and selenium balance studies were undertaken to study the effects of arsanilic acid and vitamin E on the utilization of natural and supplemental selenium. Vitamin E supplementation of swine rations is relatively expensive and determination of an optimal dietary level of vitamin E in diets supplying required levels of selenium was deemed an important area for investigation.

REVIEW OF LITERATURE

Selenium and/or Vitamin E Deficiency in Farm Animals

Experimental diet-induced deficiencies

The early investigations with selenium were achieved with diets containing some selenium and usually substantial quantities of unsaturated fat. These studies revealed a relationship between vitamin E and selenium. To induce dietary selenium deficiency, semi-purified diets containing torula yeast as the major source of protein were often used. These diets contained a very low level of vitamin E and were deficient in sulfur containing amino acids but very high in unsaturated fatty acids.

Poultry. In independent discoveries in 1957, selenium was identified as an integral part of Factor 3 active in preventing liver necrosis in rats by Schwarz and Foltz (1957) and was shown to prevent exudative diathesis in chicks fed a torula yeast diet low in vitamin E (Patterson *et al.*, 1957). The latter authors reported that exudative diathesis in chicks fed torula yeast diets could be prevented by either selenium or

vitamin E additions to the diet. In these studies selenium levels of 0.1 ppm in the form of selenocystathionine or sodium selenite prevented exudative diathesis. Rahman *et al.* (1960) reported that vitamin E, dried brewers' yeast or selenium (0.05 or 0.1 ppm) prevented exudative diathesis in chicks fed a torula yeast diet. Corn distillers' dried solubles and condensed fish solubles also prevented signs of exudative diathesis. In the absence of vitamin E, selenium promoted growth when fed at 0.05 or 0.1 ppm in the diet. In the presence of vitamin E no growth response was observed from selenium supplementation or dried brewers' yeast in the diet. Selenium, vitamin E, or the use of starch as a carbohydrate source completely prevented the development of exudative diathesis in turkey poults fed a torula yeast diet. Mathias and Hogue (1971) observed that selenium, vitamin E and ethoxyquin were effective in preventing exudative diathesis, and a significant interaction between selenium and vitamin E was observed in preventing mortality. This result was interpreted as support for a biological synergism between the two nutrients. They concluded that at relatively low levels of dietary and body stores of selenium and vitamin E, synthetic antioxidants are partially capable of preventing or curing exudative diathesis but not eventual death of chicks fed torula yeast basal diets. Nesheim and

Scott (1961) stated that the most marked nutritional effect of dietary selenium in chickens and turkeys is its effectiveness in preventing exudative diathesis. Selenium is not completely effective in preventing muscular dystrophy in chicks, and levels of selenium that partially prevent muscular dystrophy (1-5 ppm in the diet) are much higher than the levels effective against exudative diathesis. Selenium appeared to have growth promoting properties for chicks receiving diets containing torula yeast and adequate in vitamin E.

The major muscle lesions in chicks were muscular dystrophy of the pectoral and leg muscles while myopathy of the gizzard and myocardium were observed in turkey poults fed selenium and/or vitamin E deficient diets. Machlin and Shalkop (1956) observed that chickens fed casein-gelatin diets low in vitamin E and sulfur amino acids to four weeks of age developed a muscular degeneration manifested grossly as white striations of the breast and leg muscles and microscopically as a hyaline type degeneration. The addition of α-tocopheryl acetate, methionine, cystine or a high level of an antioxidant to the diets completely prevented muscular degeneration.

Walter and Jensen (1963) reported that adding 1 ppm selenium (as selenious acid) or 20 IU/kg of α-tocopheryl acetate to torula yeast diets calculated to be slightly deficient in sulfur amino acids completely protected

turkey poults against gizzard and skeletal muscular dystrophy. Skeletal muscles were affected to a lesser extent than the gizzard musculature. Supplementation of the ration with cystine (0.15%), methionine (0.4%), low levels of ethoxyquin (0.025%) or selenium (0.01% or 0.1 ppm) proved ineffective in preventing the skeletal and gizzard muscular dystrophy. A high level of ethoxyquin (0.3%) reduced the incidence but did not completely protect against the skeletal and gizzard muscular dystrophy. Selenium, vitamin E and the high level of ethoxyquin prevented anemia and reduced albumin-globulin ratios which is generally observed in cases of muscular dystrophy. Hintz and Hogue (1964b) added raw kidney beans to vitamin E supplemented torula yeast diets and increased the incidence of nutritional muscular dystrophy from 5-6% to 45-100%. This indicated to the authors that the beans contained an anti-vitamin E Factor. Two vitamin E antagonists were isolated and the authors indicated that an alcohol soluble antagonist was due to unsaturated fats present in the raw kidney bean. Hathcock and Scott (1966) fed chicks a casein-soy-torula yeast diet to study the role of cysteine as the functional compound in prevention of muscular dystrophy in chicks. Sodium selenite and ethoxyquin were included in the diet to prevent exudative diathesis and encephalomalacia while allowing production of muscular dystrophy. Their results

indicated that guanidoacetic acid accelerates the conversion of methionine to cysteine and reduces the severity of muscular dystrophy while creatinine, choline and betaine inhibit the conversion and accentuate the dystrophy. They concluded that cysteine, not methionine, was the metabolically active sulfur amino acid in the prevention of nutritional muscular dystrophy in vitamin E deficient chicks. Hathcock *et al.* (1968a) studied the effects of cysteine and several sulfhydryl compounds on nutritional muscular dystrophy in chicks. They concluded that cysteine is the functional sulfur compound in one of the pathways involved in the prevention of muscular dystrophy in the chick while vitamin E may be involved in another pathway. Hathcock (1968b) studied the role of cysteine in prevention of muscular dystrophy in the chicken with recognition that one of the normal metabolic fates of cysteine is oxidation and decarboxylation to taurine, and taurocholate is the only bile salt produced by the chicken. The rate of cysteine to taurine conversion and the incidence of muscular dystrophy were increased when cholic acid was added to the diet. Taurocholic acid and taurine additions decreased the rate of cysteine to taurine conversion and reduced the occurrence of muscular dystrophy. Vitamin E additions to the basal diet reduced the taurine excretion rate. Scott *et al.* (1967) formulated a practical type corn-soybean meal diet utilizing feedstuffs obtained from areas with known low

selenium soils and without vitamin or methionine additions to the diet. They concluded that selenium is the primary nutritional factor required to prevent myopathies in turkey poults while vitamin E was of less importance and sulfur amino acids were ineffective. They suggested that the selenium requirement of the poult on a practical type diet ranged from 0.18 ppm in the presence of vitamin E to 0.28 ppm of selenium in the absence of added vitamin E. The order of prominence of the "selenium-responsive" myopathies of the young poult appear to be first of the gizzard, second of the myocardium and third of the skeletal muscle. Thompson and Scott (1969), in an attempt to establish selenium as an essential element, reported that chicks fed semi-purified diets were protected against exudative diathesis by supplements of either 10 ppm d-α-tocopheryl acetate or 0.04 ppm selenium. Chicks given crystalline amino acid diets containing less than 0.005 ppm selenium had poor growth and high mortality even when the diet contained up to 200 ppm d-α-tocpheryl acetate. Higher levels of vitamin E prevented mortality, but, even with 1000 ppm, growth was inferior to that obtained with supplements of selenium and no added vitamin E. When diets contained 100 ppm vitamin E (d-α-tocopheryl acetate), the selenium requirement was less than 0.01 ppm, whereas with 10 ppm vitamin E it was 0.02 ppm, and with no added vitamin E it was approximately 0.05 ppm. Thompson and

Scott (1970) conducted further studies with crystalline amino acid diets, containing high levels of vitamin E but less than 0.02 ppm selenium. Selenium deficiency resulted in poor growth, poor feathering and atrophy of the pancreas which resulted in impaired fat hydrolysis and poor absorption of lipids, including vitamin E. They stated that selenium is, thus, an essential trace nutrient and one of its roles is in maintenance of the pancreas. The indirect effect of selenium deficiency on vitamin E absorption does not explain the ability of both selenium and vitamin E to prevent exudative diathesis since a more direct interrelationship is probably responsible for this effect. Noguchi *et al.* (1973a) studied selenium deficiency uncomplicated by vitamin E deficiency in chicks using a crystalline amino acid diet complete in all known nutrients except selenium. Gross signs of deficiency included growth retardation marked by particularly poor growth of muscle, liver, and pancreas. Histochemical and biochemical studies throughout the entire experimental period showed no sign of lysosomal disruption in the acinar cells or in the invading fibroblasts and macrophages, demonstrating that the function of selenium in protecting the pancreas from atrophy and fibrosis does not result from its protection of the lysosomal membranes of the pancreatic cells. Noguchi *et al.* (1973b) continued their studies using a crystalline amino acid diet very

low in selenium and vitamin E and demonstrated that the glutathione peroxidase level of chick plasma is directly related to selenium level in the diet and to the effectiveness of selenium in prevention of exudative diathesis. They suggested that the plasma glutathione peroxidase present, when the diet contains adequate selenium, acts to prevent exudative diathesis by destroying peroxides that may form in the plasma and/or cytosol of the capillary cell. Vitamin E appears to prevent exudative diathesis by acting within the lipid membrane where it neutralizes free radicals, thereby preventing a chain reaction autoxidation of the membrane lipids. Combs and Scott (1974a) determined the dietary requirements of chicks for vitamin E and selenium utilizing a semi-purified basal diet low in selenium and vitamin E. The results of this study showed that both selenium and vitamin E were required to completely protect hepatic microsome membranes from *in vitro* ascorbic acid-stimulated peroxidation. Vitamin E was required at 30 to 50 IU per kilogram diet for optimal growth and utilization of dietary vitamin E as plasma tocopherols in selenium-adequate chicks. Selenium was required at 0.06 ppm (as sodium selenite) for inhibition of peroxidation and for optimal growth in vitamin E-adequate chicks. Combs and Scott (1974b) determined the effects of high-level antioxidant feeding on selenium and vitamin E function in

vitamin E depleted chicks fed a semi-purified basal diet low in selenium and tocopherols. Ethoxyquin, ascorbic acid and vitamin A reduced selenium requirements for growth and prevention of exudative diathesis and mortality in vitamin E deficient chicks. Ethoxyquin and ascorbic acid reduced the dietary vitamin E requirement for prevention of *in vitro* ascorbic acid stimulated peroxidation in hepatic microsomes in selenium adequate chicks.

Vitamin A severely depressed the plasma concentration of tocopherols and increased microsomal peroxidation without response to dietary vitamin E. The results of this study indicated that antioxidants increased the utilization of dietary selenium without severely affecting vitamin E antioxidant function, but vitamin A appeared to antagonize vitamin E absorption directly.

Cantor *et al.* (1975a) studied the biological availability of selenium in feedstuffs and selenium compounds for prevention of exudative diathesis in chicks using a casein-soy-torula yeast basal diet deficient in vitamin E and selenium. Protection against exudative diathesis was highly correlated with plasma glutathione peroxidase activity in chicks fed sodium selenite or selenomethionine, indicating that biological availability was determined by the ability of the chick to utilize the various forms of selenium for enzyme activity. Cantor *et al.* (1975b) evaluated the efficacy of dietary supplements of selenium

for prevention of pancreatic fibrosis in chicks fed a crystalline amino acid basal diet containing .012 ppm of naturally occurring selenium and 15 IU vitamin E/kg diet. Selenomethionine was four times as effective as either selenite or selenocystine in preventing pancreatic degeneration and increasing the relative weight and selenium concentration of the pancreas. Studies on plasma and pancreatic glutathione peroxidase activities did not show any relationship between enzyme activity and prevention of pancreatic fibrosis.

Cattle and sheep. Many experiments have been conducted to study methods of controlling or preventing muscular dystrophy or white muscle disease of young ruminant farm animals in many areas where soil selenium levels are low. A reason for the prevalence of white muscle disease among cattle and sheep may be that, more than any other domestic species, they are likely to consume forages grown on low selenium soils as virtually their entire diet.

Proctor *et al.* (1958) fed a known dystrophogenic diet of raw cull kidney beans and mixed hay to ewes during gestation. Muscular dystrophy in the lambs born to these ewes was prevented by addition of 1 ppm selenium as sodium selenite to the diet or by feeding 100 IU α-tocopheryl acetate per head per day or 0.25 pounds linseed meal per head per day. The protection afforded by linseed

meal was possibly due to its high selenium content (1.18 ppm). Hogue *et al.* (1959) utilized a similar hay and cull kidney bean basal diet and observed that providing lambs every other day with 100 mg α-tocopheryl acetate or 1 mg selenium would control muscular dystrophy. Vitamin E at the rate of 100 IU/ewe/day was adequate but not as effective as 1 ppm selenium in preventing muscular dystrophy of lambs. Welch *et al.* (1960) produced muscular dystrophy in lambs by feeding fish liver oil to ewes during pregnancy and lactation. The fish liver oil lowered ewe and lamb blood plasma vitamin E concentrations and increased the incidence of muscular dystrophy. Vitamin E administration to ewes was effective in preventing and curing muscular dystrophy in lambs. Selenium decreased but did not eliminate the occurrence of muscular dystrophy in lambs. Hintz and Hogue (1964a) compared the addition of combinations of selenium and sulfur to the diet of the ewe and cystine and methionine when given to the lamb on the incidence of muscular dystrophy in the lambs. Selenium (0.17 ppm) given during lactation had no significant effect on clinical incidence of muscular dystrophy but did reduce the number of lambs with elevated SGOT values. Dietary sulfur (0.33%) increased the clinical incidence of muscular dystrophy and, when given in combination with selenium, prevented any beneficial effect of selenium. Administration of cystine or methionine to

lambs was not effective in preventing the nutritional muscular dystrophy.

Ewan *et al.* (1964) fed lambs an artificial milk diet based on stripped lard and torula yeast. Selenium significantly improved total weight gains, rate of gain and survival time. The improvement in total weight gains and survival time due to vitamin E supplementation approached significance ($P<0.1$) but little effect was noted on rate of gain. No interaction between vitamin E and selenium was observed.

Schubert *et al.* (1961) produced white muscle disease in lambs and calves by feeding the dams during gestation on native hays from low selenium areas. The characteristic clinical symptoms of erratic locomotion, cardiac failure and gross and histopathological lesions of skeletal and cardiac muscle degeneration and calcification were observed in preweaning offspring. Supplementation of the dam's ration during gestation through weaning with 0.1 ppm selenium as sodium selenite provided complete protection against the disease while vitamin E did not. Selenium and vitamin E therapy of lambs was effective in preventing grossly observable postmortem lesions but not those detectable microscopically. They suggested that sulfur antagonism could influence the biological availability of trace amounts of selenium which are required. Oldfield *et al.* (1963) reported that white

muscle disease produced in lambs by feeding their dams prenatally and through lactation on an alfalfa hay-oats diet containing less than .02 ppm selenium was prevented by raising the dietary selenium level to 0.06 ppm. Selenium provided in a single parenteral dose from a slow absorption carrier was effective in protecting against deficiency throughout pregnancy, but was less effective when a similar amount was given orally, as sodium selenite, in aqueous solution. Whole blood levels of 0.11 ppm selenium in the ewes and 0.12 ppm selenium in the lambs were compatible with prevention of white muscle disease in this study.

Buchanan-Smith *et al.* (1969) prevented selenium and/or vitamin E deficiency in sheep fed a purified diet containing urea as the sole nitrogen source by giving weekly injections of 5 mg selenium (sodium selenate) and/or α-tocopheryl acetate (700 IU vitamin E). Selenium delayed, but did not prevent, death. Growth was improved by selenium, but satisfactory reproductive performance was obtained only in ewes treated with a combination of vitamin E and selenium.

Swine. Experimental diet-induced deficiencies of selenium and/or vitamin E in swine have generally been produced by feeding diets containing torula yeast as the protein source or diets containing large quantities of unsaturated or rancid fat.

Adamstone *et al.* (1949) first reported a study based on a wheat flour-casein diet with 10% added rancid lard or 50 mg d,l-α-tocopherol/head/day. Gilts fed the basal diet or the basal diet plus rancid lard had severely affected reproductive performance, and pigs born to sows on these diets exhibited wobbly gaits and incoordination of the hind legs. The livers of pigs and their dams contained large amounts of fat and exhibited fibrosis and accumulation of blood in some of the lobules. Vitamin E additions to the basal diet resulted in superior reproductive performance, and no clinical signs or histological lesions were observed in the pigs. Hove and Seibold (1955) utilized a 6% crude protein sucrose-soybean meal diet containing 6% lard and 2% cod liver oil or this diet supplemented with 0.01% d,l-α-tocopheryl acetate to study vitamin E deficiency in pigs. They observed acute hemorrhagic necrosis of the liver in three pigs that died during the study on the vitamin E deficient diet. At slaughter two pigs on this diet exhibited post-necrotic cirrhosis. However, no muscle lesions were observed in any of the pigs. Reid *et al.* (1968) studied a combined protein-vitamin E deficiency syndrome, causing severe liver damage in the pig, by manipulating dietary levels of methionine, choline and selenium. Animals were fed a basal diet containing 3% isolated soy protein and 25% corn oil during the eight week trial. Liver damage was

completely preventable by supplementation with α-tocopherol, or selenium, or both. Choline supplementation aggravated the liver damage while methionine gave partial or complete protection against necrosis and scarring but did not prevent the appearance of hyaline bodies in the hepatocytes. The methionine effect was not related to contamination of the supplemental methionine with selenium.

Eggert *et al.* (1957) utilized a torula yeast basal diet for two to three week old pigs and reported that 67% of the pigs on the basal diet died suddenly within 53 days, and typical lesions of liver necrosis and yellow fat were observed. Supplementation of the basal diet with 1.0 ppm selenium as sodium selenite or 40 ppm α-tocopheryl acetate prevented sudden deaths and gross lesions in animals when sacrificed. Wastell *et al.* (1968) and Ewan *et al.* (1969) studied the effects of selenium and/or vitamin E deficiency in young pigs fed torula yeast or isolated soy protein diets containing 5% fortified cod liver oil. Supplements of selenium or tocopherol or both to the semi-purified diets had no effect on growth rate but did reduce mortality from 54% to 7% and prevented lesions of selenium and/or vitamin E deficiency of pigs. Wastell *et al.* (1972) continued these studies by feeding growing finishing pigs the diets previously described. Pigs fed a torula yeast diet

supplemented with both vitamin E and selenium gained significantly faster than with either vitamin E or selenium alone. Isolated soy protein diets supplemented with vitamin E or selenium alone or in combination had no effect on gain. Pigs fed the torula yeast diets supplemented with selenium alone had edema, hyalinization of skeletal muscles and a yellowish-brown discoloration of body fat and tissues. Pigs fed the torula yeast diets supplemented with both vitamin E and selenium showed liver fibrosis, hyalinized skeletal muscle fibers and degenerative heart myofibrils. No tissue changes were observed in pigs fed a torula yeast diet supplemented with vitamin E alone or in pigs fed the isolated soybean protein diets supplemented with vitamin E and selenium alone or in combination.

Michel *et al.* (1969) fed young pigs a 6% crude protein torula yeast diet containing stripped lard. Dietary hepatic necrosis occurred consistently on the basal diet or this diet supplemented with methionine or low levels of vitamin E. Selenium, high levels of vitamin E or additional protein prevented dietary hepatic necrosis. However, microscopic lesions of nutritional myopathy were observed in some experimental pigs of all singly supplemented groups except those fed vitamin E or ethoxyquin. Selenium did not prevent the myopathy. Sharp *et al.* (1970) reported that torula yeast and high moisture corn

increased the occurrence of muscular dystrophy in young growing pigs. Vitamin E alone or in combination with selenium prevented death losses on deficient diets. Mahan *et al.* (1974) fed a torula yeast semi-purified diet devoid of supplemental vitamin E and selenium to gilts. Sows fed the semi-purified diet exhibited very poor reproduction and had small litters. All piglets were fed a similar semi-purified diet from 21 to 56 days of age. Piglets from selenium supplemented sows did not exhibit clinical or histopathological deficiency signs by 56 days of age. Their results suggested that selenium fed to sows at the supplementary rate of 0.1 ppm effectively delays the onset of vitamin E-selenium deficiency lesions in their progeny.

Numerous studies have reported an induced selenium and/or vitamin E deficiency in swine fed large quantities of fish oils, fat or damaged grains. Obel (1953) observed that up to 14.5% of all diagnoses in her laboratory from 1947 to 1951 were accounted for by a naturally occurring condition she designated *hepatosis diaetetica* in swine submitted for necropsy at the State Veterinary Medical Institute of Sweden. The condition was produced experimentally by feeding a starch-brewers' yeast diet containing 6% cod liver oil. She stated that vitamin E deficiency was the major problem even though Schwarz's Factor 3 might also be involved. Lannek *et al.* (1961)

fed 20-25 kg pigs a sucrose-casein-yeast basal diet with added fat. Nutritional muscular dystrophy in pigs was not observed on the vitamin E-stripped lard diet but did occur on a diet supplemented with cod liver oil which was rich in α-tocopherol. Further supplementation with α-tocopherol prevented nutritional muscular dystrophy and sodium selenite injections lowered plasma transaminase levels in deficient pigs. Orstadius *et al.* (1963) indicated that intramuscular injections of sodium selenite or vitamin E reduced the occurrence of nutritional muscular dystrophy on grain diets containing heated cottonseed oil. They indicated that vitamin E and selenium were synergistic in curing the disease. Grant and Thafvelin (1958) fed weaned pigs a hepatonecrogenic soybean meal diet and observed the effect of supplementing this diet with sodium selenite. All pigs fed the basal diet died between the twenty-second and forty-fifth days of the experiment. The pigs had liver necrosis, massive transudations, degeneration of skeletal and heart muscle, and deposits of ceroid pigment in the adipose tissue. Those supplemented with sodium selenite survived and had normal livers, but degeneration of skeletal muscle and deposits of ceroid in the fat did occur. Thafvelin (1960) and Swahn and Thafvelin (1962) stated that when *hepatosis diaetetica* and muscular degeneration were seen in pigs fed grain, the occurrence of the lesions was associated with the properties of the cereal fat. Heating the grains

increased the peroxide value and reduced the iodine number and vitamin E level in cereal fats. The heated grains or heated maize oil produced nutritional muscular dystrophy and *hepatosis diaetetica* with increased serum transaminase levels. They theorized that heating disrupted the natural antioxidative system of the cereal fat and permitted oxidation of the fatty acids. These changes occur gradually in ground grains stored under usual conditions. Sodium selenite and vitamin E prevented transudation and *hepatosis diaetetica* while selenite decreased muscular degeneration but did not completely prevent it. Lannek *et al.* (1960) studied the effect of moldy grain on selenium-vitamin E deficiency in pigs. Supplementation of the moldy grain diet with d,l-α-tocopheryl acetate (50 ppm) or 0.2 ppm selenium as sodium selenite prevented the occurrence of muscular dystrophy.

Occurrence of deficiencies under practical conditions

Cattle and sheep. A naturally occurring deficiency of selenium and/or vitamin E deficiency in young lambs and calves designated as white muscle disease has been recognized for many years. Muth (1955) described the occurrence of white muscle disease in Oregon and the etiological and pathological changes observed. Hartley and Grant (1961) described a congenital and a delayed white muscle disease in lambs and white muscle disease in

yearling sheep. Selenium treatments improved fertility and cured ill thrift of sheep and cattle. White muscle disease was also diagnosed in calves and foals in New Zealand.

Blaxter (1963) reported that selenium deficiency was less severe in Scotland soils than in New Zealand or deficient areas of the United States. Selenium drenches or injections resulted in small improvements in weight gain in several Scottish flocks. Shirley *et al.* (1966) demonstrated that intramuscular injections of sodium selenite were ineffective in improving weaning weights of calves or rate of gain of lambs in Florida. No clinical signs of white muscle disease were observed in any of the cattle or lambs even though the selenium content of the pastures was low.

Swine. Obel (1953) first observed and reported sudden death in pigs without evidence of illness. She referred to this condition as *hepatosis diaetetica* and a large proportion of the pig deaths in Sweden resulted from this condition. The incidence of the condition occurred most frequently in 6 week old pigs during the fall of the year. Thomke *et al.* (1965) reported that vitamin E and selenium supplementation of practical skim milk and barley diets for pigs from 20 to 90 kg had no effect on growth rate, feed conversion or carcass quality. Vitamin E additions did improve the stability of

subcutaneous fat depots. Lannek *et al.* (1960) and Lindberg and Siren (1965) diagnosed the nutritional muscular dystrophy condition on several farms.

Hartley and Grant (1961) stated that *hepatosis diaetetica* was responsible for pig deaths on at least 20 swine farms in New Zealand. Most outbreaks occurred in areas where selenium-responsive diseases were diagnosed in sheep. Outbreaks usually occurred at weaning time in pigs fed barley-skim milk diets with added cod liver oil. Removal of the cod liver oil from the diet and oral administration of inorganic selenium (5 mg) prevented the occurrence of *hepatosis diaetetica*. Losses have also been controlled by selenium administration when no cod liver oil was being fed.

Michel *et al.* (1969) studied the nature and pathogenesis of dietary hepatic necrosis in pigs from seven Michigan swine herds. The diets on which deficiencies occurred were typical corn-soybean meal diets without added vitamin E. The α-tocopherol and selenium levels of feed samples from two of the farms were extremely low. The analyzed values for α-tocopherol were 4.0 and 6.5 mg per kg diet and selenium concentrations were .04 and .057 ppm. Losses in all the herds ceased following supplementation of the diets with 22 IU vitamin E per kg of diet. Trapp *et al.* (1970) diagnosed a vitamin E-selenium deficiency in 97 pigs from 37 Michigan swine herds. In

most cases, the diets were corn-soybean meal diets supplemented with minerals and vitamins A and D and the water-soluble vitamins. In addition, arsanilic acid was used as a feed additive in most cases. The deficiency was characterized by sudden deaths in feeder pigs and lesions of hepatic necrosis, icterus, edema, hyalinization of arteriole walls and skeletal and cardiac muscular degeneration. Edema was present in most tissues examined, especially in the mesentery of the spiral colon, lungs, subcutaneous tissues and submucosa of the stomach. The acute death losses due to hepatic necrosis and muscular degeneration were prevented by supplementing diets with vitamin E, or injections of selenium-vitamin E, or both, in affected herds.

Klein *et al.* (1970) utilized normal and opaque-2 corn to study the effects of added vitamin E and selenium upon weanling pigs. Supplementation of the diet with selenium and vitamin E alone or in combination had no effect on rate or efficiency of gain. Mahan *et al.* (1971), Cline *et al.* (1973) and Mahan *et al.* (1973) conducted two experiments to evaluate the efficacy of injecting selenium and/or α-tocopherol in preventing vitamin E-selenium deficiency syndrome in young swine. Pigs were obtained from sows kept under confinement conditions and fed natural ingredients. Injections of vitamin E reduced death losses in young pigs while

injections of selenium or selenium plus vitamin E completely prevented death losses. They suggested that selenium played a major role in preventing the deficiency syndrome in the progeny of sows fed natural ingredients. Groce *et al.* (1971) conducted two feeding trials to study the effects of selenium supplementation of practical diets on swine health and prevention of selenium and/or vitamin E deficiency. Classical selenium and/or vitamin E deficiency lesions were observed in two animals which died when fed the unsupplemented corn-soybean meal basal ration. Lesions attributable to selenium and/or vitamin E deficiency were not observed in any of the groups receiving supplemental selenium and/or vitamin E in the diet. Groce *et al.* (1973b) conducted experiments to define the minimum dietary selenium requirement of swine fed corn-soybean meal diets which were low (0.05 ppm) in natural selenium and contained 4.2 mg d-α-tocopherol per kg. No difference in average daily gain or gain/feed could be attributed to the additions of selenium and/or vitamin E. No death losses recognized as being due to selenium-vitamin E deficiency occurred, nor were there gross or histological lesions attributable to dietary treatments.

Ullrey (1973, 1974) reviewed the selenium deficiency problem in swine production and animal agriculture. He stated that the minimum practical level of selenium

supplementation from sodium selenite to prevent deficiencies in confined growing-finishing swine is 0.1 ppm, resulting in a total selenium level in many Midwestern swine diets of about 0.15 ppm. This is 1/50 of the lowest continuously fed dietary selenium level shown to produce toxicity in swine. This level of supplementation appears to be a safe and scientifically sound nutritional practice.

Mahan *et al.* (1974) studied the efficacy of selenium additions to sow diets over two reproductive cycles in preventing selenium deficiency in their progeny. During the second parity, sows receiving the basal corn-soybean meal diet had significantly smaller litters than sows receiving the basal diet supplemented with 0.1 ppm selenium. The histopathologic evaluation of sow tissues obtained after they had weaned their second litters revealed no abnormalities on either the basal or selenium supplemented diets.

Pathology of selenium and/or vitamin E deficiency in swine

Gross and microscopic changes. There is considerable variation in the location and appearance of gross and microscopic lesions. The signs and lesions of vitamin E-selenium deficiency in swine, whether experimentally produced or occurring naturally, have been reviewed by Andrews *et al.* (1968), Green and Bunyan (1969), Obel

(1953), Rosenfield and Beath (1964), Michel *et al.* (1969), Trapp *et al.* (1970) and Ullrey (1974). In swine, the syndrome includes hepatic necrosis, nutritional muscular dystrophy and mulberry heart disease. Pigs weighing 20 to 40 kg often die suddenly and exhibit a bilateral paleness of skeletal muscles. The skeletal muscles most affected are the *gracilis, adductor, quadriceps femoris, psoas* and *longissimus* muscles. Histologically, loss of striations, vacuolization, fragmentation and mineral deposition in muscle fibers is observed. The liver is often swollen and pale with focal lesions that give it a roughened appearance. Histological examination reveals lobules that have undergone marked degeneration and necrosis, while adjacent lobules may appear normal. Damaged lobules exhibit lysis of the hepatic cells and dilatation of sinusoids with blood which appears as extensive intralobular hemorrhage. Many of the pigs exhibit icterus and occasional mottling and dystrophy of the myocardium. Edema occurs in the mesentery of the spiral colon, lungs, subcutaneous tissues and submucosa of the stomach (Michel *et al.*, 1969; Trapp *et al.*, 1970) and are believed to be useful in diagnosing selenium and/or vitamin E deficiency in swine. The lesions of the deficiency in young pigs have been described in many of the trials discussed above when the deficiency occurred under experimental or natural conditions.

Groce *et al.* (1971) described classical selenium and/or vitamin E deficiency lesions in two animals which died when fed an unsupplemented corn-soybean meal basal ration. Chronic fibrosis of the liver was observed in another pig from the basal group at slaughter. Mahan *et al.* (1973) reported that pigs from sows kept under confinement conditions and fed natural ingredients displayed classical lesions of the vitamin E-selenium deficiency syndrome. Necropsy observations revealed a white pale striation in the skeletal muscle and in the heart muscle, a yellow discoloration in the body fat, degenerative and hobnail livers, enlarged hearts and a generalized edema of internal tissues.

Mahan *et al.* (1974) described deficiency lesions in sows and their progeny over two reproductive cycles. Vitamin E-selenium deficiency lesions were observed in the skeletal muscle and gastric area of the stomach in sows fed semi-purified diets. Esophago-gastric ulcers and histopathologic deficiency lesions occurred in progeny of the sows by 56 days of age. The progeny of sows that died during the experiment exhibited white striations of the skeletal muscles, *hepatosis diaetetica*, enlarged flaccid hearts and a generalized edema of the internal tissues and organs. Histopathologic deficiency lesions were observed in the myocardium in most but not all animals.

Clinical pathology of selenium and/or vitamin E deficiency of swine. Plasma, serum and cellular hematological measures have been studied in relation to selenium and/or vitamin E deficiency in swine as possible diagnostic criteria. Rahman *et al.* (1960) observed that serum albumin to globulin ratios were lower in chicks with exudative diathesis. This depression was completely prevented by adding 0.05 ppm selenium to the diet. The severe edema and exudation observed in exudative diathesis suggests that one might expect a lower albumin to globulin ratio but it has not been consistently demonstrated. Klein *et al.* (1970) reported that erythrocyte hemolysis tests, which have formed the basis for the biological assay of vitamin E in rats, was not an effective diagnostic criterion of selenium-vitamin E deficiency in swine. Michel *et al.* (1969) and Trapp *et al.* (1970) indicated that standard hematological measures were of little use in diagnosing selenium-vitamin E deficiency in swine.

Plasma or serum enzymes have commonly been used to assess the development and diagnosis of selenium-vitamin E deficiency in swine.

Enzyme assays have been used extensively by research groups in Sweden as diagnostic criteria of selenium-vitamin E deficiency. Wretlind *et al.* (1959) reported that skeletal and heart muscle contained large amounts of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) but were very low in ornithine

carbamyl transferase (OCT) while liver was very high in OCT. They suggested that these enzyme parameters might be useful in differentiating between muscle damage and liver damage in living animals. Orstadius *et al.* (1959) reported elevated plasma GOT, GPT and OCT values in cases of "liver dystrophy" of pigs. The elevations occurred early in the course of the disease and declined gradually with time. In cases of muscular dystrophy pigs exhibited elevated plasma GOT and GPT levels early in development of the deficiency with a gradual decrease with time as was observed in situations of "liver dystrophy." Michel *et al.* (1969) reported an increase in serum OCT levels in pigs with dietary hepatic necrosis. Lannek *et al.* (1960), Augustinssen *et al.* (1960) and Ewan and Wastell (1970) have reported similar changes of plasma GOT, GPT, and OCT enzymes. They suggested that plasma GOT and OCT were better indicators of selenium and/or vitamin E deficiency in swine since plasma GPT may be elevated in several other swine diseases. Lannek *et al.* (1961), Orstadius *et al.* (1963), Ewan and Wastell (1970), Groce *et al.* (1971), Mahan *et al.* (1971), Wastell *et al.* (1972), Groce *et al.* (1973b), Mahan *et al.* (1973) and Mahan *et al.* (1974) have used serum or plasma GOT activity to assess the stage of development of selenium-vitamin E deficiency in swine. Ewan and Wastell (1970) and Wastell *et al.* (1972) reported elevated serum lactic

dehydrogenase (LDH) activity in dietary induced selenium and/or vitamin E deficiency in young pigs. They suggested that serum LDH activity may be useful in diagnosing nutritional muscular dystrophy in swine since Blincoe and Marble (1960) and Paulson *et al.* (1968) have reported that elevated serum LDH activity is a sensitive indicator of subclinical nutritional muscular dystrophy in young lambs.

The discovery of a specific function of selenium as a component of glutathione peroxidase in animals has been described by Rotruck *et al.* (1973) and Hoekstra (1974). Decreases in gluathione peroxidase appear to explain at least some of the degenerative diseases induced by selenium deficiency. The usefulness of this enzyme as a convenient indicator of selenium adequacy has been suggested by its relation to the prevention of exudative diathesis in chicks (Noguchi *et al.*, 1973b) and by its relation to selenium status of rats (Chow and Tappel, 1974; Hafeman *et al.*, 1974) and sheep (Oh *et al.*, 1974). However, experiments with swine to determine the relationship of tissue and blood glutathione peroxidase levels to selenium adequacy or as a diagnostic aid in selenium-vitamin E deficiency have not been reported. This enzyme may become a useful diagnostic criterion of selenium adequacy of swine in the future.

Selenium levels in swine blood and tissues

In establishing the essentiality of selenium, it has been important not only to understand the metabolic role of selenium but also to assess the levels of selenium that occur in animal tissues. Lindberg and Siren (1963, 1965) reported kidney selenium concentrations for normal pigs (10.98 ppm), pigs with nutritional muscular dystrophy (3.40 ppm) and pigs with dietary hepatic necrosis (3.33 ppm) on a dry basis. Liver selenium concentrations of normal pigs ranged from 0.52 to 2.12 ppm while pigs with muscular dystrophy ranged from 0.13 to 0.28 ppm and pigs with liver dystrophy ranged from 0.13 to 0.24 ppm expressed on a dry basis. Lindberg (1968) observed similar tissue concentrations in later experiments. Lindberg and Lannek (1965) supplemented a commercial basal diet containing 0.126 ppm selenium with 1.2 ppm selenium as sodium selenite. Selenium supplementation for 78 days did not increase kidney selenium concentrations but did increase liver and muscle selenium concentrations over levels observed for pigs on the basal diet. Withdrawal of selenium for 14 days decreased muscle selenium levels back to control levels and liver selenium levels returned nearly back to control levels. Andrews *et al.* (1968) obtained liver and kidney selenium concentrations of 0.047 and 0.53 ppm, respectively, on a wet basis in necropsied pigs which had died of *hepatosis diaetetica.*

Sharp *et al.* (1970) observed that tissue selenium levels in growing-finishing pigs were comparable to tissue levels in deficient and selenium supplemented pigs previously discussed. They reported that added vitamin E increased kidney selenium levels while decreasing liver, muscle and heart selenium levels. This tendency was observed on both unsupplemented and selenium sulfide supplemented diets. A liver selenium level of 0.2 ppm selenium on a dry basis was the minimum level compatible for protection against selenium responsive diseases and liver selenium levels were a more sensitive indicator of selenium status than kidney selenium levels were.

Ewan (1971) studied the effects of vitamin E and selenium supplementation on tissue composition of young pigs fed torula yeast diets. Muscle and liver selenium concentrations of pigs receiving the deficient diet decreased significantly during the eight week feeding period. Muscle selenium concentrations were higher in pigs fed selenium supplemented diets than pigs on unsupplemented diets but all diets resulted in lower concentrations than the initial muscle selenium concentrations.

Liver selenium levels followed a similar pattern. Kidney selenium concentrations were highest (7.50 ppm) in selenium fed animals. Vitamin E had no significant effect on the level of selenium in the tissues, but deficiency symptoms were completely prevented by supplementation with vitamin E.

Groce *et al.* (1971) reported that selenium supplementation (1.0 ppm) of a basal corn-soybean meal diet significantly increased muscle selenium concentrations even though the levels on selenium supplemented diets were lower than levels reported for porcine muscle in selenium adequate areas. Muscle selenium levels did not decrease significantly in 30 days but did increase significantly by 65 days after the termination of selenium suppplementation.

Ku *et al.* (1972) reported a significant linear correlation of 0.95 between dietary selenium level and the selenium concentration of *longissimus* muscle of pigs from each of 13 locations in the United States. The selenium values of the diets ranged from 0.027 ppm to 0.493 ppm on an air dry basis while selenium concentrations of the *longissimus* muscle ranged from 0.046 to 0.521 ppm on a fresh basis. No relationship was apparent between α-tocopherol concentration and tissue selenium concentration. Ku *et al.* (1973) studied the effects of supplementation of naturally high selenium swine diets with 0.1 ppm of selenium from sodium selenite. Sodium selenite additions to the naturally high selenium diets did not significantly increase *longissimus* muscle or kidney selenium concentrations and increased liver selenium concentrations only slightly. Tissue selenium levels resulting from adding 0.40 ppm of selenium from

sodium selenite to a naturally low selenium diet (0.04 ppm) were significantly lower than when a naturally high selenium diet (0.44 ppm) was fed. They concluded that the dietary level of naturally occurring organic selenium compounds is much more significant in influencing the tissue selenium concentration of the pig than is supplemental selenium from sodium selenite. Groce *et al.* (1973a) observed that serum and whole blood selenium levels on seleniferous corn diets were significantly lower than on selenite selenium diets. Supplemental vitamin E had no significant effect on serum erythrocyte or whole blood selenium concentration.

Groce *et al.* (1973b) conducted an experiment to define the minimum dietary selenium requirement of swine fed corn-soybean meal diets which were low (0.05 ppm) in natural selenium and contained 4.2 mg d-α-tocopherol per kilogram. Selenium concentrations increased significantly in *longissimus* muscle and liver with increasing dietary selenium to the supplemental level of 0.1 ppm. Myocardium selenium was highest with 0.2 ppm supplemental selenium while kidney selenium did not increase above that resulting from 0.05 ppm supplemental selenium. Selenium in whole blood, erythrocytes and serum was significantly increased by selenium supplementation, but there were no differences between supplemental levels. Selenium withdrawal reduced selenium levels in all

tissues as compared to those from pigs fed selenium to slaughter. Vitamin E supplementation decreased erythrocyte selenium concentration and increased kidney selenium concentrations.

Selenium Balance in Swine

There are many factors that affect the absorption and excretion of selenium. Lindberg and Lannek (1965) stated that studies with physiologically compatible levels of selenium indicated that animals will retain inorganic selenium in proportion to their physiological needs. Once these physiological stores are filled, the animal will excrete the remainder unless detoxification and excretory mechanisms are overwhelmed, in which case significant quantities of selenium will be retained and selenium intoxication may result. Groce (1972) has reviewed the literature on selenium balance in animals. Much of the work reported on absorption and tissue distribution, excretion and secretion of selenium was cited in this review. The amount of research conducted on selenium balance in swine has been extremely limited.

Grant *et al.* (1961) reported that orally ingested inorganic selenium followed generally the same pattern of tissue distribution and excretion as parenterally-administered selenium materials. Wright and Bell (1966), using radioactive sodium selenite, found that selenium was resecreted into the first section of the small

intestine and then reabsorbed from succeeding sections of the small intestine to result in a net absorption of selenite selenium of 86%.

Buescher *et al.* (1961) reported that excessive calcium levels in normal selenium diets did not alter the excretion or tissue distribution of oral ^{75}Se in swine. The urinary route of excretion appears to be more important with inorganic forms than with naturally occurring selenium in food.

Groce *et al.* (1971) studied the influence of added dietary selenium upon selenium excretion and retention. Their results indicated that young pigs retained a decreasing percentage of dietary selenium as levels of supplemental selenium increased but the absolute daily retention of selenium was similar for both 0.1 and 0.5 ppm of added selenium. Urinary excretion of selenium increased markedly at the higher level of selenium supplementation. Groce *et al.* (1973a) conducted balance trials with young pigs to compare supplements (0.2 ppm Se) of natural selenium (from seleniferous corn) or supplements of selenite selenium and a supplement of 22 IU of vitamin E per kg of diet versus no E supplementation. A higher proportion of selenium from seleniferous corn was excreted in the urine as compared to selenium from sodium selenite. Vitamin E supplementation significantly reduced fecal selenium excretion of pigs fed seleniferous

corn. Groce *et al.* (1973b) studied selenium absorption and retention on diets containing several levels (0, 0.05, 0.1 and 0.2 ppm) of added selenium from sodium selenite. The effect of a supplement of 22 IU of vitamin E per kg of diet was also studied. Selenium retention was maximized at 0.1 ppm of supplemental selenium. Vitamin E supplementation appeared to increase urinary selenium excretion at the supplemental selenium level of 0.2 ppm. Therefore, based on tissue selenium levels and selenium retention and excretion patterns, 0.15 ppm selenium was required in a corn-soybean meal diet unsupplemented with vitamin E. Serum selenium levels plateaued at the 0.1 ppm supplemental selenium level, indicating the possible existence of tissue and serum thresholds for selenium from sodium selenite in the pig.

Interrelationships of Selenium with Sulfur, Vitamin E and Other Dietary Factors

It is apparent from the literature already cited that the metabolic role of selenium in animals is linked with that of vitamin E, sulfur amino acids and other dietary factors.

Vitamin E

The literature discussed in previous sections indicates that some diseases associated with low concentrations of selenium in the diet (less than 0.1 ppm) are analogous to those of vitamin E deficiency and are

usually observed under conditions of low intakes of vitamin E. In some cases these syndromes respond fully to administration of vitamin E while in others selenium was substantially more effective or induced a further response. Many of the studies indicate that, although selenium cannot replace vitamin E in nutrition, it reduces the amount of tocopherol required and delays the onset of symptoms of deficiency.

Sulfur amino acids

Sulfur amino acids may also influence the need for vitamin E and selenium. Obel (1953) reported that sulfur-containing amino acids prevented liver dystrophy in pigs. However, many of Obel's studies that reported a protective effect of sulfur amino acids were questioned because the commercial L-cystine she used was usually contaminated with selenium. Schwarz (1965) used uncontaminated sulfur amino acids to show that sulfur amino acids delayed the onset of dietary necrotic liver degeneration in rats. He concluded that this effect was mediated by a sparing action of sulfur amino acids in the vitamin E requirement. Reid *et al.* (1968) reported that methionine partially prevented liver damage in pigs and that selenium contamination was not a significant factor in the protective action of methionine. On the other hand, sulfur amino acids do not appear to prevent the development of muscular dystrophy in lambs (Erwin *et al.*, 1961). In general,

sulfur amino acids moderate the effects of feeding diets deficient in these nutrients but cannot substitute for them. The efficacy of these amino acids may be explained by their contribution to the antioxidant activity of tissues.

Polyunsaturated fat

The muscular dystrophy producing property of polyunsaturated fatty acids has been experimentally demonstrated by several workers (Obel, 1953; Lannek *et al.*, 1961; Lindberg and Orstadius, 1961; Orstadius *et al.*, 1963). Thafvelin (1960) and Lannek *et al.* (1960) produced nutritional muscular dystrophy experimentally in pigs fed grain obtained from areas where natural outbreaks of the disease had occurred. Thafvelin (1960) suggested that the fats of the grains were the cause of the deficiencies and that in grain causing nutritional muscular dystrophy, the vitamin E content was reduced. Swahn and Thafvelin (1962) demonstrated that in grain which had induced nutritional muscular dystrophy, the fat was unstable under oxidizing conditions. Oksanen (1967) indicated that the changes in fatty acid composition, fat quality and vitamin E content of grain influence the occurrence of nutritional muscular dystrophy in pigs and that a low selenium concentration is a prerequisite for a spontaneous occurrence of nutritional muscular dystrophy.

Synthetic antioxidants

Mertz and Schwarz (1958) reported that the synthetic antioxidant N,N'-diphenyl-p-phenylenediamine was highly active in preventing "respiratory decline" (indicative of liver necrosis) when administered intraportally to rats deficient in selenium and vitamin E. Machlin *et al.* (1959) demonstrated that certain synthetic antioxidants are also capable of preventing exudates in chicks, even though they were significantly lower in efficacy than vitamin E. Hill (1963) reported that ethoxyquin, a synthetic antioxidant, protected the tissues of the pig from increased thiobarbituric acid test values and from increased hemolysis which are usually associated with low vitamin E status. Tollerz and Lannek (1964) reported that synthetic antioxidants restored resistance to iron toxicity under vitamin E deficiency situations.

Iron. Lannek *et al.* (1962) produced iron hypersensitivity experimentally in piglets by feeding the sows a vitamin E deficient diet during pregnancy and lactation. The LD_{50} of iron preparations was reported to be greatly lowered in vitamin E deficient baby pigs. The resistance to iron toxicity may be restored by the administration of α-tocopherol (Lannek *et al.*, 1962) and selenium (Arpi and Tollerz, 1965). Miller *et al.* (1973) reported that the administration of a second intramuscular injection of 100 mg of iron from iron dextran gave no evidence of iron

toxicity or anaphylaxis. Feeding of oral iron at levels up to 600 ppm in the diet showed no evidence of iron toxicity. An intramuscular injection of 1000 mg iron from iron dextran gave no evidence of iron toxicity. In these experiments, iron toxicity was not demonstrated in pigs having low vitamin E-selenium reserves, raised on diets low in vitamin E and selenium.

Arsenic. Underwood (1971) stated that the beneficial effects of various organic arsenicals on the growth, health and feed efficiency of poultry and swine have been thoroughly established. These effects have been reviewed by Frost and his associates (Frost, 1953, 1967; Frost *et al.*, 1955). Arsanilic acid, 4-nitrophenylarsonic acid, 3 nitro-4-hydroxyphenylarsonic acid and arsenobenzene are the four organic arsenic compounds which have been used and are of value in animal production. No clearly evident relationship exists between structure and the ability to promote growth. The arsonic acids are recognized as growth stimulants for pigs and poultry, but the phenylarsenoxides are more potent than the arsonic acids as coccidiostats. The action of arsonic acids closely resembles that of antibiotics and is partially complementary to it, but the exact mechanism is not known.

Frost *et al.* (1955) stated that the arsonic acids differ markedly in tolerance, in coccidiostatic power and in power to promote growth. In general, toxicity appears

to be related to the amount of arsenic that was deposited in the tissues.

Hanson *et al.* (1955) studied the value of arsanilic acid as a growth stimulant for pigs and measured the arsenic retention in the tissues. Small amounts of arsenic were retained in the tissues and the amount retained was related to the level fed. Arsenic retention in the liver was 1.5 to 2.0 times as great as the storage in the kidneys. The amount retained in the muscle, fat or skin was low at all levels of arsanilic acid fed. Removal of arsanilic acid from the diet resulted in rapid excretion of arsenic from the liver and kidneys. The rate of excretion from muscle was less rapid.

Mogareidge (1963) studied the differences in availability to the growing rat of protein bound arsenic and the inorganic trivalent form. At 16 ppm of dietary arsenic, both sources gave rise to significant tissue storage although that from arsenic trioxide was somewhat higher. The protein bound source resulted in equal urinary and fecal excretions but with the inorganic source urinary was greater than 2 times the fecal excretion.

Overby and Frost (1960) studied the rate of arsenic excretion of swine receiving arsanilic acid in the diet. Much more arsenic was excreted in the feces than in the urine. After the arsanilic acid was withdrawn from the

ration, the characteristic excretion level continued for 2 days and then decreased rapidly. This was in agreement with knowledge of the rate of disappearance of arsenic from tissues of animals fed arsanilic acid. Unchanged arsanilic acid was not detected in the urine, but was present in the feces in an amount representing about 5% of the arsanilic acid consumed.

Thus it appears that toxic arsenic compounds are retained in the tissues in greater amounts and are excreted more slowly than the less toxic forms. The arsenic of organic compounds such as arsanilic acid is well absorbed and deposited in tissues of pigs and chicks in amounts proportional to the level fed. However, it rapidly disappears from the tissues upon withdrawal and most is excreted in the feces.

Arsenic has been successfully used to alleviate selenium poisoning in cattle, dogs, chicks and swine for many years.

Moxon (1938) first demonstrated that 5 ppm As in the drinking water would completely prevent all signs of selenosis in rats. Moxon (1944) reported that 25 ppm as sodium arsenate when added to salt partially protected cattle on seleniferous ranges.

Levander and Baumann (1966a,b) observed that the excretion of selenium into the gastrointestinal tract via the bile fluid was markedly increased and retention in

the liver, blood and carcass was greatly decreased, when subacute injections of arsenic were given with an injection of selenium.

Ganther and Baumann (1962) reported that arsenic administration increased the excretion of an injected dose of selenium into the gastrointestinal tract within an hour. However, administration of the arsenic and selenium, more than one hour apart, would not show this effect (Palmer and Bonhorst, 1957).

Levander and Argrett (1969) compared the effects of arsenic, mercury, thallium and lead on the metabolism of selenium. Arsenic inhibited the pulmonary excretion of volatile selenium compounds by rats injected with subacute doses of sodium selenate. The biliary excretion of selenium was stimulated sevenfold by the administration of arsenic. Arsenic improved the growth of rats chemically poisoned by selenium, largely prevented the liver damage caused by selenium and decreased the amount of selenium retained in the tissues. Urinary selenium excretion was not affected by As injection in these studies.

With high levels of selenium, arsenic reduces the loss of selenium in expired air (Olson *et al.*, 1963).

Sodium arsenite and arsenate have been found to be equal in effectiveness of prevention of selenosis. The arsenic sulfides are not effective and various organic arsenic compounds have been found to provide only a

partial protection against selenosis (Hendricks *et al.*, 1953; Kuttler and Marble, 1961; Wahlstrom *et al.*, 1955; Wright, 1940).

The beneficial effects of dietary arsenic additions in selenium toxicities produced by selenium salts or seleniferous grains have been demonstrated in swine by Wahlstrom *et al.* (1955, 1956), and Wahlstrom and Olson (1959a,b) and in poultry (Carlson *et al.*, 1954; Carlson *et al.*, 1962; Thapar *et al.*, 1969).

The mechanism by which arsenic provides protection against selenium toxicity is not yet well established.

Muth *et al.* (1971) suggested that a selenium-arsenic complementary effect exists in selenium deficiency situations. The feeding of 1.00 ppm arsenic in the form of sodium arsenate, when added to a low selenium ration for pregnant ewes, gave a significant marked protection against the myopathy commonly associated with selenium deficiency in lambs.

Although arsenic may be beneficial at low levels, arsenic can be very toxic and requires proper handling and use at all times. Frost (1967) and Schroder and Balassa (1966) have reviewed the literature on arsenic toxicity. These reviews emphasize that arsenicals are non-carcinogenic and that wide differences in the toxicity of different chemical forms of arsenic exist. In general, inorganic arsenicals are more toxic than the organic forms. It seems that enzymes are far more susceptible to

trivalent than to pentavalent arsenicals and bind similar levels of As at the point of death. Arsenicals are not cumulative in most animal tissues. The differences in toxicity are related to their rate of excretion.

Vitamin E Studies in Swine

Although vitamin E is stored in the liver and other tissues, it is sometimes possible but often difficult to produce unmistakable deficiency signs by long periods of dietary vitamin E depletion. For many years it was considered that swine fed diets composed mainly of corn or cereal grains had no danger of developing vitamin E deficiency, since high-quality green feeds, whole cereal grains and the germ of cereal grains are good sources of vitamin E (tocopherols). The various tocopherols differ in their biological activity, with d-α-tocopherol being the most active. Cereal grains contain about equal amounts of α-tocopherol and other tocopherols and since vitamin E is easily oxidized, its concentration deteriorates in ground feeds.

In recent years, many cases of vitamin E deficiency have been reported in swine fed diets composed of feedstuffs grown over a wide geographic area. Specific information regarding the significance of vitamin E in farm-animal nutrition is especially limited and the dietary needs for various species are not well defined.

Bratzler *et al.* (1950) mantained 5 barrows on a vitamin E-low, purified ration for 75 days. Three of the animals received a supplement of a concentrate of mixed tocopherols at levels of 2.87, 55.12 and 110.2 mg daily per kg liveweight. Tocopherol supplementation increased significantly the total tocopherol content in all organs and tissues studied. The increases were most marked in the case of liver and body fat. The gamma-plus delta-tocopherol content increased significantly only in the case of whole blood. Tocopherol supplementation markedly affected the fatty acid composition of the body fats by increasing the percentage of oleic acid at the expense of the saturated fatty acids. Hove and Seibold (1955) reported that 0.01% dl-α-tocopheryl acetate (150 mg of tocopherol per day) added to a synthetic semi-purified diet had no effect on the growth rate of pigs. Fatal liver necrosis was observed in growing pigs fed the basal diet but pigs receiving diets supplemented with α-tocopheryl acetate failed to show appreciable liver damage at slaughter.

Rousseau *et al.* (1957) fed graded levels of tocopherol 0, 1.0, 3.0 and 9.0 mg per lb of liveweight daily added to basal rations of calves, lambs and pigs for 12 weeks. Plasma tocopherol was found to increase at diminishing rates with tocopherol intake whereas liver tocopherol increased at constant rates with tocopherol intake.

Tocopherol intake was found to have no measurable effect on level of daily gain. Eggert *et al.* (1957) fed a purified-type, torula yeast diet or the basal diet supplemented with 40 ppm tocopheryl acetate. Four pigs receiving the basal diet died within 53 days and showed a marked necrosis of the liver. No deaths occurred among the pigs receiving vitamin E and no marked difference in growth rate was noted in favor of vitamin E supplementation. Forbes and Draper (1958) utilized 37 baby pigs to produce and study a deficiency of vitamin E. Only under the conditions of a stress provided by at least 5% cod liver oil in the diet was an unmistakable deficiency produced. The deficiency was not accompanied by changes in EKG or by ability of dialuric acid to hemolyze red blood cells. Supplementation of vitamin E (10 mg of dl-α-tocopherol acetate per 100 grams dry matter) prevented the appearance of vitamin E deficiency symptoms. Garton *et al.* (1958) reared weanling pigs to about 130 pound liveweight on a basal diet supplemented with 5 or 10 mg tocopheryl acetate/kg body weight/day. The animals given tocopherol did not exhibit changes in serum levels of the vitamin and no liver lesions were observed in any of the pigs. The serum tocopherol levels were lower in pigs slaughtered at 200 pounds liveweight.

Johnson and Alaupovic (1960) administered alpha tocopherol intraperitoneally to 175 pound pigs. They

isolated two metabolites of tocopherol from liver. Neither metabolite was identical with alpha tocopheryl metabolites commonly found in urine. Duncan *et al.* (1960) fed weanling pigs for 8-10 weeks on a basal low-fat, low-tocopherol diet or a similar diet containing lard (5%) and each diet was given with or without supplementary dl-α-tocopheryl acetate. The supplemented pigs received 20 mg tocopheryl acetate per kg bodyweight per day. At slaughter, the plasma tocopherol values of the pigs given supplementary tocopherol were significantly higher than those of unsupplemented animals. The effect of tocopherol supplementation did not depend on whether or not lard was included in the diet. Thus the absorption of the dietary tocopherol was not dependent on the simultaneous digestion and absorption of fat. Histological examination of the livers of animals, not given supplementary tocopherol, showed no significant signs of deficiency.

Leat (1961a) investigated the role of tocopherol in the nutrition of the pig reared from weaning to slaughter on a low-fat, low-tocopherol diet or this diet supplemented with 5 or 10 mg per 100 g diet of α-tocopheryl succinate. There was no difference in growth rate between the groups and no abnormality in any organs or carcasses at slaughter. Plasma-tocopherol levels were two to thirteen times higher in the animals given tocopherol than in the controls. There was no difference between groups in the content of

unsaturated fatty acids of back fat, plasma, liver or heart. The peroxide levels in the back fat and liver lipids of the control animals were four to fifty times greater than that found in the animals given tocopherol. He concluded that the major function of tocopherol in the pig is to protect body lipids from oxidation. Leat (1961b) reported that supplementation of a basal diet with 10 mg α-tocopheryl succinate per 100 grams diet significantly decreased the peroxide content of the body lipids. He suggested that although the pig does not require dietary tocopherol for optimum growth up to 200 pounds, its presence will lessen the susceptibility of body fats to oxidative rancidity.

EFFECT OF ARSANILIC ACID, SELENIUM AND VITAMIN E ON PRODUCTIVE PERFORMANCE, TISSUE SELENIUM CONCENTRATIONS AND SELENIUM BALANCE IN GROWING-FINISHING SWINE

Introduction

Selenium-vitamin E deficiency in swine has been recognized as a practical field problem in confinement-reared swine in Michigan and other Midwestern states. The problem has been described by Michel *et al.* (1969), Trapp *et al.* (1970), Groce *et al.* (1971, 1973b), and Mahan *et al.* (1971, 1973, 1974). Many pigs are being raised in complete confinement without access to pasture. Corn and soybean meal are the primary ingredients used in formulating swine feeds in the midwestern United States. Swine diets made of corn and soybean meal have resulted in low intakes of α-tocopherol. The selenium content of feedstuffs varies widely in different geographical areas of the United States (Patrias and Olson, 1969; Ku *et al.*, 1972). Groce *et al.* (1971) demonstrated the efficacy of low levels of supplementary selenium from sodium selenite in preventing death losses and clinical signs or lesions of selenium-vitamin E deficiency in the growing pig. Groce *et al.* (1973b) attempted to define the minimum dietary selenium requirements of

growing-finishing swine fed corn-soybean meal diets. They concluded that the minimum practical level of selenium supplementation from sodium selenite to prevent deficiencies in confined growing-finishing swine is 0.1 ppm, resulting in a total selenium level in many midwestern swine diets of about 0.15 ppm.

Trapp *et al.* (1970) observed natural selenium-vitamin E deficiency cases in swine and reported that most herds involved were feeding corn-soybean meal diets supplemented with arsanilic acid as a feed additive. Arsanilic acid has been demonstrated to counteract selenium toxicity in swine (Wahlstrom *et al.*, 1955). Trapp *et al.* (1970) suggested that arsanilic acid may have been binding selenium and thus enhanced the occurrence of a natural selenium-vitamin E deficiency.

The purpose of this investigation was to study the effects of supplemental arsanilic acid, selenium and vitamin E on productive performance, deficiency symptoms, hematology, tissue selenium and selenium balance in growing-finishing pigs fed diets low in vitamin E and selenium.

Experimental Procedure

Three experiments were conducted in this investigation. Experiment 1 was designed to study the response of growing pigs to added dietary selenium (Se), vitamin E and arsanilic acid. Experiments 2 and 3 were selenium

balance studies conducted with young pigs (5 weeks of age) to study the effects of added vitamin E and arsanilic acid on the retention and excretion of selenium.

Experiment 1

Eighty Yorkshire, Hampshire and Yorkshire x Hampshire barrows and gilts weighing an average of 8.4 kg were randomly allotted to eight dietary treatments and housed 10 per pen in a completely enclosed, slotted floor, environmentally controlled building. The pigs utilized in this study were offspring of sows which had received no supplemental vitamin E or selenium during gestation or lactation. The basal diet (Table 1) was a 16% crude protein fortified corn-soybean meal diet which analyzed 0.036 ppm Se and contained no supplemental vitamin E, selenium or antibiotics. The following diets were fed *ad libitum*: (1) basal (Table 1) (0.036 ppm Se); (2) basal + 0.1 ppm Se as sodium selenite;[1] (3) basal + 22 IU E[2] per kilogram of diet; (4) basal + 99 ppm arsanilic acid; (5) basal + 0.1 ppm Se + 22 IU E per kilogram; (6) basal + 0.1 ppm Se + 99 ppm arsanilic acid; (7) basal + 22 IU E per kilogram + 99 ppm arsanilic acid; and (8) basal + 0.1 ppm Se + 22 IU E per kilogram + 99 ppm

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[1]Alfa Inorganics, Ventron Corporation, Beverly, Massachusetts.

[2]Myvamix-Type 125. Vitamin E as d-α-tocopheryl acetate with dextrin. Distillation Products Industries, Rochester, New York.

TABLE 1. COMPOSITION OF BASAL DIETS (EXPERIMENT 1)

Ingredient	International ref. no.	Grower[d]	Finisher
Corn, dent yellow, grain, gr 2 US mn 54 wt (4)	4-02-931	79.2	86.7
Soybean, seed wo hulls, solv-extd grnd, mx 3 fbr (5) (soybean meal)	5-04-612	17.9	10.4
Calcium phosphate, dibasic, comm(6)	6-01-080	1.0	1.1
Limestone, grnd, mn 33 Ca (6)	6-02-632	0.9	0.8
Salt, plain white		0.5	0.5
Vitamin-trace mineral premix[a]		0.5	0.5
		100.0	100.0
Crude protein, %[b]		16.0	13.0
Calcium, %[b]		0.64	0.60
Phosphorus, %[b]		0.50	0.50
Se, ppm as fed[c]		0.036	0.040

[a]Provided the following per kilogram of diet: vitamin A, 3,300 IU; vitamin D, 660 IU; riboflavin, 3.3 mg; nicotinic acid, 17.6 mg; d-pantothenic acid, 13.2 mg; choline chloride, 110 mg; vitamin B_{12}, 19.8 µg; zinc, 74.8 mg; manganese, 37.4 mg; iodine, 2.7 mg; copper, 9.9 mg; iron, 59.4 milligrams.

[b]Calculated

[c]By analysis

[d]Basal diet for experiments 2 and 3 also

arsanilic acid. After 12 weeks, the pigs weighed an average of 52 kg and the basal diet was changed to the 13% crude protein corn-soybean meal diet shown in Table 1. Selenium was withdrawn from diets being supplemented at the end of 12 weeks of the trial since the Food and Drug Administration of the U.S. Department of Health, Education and Welfare had specified a 60-day minimum withdrawal period before carcasses of those pigs fed supplemental Se could be utilized for human consumption. Arsanilic acid was withdrawn from diets being supplemented 8 days prior to slaughter. The pigs were weighed and feed consumption determined at biweekly intervals. Tap water (1 part per billion [ppb] Se) was offered *ad libitum*.

All animals were observed closely for clinical signs of selenium-vitamin E deficiency throughout the course of the experiment. Blood samples were collected from the anterior vena cava from two pigs from each of the 8 treatment groups initially, at two weeks and 8 weeks and from all pigs two days prior to slaughter. Hemoglobin (Crosby *et al.*, 1954) and microhematocrit (McGovern *et al.*, 1955) determinations were made on heparinized whole blood. Serum glutamic-oxaloacetic transaminase (SGOT) (Sigma Technical Bulletin, 1964) was determined on all serum samples collected. Whole blood and serum samples were stored at -20 C until Se analyses could be performed.

Liver, kidney and diaphragm muscle samples were obtained from all pigs at slaughter, placed in plastic bags and frozen at -20 C until analyzed for Se. Diaphragm muscle was homogenized with twice its weight of deionized, distilled water to facilitate uniform sampling and pipetting. Liver and kidney were homogenized with three and five times their weight of water, respectively. Homogenates were stored at -20 C until analyzed for Se. The diets were ground twice through a stainless steel screen with 2 mm diameter openings in a Wiley mill[3] and stored at -20 C until analyzed for Se. Selenium analyses of blood, serum, tissues and diets were conducted fluorometrically according to the method of Hoffman *et al.* (1968) with the following modifications described by Groce *et al.* (1971). The glassware used for Se analyses was segregated and used only for this procedure. Upon completing a set of analyses, the glassware was rinsed several times in flowing distilled water, rinsed with a 1:1 mixture of concentrated nitric and sulfuric acids, rinsed 3 or 4 times with flowing deionized distilled water, and then inverted for drying in a 37 C forced air warm room. The digestion was carried out in 100 ml semi-micro Kjeldahl flasks[4] as described by Hoffman *et al.*

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[3]Thomas-Wiley Mill, Model ED-5. Arthur H. Thomas Company, Philadelphia, Pennsylvania.

[4]Aminco No. 4-1874, American Instrument Company, Silver Spring, Maryland.

(1968) after a 12 to 14 hour predigestion in the complete digestion acid mixture as described by Olson (1969). This predigestion period reduces charring problems during subsequent digestion. The 2,3-diamino-naphthalene solution and all succeeding preparations containing it were handled in dim or yellow light and produced a more stable preparation. In the complexing step the various reagents were combined in a 250 ml Phillips beaker and swirled by hand. After the one hour incubation period, the reaction mixture was extracted with 6 ml of cyclohexane in a 125 ml separatory funnel for 15 minutes. The fluorescence was read using an Aminco-Bowman Spectrophotofluorometer[5] with an excitation monochromator setting of 372 mμ and an emission monochromator setting of 516 millimicrons. Standards were prepared from Se metal[6] dissolved in a minimal quantity of redistilled concentrated nitric acid and made up to volume with 0.1 N sulfuric acid. The fluorometer was zeroed against a reagent blank carried through the entire procedure.

This method resulted in a linear standard curve up to 0.5 μg Se which consistently passed through the origin. A more thorough and complete description of the selenium analytical procedure is reproduced in Appendix A. The

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[5]American Instrument Co., Inc., Silver Spring, Maryland.

[6]Alfa Inorganics, Inc., Beverly, Massachusetts.

percent of dry matter was determined on diets and tissue homogenates by drying for 15 hr at 60 C with a vacuum of 740 millimeters.

Experiment 2

Six male and six female crossbred and Yorkshire pigs whose dams had received no supplemental Se or vitamin E during gestation and lactation were used to study the effect of added dietary selenium as sodium selenite, vitamin E as d-α-tocopheryl acetate and arsanilic acid upon Se excretion and retention. The basal diet was the 16% crude protein corn-soybean meal diet (Table 1) which analyzed 0.036 and 0.040 ppm Se for phase 1 and 2, respectively, of this balance study. The pigs were randomly allotted to the following treatments: (1) basal (Table 1); (2) basal + 0.1 ppm Se; (3) basal + 99 ppm arsanilic acid; (4) basal + 0.1 ppm Se + 99 ppm arsanilic acid. The pigs weighed an average of 8.3 kg initially and 8.7 kg at the beginning of phase 2. The pigs were housed in stainless steel metabolism cages for an 11 day adjustment period and a subsequent 3 day collection period when consuming a constant, near *ad libitum* intake of feed offered as a gruel with deionized water in three meals per day. Phase 2 began immediately upon ending of the first collection period. At this time, 22 IU of E were added per kilogram of all diets and the dietary treatments were identical as in phase 1. A 6 day

adjustment period to the diets was followed by a second 3 day collection of urine and feces. Urine was collected in 6 N hydrochloric acid separate from the feces. The feces were air dried on trays and were ground before analysis. Blood and serum were collected initially and at the end of the first and second collections and stored at -20 C until analyzed for Se and SGOT. The feces, urine, whole blood, serum and diets were analyzed for Se content as described in experiment 1.

Experiment 3

Four male and eight female crossbred and Hampshire pigs whose dams had received no supplemental Se or vitamin E during gestation and lactation were used to study the effects of added dietary selenium as seleniferous corn, vitamin E as d-α-tocopheryl acetate and arsanilic acid upon Se excretion and retention. The basal diet was the 16% crude protein corn-soybean meal diet (Table 1) which analyzed 0.054 and 0.056 ppm Se, respectively, for phase 1 and 2 of this experiment. The pigs were assigned to one of four dietary treatments: (1) basal (Table 1); (2) basal + 99 ppm arsanilic acid; (3) basal + 0.1 ppm Se from seleniferous corn; and (4) basal + 0.1 ppm Se from seleniferous corn + 99 ppm arsanilic acid. The seleniferous corn was provided by Dr. Oscar Olson, Department of Agricultural Chemistry, South Dakota State University, Brookings, and had been

harvested in 1960 in a seleniferous area of that state. Selenium analysis indicated that this corn contained 24.8 ppm Se which agreed well with the values reported for this corn by Groce *et al.* (1973a). The seleniferous corn was finely ground and substituted for 0.4% of the basal diet to provide the supplemental Se in diets 3 and 4. The pigs weighed an average of 7.6 kg initially and 8.1 kg at the beginning of phase 2. The pigs were housed in stainless steel metabolism cages for a 10 day adjustment period followed by a 3 day collection of excreta.

The diets were finely ground and fed three times daily in near *ad libitum* quantities with deionized water to form a gruel. Phase 2 began upon completion of the first collection period. All diets were supplemented with 22 IU E per kilogram and the dietary treatments remained the same as in phase 1. An adjustment period to the diets of 4 days was followed by a second 3 day collection of excreta. Urine was collected in 6 N hydrochloric acid separate from the feces. The feces were air dried on trays and were ground before analysis. Blood and serum were collected initially and at the end of the first and second collections. Hemoglobin and microhematocrits were determined on heparinized whole blood as described in experiment 1. Serum samples were stored at -20 C until analyses for Se could be conducted. Selenium analyses of diets, feces, urine and serum

samples were conducted fluorometrically according to the method described in experiment 1.

Statistical analyses were conducted utilizing least squares analysis of variance and two-way and three-way analysis of variance as outlined by Steel and Torrie (1960).

Results and Discussion

Experiment 1

The performance of pigs in experiment 1 is summarized in Table 2. No significant differences in final weight, average daily gain or feed conversion could be attributed to the dietary treatments in this experiment. Similar results were obtained by Groce *et al.* (1971, 1973b). Arsanilic acid supplementation increased final weights and average daily gains in pigs in these treatments but the main effect was not significant. During the course of this experiment two male pigs on the basal + 99 ppm arsanilic acid treatment died suddenly during the 8th week of the experiment exhibiting symptoms of selenium-vitamin E deficiency. In addition, after 12 weeks on experiment, one male and one female pig on the basal diet (0.036 ppm Se) died suddenly with lesions of selenium-vitamin E deficiency. The lesions observed in these pigs will be discussed briefly. The gross lesions observed upon necropsy of these pigs were a paleness and

TABLE 2. EFFECT OF DIETARY SELENIUM, VITAMIN E AND ARSANILIC ACID SUPPLEMENTATION UPON PIG PERFORMANCE AND LIVER WEIGHT (EXPERIMENT 1)

Item	Dietary Treatments							
	Basal	Basal +Se[a]	Basal + E[b]	Basal +As.a[c]	Basal +Se +E	Basal +Se +As.a	Basal +E +As.a	Basal +Se +E +As.a
No. of pigs	8	10	8	8	10	9	10	10
Initial wt., kg	7.9	8.4	8.3	8.4	8.4	8.5	8.4	8.4
Final wt., kg	93.0	87.6	90.9	95.0	86.1	90.2	89.3	93.8
Daily gain, kg	0.57	0.57	0.56	0.58	0.55	0.55	0.58	0.61
Daily feed, kg	1.71	1.65	1.66	1.73	1.56	1.56	1.70	1.76
Gain/feed	0.34	0.34	0.34	0.34	0.36	0.35	0.34	0.35
Liver wt., kg	1.35	1.51	1.44	1.44	1.43	1.49	1.35	1.45
Liver wt., % of live weight[d]	1.34	1.50	1.47	1.40	1.46	1.52	1.38	1.38

[a] 0.1 ppm Se

[b] 22 IU vitamin E

[c] 99 ppm arsanilic acid

[d] Liver wt., % of live weight significantly ($P<.05$) lower on unsupplemented selenium diets and a significant ($P<.05$) selenium x vitamin E interaction.

edematous condition in the skeletal muscles. There were pale areas in the heart muscle and diffusely mottled and focal hemorrhages or areas of extensive necrosis in the liver. Esophagogastric ulcers, hyperemia and hemorrhage of the fundic portion of the stomach were observed. Mucous membranes had icterus and the spiral colon and lungs were markedly edematous. Histopathologic examination revealed necrotic skeletal muscle fibers with macrophages, mineralization, swelling and fragmentation. The myocardium exhibited subepicardial hemorrhage, fragmentation, loss of striations, focal areas of necrosis and mineral deposition and excess extrafibrillar and perivascular mononuclear cells. The most apparent microscopic lesions were those in the liver, consisting of necrosis and hemorrhage of clusters of adjacent lobules with increased interlobular fibrous connective tissue deposition. Interlobular edema was observed in histologic sections of lung tissue. The cardia of the stomach exhibited loss of stratified squamous epithelium with necrosis, fibrin, polymorphonuclear cells and thrombi in the submucosa. The postmortem diagnostic conclusion in all cases was *hepatosis diaetetica* (vitamin E-selenium deficiency) and esophagogastric ulceration. No sudden death losses occurred in pigs fed diets containing supplemental selenium and/or vitamin E and no gross lesions attributable to treatment were observed at

slaughter. Carcass data collected at slaughter were not significantly affected by any of the dietary treatments and were considered normal values for present day growing-finishing swine. Liver weight (Table 2) was not significantly affected by the dietary treatments in this study but liver weight expressed as a percent of live weight was significantly ($P<.05$) lower on unsupplemented selenium diets. A significant ($P<.05$) interaction between selenium and vitamin E with regard to liver weight as a percent of live weight did occur. Added vitamin E to unsupplemented selenium diets increased liver weight as a percent of live weight but decreased this parameter when vitamin E was added to selenium supplemented diets.

The effects of supplemental, vitamin E and arsanilic acid on initial, 2 week and 8 week blood parameters are presented in appendix Table 1. Initial, 2 week and 8 week hemoglobins, hematocrits and SGOT values were not different among treatments. Serum selenium at 2 and 8 weeks was ($P<.01$) higher on selenium supplemented diets.

The effects of supplemental selenium, vitamin E and arsanilic acid on the final blood parameters are presented in Table 3. Selenium supplementation ($P<.01$) increased serum selenium levels at the final bleeding even though selenium had been withdrawn from the diets for 60 days. This finding is contrary to the results reported by

TABLE 3. EFFECT OF ADDED DIETARY SELENIUM, VITAMIN E AND ARSANILIC ACID ON FINAL BLOOD PARAMETERS (EXPERIMENT 1)

Item	Supplemental Se, ppm		Supplemental E		Arsanilic acid, ppm		EMS[a]
	0	0.1	0	22 IU/kg	0	99	
No. of pigs	26	37	29	34	32	31	
Hemoglobin, g/100 ml	12.7	13.3	13.2	12.9	13.8[c]	12.3	4.3
Hematocrit, %	36.1	37.9	36.5	37.4	40.4[d]	33.6	32.4
No. of pigs	34	39	35	38	36	37	
SGOT, S-F units/ml	39.2	35.8	38.7	36.4	35.6	39.4	103.7
Serum Se, ppm	.035[b]	.058	.046	.047	.046	.046	.0001

[a]Overall error mean square

[b]Serum Se were significantly (P<.01) higher on Se supplemented diets

[c]Hemoglobin was significantly (P<.01) lower on arsanilic acid supplemented diets

[d]Hematocrit significantly (P<.01) lower on arsanilic acid supplemented diets

Groce *et al.* (1973b), who reported that serum Se levels of pigs receiving the basal diet were not different from those of pigs receiving Se supplemented diets from which Se was withdrawn 60 days before slaughter. Arsanilic acid supplementation decreased ($P<.01$) hemoglobin and hematocrit values at the final bleeding. The significance of this finding is uncertain at this time. SGOT values were not affected by dietary treatment in this experiment. All values were in the range of normal values reported by Wretlind *et al.* (1959) and were lower than values reported by Orstadius *et al.* (1959) for deficiency symptoms in pigs. Supplemental vitamin E had no effect on any of the blood parameters measured in this experiment.

Tissue selenium levels as influenced by supplementary selenium, vitamin E and arsanilic acid are presented in Table 4. Selenium supplementation increased ($P<.01$) liver, kidney and diaphragm muscle Se concentrations on both a wet and dry basis even though selenium had been withdrawn from supplemented diets for 60 days. These tissue Se values are lower than those reported by Groce *et al.* (1973b) for pigs having received Se followed by a 60 day withdrawal period. Lindberg and Lannek (1965) supplemented a Se adequate diet (0.126 ppm natural Se) with 1.2 ppm Se from sodium selenite which was fed to growing pigs for 78 days. Skeletal muscle Se

TABLE 4. EFFECT OF ADDED DIETARY SELENIUM, VITAMIN E AND ARSANILIC ACID ON TISSUE SELENIUM, PPM (EXPERIMENT 1)

Item	Supplemental Se, ppm		Supplemental E		Arsanilic acid, ppm		EMS[a]
	0	0.1	0	22 IU/kg	0	99	
No. of pigs	34	32	32	34	31	35	
Liver							
Wet	0.134[b]	0.197	0.167	0.165	0.162	0.170	.0005
Dry	0.468[b]	0.697	0.584	0.581	0.567	0.599	.0054
Kidney							
Wet	1.063[b]	1.386	1.235	1.214	1.232	1.217	.0793
Dry	4.685[b]	6.411	5.557	5.539	5.524	5.572	1.4141
No. of pigs	16	22	19	19	20	18	
Diaphragm muscle							
Wet	0.049[b]	0.078	0.065	0.062	0.058[c]	0.069	.0001
Dry	0.174[b]	0.277	0.229	0.223	0.205[c]	0.247	.0022

[a]Overall error mean square

[b]Selenium levels for all tissues analyzed were significantly ($P<.01$) higher on selenium supplemented diets.

[c]Diaphragm muscle selenium levels were significantly ($P<.05$) higher on arsanilic acid supplemented diets on both a wet and dry basis.

concentrations were 0.52 and 0.76 ppm for the basal and supplemental groups, respectively. Supplemental Se was withdrawn for 14 days and muscle Se concentrations declined to levels similar to those from pigs on the basal diet. Grant *et al.* (1961) observed no difference in muscle Se concentrations of pigs fed 0.2 ppm supplemental sodium selenite and those of pigs fed no Se supplement. They observed little effect of duration of Se supplementation or length of withdrawal period on tissue Se concentration. Similar tissue Se values have been reported by Lindberg and Siren (1963, 1965) and Sharp *et al.* (1970). Andrews *et al.* (1968) and Sharp *et al.* (1970) reported tissue Se levels for pigs dying of selenium-vitamin E deficiency that were lower than those of pigs on the basal diet in this experiment. In addition Se supplementation decreased ($P<.05$) kidney dry matter percentage. Kidney dry matter was ($P<.05$) lower on selenium supplemented versus unsupplemented diets (21.7 vs. 22.6%). The significance of this observation is not apparent, but this difference in tissue dry matter could be due to minor fluctuations of fluid balance in the animal.

Arsanilic acid supplementation increased ($P<.05$) diaphragm muscle selenium levels on both a wet and dry basis. Supplements of vitamin E had no significant effect on tissue Se levels in this experiment.

Experiment 2

The main effects of supplemental Se and arsanilic acid on Se balance and blood Se levels for the two collection periods are presented in Table 5. In the first collection period, selenium supplementation increased ($P<.01$) Se intake, percent Se retention and absolute Se retention. This increase in Se retention resulted primarily because fecal Se excretion (as a percent of intake) was markedly ($P<.01$) reduced, and secondly because urinary Se excretion was lower on selenium supplemented diets, but this difference was not statistically significant. Arsanilic acid supplementation significantly increased ($P<.05$) percent Se retention and there was a significant ($P<.01$) interaction between selenium and arsanilic acid. The addition of arsanilic acid to unsupplemented Se diets increased percent Se retention but decreased percent Se retention when added to selenium supplemented diets. This interaction can be attributed to the ($P<.05$) interaction between selenium and arsanilic acid in percent fecal Se excretion on unsupplemented Se diets but increased percent fecal Se excretion on Se supplemented diets. Serum and whole blood Se levels were higher ($P<.01$) on selenium supplemented diets at the end of phase 1.

In the second collection period (following the addition of 22 IU of vitamin E per kg of diet), Se intake

TABLE 5. EFFECT OF ADDED DIETARY SELENIUM, VITAMIN E AND ARSANILIC ACID ON SELENIUM BALANCE IN THE YOUNG PIG (EXPERIMENT 2)

Item	Supplemental Se, ppm		Arsanilic acid, ppm		S.E.[a]
	0	0.1	0	99	
No. of pigs	6	6	6	6	
Phase 1 - No added vit. E					
Se intake, µg/day	8.5[b]	25.2	16.9[c]	16.8	2.6
Se retention, % of intake	34.0[b]	50.6	36.9[c]	47.6	2.7
Se retention, µg/day	3.0[b]	12.8	7.6	8.1	0.8
Se excretion, % of intake					
Fecal	52.0[d]	37.1	48.7	40.5	2.8
Urinary	14.0	12.3	14.4	11.9	0.9
Phase 2 - 22 IU vit. E/kg diet					
Se intake, µg/day	11.6[e]	36.3	24.0	23.9	0.5
Se retention, % of intake	32.2	45.8	33.5	44.5	5.0
Se retention, µg/day	3.7[e]	16.6	9.2	11.1	1.3
Se excretion, % of intake					
Fecal	54.2	39.7	51.3	42.7	5.1
Urinary	13.5	16.0	16.7	12.8	2.3
Serum Se, ppm					
Initial	.032	.027	.030	.030	.003
End of phase 1	.033[e]	.079	.059	.053	.005
End of phase 2	.028[e]	.084	.056	.056	.004

TABLE 5 (CONTINUED)

Item	Supplemental Se, ppm		Arsanilic acid, ppm		S.E.[a]
	0	0.1	0	99	
Whole blood Se, ppm					
Initial	.054[e]	.048	.051	.050	.005
End of phase 1	.054[e]	.087	.072	.069	.004
End of phase 2	.048[e]	.099	.073	.074	.004

[a]Standard error

[b]Se intake, Se retention (% of intake) and Se retention (μg/day) significantly (P<.01) higher on Se supplemented diets

[c]Se retention (% of intake) significantly (P<.05) higher on arsanilic acid supplemented diets and there was a significant (P<.01) interaction of selenium and arsanilic acid

[d]Fecal Se excretion significantly (P<.01) lower on selenium supplemented diets and there was a significant (P<.05) interaction of selenium and arsanilic acid

[e]Se intake, retention (μg/day), serum Se and whole blood Se significantly (P<.01) higher on selenium supplemented diets at the end of phases 1 and 2.

and absolute selenium retention were increased ($P<.01$) by selenium supplementation of the diet. There were no significant differences in the proportion of Se intake excreted in the feces or urine. However, the observed increase in absolute retention must have resulted from the reduced but not significant ($P=.077$) reduction in percent fecal Se excretion resulting from dietary Se supplementation. Serum and whole blood Se levels were increased ($P<.01$) by selenium supplementation of the diets at the end of the second collection period.

In comparing the data from the two collection periods in this experiment, the addition of supplemental dietary vitamin E tended to increase percent urinary excretion of supplemental dietary selenium and thus lowered the percentage of dietary Se retained. The absolute retention of Se increased slightly from the first to second collections from 12.3 to 16.6 µg per day on Se supplemented diets which may be a reflection of the increase in Se intake from 25.2 to 36.3 µg/day. The comparison of the effect of vitamin E involves a time span, and thus the effects of supplemental vitamin E are confounded with the effect of period. These results are similar to those of Groce *et al.* (1973b) in which diets were supplemented with 0.1 ppm Se. Groce *et al.* (1973a) reported research that supported the hypothesis that these differences may truly represent a vitamin E effect

since supplemental dietary vitamin E lowered selenite Se retention and a trend for increased urinary Se loss was evident in all trials reported. Comparison of the data from the two collection periods revealed that the effect of arsanilic acid supplementation of the basal diet on Se excretion and retention was similar to and parallels the changes discussed attributable to selenium supplementation of the diet.

Experiment 3

The main effects of dietary Se supplementation (as seleniferous corn) and arsanilic acid supplementation on Se balance and serum Se levels for the two collection periods are presented in Table 6. In the first collection period, selenium supplementation increased ($P<.01$) Se intake, percent Se retention and absolute Se retention. The increase in Se retention can be attributed to the decreased ($P<.01$) urinary Se excretion on Se supplemented diets and the lower non-significant fecal Se excretion. Arsanilic acid increased ($P<.01$) absolute Se retention and there was a significant ($P<.01$) interaction between selenium and arsanilic acid. Arsanilic acid supplementation decreased absolute Se retention on unsupplemented Se diets while it increased absolute Se retention on Se supplemented diets. Serum Se levels had increased ($P<.01$) on selenium supplemented diets by the end of the first collection period.

TABLE 6. EFFECT OF ADDED DIETARY SELENIUM (SELENIFEROUS CORN), VITAMIN E AND ARSANILIC ACID ON SELENIUM BALANCE IN THE YOUNG PIG (EXPERIMENT 3)

Item	Supplemental Se, ppm		Arsanilic acid, ppm		S.E.[a]
	0	0.1	0	99	
No. of pigs	6	6	6	6	
Phase 1 - no added vit. E					
Se intake, µg/day	14.2[b]	38.2	25.3	27.2	3.7
Se retention, % of intake	38.3[b]	50.7	42.4	46.7	2.9
Se retention, µg/day	5.5[c]	19.3	11.1[c]	13.7	0.5
Se excretion, % of intake					
Fecal	50.7	42.9	49.3	44.3	3.1
Urinary	11.0[d]	6.4	8.4	9.0	0.5
Phase 2 - 22 IU vit. E/kg diet					
Se intake, µg/day	16.9[e]	46.4	28.4	34.9	7.2
Se retention, % of intake	47.4	55.7	51.0	52.1	3.2
Se retention, µg/day	8.0[f]	25.9	14.6[f]	19.3	1.4
Se excretion, % of intake					
Fecal	43.6	38.3	41.6	40.3	3.0
Urinary	9.0[g]	6.0	7.4	7.6	0.5

TABLE 6 (CONTINUED)

Item	Supplemental Se, ppm		Arsanilic acid, ppm		S.E.[a]
	0	0.1	0	99	
Serum Se, ppm					
Initial	.028[h]	.025	.027	.026	.002
End of phase 1	.024[h]	.040	.031	.032	.002
End of phase 2	.020[h]	.050	.036	.035	.003

[a]Standard error

[b]Se intake and Se retention (% of intake) ($P<.01$) higher on Se supplemental diets

[c]Se retention (μg/day) significantly ($P<.01$) affected by Se supplementation, arsanilic acid supplementation and a selenium x arsanilic acid interaction

[d]Urinary excretion lower ($P<.01$) on selenium supplemented diets

[e]Se intake ($P<.01$) higher on Se supplemented diets

[f]Se retention (μg/day) ($P<.01$) higher from Se supplementation and higher ($P<.05$) on arsanilic acid diets and there was a significant interaction ($P<.05$) of selenium and arsanilic acid

[g]Urinary excretion ($P<.01$) lower on Se supplemented diets and a significant ($P<.05$) interaction of selenium and arsanilic acid

[h]Serum Se ($P<.01$) higher on Se supplemented diets at end of phases 1 and 2

In the second collection period (after adding 22 IU vitamin E per kg of diet) Se intake and absolute Se retention were higher ($P<.01$) on selenium supplemented diets. This increase in absolute Se retention is attributable to the decreased ($P<.01$) urinary Se excretion on Se supplemented diets and the lower but non-significant fecal excretion of Se. Arsanilic acid increased ($P<.05$) absolute Se retention and a selenium x arsanilic acid interaction ($P<.05$) was observed in that arsanilic acid supplementation resulted in the same absolute Se retention on unsupplemented Se diets while increasing absolute Se retention on Se supplemented diets. Furthermore, there was a significant ($P<.05$) interaction of selenium and arsanilic acid in regard to urinary Se excretion. Arsanilic acid decreased urinary Se excretion on unsupplemented Se diets but increased urinary Se excretion on Se supplemented diets. Serum Se levels were higher ($P<.01$) on Se supplemented diets at the end of the second collection period.

Comparison of the data from the two collection periods in this experiment revealed that dietary vitamin E decreased urinary and fecal Se excretion on supplemented Se (seleniferous corn) diets and accordingly increased the percent Se retention and absolute Se retention. The effects of vitamin E supplementation are confounded with the effect of period since the two collections were conducted at different times. However, Groce *et al.* (1973a) reported similar results in that added vitamin E increased

absolute retention of Se from seleniferous corn (22.5 vs. 31.7 μg per day). The increased Se retention from seleniferous corn in their study, when vitamin E was added to the diet, was due primarily to decreased fecal Se excretion. The effect of arsanilic acid supplementation of the basal diet on Se excretion and retention paralleled the changes observed due to selenium supplementation of the diet for the two collection periods in this experiment.

The results of experiments 2 and 3 are in excellent agreement with the results reported by Groce *et al.* (1973a). When supplemental Se was from seleniferous corn, a significant higher percentage of Se ingested was excreted via the feces and less via the urine as compared to Se from sodium selenite. They reported a significant interaction in which added vitamin E reduced the absolute retention of selenite Se and increased the absolute retention of Se from seleniferous corn. The increased Se retention from seleniferous corn, when vitamin E was added to the diet, was due to decreased fecal Se excretion in their study but primarily due to decreased urinary and fecal Se excretion in my experiment 3. Fecal Se could be non-absorbed dietary Se or endogenous Se secreted into the lumen of the intestine (Wright and Bell, 1966) and therefore increased Se absorption or reduced endogenous fecal Se secretion may have been altered by supplemental vitamin E to cause an increase in Se retention from seleniferous corn. In addition they observed that serum

Se levels were lower on seleniferous corn as compared to selenite Se and supplemented vitamin E had no significant effect on serum Se levels. The decrease in serum Se levels resulting from seleniferous corn may have been due to decreased intestinal absorption or to increased clearance of serum Se to tissues. McConnell and Hoffman (1972) have demonstrated that selenium in the form of selenomethionine is readily incorporated into rat hepatic proteins via the methionine pathway. Groce *et al.* (1971, 1973a,b) and Ku *et al.* (1972, 1973) have shown that a given concentration of Se naturally occurring in feeds will result in greater Se retention and higher tissue Se levels than similar dietary Se levels from sodium selenite.

The results of experiment 2 also provide additional support for the observation of Groce *et al.* (1973b) that supplemental dietary vitamin E lowers selenite selenium retention primarily by promoting the urinary excretion of selenite Se, since a trend for increased urinary loss was evident in their trials and in experiment 2 of this study. Sharp *et al.* (1970) reported that supplements of vitamin E to selenite containing diets increased kidney Se levels and noted that decreased liver and muscle Se concentrations were associated with the elevation of kidney Se.

Arsanilic acid was not antagonistic to physiological requirement levels of dietary selenium in this study.

Although hemoglobin and hematocrit values were lower on arsanilic acid supplemented diets (Exp. 1), the physiological significance of this observation is not clear. Arsanilic acid increased diaphragm muscle Se concentrations (even though Se had been withdrawn for 60 days) and had no effect on liver or kidney Se concentrations. The balance studies demonstrated that arsanilic acid decreases Se excretion and increased Se retention. These data indicate that a normal usage level of arsanilic acid is not antagonistic to selenium at nutritional levels and support the suggestion by Muth *et al.* (1971) that a selenium-arsenic complementary effect exists in selenium deficiency situations. In their study, feeding 1.00 ppm arsenic in the form of sodium arsenate, when added to a low selenium ration for pregnant ewes, gave a significant marked protection against the myopathy commonly associated with selenium deficiency in lambs. The data from this study suggest that discrimination against the use of arsanilic acid as a feed additive in rations containing nutritional levels of natural selenium or in rations supplemented with 0.1 ppm Se from inorganic sodium selenite for growing-finishing pigs in low selenium areas of the United States is unwarranted.

Summary

One feeding trial and two balance studies were conducted to study the effects of arsanilic acid, selenium

(Se) and vitamin E (E) on productive performance, tissue selenium and selenium balance in growing-finishing pigs fed diets low in vitamin E and selenium. Eighty weanling pigs were assigned to eight dietary treatments in a 2 x 2 x 2 factorial design to evaluate the effects of supplementing a basal corn-soybean meal diet (0.036 ppm Se) with 0.1 ppm Se (as sodium selenite) and/or 22 IU E per kg of diet and/or 99 ppm arsanilic acid. Selenium supplements were withdrawn from Se supplemented diets 60 days before slaughter. Classic Se and/or vitamin E deficiency lesions were observed in four animals fed diets not supplemented with Se or vitamin E. Selenium supplementation increased ($P<.01$) liver, kidney and diaphragm muscle Se concentrations, while arsanilic acid supplementation increased ($P<.05$) diaphragm muscle selenium concentrations. Vitamin E supplements had no effect on tissue Se levels. The two balance studies were concerned with Se absorption and retention on diets supplemented with Se as sodium selenite or seleniferous corn as determined by measurement of Se intake and fecal and urinary Se excretion. The effects of arsanilic acid and vitamin E (22 IU E/kg diet) were also studied. Supplemental Se from seleniferous corn increased percent fecal Se excretion and decreased percent urinary Se excretion as compared to Se from sodium selenite. Vitamin E supplementation increased Se retention from seleniferous

corn by decreasing urinary and fecal Se excretion. Arsanilic acid decreased Se excretion and increased Se retention. Arsanilic acid was not antagonistic to physiological requirement levels of dietary selenium in this study.

EFFECT OF DIETARY VITAMIN E LEVEL ON PERFORMANCE, TISSUE SELENIUM AND SELENIUM BALANCE IN GROWING-FINISHING SWINE

Introduction

Natural deficiencies of vitamin E and selenium (Se) have been reported by Michel *et al.* (1969), Trapp *et al.* (1970), Groce *et al.* (1971, 1973b) and Ullrey (1973, 1974). When vitamin E was shown to be essential for small laboratory animals, investigations were carried out with large animals to determine whether supplementary tocopherols were required under practical conditions. Carpenter and Lundberg (1949) reported that swine experiments up to 1949 had shown that tocopherols were not a limiting dietary factor under field conditions. Prior to 1973, the National Research Council stated that practical swine diets are not likely to be deficient in vitamin E unless the diet contains excessive amounts of highly unsaturated fatty acids or oxidized fats. Furthermore, since tocopherols are present in almost all cereal grains, little research has been conducted to determine the effects of supplemental dietary vitamin E in swine. The National Research Council (1973) suggests that 11 IU of vitamin E be added per kg of diet until more specific

information is obtained on vitamin E and selenium requirements of swine.

Preliminary estimates of the dietary selenium requirement for the pig have been reported by Groce *et al.* (1973b). These studies indicated that the dietary selenium requirement for the pig when supplemented vitamin E does not exceed 11 IU/kg is 0.15 ppm Se. These studies also indicated a need for further research to study the interrelationship of Se with vitamin E. Although vitamin E-selenium deficiencies primarily affect the young growing pig, Hartley and Grant (1961) and Mahan *et al.* (1974) have reported that deficiencies also occur in the finishing pig and the sow.

The purpose of this investigation was to study the effects of supplemental Se and 4 dietary levels of vitamin E supplementation on productive performance, hematology, tissue selenium and selenium balance in growing-finishing pigs fed diets low in vitamin E and selenium.

Experimental Procedure

Two experiments were conducted in this investigation. Experiment 1 was designed to study the response of growing pigs to added dietary selenium and 4 levels of vitamin E. Experiment 2 was a selenium balance study conducted with young pigs to study the effects of 4 added vitamin E levels on the retention and excretion of selenium.

Experiment 1

Sixty-four Yorkshire, Hampshire and Yorkshire x Hampshire barrows and gilts weighing an average of 11.7 kg were randomly allotted to eight dietary treatments and housed eight per pen in a completely enclosed, slotted floor, environmentally controlled building. The pigs were offspring of sows which had received no supplemental vitamin E or selenium during gestation or lactation. The basal diet (Table 7) was a 16% crude-protein fortified corn-soybean meal diet which analyzed .054 ppm Se and contained no supplemental vitamin E or selenium. The pigs were fed this basal diet or the basal diet supplemented with 0.1 ppm Se (as sodium selenite) and vitamin E at 0, 5, 10 or 15 IU vitamin E per kg of diet in a 2 x 4 factorial design of treatments. The pigs were weighed and feed consumption determined at biweekly intervals. Tap water (1 part per billion [ppb] Se) was offered *ad libitum*. All animals were observed for clinical signs of selenium-vitamin E deficiency throughout the course of the experiment. Blood samples were collected from the anterior vena cava from 5 pigs from each of the 8 treatment groups initially, at 10 weeks and 2 days prior to slaughter. Microhematocrit (McGovern *et al.*, 1955) and hemoglobin (Crosby *et al.*, 1954) determinations were made on heparinized whole blood and serum glutamic-oxaloacetic transaminase (SGOT) (Sigma Technical Bulletin 505, 1964) were determined

TABLE 7. COMPOSITION OF BASAL DIET (EXPERIMENT 1)

Ingredient	International ref. no.	Percent
Corn, dent yellow, grain, gr 2 US mn 54 wt (4)	4-02-931	79.2
Soybean, seed wo hulls, solv-extd grnd, mx 3 fbr (5) (soybean meal)	5-04-612	17.9
Calcium phosphate, dibasic, comm (6)	6-01-080	1.0
Limestone, grd, mn 33 Ca (6)	6-02-632	0.9
Salt, plain white		0.5
Vitamin-trace mineral premix[a]		0.5
		100.0
Crude protein, %[b]		16.0
Calcium, %[b]		0.64
Phosphorus, %[b]		0.50
Se, ppm[c]		.054

[a]Provided the following per killogram of diet: Vitamin A, 3,300 IU; vitamin D, 660 IU; riboflavin, 3.3 mg; nicotinic acid, 17.6 mg; d-pantothenic acid, 13.2 mg; choline chloride, 110 mg; vitamin B_{12}, 19.8 µg; zinc, 74.8 mg; manganese, 37.4 mg; iodine, 2.7 mg; copper, 9.9 mg; iron, 59.4 milligrams.

[b]Calculated

[c]Analyzed

on all serum samples collected. Serum samples were stored at -20 C until Se analyses were performed. *Rectus abdominus* muscle, liver, and kidney samples were obtained from 5 pigs from each treatment group at slaughter, placed in plastic bags and frozen at -20 C until analyzed for Se content. Preparation of tissue samples for selenium analyses was as described in Chapter III; Experimental Procedure - Experiment 1. Selenium analyses of serum, tissues and diets were conducted fluorometrically according to the method of Hoffman *et al.* (1968) with the modifications described by Groce *et al.* (1971) and which were previously discussed in Chapter III - Experimental Procedure, Experiment 1.

Experiment 2

Seven male and five female crossbred pigs whose dams had received no supplemental Se or vitamin E during gestation or lactation were used to study the effect of dietary vitamin E level as d-α-tocopheryl acetate upon Se excretion and retention. The basal diet was the 16% crude protein fortified corn-soybean meal diet (Table 7) supplemented with 0.1 ppm selenium as sodium selenite (total Se, 0.172 ppm). The pigs were randomly allotted to the basal diet or the basal diet supplemented with 5, 10 or 15 IU vitamin E per kg of diet. Average weight of the 12 pigs initially was 9.2 kilograms. The pigs were housed in stainless steel metabolism cages for an 8 day

adjustment period and a subsequent 3 day collection period when consuming constant near *ad libitum* daily intakes (375 grams) of the test diets offered as a gruel with deionized water in three meals per day. Urine was collected in 6 N hydrochloric acid separate from the feces. The feces were air dried on trays and were ground prior to analysis. Blood and serum were collected initially, at the end of the collection and prior to sacrificing the pigs. Pigs were sacrificed to obtain samples of *quadriceps femoris* muscle, myocardium, liver and kidney for Se analyses one day following the completion of the urine and fecal collection. Serum was analyzed for SGOT, lactic dehydrogenase (LDH) (Sigma Technical Bulletin 500, 1969) and creatine phosphokinase (CPK) (Sigma Technical Bulletin 520, 1971) and then stored at -20 C until analyzed for Se. Hemoglobin and microhematocrit were determined on heparinized whole blood as previously described. Serum, tissues, diets, feces and urine were analyzed for Se content as described in experiment 1.

Statistical analyses of the data were conducted utilizing one-way and two-way analysis of variance as described by Steel and Torrie (1960).

Results and Discussion

Experiment 1

During the first 14 weeks of this experiment no significant differences in performance of the pigs were

observed. However, at 16 and 18 weeks of the experiment, dietary supplementation of 5 IU vitamin E per kg of diet increased ($P<.05$) pig weight and total gain over the groups fed 0, 10 or 15 IU vitamin E per kg of diet. Nevertheless, this observed effect of 5 IU vitamin E per kg of diet disappeared during the last 4 weeks of the trial. The performance of pigs for the entire experiment is summarized in Table 8. No significant differences in final weight, average daily gain or feed conversion could be attributed to the dietary supplements of Se or vitamin E in this experiment. Groce *et al.* (1971, 1973b), Ku *et al.* (1973) and Wastell *et al.* (1972) have reported similar results of supplemental Se and vitamin E on growth and feed conversion of pigs. During the experiment, one pig on the basal + 15 IU of vitamin E/kg of diet treatment died suddenly during the 18th week of the trial. Postmortem diagnosis indicated acute bronchopneumonia as the cause of death. There were no lesions indicative of vitamin E-selenium deficiency and no gross lesions of vitamin E-Se deficiency were observed in any pig at slaughter. The effects of supplemental selenium and vitamin E on 10 week and final hemoglobin and hematocrit are presented in Table 9. Selenium supplementation ($P<.05$) decreased 10 week hemoglobin values from 11.9 to 10.9 g/100 ml. At the final bleeding, hemoglobin values were unaffected by selenium supplementation of the diet but

TABLE 8. EFFECT OF DIETARY SELENIUM AND VITAMIN E SUPPLEMENTATION UPON PIG PERFORMANCE (EXPERIMENT 1)

Selenium, ppm	0				0.1			
Vitamin E, IU/kg[a]	0	5	10	15	0	5	10	15
No. of pigs	8	8	8	7	8	8	8	8
Initial wt., kg	10.9	12.1	11.4	12.1	11.8	12.6	11.5	11.5
Final wt., kg	94.0	100.8	98.1	94.5	100.2	103.7	96.2	91.2
Daily gain, kg	0.59	0.64	0.62	0.59	0.63	0.65	0.60	0.57
Daily feed, kg	1.90	1.94	1.81	1.77	1.85	1.91	1.87	1.73
Gain/feed	0.31	0.33	0.34	0.33	0.34	0.34	0.32	0.33

[a] d-α-tocopheryl acetate with dextrin, myvamix-type 125. Distillation Products Industries, Rochester, New York

TABLE 9. EFFECT OF ADDED DIETARY SELENIUM AND VITAMIN E ON 10 WEEK AND FINAL HEMOGLOBIN CONCENTRATION AND HEMATOCRIT (EXPERIMENT 1)

Item	Supplemental Se, ppm	Vitamin E, IU/kg 0	5	10	15	Mean	EMS[a]
Hemoglobin, g/100 ml[b]							
10 week	0	12.0	11.3	12.7	11.6	11.9[c]	
	0.1	11.1	11.2	11.1	10.1	10.9	1.7
	Mean	11.6	11.3	11.9	10.9		
Final	0	12.7	12.8	10.8	9.5	11.4	
	0.1	12.9	13.2	10.5	12.0	12.1	2.8
	Mean[d]	12.8	13.0	10.7	10.7		
Hematocrit, %[b]							
10 week	0	36.5	36.2	34.0	36.3	35.7	
	0.1	35.9	34.6	33.7	34.0	34.6	5.5
	Mean	36.2	35.4	33.8	35.1		
Final	0	41.0	41.6	36.2	33.4	38.1	
	0.1	41.6	41.1	35.6	40.0	39.6	28.5
	Mean[e]	41.3	41.4	35.9	36.7		

[a]Overall error mean square

[b]Bled 5 pigs per treatment on each level of Se supplementation

[c]10 week hemoglobin lower ($P<.05$) on Se supplemented diets

[d]Final hemoglobin higher ($P<.01$) on 0 and 5 IU E/kg treatments than 10 and 15 IU/kg diet

[e]Final hematocrit higher ($P<.05$) on 0 and 5 IU E/kg treatments than on 10 IU E/kg treatment

vitamin E supplementation of 10 or 15 IU per kilogram of diet depressed ($P<.01$) blood hemoglobin levels.

Ewan and Wastell (1970), Groce *et al.* (1971, 1973b) and Wastell *et al.* (1972) reported that the addition of vitamin E and selenium alone or in combination to deficient diets had no effect on the blood hemoglobin concentration. Selenium and vitamin E had no significant effect on 10 week hematocrits. The hematocrit of blood samples obtained at the final bleeding were increased ($P<.05$) by dietary supplements of 0 and 5 IU of vitamin E/kg of diet over the hematocrit values of pigs receiving 10 IU per kg of diet.

The effects of supplemental Se and vitamin E on serum Se levels during this experiment are presented in Table 10. Selenium supplementation markedly increased ($P<.01$) serum Se concentrations at 10 weeks and at the bleeding prior to slaughter. Vitamin E supplementation had no effect on serum selenium concentrations at either the 10 week or final bleeding which adds additional support to the observations of Groce *et al.* (1973a,b) that supplemental vitamin E had no effect on serum selenium levels. Serum SGOT values were not affected by the dietary treatments in this experiment, but were similar to the normal values reported by Wretlind *et al.* (1959).

Tissue selenium levels as influenced by supplemental Se and vitamin E are presented in Table 11. Selenium

TABLE 10. EFFECT OF ADDED DIETARY SELENIUM AND VITAMIN E ON SERUM Se LEVELS (EXPERIMENT 1)

Sampling time	Supplemental Se, ppm	Vitamin E, IU/kg 0	5	10	15	Mean	EMS[a]
		Serum Se, ppm[b]					
Initial	0	.028	.025	.023	.024	.025	
	0.1	.024	.020	.022	.029	.024	
	Mean	.026	.022	.022	.026		.00005
10 weeks	0	.024	.018	.017	.017	.019[c]	
	0.1	.142	.129	.122	.122	.129	
	Mean	.083	.074	.070	.069		.0003
Final	0	.046	.027	.030	.030	.034[d]	
	0.1	.174	.167	.160	.175	.169	
	Mean	.110	.097	.095	.103		.0003

[a]Overall error mean square

[b]Bled 5 pigs per treatment on each level of Se supplementation at each time

[c]10 week serum Se levels higher ($P<.01$) on Se supplemental diets

[d]Final serum Se levels higher ($P<.01$) on Se supplemental diets

TABLE 11. EFFECT OF ADDED DIETARY SELENIUM AND VITAMIN E ON TISSUE Se LEVELS (EXPERIMENT 1)

Tissue	Supplemental Se, ppm	Vitamin E, IU/kg 0	5	10	15	Mean	EMS[a]
		Tissue Se (ppm)[b]					
Rectus abdominus muscle							
Wet[c]	0	.070	.054	.053	.049	.056[e]	
	0.1	.108	.104	.109	.116	.109	
	Mean	.089	.079	.081	.082		.0001
Dry[d]	0	.273	.210	.213	.197	.223[f]	
	0.1	.435	.418	.438	.456	.437	
	Mean	.354	.314	.326	.326		.0018
Liver							
Wet	0	.137	.114	.129	.122	.125[g]	
	0.1	.518	.496	.491	.496	.500	
	Mean	.327	.305	.310	.309		.0012
Dry	0	.481	.404	.437	.426	.437[g]	
	0.1	1.789	1.790	1.734	1.702	1.754	
	Mean	1.135	1.097	1.085	1.064		.0155

TABLE 11 (CONTINUED)

Tissue	Supplemental Se, ppm	Vitamin E, IU/kg 0	5	10	15	Mean	EMS[a]
Kidney							
Wet	0	1.226	1.058	1.019	0.994	1.074[g]	
	0.1	2.244	2.063	1.914	1.984	2.051	
	Mean	1.735	1.561	1.466	1.489		.0730
Dry	0	5.611	4.844	4.547	4.505	4.877[g]	
	0.1	9.482	9.536	8.565	9.131	9.178	
	Mean	7.546	7.190	6.556	6.818		.7979

[a]Overall error mean square

[b]Tissue samples from 5 pigs per treatment on each selenium level

[c]ppm Se on a fresh tissue basis

[d]ppm Se on a dry matter basis

[e]Muscle Se lower ($P<.01$) on unsupplemented diets; interaction ($P<.05$) between selenium and vitamin E

[f]Muscle Se lower ($P<.01$) on unsupplemented Se diets

[g]Liver Se and kidney Se lower ($P<.01$) on unsupplemented Se diets on both a wet and dry basis

supplementation increased (P<.01) *rectus abdominus* muscle selenium concentrations on both a wet and dry basis. There was a significant (P<.05) interaction between selenium and vitamin E in regard to *rectus abdominus* muscle selenium concentration on a wet basis. The addition of increasing levels of vitamin E to unsupplemented Se diets decreased muscle Se concentrations while on Se supplemented diets, muscle Se concentrations increased with increasing levels of vitamin E. Selenium supplementation increased (P<.01) liver and kidney Se concentrations on both a wet and dry basis. The dry matter percentages of all tissues were unaffected by dietary supplementation of selenium or vitamin E in this experiment. The vitamin E main effect was not significantly different for any of the tissue Se concentrations or dry matter percentages. Ewan (1971) reported similar findings in that the addition of selenium resulted in a significant increase in the selenium content of tissues compared with dietary treatments containing no added selenium and that vitamin E had no significant effect on the level of selenium in the tissues. However, the observation made by Groce *et al.* (1971, 1973b) that supplemental vitamin E increased kidney Se levels was not observed in this experiment. They also reported that supplemental vitamin E had no apparent effect on Se levels of other tissues analyzed which is consistent with the findings of this experiment.

Experiment 2

The main effects of supplemental vitamin E level on selenium balance are summarized in Table 12. Supplemental levels of vitamin E had no significant effects on selenium excretion or retention in this experiment. Increasing levels of vitamin E tended to increase fecal Se excretion and thus decrease absolute and percent Se retention. Groce *et al.* (1973a,b) have reported similar results in that added vitamin E reduced the absolute retention of selenite Se by increasing fecal and urinary Se excretion.

The effect of dietary vitamin E level on serum Se and blood parameters are presented in Table 13. The serum Se and blood hemoglobin levels were not significantly affected by the supplemental levels of vitamin E at any sampling time. In addition, initial and final hematocrits were unaffected by supplemental level of E, but prior to being sacrificed pigs receiving 15 IU vitamin E/kg of diet had lower ($P<.05$) hematocrits than pigs receiving diets supplemented with 0 or 10 IU vitamin E/kg of diet.

The serum enzyme activities are reported in Table 14. Serum SGOT values were unaffected by supplemental level of vitamin E but were in the normal range of SGOT values reported by Wretlind *et al.* (1959). Lactic dehydrogenase activity was higher ($P<.05$) initially in pigs allotted to the 5 IU vitamin E/kg of diet treatment.

TABLE 12. EFFECT OF DIETARY VITAMIN E LEVELS OF SUPPLEMENTATION ON SELENIUM BALANCE (EXPERIMENT 2)

Item	Vitamin E, IU/kg[a]				S.E.[b]
	0	5	10	15	
No. of pigs	3	3	3	3	
Se intake, µg/day	61.5	63.0	60.2	62.6	3.0
Se retention, % of intake	67.1	62.4	61.3	64.9	2.6
Se retention, µg/day	41.3	39.3	36.9	40.7	1.8
Se excretion, % of intake					
Fecal	24.4	27.1	30.6	26.8	2.0
Urinary	8.5	10.5	8.1	8.3	0.9

[a]Vitamin E as d-α-tocopheryl acetate. Distillation Products Industries, Rochester, New York.

[b]Standard error

TABLE 13. EFFECT OF DIETARY VITAMIN E LEVELS OF SUPPLEMENTATION ON BLOOD PARAMETERS (EXPERIMENT 2)

Item	Vitamin E, IU/kg 0	5	10	15	S.E.[a]
No. of pigs	3	3	3	3	
Serum Se, ppm					
Initial	.017	.022	.017	.019	.006
Final	.108	.118	.107	.105	.007
Pre-sacrifice	.093	.095	.097	.105	.005
Hemoglobin, g/100 ml					
Initial	10.9	9.8	10.6	9.8	0.72
Final	10.9	10.3	12.5	10.9	0.99
Pre-sacrifice	9.9	9.6	10.5	11.3	1.12
Hematocrit, %					
Initial	35.7	32.4	35.8	33.1	2.40
Final	34.4	32.9	35.1	30.1	1.77
Pre-sacrifice	32.8[b]	31.0[bc]	34.1[b]	26.8[c]	1.55

[a]Standard error

[b,c]Means in the same row with different superscripts are significantly different ($P<.05$).

TABLE 14. EFFECT OF DIETARY SUPPLEMENTAL LEVEL OF VITAMIN E ON SERUM ENZYME ACTIVITY (EXPERIMENT 2)

Item	Vitamin E, IU/kg 0	5	10	15	S.E.[a]
No. of pigs	3	3	3	3	
SGOT, S-F units[b]					
Initial	39.0	58.0	36.7	44.3	6.1
Final	32.3	34.0	31.7	34.0	3.7
Pre-sacrifice	33.0	32.0	26.3	30.7	3.0
LDH, B-B units[c]					
Initial	779[e]	1311[f]	834[e]	795[e]	124
Final	661	759	733	921	61
Pre-sacrifice	637	711	570	707	37
CPK, Sigma units[d]					
Initial	22.7[g]	63.3[h]	21.7[g]	23.3[g]	7.3
Final	6.0	14.0	6.7	10.7	2.3
Pre-sacrifice	10.3	14.7	9.0	13.7	3.9

[a]Standard error

[b]Sigma Technical Bulletin 505, 1964

[c]Sigma Technical Bulletin 500, 1969

[d]Sigma Technical Bulletin 520, 1971

[e,f]Means in the same row with different superscripts are significantly different (P<.05)

[g,h]Means in the same row with different superscripts are significantly different (P<.01)

This can be accounted for by the fact that 2 pigs assigned to this treatment had very high LDH levels in serum. Both of them being assigned to the same treatment was a random chance occurrence. LDH values at the final bleeding and prior to being sacrificed were unaffected by supplemental level of vitamin E. Ewan and Wastell (1970) stated that the activity of LDH enzyme of pigs fed diets supplemented with vitamin E alone remained essentially the same as the enzyme activity of blood of pigs fed both vitamin E and selenium. Creatine phosphokinase (CPK) enzyme activities initially were higher ($P<.01$) on the 5 IU vitamin E/kg treatment due to the two pigs which had very high serum CPK levels that happened to be randomly allotted to this treatment as discussed for LDH values. These two pigs must have been in a marginal state of selenium and/or vitamin E deficiency since they had elevated levels of SGOT, LDH and CPK enzymes. However, CPK levels at the final bleeding and prior to being sacrificed were not affected by supplemental level of vitamin E.

Tissue Se levels as influenced by supplemental level of vitamin E are summarized in Table 15. Dietary levels of supplemental vitamin E had no significant effect on Se concentration of any of the tissues analyzed on either a wet or dry basis. This observation may be partially explanable since this experiment was only a

TABLE 15. EFFECT ON DIETARY SUPPLEMENTAL LEVEL OF VITAMIN E ON TISSUE Se CONCENTRATIONS (EXPERIMENT 2)

Item	Vitamin E, IU/kg 0	5	10	15	S.E.[a]
No. of pigs	3	3	3	3	
Quadriceps muscle Se, ppm					
Wet[b]	.059	.058	.059	.057	.003
Dry[c]	.282	.290	.274	.266	.013
Myocardium Se, ppm					
Wet	.106	.128	.112	.132	.011
Dry	.551	.653	.559	.654	.059
Liver Se, ppm					
Wet	.257	.273	.263	.259	.017
Dry	.938	.990	.943	.915	.058
Kidney Se, ppm					
Wet	.988	.968	.874	.950	.085
Dry	5.334	5.008	4.622	5.171	.440

[a]Standard error

[b]ppm Se on a fresh tissue basis

[c]ppm Se on a dry matter basis

13 day experiment and one might not expect to see significant changes in tissue Se concentrations in this short period of time. Groce *et al.* (1971, 1973b) reported that supplemental vitamin E had no apparent effect on Se levels of tissues except for an increase in kidney Se levels. Ewan (1971) also reported similar observations in that vitamin E had no significant effect on the level of selenium in the tissues.

Summary

One feeding trial and one balance trial were conducted to study the effects of supplemental Se and dietary supplemental levels of vitamin E on performance, hematology, tissue selenium and selenium balance in growing-finishing pigs fed diets low in vitamin E and selenium. Sixty-four weanling pigs were assigned to eight dietary treatments in a 2 x 4 factorial design to evaluate the effects of supplementing a basal corn-soybean meal diet (0.054 ppm Se) with 0.1 ppm Se (as sodium selenite) and vitamin E at 0, 5, 10 or 15 IU per kg of diet. There were no lesions indicative of vitamin E-selenium deficiency and no deaths due to the deficiency were observed in this study.

No significant differences in overall performance of pigs during this experiment could be attributed to the dietary supplements of Se or vitamin E. Selenium supplementation decreased ($P<.05$) 10 week hemoglobin

values by 1 g/100 ml. Blood hemoglobin level was depressed ($P<.01$) when the diets were supplemented with 10 or 15 IU of vitamin E per kilogram of diet at the final bleeding. Hematocrits of blood samples obtained at the final bleeding were increased ($P<.05$) by dietary supplements of 0 and 5 IU vitamin E/kg of diet over pigs receiving 10 IU vitamin E/kg of diet. Selenium supplementation increased ($P<.01$) serum Se concentrations, while vitamin E supplementation had no effect on serum Se concentrations. Selenium supplementation increased ($P<.01$) *rectus abdominus* muscle, liver and kidney Se concentrations on both a wet and dry basis. There was a significant ($P<.05$) interaction between selenium and vitamin E on muscle selenium concentrations on a wet basis. Dietary level of vitamin E supplementation had no significant effect on tissue Se concentrations.

The balance study was concerned with Se absorption and retention on a selenium supplemented basal diet (0.172 ppm Se) with dietary vitamin E added at levels of 0, 5, 10, or 15 IU vitamin E/kg of diet. Supplemental levels of vitamin E had no significant effects on selenium excretion or retention, serum Se, hemoglobin, SGOT, LDH or CPK enzyme activities, or on Se concentrations of *quadriceps femoris* muscle, myocardium, liver or kidney on either a wet or dry basis.

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APPENDICES

APPENDIX A

FLUOROMETRIC SELENIUM ANALYSIS

Foreword

If one is to have reasonable success with this method he has to accept the "rules" dictated by the procedure. This is a demanding procedure: in time, care, and a certain amount of technique. The entire analysis, from the start of digestion to completion of reading the fluorescence of the samples requires a minimum of 5 1/2 to 6 1/2 hours depending on the agility and adeptness attained by the technician. There is a 1-1 1/2 hour incubation period in the procedure; otherwise the procedure requires *constant* attention.

If this is your first experience with fluorometric analysis as it was ours, you may tend to take lightly some of the precautions herein. You will have much less trouble if you take them seriously. Assume that nothing is clean unless you cleaned it. Reagents should be standardized as much as possible and each new batch purchased used with caution until it has proven to be acceptable.

Materials

Apparatus:

(a) Eberbach shaker -

Fitted with an improvised rack for holding 12 125-ml separatory funnels in an upright position (made from a sulfuric acid box which fits perfectly on the platform - wooden rack was too heavy). A cardboard "spacer" is used over the funnel necks to keep the funnel stems from touching and breaking. Shaker is set at a maximum or nearly so.

(b) Fluorometer -

Aminco-Bowman Spectrophotofluorometer - This machine is used "manually", i.e., the scanner is not used and the percent transmittance is read directly off the meter -

READ THE INSTRUCTION MANUAL BEFORE OPERATING FLUOROMETER.

Settings:

Excitation monochromator	372 mμ
Emission monochromator	516 mμ

Slit widths:

Excitation	3 mm
Emission	3 mm
Photomultiplier	3 mm
Meter multiplier range	.01
Meter sensitivity	50

Use the small round cuvettes. These are cleaned with alcohol and dried with acetone between samples. Periodically they should be cleaned with the cleaning solution. *Never*, repeat *never* touch any part of the cuvette except the extreme lips with the bare fingers. Don't spare the kimwipes when handling and drying cuvettes. They cost $25.00 each and cannot be used if etched or scratched.

For stable operation, the instrument and lamp should be allowed to warm up for 30-45 minutes before making readings. When finished, turn off the lamp and photometer; but do not turn off the blower until the lamp is completely cooled. Remove the slot holders from the machine since they will rust in tight if left in position.

(c) Glassware -

12	microKjeldahl digestion flasks (100 ml with expansion bulb, Aminco 4-1874)
12	Phillips beakers with watchglasses
12	125 ml separatory funnels with *Teflon* stopcocks
1	250 ml separatory funnel with *Teflon* stopcock
2	100 ml beakers
1	100 ml graduated cylinder
1	10 ml graduated cylinder
1	5 ml volumetric pipette for DAN solution
1	1 ml volumetric pipette for standards

1 2 ml volumetric pipette for standards

1 25 ml burette with *Teflon* stopcock

2 50 ml burettes with *Teflon* stopcock

1 250 ml burette with *Teflon* stopcock

1 10 ml repipette to greatly speed the adding of the water and EDTA solution.

The ground glass stoppers of the separatory funnels *must* be replaced with No. 4 soft rubber stoppers (wrapped with a fresh piece of Saran wrap for each run). Use only *Saran* wrap. We lost a whole week trying to figure out the source of interference when it was only a new batch of brand X plastic wrap we had just begun using.

The above listed glassware should be segregated from circulation with the general lab glassware to avoid problems of contamination. Once this glassware has been initially cleaned with Alconox and acid washed, one should not use soap on it again. Don't worry about the outside of glassware, it's the inside that counts. The washing procedure is as follows: (1) Rinse at least 3 times in flowing distilled water (use a segregated nylon brush only if necessary - *no soap*). (2) Pour 250 ml of cleaning solution into a Phillips beaker, use this to acid wash all glassware once and then discard. (3) Rinse all acid washed items 3 to 4 times in *flowing* deionized water. (4) Invert and dry in a reserved section of the drying room or a forced air oven. Whenever possible

wash the items in this order: digestion flasks, Phillips beakers, separatory funnels.

Reagents:

(1) Nitric acid - sp. gr. 1.42 - Redistilled from glass-use lots of acid-washed boiling beads, discard first portion of distillate and do not boil down too low in distillation flask. One can use Baker nitric acid without redistilling. This material is satisfactory for all except very low Selenium samples (<0.01 µg in a 1 gram sample).

(2) Sulfuric acid solution - Add 140 ml selenium-free H_2SO_4 - sp. gr. 1.85 - to deionized distilled water and dilute to 1 liter.

(3) Ammonium hydroxide solution - Dilute 400 ml NH_4OH - sp. gr. 0.90 - to 1 liter with deionized distilled water.

(4) Disodium ethylenedinitrilotetraacetate solution (EDTA) - 0.01M; dissolve 7.4450 g dihydrate in water and dilute to 1 liter.

(5) 2,3-Diaminonaphthalene (DAN) solution. Reagent from Aldrich Chemical Company, Milwaukee, Wisconsin, or G. Frederick Smith Chemical Co. Grind finely with mortar and pestle, store in brown bottle in the dark. We store excess DAN in 1 gram vials in the freezer. Weigh in

reduced light. Preparation: Insert a glass wool plug in stem of 250 ml separatory funnel. At point indicated in Procedure transfer 0.0650 g DAN reagent to separatory funnel. Wash in with 65 ml of H_2SO_4 solution. Place on shaker for 15 min.; then add 65 ml of cyclohexane and shake for another 15 min; let phases separate for 5 min. and draw off lower phase for immediate use. Note: The shaker should be kept in the darkroom, and all operations with the DAN solution performed in dim light. The samples after extraction into cyclohexane should be protected from light also.

(6) Cyclohexane-Eastman Kodak-Reagent grade.

(7) Digestion acids:

Concentrated nitric acid-reagent grade redistilled over glass or J. T. Baker nitric acid may be used without redistilling.

Perchloric acid (70% reagent grade) (G. Frederick Smith)

Selenium-free sulfuric acid - Dilute concentrated sulfuric acid (J. T. Baker) with an equal volume of deionized distilled water. Add 15 ml of reagent grade 48% hydrobromic acid per 200 ml of the mixture. Heat strongly

until volume is the same as that of the original concentrated sulfuric acid. This process requires several hours on a large hotplate.

(8) Cleaning solution - 1:1 mixture of concentrated nitric and concentrated sulfuric acids. Add H_2SO_4 to HNO_3. Do not mix in a soft glass bottle as considerable heat is generated. This is *bad stuff* - use under a hood; wear *heavy* rubber gloves and keep bicarbonate solution handy. Wash it off your gloves with deionized distilled water as it will slowly eat through them too.

(9) Standard selenium solution -
Stock solution - Dissolve 100 mg selenium metal (Alfa Inorganics - 99.999% pure) in 10 ml concentrated redistilled nitric acid. Add 28.0 ml concentrated sulfuric acid and dilute to 1 liter to give 100 µg Se/ml in 1 N sulfuric acid. Store in cold room. To prepare a working solution dilute 1.0 ml of stock solution to 1 liter with 0.1 N H_2SO_4 to give 0.1 µg Se/ml in 0.1 N H_2SO_4.

(10) Hydrogen peroxide (30%) - Reagent grade (store in cold room or refrigerator).

Procedure

Sample Preparation:

Grain, feed, feces and other dry samples should be finely ground to insure good mixing and efficient predigestion and digestion. Tissues are homogenized with deionized water on either the Polytron or the Virtis homogenizer to facilitate pipetting. In general, the following dilutions have worked well for us: Skeletal muscle and heart are homogenized with twice their weight of deionized water. Liver and kidney are homogenized with three and five times their weight of deionized water, respectively. Clean all glassware, including storage vials, with cleaning solution and rinse them well with deionized water. The disposable plastic Whirl-paks seem to work well for storing. Freeze homogenates until you are ready to analyze them. Tissue homogenates keep better if they are thawed rapidly in a container of warm water than they do if allowed to thaw slowly at room temperature. This rapid-thaw procedure works especially well with the Whirl-paks.

Sampling error seems to be the greatest source of replication deviation with this method, so due care in sample preparation is warranted.

Predigestion:

All samples (feeds, grains, tissue homogenates, etc.) except strictly liquid samples (e.g., urine and

standards) should be predigested for *at least 12 hours in the complete digestion acid mixture.*

Place accurately weighed sample (containing up to 0.4 µg selenium and preferably not greater than 1.0 g dry matter) along with 3 glass beads in a 100 ml Kjeldahl flask. Add *in sequence* 6.0 ml redistilled nitric acid, 2.0 ml 70% perchloric acid, and 5.0 ml H_2SO_4 (sp. gr. 1.84). Burettes will greatly speed the addition of these many reagents. Use *Teflon* stopcocks only. Cover the flasks with a Nalgene centrifuge tube to prevent contamination during the predigestion period.

Digestion:

CAUTION: Since this is a modified perchloric acid digestion, there is a potential danger for explosive perchlorates to be formed. One should be especially careful with samples containing fat; always swirl them to mix before putting on heat. Keep the hood glass pulled down level with the top edge of the heaters. This protects you and makes a draft over the flasks giving more efficient condensation action. Turn on showers in the perchloric hood during digestion.

Always work in an efficient hood. Use the Aminco digestor with individual rheostats for each pair of heating elements. Heat carefully in initial stages to avoid loss by foaming. Increase heat as digestion proceeds.

Turn the flasks 180° occasionally to "wash" down all sides with condensate. Starting with cold heaters, set rheostat at 5, for 20 minutes, increase to 7 for 20 minutes, then to 9 to finish. Take back to 7 for H_2O_2 addition. Add *small* amounts of nitric acid with a Pasteur pipette at first signs of darkening due to charring. As digestion proceeds, the dark brown nitric acid fumes will dissipate and eventually be replaced by white fumes. The liquid will become bluish-green and then slowly yellow. This is the stage that samples will char if they are going to; *watch them closely*. The yellow color will slowly disappear and the liquid become water clear in most cases. With feces and mixed feeds or high ash samples, precipitation will occur before the solution becomes water clear. These samples are cooked until turbid rather than water clear. Once they are water clear (or turbid) remove flask from heat and swirl contents to wet entire bulb area and lower neck of flask. Some yellow coloring will reappear. This will be a "healthier" yellow in the case of standards and tissue homogenates than it will be for feeds and grains. Replace flask on heater and continue heating until solution becomes water clear and white fumes appear. *Make a check list*, with flask number and columns for "yellow" and "washed" to check off when you note yellowing or wash down the flask; otherwise you will not

know if a flask has yellowed and cleared or just cleared.

When flasks become water clear and white fumes appear remove them from heat and place in rack until all are cleared. Turn the heaters down to 7 and replace flasks on heater. Remove second flask from heater and let cool in rack while you are working with first flask. This allows the flasks to cool just enough; they need to be fairly warm to react with the H_2O_2. Take the partially-cooled flask, swirl contents, and add 1.0 ml of 30% hydrogen peroxide *slowly* down walls of flask, swirl until all action ceases, and resume heating until contents are boiling briskly and white fumes appear. By cooling one and working with another this works well to go around all 12 flasks and start over again. Add 1.0 ml hydrogen peroxide twice more and continue final heating for 5 minutes after appearance of white fumes. Set flasks aside to cool while you are getting materials ready for the next steps in the procedure. Keep them near the hood to keep fumes out of the lab.

NOTE: Turn on hot plate *NOW*!

Use a repipette to add 20 ml of deionized distilled water to the digested material, *carefully*. Mix and quantitatively transfer the contents of the flask to a 250 ml Phillips beaker using two 10 ml washings and cover with a watchglass. After you have transferred all

12 samples to the Phillips beakers add *in turn, with mixing*, 10 ml disodium EDTA solution, 25.0 ml ammonium hydroxide solution and 5.0 ml DAN solution. The time one begins the DAN preparation will vary with how long it takes him to transfer the flask contents to the beakers and add the other two reagents. You should time it so that the DAN is ready for use when you are ready to add it to the samples. In other words, do not let it sit around too long after preparation.

NOTE: The shaker should be in the darkroom, and all operations with the DAN solution or the complete reaction mixture should be performed in dim light. The samples after extraction should be protected from light also. Give the beaker and contents several seconds of healthy swirling after the addition of *each* reagent. Boil the flask contents for 2 minutes on the hotplate. Place on cart and roll into darkroom and let them sit undisturbed *in the dark* for 1 hour. You have about 20-30 minutes leeway here, i.e., the samples may be incubated up to 1 1/2 hours *if* you don't mess around in the extraction of such samples.

After incubation quantitatively transfer the flask contents to a 125 ml separatory funnel and add 6.0 ml cyclohexane. Stopper with a Saran-wrapped rubber stopper and shake for 15 minutes. Let stand for 5 minutes to allow phases to separate. Discard lower

aqueous phase and drain cyclohexane solution into 15 ml centrifuge tube. Centrifuge for 5 minutes, place tubes in a light tight container for trip to fluorometer.

Read fluorescence against deionized water blank taken through entire procedure. Do not leave the blank in the cuvette holder for any longer than necessary, as exposure to the excitation light causes fluorescence of the solutions to change. Plot standard graph from two standards. Plot is linear up to at least 0.5 μg selenium and passes through the origin consistently.

Additional notes:

The clean work bench is used in our lab to store reagents and some glassware. It is essential that all items be kept rust-free and dust-free. Metal contamination as well as grease contamination must be avoided. Any fluorescing material is a potential problem also.

It has been our experience that when fluorescent contamination has been a problem (e.g., with the Brand X plastic film) the samples have a definite blue fluorescence when placed in the cuvette well rather than the normal bright green fluorescence.

Once the digested material has cooled enough to work with, it should be carried through the rest of the procedure. We have found that we cannot get consistent results by digesting samples one day and working them up the next.

In conclusion, I would say that this may seem like a very lengthy treatment of a relatively simple procedure. You were forewarned: This *is not* a routine or simple procedure. You will spare yourself much agony and frustration if someone familiar with the analysis walks you through the first several times.

APPENDIX B

TABLE 1. EFFECT OF ADDED DIETARY SELENIUM, VITAMIN E AND ARSANILIC ACID ON INITIAL AND INTERMEDIATE BLOOD PARAMETERS (EXPERIMENT 1)

Item	Supplemental Se, ppm		Supplemental E		Arsanilic acid, ppm		S.E.[a]
	0	0.1	0	22 IU/kg	0	99	
No. of pigs	8	8	8	8	8	8	
Hemoglobin, g/100 ml							
Initial	11.6	11.2	11.5	11.4	11.2	11.7	.340
2 weeks	11.1	10.8	11.3	10.7	10.8	11.1	.523
8 weeks	11.6	12.7	12.3	12.0	11.6	12.8	.491
Hematocrit, %							
Initial	35.5	34.4	35.1	34.8	34.0	35.8	.884
2 weeks	34.6	32.6	34.2	33.0	33.2	34.0	1.354
8 weeks	37.0	36.5	37.8	35.7	36.0	37.4	1.273
SGOT, S-F units/ml							
Initial	50.4	45.5	47.0	48.9	45.8	50.1	2.097
2 weeks	46.1	49.8	45.9	50.0	46.8	49.1	3.622
8 weeks	51.8	37.6	44.9	44.5	46.4	43.0	6.722

APPENDIX B (CONTINUED)

Item	Supplemental Se, ppm		Supplemental E		Arsanilic acid, ppm		S.E.[a]
	0	0.1	0	22 IU/kg	0	99	
Serum Se, ppm							
Initial	.030[b]	.025	.030	.026	.028	.028	.002
2 weeks	.021[b]	.071	.045	.047	.047	.045	.004
8 weeks	.014[b]	.127	.073	.067	.074	.067	.005

[a]Standard error

[b]Serum Se were significantly (P<.01) higher on selenium supplemented diets