This is to certify that the

thesis entitled

IMPORTANCE OF COLOSTRUM TO THE BIOLOGICAL ANTIOXIDANT STATUS OF THE NEONATAL PIG AND HIS COMPETENCE TO MAINTAIN HOMEOSTATIS FOLLOWING PARENTERAL IRON ADMINISTRATION

presented by

MARILYN JEAN LOUDENSLAGER

has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

E. R. Miller

Major professor
Dr. E. R. Miller

Date June 15, 1984

IMPORTANCE OF COLOSTRUM TO THE BIOLOGICAL ANTIOXIDANT STATUS OF THE NEONATAL PIG AND HIS COMPETENCE TO MAINTAIN HOMEOSTASIS FOLLOWING PARENTERAL IRON ADMINISTRATION

BY

Marilyn Jean Loudenslager

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Science

1984

ABSTRACT

IMPORTANCE OF COLOSTRUM TO THE BIOLOGICAL ANTIOXIDANT STATUS OF THE NEONATAL PIG AND HIS COMPETENCE TO MAINTAIN HOMEOSTASIS FOLLOWING PARENTERAL IRON ADMINISTRATION

By

Marilyn Jean Loudenslager

Fifteen second parity sows were used to determine the effects of vitamin E and selenium supplementation of the sow's diet and to examine the importance of this diet and/or colostrum consumption for the neonatal pig's tolerance to parenteral iron. Supplementation of the sow's diet increased her plasma vitamin E and selenium, but did not increase plasma glutathione peroxidase activity. Colostrum had greater concentrations of vitamin E (primarily α-tocopherol) and selenium than mature milk. Pig's pre-colostral biological antioxidant status was very low, but by two days of age had increased, especially in vitamin E. Pigs performance was not affected by the pre-colostral iron injection, however, their plasma vitamin E was lower and their plasma selenium and glutathione peroxidase activity tended to be higher at two days than that of pigs not receiving iron. Supplementation of the dam's diet maintained a higher vitamin E and selenium level in colostrum and milk and a higher biological antioxidant status in pigs throughout lactation.

ACKNOWLEDGEMENTS

Upon completion of my Masters of Science Degree I wish to extend sincere thanks to Dr. E.R. Miller. His knowledge, patience and subtle motivational support have made the completion of this degree possible.

I am also grateful to my committee members, Dr. J.L. Gill, Dr. M.G. Hogberg, Dr. H.D. Stowe and Dr. D.E. Ullrey. The willingness of the committee to give freely of their time, resources and professional expertise were deeply appreciated.

I wish to thank Dr. W.T. Magee and Dr. C.K. Whitehair for their input into the finalization of this project.

I am indebted to Dr. P.K. Ku and Phyllis Whetter for the professional assistance and advice given in the laboratory.

The diligent typing and exceptional cooperation given by Judy Witwer in the preparation of this manuscript was sincerely appreciated.

I also wish to thank my fellow graduate students who assisted in the activities involved in this project. I am especially grateful to Patty Dickerson and Kris Johnson for giving so much of their time, knowledge and understanding to this project.

The final thank you is extended with a great deal of love and respect to my family. The understanding and appreciation of the livestock industry brought with me from home have played a important role in my education, but of even greater value has been the love and continuous support I received from my family making the completion of this degree a reality.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Parenteral or oral administration of iron to young, nursing pigs is a common management practice in the swine industry. However Scandanavian researchers have reported toxic effects, including waxy muscle degeneration and transudations into the pericardium and thoracic cavities following iron administration to nursing pigs. Death losses, often involving the majority of the litter, occurred 8-12 hours after the administration of a commonly accepted form and level of iron (Lannek et al., 1962; Tollerz, 1973). The toxic effects of iron are thought to be a result of concomitant vitamin E and selenium deficiency in the sow's diet (Tollerz and Lannek, 1964). Tollerz (1973) reported that adequate vitamin E and selenium levels and low quantities of polyunsaturated fatty acids in sow's diet prevented iron toxicosis in the nursing pigs.

Iron toxicosis has been difficult to produce in the United States when levels of vitamin E and selenium are low in the sow's diet unless massive doses of iron are given to the pigs (Cook et al., 1981). However, there has been a concern that pre-colostral pigs may be especially sensitive to parenteral iron administration. This study was designed to determine the neonatal pigs' ability to deal with a parenteral iron injection and the role of colostrum in the biological antioxidant status of the newborn pig.

Literature Review

I. Colostrum

A. Importance for immunocompetence for the newborn.

The neonatal pig is devoid of immunoglobulins and antibodies against specific pathogens because the epitheliochorial placentation of the fetal pig does not permit their transfer from the dam (Miller, 1982). Furthermore, pigs are not capable of producing their own antibodies until they are two (Wilson, 1974) to three weeks of age (Brown et al., 1961, Miller et al., 1962). Therefore, pigs are dependent upon a supplemental source of immunoglobulins, provided to them through colostrum, to protect them from disease during the first weeks of life.

Colostrum is known to have a high immunoglobulin content (Jensen and Pederson, 1979). The immunoglobulins and antibodies present in colostrum are produced by the sow's immune system. Therefore, factors affecting the sows immunity in the weeks prior to parturition will determine the antibody content of colostrum (Wilson, 1974). These factors may be exposure of the sow to viruses or vaccination, either of which will cause the sow's immune system to produce antibodies against specific pathogens. The nutritional regimen of the sow may also affect her ability to produce antibodies. A recent review by Nockels (1980) suggests that vitamins A and E play a role in

increasing the synthesis of antibodies in animals.

Colostral antibodies have an enteric effect (Wilson, 1974), but are also known to be passed into the portal system unchanged (Miller et al., 1962; Jensen and Pederson, 1979; Brown et al., 1961) thereby increasing the serum antibody titer of the pig.

For the pig to obtain maximum immunological protection, it must consume colostrum within the first 21-36 hours after birth (Miller et al., 1962; Asplund et al., 1962; Lecce, 1966; and Wilson, 1974). After this time the composition of the colostrum and the physiology of the gut have changed and limit absorption of intact immunoglobulins (Jensen and Pederson, 1979).

The importance of the consumption of sufficient amounts of colostrum by the neonate within the first hours of life has been demonstrated by Perry and Watson (1967) who reported increased growth rates and lower mortality in 12-hour old pigs with higher serum antibody levels. Blecha and Kelly (1981) supported these data in a study which found that pigs which died prior to 21 days of age had lower serum γ-globulin concentration in their first day of life than those which lived.

Colostrum as a source of immunoglobulins to the neonate is very important for both growth and survival. However, it is important to be aware of the other components of colostrum, and to investigate the role they play in

enhancing the survival of the neonatal pig.

B. Composition and compositional changes of colostrum.

The composition of colostrum and milk is highly variable. This variability can be attributed to genetics, stage of lactation, nutrient levels in gestation and lactation diets as well as environmental factors (Pond and Houpt, 1978).

1. Proteins

As one would expect in regard to the earlier discussion of colostral immunoglobulins, the protein content of colostrum is much higher than that of mature milk. Perrin (1955) reported 19% protein in sow colostrum as compared to 6% found in milk by the second week of lactation.

2. Fat

The fat content of colostrum and milk are quite variable (2.7-7.7% in colostrum and 3.5-10.5% in milk) as reported by Bowland (1966). Although the total fat content of colostrum is lower than the percent fat in milk, there are several long chain fatty acids that are at a higher level in sow's colostrum. Oleic and linoleic acid are typically high in sow's colostrum then decline as lactation proceeds. Supplementing the sow's diet with corn oil will increase the proportion of linoleic acid in colostrum. Continuing corn oil supplementation throughout lactation will prevent the level of linoleic acid from dropping as

colostrum turns to milk (Miller et al., 1971). This is in agreement with the recent finding of increased levels of linoleic acid in human milk which has been attributed to increased consumption of margarines by the mothers (Jansson et al., 1981). The increased level of polyunsaturated fatty acids in colostrum and milk may increase the neonate's need for vitamin E. Hasson et al. (1966) reported a syndrome in premature infants associated with low plasma vitamin E values and high dietary polyunsaturated fatty acids, possibly indicating a need for supplementary vitamin E, whether through dietary sources or injection. This is further emphasized by the recommendation of the Committee on Nutrition, Academy of Pediatrics (1976) that 0.7 IU of vitamin E be provided for every gram of linoleic acid in the infant formula.

2. Vitamins

Vitamin A

Only small quantities of vitamin A are transferred from the dam to the fetus (Hjarde et al., 1961). Therefore, the newborn is dependent upon the vitamin A content of colostrum and milk for its needs. Colostrum is an important source of vitamin A for the newborn pig as it has been reported to contain 4 to 7 times as much of the vitamin as mature milk (Braude et al., 1947; Evans, 1959; Hjarde et al., 1961 and Nielsen et al., 1965). The wide variation may be attributed to both the source and quantity of vitamin A in the sow's diet. Nielsen et al. (1965) reported more efficient

transfer of the vitamin from the sow's diet to colostrum and milk if it was supplemented as the synthetic form rather than as cod liver oil.

Vitamin D

Cholecalciferol (vitamin D_3) is not transferred to the pigs via the placenta, but does increase in the plasma of the pigs upon consumption of the dam's milk. The amount of cholecalciferol in milk varies with the amount of supplementation of the dam (Goff et al., 1984).

Vitamin E

The extent to which vitamin E is placentally transfered from the sow to the pigs is unclear. Malm et al. (1976) reported pre-suckled pigs had several fold higher serum tocopherol, (3.6-6.2 µg/ml), levels than their dam, suggesting efficient transfer of the vitamin across the placental membrane. Loosli (1949) also indicated that placental transfer of vitamin E occurred in swine, but the values reported were much lower than those reported by Malm et al. (1976). Young et al. (1977) suggested that there was a relatively low rate of transfer accross the placenta, as he found 0.75 µg/ml and 1.16 µg/ml in the serum of unsuckled newborn pigs who were from vitamin E-deficient or supplemented sows, respectively.

Placental transfer of vitamin E in humans and rats is low, but recent studies indicate that some transfer does occur. Pazak's (1983) work with rats indicates placental

transfer of vitamin E, but it is preferentially incorporated into heart and lung tissue, resulting in very low circulating levels. Martinez et al. (1981) found that human infants have one-half the concentration of plasma tocopherol as either placental or maternal plasma. They also indicated that plasma total lipids in the infants were very low. From this evidence they suggested that placental transfer of vitamin E is inefficient and the low tocopherol level may be a consequence of low plasma total lipids.

Vitamin E deficiency in the newborn and especially in the premature infant can be a very serious problem (Bell et al., 1979; Harris et al., 1952; Jansson et al., 1978; Jansson et al., 1981) possibly leading to hemolytic anemia (Oski and Barness, 1967). Jansson et al. (1978) showed that dietary supplementation of low birth weight (<200 g) or pre-term (35 wks, gestation) infants with 16.5 mg tocopheryl acetate/day resulted in higher hemoglobin and lower reticulocyte counts at 8-10 weeks of age than infants supplemented with only 1.5 mg tocopheryl acetate/day, indicating that adequate vitamin E in the diet is important to the neonate.

Colostrum is a concentrated source of many essential nutrients to help the newborn through the first few days of life. High concentrations of vitamin E in colostrum have been reported in many species, with a dramatic decline as milk is secreted (Jagadeesan and Prema, 1980; Jannson et

al., 1981; Malm et al., 1976; Nielsen et al., 1973).

The development of high prasure liquid chromatography (HPLC) analytical techniques has allowed separation of alpha- and, beta/gamma-tocopherols in colostrum and milk. Jansson et al. (1981) found high levels of alpha- and total-tocopherol in human colostrum and reported a decline in these levels as the composition changed to milk. Total tocopherol levels have been reported to follow the same pattern in human (Jagadeesan and Prema, 1980) and sow colostrum and milk (Loosli, 1949; Malm et al., 1976; Neilsen et al., 1973).

There is a great deal of variation in the tocopherol concentrations of sow's milk. This variation can be attributed to the composition of the sow's diet. Increasing the vitamin E in the diet of the sow (Loosli, 1949; Malm et al., 1976; Neilsen et al., 1973) or cow (Dunkley et al., 1967) has resulted in an increase of tocopherols in colostrum and milk. Supplementing selenium in the ewe's diet has also been shown to increase the tocopherol level in milk (Gardner and Hogue, 1967).

Polyunsaturated fatty acid supplementation of the dam's diet has a negative effect on tocopherol levels in colostrum (Malm et al., 1976; Neilsen et al., 1973).

Water soluble vitamins

Vitamin C and thiamin have been found in higher concentration in colostrum (Braude et al., 1947; Evans, 1959; Pond and Houpt, 1978) than in milk while pantothenic

acid and niacin are found in higher levels in milk than in colostrum (Evans, 1959; Pond and Houpt, 1978). Riboflavin is maintained at a constant high level in both colostrum and milk (Braude et al., 1974; Evans, 1959).

4. Minerals

Selenium

Placental transfer of selenium has been reported in dogs (McConnell and Roth, 1964) when a subcutaneous injection of selenium was given to the dam, and in rats (Pazak, 1983) through dietary selenium supplementation of the dam.

Selenium is also made available to the offspring through colostrum and milk. Mahan et al. (1975) reported colostrum selenium values ranging from 0.043 ppm to 0.106 ppm and milk selenium values of 0.013 to 0.029 ppm. These values represent a four fold greater concentration of colostral selenium than the selenium concentration of milk. The wide variation in both colostrum and milk selenium values can be attributed to a lack of selenium supplementation in gestation and lactation diets, for the low values, and 0.1 ppm dietary selenium supplementation over the same period for the high values.

The source of supplemental selenium in the dam's diet affects how efficiently the selenium is incorporated into milk. In ewes, plant forms of selenium were found to be

absorbed and incorporated into milk more efficiently than inorganic selenium (Gardner and Hogue, 1967). Jones and Godwin (1963) showed that radioactive selenium which had been incorporated into plants was absorbed by the dam, incorporated into her milk and could be detected in the pup's stomach just four hours after the plant was fed to the dam. They suggested that selenium complexes in plants are readily metabolized by the dam and are normal constituents of milk. The radioactive selenium was found primarily in the protein fraction of the milk. McConnell and Roth (1964) found that a subcutaneous injection of an inorganic selenium was converted to an organoselenium prior to being incorporated, primarily, into the protein fraction of the milk.

Several studies have indicated that the dam will maintain nutrient levels in her colostrum and milk at the expense of her own body stores (Gardner and Hogue, 1967; Lane et al., 1984; and Mahan et al., 1976). Mahan et al. (1976) reported that sows had decreased serum selenium values at the end of lactation when no supplemental selenium was given. Gardner and Hogue (1967) observed a decrease in milk selenium levels three to six weeks into lactation of unsupplemented sheep, suggesting that they had depleted their body stores. They also reported a decrease in milk production at this time. Lane et al. (1984), who analyzed the mammary gland of rats on high (1.5 ppm) or low (.03 ppm) selenium, found that the selenium content of lactating

mammary glands in rats on low selenium diets was high, but glutathione peroxidase activity was low. They suggested that the selenium was being partitioned into the milk compartment of the mammary gland, thus making it unavailable as a prosthetic group for glutathione peroxidase.

Other minerals

Calcium and phosphorus are found in higher concentrations in milk then in colostrum (Braude et al., 1947; Perrin, 1955). In fact, Braude et al. reported an increase in calcium, phosphorus and total ash as lactation progressed.

Copper and iron levels are considered deficient in milk and are not responsive to supplementation of these elements in the sow's diet (Pond and Houpt, 1978).

5. Others

Lactose is very low in colostrum (Braude et al., 1974; Perrin, 1955), but increases as mature milk is secreted. The lactose content tends to decline as lactation proceeds (Braude et al., 1947).

Carnitine. Pigs are born with suboptimal levels of carnitine as indicated by the blood and liver of the newborn. Colostrum has a high level of carnitine. In fact, the sow concentrates carnitine in colostrum as indicated by the six to seven fold greater concentration of carnitine in colostrum in relation to the sow's serum level (Kerner et al., 1983).

II. Biological Antioxidants

Vitamin E and selenium, as an integral part of glutathione peroxidase, serve as biological antioxidants in animals. An understanding of the cellular and subcellular role of these two nutrients is needed to understand the lesions seen with vitamin E and selenium deficiency in pigs. The understanding of the function of vitamin E and selenium should also explain why various dietary and managerial practices may have a detrimental effect on pigs fed diets with low levels of these nutrients.

A. Functions

It is well known that oxygen is essential for aerobic organisms to live, but certain forms of this element are lethal to anaerobic organisms (McCord et al., 1971). Witting (1980), suggests that this difference in tolerance to oxygen between the two types of organisms can be attributed to a defense mechanism in the aerobic organism which allows it to control the level of oxygen breakdown products. This defense mechanism has both enzymatic and non-enzymatic components and can be classified as a biological antioxidant system.

A series of reactions, occuring naturally in animal cells, will produce superoxide (O_2^-), hydrogen peroxide peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radicals (HO·), (Diplock, 1981; Ullrey, 1981). The role these oxygen breakdown products play in the initiation of lipid peroxidation, which may lead to tissue damage, has

been reviewed by several researchers (Schwarz, 1976; Witting, 1980; Diplock, 1981; Ullrey, 1981). With the production of potentially damaging free radicals in the animal cell, the importance of a properly functioning defense system is clear. To understand the role of biological antioxidants in maintaining this defense system, it is important to understand the proposed mode of action at the cellular level.

1. Production of Free Radicals

Naturally occurring.

Various enzymatic reactions in the normal metabolic cycle of the cell will produce superoxide and hydrogen peroxide (Fridovich, 1978; Witting, 1980). However, Schwarz (1976) stated that these two breakdown products are not destructive to tissue components or cellular membranes. Hydrogen peroxide and superoxide are thought to react through a Fenton-type reaction catayzed by chelated iron to form the extremely reactive hydroxyl radical (Halliwell, 1978; Thomas et al., 1978; Witting, 1980). Witting (1980) also suggested that superoxide and the hydroxyl radical may react to form singlet oxygen. Hydroxyl radicals and singlet oxygen are thought to initiate lipid peroxidation (Kellog and Fredovich, 1975; Witting, 1980; Diplock, 1981) and therefore are destructive to tissue (Schwarz, 1976).

Iron-induced free radicals

Iron has been reported by many researchers to cause vitamin E deficiency-like lesions often resulting in death in young pigs (Tollerz and Gunnar, 1973; Cook et al., 1981; Lannek et al., 1962). The in vivo mechanism of iron's involvement in vitamin E deficiency-like lesions and death is unclear. However Patterson et al. (1967) suggested that the initial effect of iron on muscle tissue was to potentiate lipid peroxidation. Galberg et al. (1960) and Dillard's et al. (1983) work supports this by showing an increase in lipid peroxidation in iron loaded rats. Cook et al. (1981) found on microscopic examination of muscle tissue extensive degeneration. Histochemically, the degenerated lesions involved mainly the aerobic red muscle fibers. The degeneration of red muscles has previously been described as vitamin E-selenium myopathy in weanling pigs (Ruth and VanVleet, 1974).

There are several proposed mechanisms linking iron to lipid peroxidation and cell wall destruction. In a review by Diplock (1981), Cohen (1977) suggested that iron was a catalyst in a series of oxygen related reactions where hydroxy radicals (OH^-) are produced. He also suggested that $Fe^{2+} + O_2 \rightarrow [FeO_2^-]^+$ may occur where the product of this reaction is known to be a powerful oxidant. Ascorbic acid and Fe^{+2} were shown to be powerful initiators of lipid peroxidation in vitro (Fukuzawa et al.,

1981) but there is controversy whether these reactions actually occur in vivo.

Defense Mechanisms

To prevent tissue damage it is important to keep the concentrations of hydroxyl radical and singlet oxygen low. This is accomplished by enzymatic biological antioxidants. Superoxide dismutase decreases the level of superoxides by this reaction; $2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$, therefore rendering O_2 unavailable for conversion to more destructive forms (Witting, 1980). Glutathione peroxidase and catalase are responsible for the removal of hydrogen peroxide from the reaction pool, so it will not be converted to the hydroxyl radical.

Glutathione peroxidase is a selenium dependent enzyme which was shown in 1973 by Rotruck et al. to catalyze, $2GSH+H_2O_2 \rightarrow GSSG + 2H_2O$. This reaction serves an essential biochemical role in an organism, as well as providing a means of keeping the level of H_2O_2 low.

Dietary imbalances have been shown to affect the activity of superoxide dismutase and glutathione peroxidase. Dietary levels of copper and iron affect superoxide dismutase activity (Williams et al., 1975; Witting, 1980) whereas deficient levels of selenium (Rotruck et al., 1973; Chow and Tappel, 1974; Witting, 1980; Burk, 1983) and riboflavin (Brady et al., 1979) will decrease glutathione

peroxidase activity. Sklan et al. (1981) found that chicks fed diets deficient in vitamin E had an increase in both superoxide dismutase and glutathione peroxidase. It is important to note that any factor, dietary or otherwise, which decreases the activity of these enzymes may result in an increased level of hydroxyl radicals and singlet oxygen and therefore promote tissue damage.

2. Lipid Peroxidation

Lipid peroxidation involves a cyclic reaction:

$$RH \xrightarrow{r1} R\cdot$$

$$R\cdot + O_2 \rightarrow RO_2\cdot$$

$$RO_2\cdot + RH \rightarrow ROOH + R\cdot \quad \text{(Witting, 1980)}$$

where RH is a fatty acid in a membrane structure.

Initiation

The rate of the production and removal of the non-lipid free radical will determine the rate (r_1) of the first step. The variation rate constant is low and less likely to initiate production of organic free radicals when the level of hydroxyl radicals and singlet oxygen is low.

Secondary Initiation

As the cyclic peroxidation reaction proceeds there will also be a steady increase in organic peroxides (ROOH). A chain branching reaction, $2ROOH \rightarrow RO_2\cdot + RO\cdot + H_2O$,

will produce organic free radicals which will result in secondary initiation (Rui, 1961; Witting, 1980).

Defense Mechanism

To minimize the extent of secondary initiations the organic peroxides can be removed from the system by the following reaction $2ROOH + 2GSH \rightarrow 2ROH + GSSG + H_2O$ (Lawrence and Burk, 1976; Witting, 1980). This reaction is very similar to the one described earlier for the removal of hydrogen peroxides, and as one might expect, it is catalyzed by the same enzyme, glutathione peroxidase. Therefore, the reaction may be affected by the same dietary changes as were described earlier. Burk (1983) stated that glutathione-S-transferase is also capable of catalyzing this reaction. This enzyme does not have selenium as an active site and is therefore not restricted in selenium deficiency. Glutathione-S-transferase activity has in fact been shown to increase in selenium deficiency, possibly in an attempt to compensate for the decrease in glutathione peroxidase activity (Lee et al., 1981; Buck, 1983).

Cyclic Propagation

The initiation of lipid peroxidation may not be dependent upon the structure of the fatty acids making up the membrane. However, once peroxidation has begun, polyunsaturated fatty acids (PUFA) tend to oxidize at a

faster rate as compared to saturated fatty acids (Uri, 1961; Witting, 1980). Tinberg and Barber (1970) showed that peroxidation in microsomal membranes leads to a decrease in oleic (18:1) and linoleic acids (18:2) and a total disappearence of arachidonic acid (20:4), suggesting a greater peroxidation of fatty acids with a greater degree of unsaturation.

Defense Mechanisms

Vitamin E

Several roles have been suggested for vitamin E in the protection of cellular membranes against peroxidative damage. Diplock (1973) suggested that α-tocopherol functions as a membrane bound redox substance. Uri (1961) and Witting (1980) stated that α-tocopherol is an antioxidant and functions by competing with the lipids for a reaction with the lipid peroxy free radicals (RO·), therefore removing the free radicals from the peroxidation reaction. The protective relationship vitamin E has with PUFA is thought to be a consequence of specific complexes between vitamin E and especially arachadonic, but also linolenic and linoleic acids. The complex stabilizes the membranes and prevents degradation of the PUFA (Diplock and Lucy, 1973). Support for this theory can be found in work by Vos et al. (1973) which showed reduced levels of linoleic and arachadonic acids in the mitochondrial membranes of vitamin E-deficient chicks.

Dillard et al. (1983) used iron to induce lipid peroxidation in rats and found that dietary supplementation of 40 mg/kg diet α-tocopherol or 50 mg/kg diet γ-tocopherol would decrease peroxidation. They also found γ-tocopherol was only 31% as effective in preventing lipid peroxidation as α-tocopherol.

Other membrane components may affect the activity of vitamin E. Fukuzawa et al. (1981) found that when cholesterol was incorporated into the membrane in large amounts it increased the antioxidant efficiency of α-tocopherol.

Other antioxidants

Other antioxidants have been shown to be effective in vivo. Follerz (1973) found that ethoxyquin, diphenyl-B-phenylenediamine and methylene blue are effective antagonists against iron induced peroxidation. β-carotene (Ullrey, 1981), BHT, and nitroxide radicals (Hicks and Gebicke, 1981) have also been suggested as antioxidants capable of helping membranes withstand oxidative stress. However Tollerz (1973) found BHT not to be as effective as ethoxyquin, diphenyl-β-phenylenediamine and methylene blue.

B. Importance of vitamin E and selenium for swine

Vitamin E and selenium play their roles as biological antioxidants at the cellular and subcellular levels. So how does this phenomenon relate back to the pig? What are the

typical signs of a failure of the biological antioxidant system?

Several articles have discussed the lesions associated with vitamin E and selenium deficiencies in swine (Ullrey et al., 1970; Ullrey, 1973; Trapp et al., 1970; Young et al., 1980). The signs which may be observed in pigs indicating a possible E-selenium deficiency are; sudden death, hepatosis dietetica, edema, nutritional muscular dystrophy, possible decrease in reproductive efficiency and increased incidence of mastitis, metritis and agalactia (MMA).

Sudden death in weaned pigs, commonly weighing 20-40 kg, may be induced by environmental stress, fighting, or rapid growth. Many times gross lesions are not seen but upon microscopic examination E-selenium deficiency lesions may be seen in the tissue (Ullrey et al., 1970; Ullrey, 1970; Trapp et al., 1970). This is supported by Adkins and Evans in Goihl (1984), who found that pigs died within the first two weeks after weaning when vitamin E was adequate but the selenium level in the diet was only 0.025 ppm. Sudden death may also be seen in newborn pigs and finishing pigs if they are severely stressed (Ullrey, 1973). Sudden death following iron administration, for the prevention of anemia, to suckling pigs has also been studied (Tollerz, 1973).

The economic implications of E-selenium deficiency were pointed out by Ullrey (1973). He indicated that growing pigs fed a corn-soy diet unsupplemented with vitamin E and

selenium had a 15-20% mortality and a 25% morbidity rate.

Nutritional muscular dystrophy (NMD), resulting in stiff-gated, sore-muscled pigs, also may occur in E-selenium deficiency. Though NMD may occur in pigs of all ages it is most prominent in fast growing pigs which were 3-5 months old (Young et al., 1980). Most investigators found E-selenium supplementation does not improve growth rate and feed efficiency (Young et al., 1980). This was supported by recent Iowa State work which found no significant effect on average daily gain, feed intake or feed efficiency when selenium was supplemented (Goihl, 1984).

The clinical signs of E-selenium deficiency in living pigs are often difficult to detect. When the pig is necropsied typical E-selenium deficiency lesions can be seen. Any animal may have one, or a combination of the lesions described. Liver necrosis is signified by a pale, swollen appearance, with focal lesions that give the surface a rough texture. Microscopic examination of the liver usually reveals both normal and degenerative regions. The damaged area of the liver exhibits cell lysis and dilatation of sinusoids with blood giving the appearance of massive intralobular hemorrhage (Ullrey et al., 1970; Ullrey, 1973).

Other lesions typical of E-selenium deficiency are mulberry heart disease and NMD, both resulting from atrophy

of the muscle fiber as well as generalized edema. More extensive edema is found in the lungs, mucosa of the spiral colon, subcutaneous tissue and submucosa of the stomach (Trapp et al., 1970; Ullrey et al., 1970; Ullrey, 1973). Trapp et al. (1970) and Ullrey et al. (1970) also reported some incidence of anemic, jaundiced pigs or pigs with esophagogastric ulcers associated with E-selenium deficiency.

Reproduction

Vitamin E and selenium supplementation of the sow has been stated in several reviews to decrease the incidence of mastitis-metritis-agalactia (MMA), spraddle-legged newborn pigs and impaired fertility (Ullrey, 1981, Lannek, 1973). Ullrey (1971) in a two year study found that supplementing the sow's diet with vitamin E, and in some cases selenium, significantly decreased the incidence of MMA. This is in agreement with Vale (1983) who saw signs of MMA in five of ten sows fed a vitamin E and selenium unsupplemented diet and no signs of MMA in the sows receiving the supplemented ration. Nielsen et al. (1973) found that when oxidized herring oil was added to a diet, which was not supplemented with vitamin E, a very pronounced reduction in milk yield occurred, however mastitis and metritis symptoms were not seen.

There is a great deal of disagreement in the literature concerning the effect of vitamin E and selenium on litter size at birth and weaning. Vale (1983) found that sows which were supplemented with vitamin E and selenium throughout gestation and lactation had larger litters, weaned heavier pigs and tend to have increased livability of the pigs.

In a two year study conducted by Ullrey et al. (1971) sows were fed diets supplemented with vitamin E, vitamin E + selenium, or vitamin E, selenium and choline. Supplementing vitamin E tended to increase the number of pigs surviving at three weeks and to reduce the incidence of MMA. Mahan et al. (1975) fed sows one of two diets for two successive parities. One of these diets was deficient in selenium, the other was supplemented with 0.1 ppm selenium. By the second parity the sows on the deficient diet had significantly smaller litters. Malm et al. (1976) and Young et al. (1977) found no effect of vitamin E and selenium supplementation on the sow's reproductive performance.

In cattle a prepartum selenium injection was effective in reducing the incidence of metritis and cystic ovaries during the early postpartum period (Harrison et al., 1984). Work of this type is limited for swine, however, Young et al. (1977) reported that dietary levels of vitamin E and selenium did not effect rebreeding of sows.

Although the exact effect of low vitamin E and selenium on reproductive performance is not clear, it is certain that vitamin E and selenium are required in the sow's diet. Young et al. (1977) using a basal diet which consisted of high moisture corn and Malm et al. (1974) feeding a semi-purified diet, (both diets containing very low levels of natural vitamin E and selenium with no supplementation of these nutrients), reported heavy death losses. Necropsies of the animals showed various vitamin E and selenium deficiency lesions. Supplementation of either vitamin E (60 IU/kg) or selenium (0.6 ppm) to the high moisture corn diet prevented the death losses and the lesions (Young et al., 1977).

Baby pig survival

The survival rate of pigs between birth and weaning need to be improved upon. Although there are many factors which contribute to death losses among pigs during these first few weeks of life, there is reason to believe that adequate vitamin E and selenium may enhance the pig's livability.

Young et al. (1977) found that supplementation of the sow's diet with E-selenium in gestation and lactation increased the E-selenium levels of colostrum and milk. Through placental transfer and consumption of colostrum the serum levels of E-selenium were in turn increased in the pigs. If E-selenium were not supplemented in the sow's diet the levels remained very low in the pig. Environmental

stress, to which newborn pigs may be exposed, has been suggested to induce E-selenium deficiency lesions in low to borderline pigs (Ullrey et al., 1970). An Iowa State study supports the livability theory in their finding of a 20% increase in livability from birth to weaning when pigs from sows on selenium-deficient diets were given 100 IU dl-α-tocopherol, IM, at birth (Goihl, 1984).

In Great Britain and Scandanavian countries E-selenium deficiency has been attributed to the increased sensitivity of pigs to recommended doses of oral or parenteral iron (Lannek et al., 1962; Patterson et al., 1967; Tollerz, 1973). The iron toxicity occured when pigs from sows fed diets high in PUFA and low in vitamin E, were given oral iron (ferrous sulfate or ferrous fumarate) or parenteral iron (iron dextran) in recommended dosages. Affected pigs have clinical signs 8-12 hours after the iron was given. Death follows a few hours later. Not all pigs showing signs of iron toxicosis died (Tollerz, 1973). Postmortem histological examination of the pigs showed waxy muscle degeneration and occasionally pericardium and thoracic edema (Arpi & Tollerz, 1965; Lannek et al., 1962).

Tollerz (1973) suggested that iron-induced muscle damage allowed the contents of the cells to enter the blood. This is in agreement with Patterson et al. (1967) who suggested that the initial effect of iron was to potentiate lipid peroxidation. Above a limiting amount of muscle

peroxides (possibly indicating cell membrane destruction) there was a rapid release of muscle potassium. Death resulted from cardiac arrest and hypercalcemia. Tollerz (1973) found that α-tocopherol and sodium selenite were only effective in preventing iron toxicosis if they were given 24 hours (vitamin E) or several days (selenium) prior to the iron treatment. Synthetic antioxidants, ethoxyquin, diphenyl-β-phenylenediamine and methylene blue were all effective in preventing iron toxicosis when given along with the iron.

Iron toxicosis in nursing pigs is much more difficult to produce in the United States. Cook et al. (1981) was able to produce some muscle lesions in pigs from sows on an E-selenium inadequate diet, supplemented with 5% cod liver oil when the pigs were given 750 mg of iron from iron-dextran IM. However, it should be noted that no deaths occurred, muscle lesions were seen in only 2 of the 8 pigs treated and the iron injection given was much larger than the recommended dose. Given a recommended dosage of iron as either iron dextran (IM) or ferrous sulfate (oral) no iron toxicosis was seen in piglets born to sows on a low E-selenium diet (Miller et al., 1973).

C. Source

1. Vitamin E

Tocopherols are not synthesized by mammals and therefore become part of mammalian tissue primarily through ingestion of natural occurring tocopherols (found mostly in plants), or from synthetic forms of tocopherol (Machlin, 1980). There are eight naturally occurring vitamin E compounds. Of these eight compounds d-α-tocopherol is biologically the most active. The biopotency of the remaining compounds decrease dramatically with d-γ-tocopherol 40%, d-β-tocopherol 10% and d-α-tocotrienol 25% as active as d-α-tocopherol (Bieri and McKenna, 1981). Forages are a good source of vitamin E. In fact, before many producers began raising pigs in total confinement, the NRC (1968) stated: "It is unlikely that practical swine diets would be deficient in vitamin E unless the diet contained excessive amounts of highly unsaturated or oxidized fats." Ullrey (1981) attributes two factors to the increased incidence of E-selenium deficiencies in swine. These are (1) the increased practice of having the entire life-cycle of swine in confinement and (2) the low level of E-Se in corn-soy diets in the Midwestern United States.

Tocopherol values for corn vary. Bauernfeind and Cort (1974) cited by Ullrey, (1981) reported α-tocopherol at 6 mg/kg and γ-tocopherol at 38 mg/kg. Pond et al. (1971) published average values of 1.5 mg/kg and 20.6 mg/kg for α- and γ-tocopherol, respectively, and Young et al. (1975)

published a value for corn of 9.3 mg/kg of α-tocopherol. A great deal of variation seen in corn tocopherol values may be due to the post-harvest handling of the corn. Drying corn at either high or low temperatures did not seem to affect the actual tocopherol content of the corn. However, the high temperature did decrease the biopotency of the tocopherol when selenium levels in the corn were low (Pond et al., 1971). This is supported by Young et al. (1975) as well as Chow and Draper (1969) who reported little to no destruction of tocopherol in corn with artificial drying. However Chow and Draper (1969) found that both unsaturated fatty acids and vitamin E can be destroyed in corn if it is over-dried, particularly by over-drying at high temperatures. High moisture corn treated with propionic acid or acetic-propionic acid and stored for 230 days showed a dramatic decrease in α-tocopherol, from 9.3 mg/kg to 1.2 mg/kg (Young et al., 1975). Chow and Draper (1969) suggested and Young et al. (1973) agreed that the high moisture content may be the main contributing factor to the decrease in α-tocopherol.

Soybean meal is relatively low in α-tocopherol. Both 44 and 48% soybean meals have been reported to have 3 mg/kg (Brunnel et al., 1968; Ullrey, 1981).

Commercial sources of vitamin E are either the acetate or the hydrogen succintate esters of d-α-tocopherol or the acetate ester of dl-α-tocopherol (Ames, 1979). Young et al. (1975) checked the stability of α-tocopherol acetate in

feeds held under various storage conditions and found that the α-tocopherol potency was retained for at least 70 days in artificially dried corn and corn treated with acetic-propionic acid. The potency of α-tocopherol was retained for at least seven days in high moisture ensiled corn.

2. Selenium

Selenium can be incorporated into swine diets in a number of different forms ranging from the inorganic sodium selenite ($Na_2 SeO_3$) and sodium selenate ($Na_2 SeO_4$) (NRC, 1979), to the organic seleniferous compounds found in plants. Selenium in grain is primarily associated with the protein (Selenium and Nutrition, NRC, 1983).

Scott (1973) found that the bioavailability of selenium from naturally occurring feeds was only 30 to 80% that of the Se in sodium selenite for the prevention of exudative diathesis in chickens. The bioavailability of selenium in corn and soybean meal was reported as 83 and 64%, respectively. Groce (1973) found lower serum blood levels of selenium when pigs were fed seleniferous corn as compared to those in pigs fed the same level of Se as sodium selenite. This may be explained by the greater retention of selenium in the tissues when supplied by seleniferous corn (Ku et al., 1972; Groce et al., 1971, 1973).

The low level of selenium in grain is a problem in many areas. The areas of the United States which are typically

low in selenium are the Northwest, Northeast, Southeast and regions surrounding the Great Lakes, which includes most of Michigan, Ohio, Indiana, Illinois, and Wisconsin. (Selenium in Nutrition, 1983). The concentration of selenium in grain is dependent upon the type of soil. Sandy loam soil with a low pH will normally produce grain low in selenium. Variety of grain produced may also be a factor in the Se concentration.

Across the United States there is a great deal of variation in selenium content of grains. The ranges reported in Selenium in Nutrition (NRC, 1983) for ingredients typically used in swine diets were: soybean meal, .06-1.00 ppm, whole soybeans 0.07-0.90 ppm, corn .01-1.00 ppm and dicalcium phosphate .15-1.00 ppm. Selenium content of Michigan corn normally fall at the lower end of the range with a mean value of .033 ppm and a range of .013-.089 ppm (Groce, 1972).

Groce et al. (1973) found that selenium in a stored selenite-glucose premix was not retained as effectively as that in a fresh premix. They suggested that the selenite was changed to elemental selenium and was not absorbed as efficiently.

D. Requirement

The NRC (1979) recommended .15 mg selenium and 10-15 IU vitamin E per kilogram of diet for bred gilts and sows and lactating sows. However, it should be recognized that the

levels of vitamin E and selenium required are dependent upon a number of dietary factors as well as environmental factors.

The common function of vitamin E and selenium as biological antioxidants explains the relationship between the required levels of the two nutrients. A sparing effect was demonstrated by Groce et al. (1973) who found increased amounts of selenium excreted in the urine when vitamin E was added to the diet. This suggests that selenium is retained to meet the requirement and the excess is excreted in the urine. Feeding diets high in PUFAs will increase the requirement for vitamin E. It has been suggested that the lability of cell membranes varies with the PUFA content of the constituent phospholipids, and that this composition is sensitive to the dietary level of PUFAs. As more PUFAs are incorporated into the membrane, the requirement for membrane-bound tocopherol is increased and therefore the dietary requirement is increased (NRC, 1979). Dam (1962), on the otherhand, suggested that dietary PUFAs accelerate the depletion of tocopherols in feeds and therefore increase the requirement.

The presence of kidney beans or peas in the diet inhibits absorption of vitamin E and thereby increases the dietary requirement (Desai, 1966; Huntz and Hogue, 1964; Machlin, 1980), while the addition of synthetic biological antioxidants to the diet will decrease the requirement (NRC, 1979). Excessive heat, cold or other stresses may also

increase the dietary requirements for these nutrients (NRC, 1979).

Materials and Methods

A. Sows

Nineteen Yorkshire X Landrace gilts were balanced by litter, placed on two dietary treatments and bred as they came into heat. The diets were formulated from dried high moisture corn. The analysis of the basal diet found 0.78 µg/g DM vitamin E (E) and 0.04 ppm selenium (Se), while the vitamin E and selenium supplemented diet had analyzed values of 56.1 µg E/g DM and 0.12 ppm Se respectively. The gilts were housed in total confinement during gestation and lactation. Five of the sows from the supplemented group and ten of the sows from the basal group remained in the second parity gestation period. These animals were kept on their respective dietary treatments but were switched to a diet formulated with dried shelled corn (Table 1). By analyses the basal and supplemented gestation diets contained 7.1 and 44.6 µg E/g DM and 0.06 and 0.18 ppm Se, respectively whereas the basal and supplemented lactation diets contained 4.7 and 14.9 µg E/g DM and 0.05 and 0.26 ppm Se, respectively. The sows were on the shelled corn diet from their second parity gestation through their third parity lactation. There was three sows on each dietary treatment in the third parity. This thesis reports only second and third parity results.

Table 1. Composition of Diets

Ingredients	International Ref. No.	Gestation Basal	Gestation +Vitamin E & Se
Ground shelled corn	4-02-931	85.3	85.24
Soybean Meal (44)	5-04-607	11.0	11.0
Mono-Dicalcium Phosphate	6-01-080	1.5	1.5
Limestone	6-02-632	1.2	1.2
Salt		0.5	0.5
VTM (premix)[a]		0.5	0.5
Selenium 90 (premix)[b]		0.0	0.05
Vitamin E (50%)[c]		0.0	0.01
Total		100.00	100.00

Ingredients	International Ref. No.	Lactation Basal	Lactation +Vitamin E & Se
Ground Shelled Corn	4-02-931	72.86	72.36
Soybean Meal (44)	5-04-607	23.5	23.5
Mono-Dicalcium Phosphate	6-01-080	1.5	1.5
Limestone	6-02-632	1.1	1.1
Salt		0.5	0.5
VTM (premix)[a]		0.5	0.5
Selenium, Vitamin E premix[d]		0.0	0.5
Chlorachel 50[e]		0.04	0.04
Total		100.000	100.000

[a]Supplying the following per kg of diet: Vitamin A, 3300 IU; Vitamin D_3, 660 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; d-pantothenic acid, 13.2 mg; choline, 110 mg; vitamin B_{12}, 19.8 µg, zinc, 75 mg; iron, 60 mg; manganese, 37 mg; copper, 10 mg and iodine, 0.5 mg.

[b]Contained 200.2 mg Se/kg and was supplemented to provide 0.1 ppm Se in the sow's diet.

[c]Supplied 500 IU/g and was supplemented to provide 50 IU/kg of diet.

[d]Consists of 89.3% finely ground corn, 10% Selenium 90 Premix and Vitamin E 50% and was supplemented in the sow's diet to provide 17.5 IU vitamin E/kg and 0.1 ppm Se.

[e]Chlorachel 50 is a trade name for Chlortetracycline, Calcium Complex, Rachelle Laboratories, Inc.

Blood samples were taken from sows in late gestation, immediately post-farrowing and at twenty-one days of lactation. Ten milliliters of blood were drawn from the anterior vena cava with a sterilized 3.5 inch, 18-gauge needle and 20 ml syringe. The blood was transferred to 10 ml heparinized centrifuge tubes and covered until transported to the lab for further processing.

Weights were taken on the sows when they were moved into the farrowing house, after parturition and at 21 days post-farrowing.

At parturition the sows were carefully monitored. If farrowing intervals were greater than 20 minutes, 60 U.S.P. units of oxytocin were given IM. If the oxytocin had no effect, the sow was assisted in delivering the pigs. The numbers of mummies, stillbirths and live pigs were recorded at the end of farrowing.

Colostrum samples were collected on all sows while farrowing. Milk samples were taken at 2 and 21 days post-farrowing by administering 60 U.S.P. of oxytocin IM, washing the udder with warm water and drying with paper toweling, to stimulate milk ejection. Uniform amounts of milk were stripped from all functional nipples on one side of the sow's udder. Enough milk was obtained at each sampling to fill a one ounce, Lermer poly-opal plastic ointment jar. The full containers were capped and frozen at -20C until analyzed. Jansson et al. (1981) reported no

significant effect of freezing (-20C) on human milk tocopherol levels.

B. Pigs

The pigs were separated from the sows immediately after birth, to prevent suckling. At the end of the farrowing process the pigs were weighed, a 10 ml blood sample was taken and one half of the litter was given 200 mg parenteral iron, in the form of gleptoferron IM[1]. Blood samples were taken by laying the pigs on their backs in a V-shaped holder and as restraint was provided by one person, the second person drew the blood from the anterior vena cava using a 1 inch, 20-gauge needle and a 10 ml glass syringe. Blood was put in 10 ml heparinized centrifuge tubes and covered until they could be taken to the laboratory for further processing.

At 2 days of age the pigs were weighed, a 10 ml blood sample was taken in the same manner as described earlier, and the pigs not receiving iron previously were given 200 mg iron in the form of gleptoferron IM to prevent anemia.

The pigs were again weighed and bled at 21 days of age. The procedure for bleeding was similar to that described for the younger pigs, but in this case an 18-gauge, 1 1/2 inch needle was used.

[1]A polysacharide complex of beta-ferric oxyhydroxide and dextran glucoheptonic acid, Burns-Biotec Laboratories, Inc.

C. Laboratory Analyses

Hematology

Packed cell volume (PCV) or hematocrit was determined on heparinized blood samples using the method described by McGovern et al. (1955). Each blood sample was drawn into a microhematocrit tube, the tube was sealed at one end using a flame, then it was centrifuged in an International Model MB microhematocrit centrifuge at 13,000 X g for 5 minutes. Spun samples were immediately read on an International microhematocrit reader.

Hemoglobin (Hgb) concentration was determined by putting 0.02 ml of whole blood into 5 ml of Drabkins solution. The blood cells lyse, and ferricyanide converts the iron of hemoglobin from the ferrous to the ferric state to form methemoglobin. Methemoglobin reacts with potassium cyanide to form cyanmethemoglobin, a stable pigment with an absorbance which can be measured by a spectrophotometer at a wavelength of 540 nm (Wintrobe, 1980). The absorbance value is then multiplied by a conversion factor, characteristic of the specific Drabkins solution, to obtain hemoglobin concentration in grams per deciliter (g/dl).

Mean corpuscular hemoglobin concentration (MCHC) may be calculated using the Hgb and PCV values for each blood sample as shown: MCHC% = (Hgb(g/dl)/PCV%) X 100. The percentage MCHC represents the concentration of hemoglobin in the red blood cells.

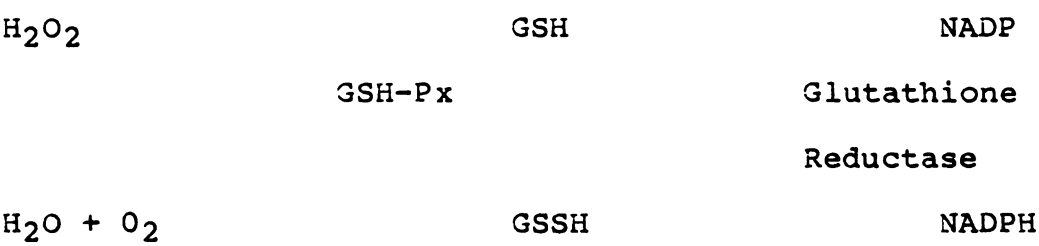
Sample storage

Heparinized blood was centrifuged for 15 minutes at 3000 rpm. The plasma was harvested into 5 ml falcon tubes, the air was removed from the tubes with a gentle stream of nitrogen and caps were snapped onto the tubes immediately. The tubes were stored in the freezer (-20C) until further analyses could be run.

Plasma Analyses

Plasma glutathione peroxidase (GSH-Px) activity was determined by the coupled assay of Paglia and Valentine (1967) as revised by Lawrence et al. (1974). In this assay known amounts of NADPH and glutathione reductase were placed in a 1 ml cuvette in combination with a potassium phosphate buffer solution. Reduced glutathione (GSH) solution (0.05 ml) was added to the cuvette along with 0.04 ml of plasma which contains an unknown amount of GSH-Px. Finally hydrogen peroxide (H_2O_2) was added to the cuvette to initiate the reaction (Figure 1). The decrease in activity

Figure 1. Reaction Involved in Measuring GSH-Px Activity



of NADPH (A_{340}) is measured at a wavelength of 340 nm on the Varian 634 spectrophotometer and recorded on the Varian model 9176 chart recorder for five minutes. Since there were constant amounts of all the reaction components except GSH-Px any change in NADPH activity was reflective of the amount of GSH-Px in the plasma. Glutathione peroxidase activity was reported in enzyme units (EU) which are moles of GSH oxidized per minute. The EU for a plasma sample were calculated by: (the change in A_{340} of plasma/min. - the change in A_{340} of blank/min.) X 8.0386. The factor 8.0386 is determined by sample size, the molar extinction coefficient of 6.22×10^3 for NADPH and the stoichiometry for the reaction, 2 moles GSH per mole of NADPH oxidized.

Alpha-tocopherol was determined in plasma samples by a fluorometric procedure developed by Whetter and Ku (1982) from a tissue α-tocopherol procedure (Taylor, 1976). The α-tocopherol in the sample can be dramatically reduced by oxidation. Therefore, careful precautions were taken to minimize the oxidation process. This was accomplished by extracting the samples in acid washed glassware, keeping the samples on ice throughout the extraction process and displacing the air in the test tubes with nitrogen (N_2) prior to vortexing.

Duplicate standards were prepared from a stock standard

solution[1] in absolute ethanol (AR grade) to obtain standards of 0, 1, 2, and 4 α-tocopherol/ml. Two millilters of absolute ethanol were added to the test tubes prepared for the plasma samples followed by 1 ml of plasma. One milliliter of deionized distilled water (DDH_2O) was added to each of the standards to bring them to equal volume with the sample and all tubes were vortexed for 5 seconds to precipitate the protein.

Cyclohexane (2 ml, Eastman Kodak, AR grade) was added to each preparation followed by 20 seconds of vortexing to extract the α-tocopherol from the sample. Centrifugation of the samples in a Damon/IEC Model PR-6000 refrigerated centrifuge at 2070 X g for 15 min. provided complete sedimentation of the sample debris from the cyclohexane X α-tocopherol layer. The α-tocopherol in the cyclohexane will fluoresce at excitation 296 mμ and emission 330 mμ. This layer was carefully transferred to 2 dram vials, and read in the Aminco-Bowman spectrophotofluorometer set at the appropriate wavelengths. The reading from the spectrophotoflourometer was percent transmission (%T). To calculate the concentration of α-tocopherol in the plasma, the known concentration of α-tocopherol in the standards and the %T for the standards were used to formulate a curvilinear regression line. The %T for the samples were

[1]Stock standard was 2 mg α-tocopherol/ml in hexane which was stored in the freezer (-20C).

then read off of this line to determine plasma α-tocopherol in μg/ml.

Plasma selenium (Se) concentration were determined by a spectrofluorometric procedure (Whetter and Ullrey, 1978). Duplicate plasma samples (1 ml) and duplicate standards of 0, 0.05, 0.1, 0.2 μg of Se/ml were digested in 3 ml of nitric acid (HNO_3) and 2 ml of perchloric acid ($HClO_4$, and the HNO_3 was driven off. Nine milliliters of ethylene diamino tetraacetic acid (EDTA) was used to wash down the sides of the digestion flask and prevent contamination of the sample with other metals. Approximately 1 ml of concentrated ammonium hydroxide (NH_4OH) was added to each sample to neutralize the remaining $HClO_4$. Cresol red was added to indicate the proper acid:base balance of the sample. Any adjustment in pH was made by adding drops of NH_4OH or HCl (1:9).

Five milliliters of 2,3,Diaminonaphthalene (DAN) added to each sample complexed with the Se to form diazoselenol, a light sensitive complex. This complex was then extracted into cyclohexane (5 ml), and transferred to a test tube to allow its %T to be read in the Aminco-Bowman spectrophotoflourometer (excitation 376 mμ and emission 510 mμ). Plasma Se concentration (μg/ml) were read off a curvilinear regression line calculated from similarly processed standards.

Plasma iron concentrations were determined by an atomic absorption spectrophotometric procedure (Olson and Hamlin, 1969). Plasma (1 ml) and 20% trichloroacetic acid (TCA) (2 ml) were combined in plastic centrifuge tubes incubated at 90C for 15 min. in a waterbath to precipitate the plasma proteins. The cooled samples were centrifuged at 2070 x g for 15 min. and the clear supernatant was transferred to 5 ml falcon tubes and read on the IL951 atomic absorption emission spectrophotometer at a wavelength of 248.3 nm. Iron concentration was expressed in µg/ml supernatant. A simple formula was used to correct this volume for volume as well as absorption due to the TCA. Formula: (sample µg/ml - standard µg/ml) X 300 = plasma iron in µg/dl.

Sow Colostrum and Milk Analysis

Alpha- and beta/gamma- tocopherols were determined in sow's colostrum and milk by reverse phase HPLC (Whetter and Loudenslager, 1984).

Ascorbic acid (0.5 g) was weighed into acid washed test tubes to prevent oxidation of the tocopherols. Duplicate standards of 1, 2, 4 and 8 µg of γ-and α-tocopherol/ml were prepared from the stock standards[1], methanol and DDH_2O,

[1]The stock standard solutions were 2 mg γ-tocopherol in methanol and 2 mg α-tocopherol/ml in methanol. Both standards were prepared from Eastman products prior to any analysis and stored in a freezer (-20C) for use in all milk-tocopherol analytical procedures.

at the beginning of an analytical run and were processed as the samples. Samples were weighed into test tubes, and two milliliters of methanol (HPLC grade) (2.5 for colostrum) were added to each sample. The air in the test tube was displaced with nitrogen (N_2) and the samples were vortexed (60 sec). Deionized distilled water (1.5 ml) was added to each sample, air was displaced from the sample and the sample was vortexed (10 sec) and allowed to set for 5 min. This procedure precipitated the protein in the milk and allowed the fat soluble tocopherols to be extracted more uniformily. Potassium hydroxide (1 ml for milk, 1.5 ml for colostrum) was added to each sample, and the samples were incubated for 15 min. in a 60C waterbath to saponify the fat. The samples were cooled in ice water for 3 min., 3 ml of HPLC grade n-hexane was added and the samples vortexed (60 sec.) to extract the tocopherols and stop the saponification reaction. Care was taken in the saponification step to assure equal time of exposure of each sample to the KOH. Prolonged exposure to KOH could be very damaging to the tocopherols and may cause undue variation between samples if unequal exposure times existed. The samples were centrifuged for 15 min. at 2070 X g to remove debris from the hexane layer. The hexane X tocopherol layer was quantitatively transferred from the sample to a 25 ml Erlenmeyer flask. The hexane was evaporated off in an

evaporation oven leaving only the tocopherols in the flask. The tocopherols were picked up with 1 ml of methanol and filtered through Millex-HV filter units (0.45 µm) into 2 dram, screw capped vials. The samples at this point could be stored under N_2 in the freezer for up to two days prior to reading them on the HPLC. The samples were read on HPLC instrumentation from Waters and Associates, Inc. (Milford, Mass). The system consisted of a model 45-M solvent delivery system, a model U6K universal liquid chromatograph injector, and a model 440 absorbance detector. The detector was connected to a Servogor 120 recorder set at 0.25 cm/min. A RCSS Guard-Pak (C_{18}) used as a pre-column, and a Bondapak C_{18} reverse phase, 3.9 mm X 15 cm column were used on the system. The solvent mobile phase was 95% methanol: water, and was pumped through the system at 1.5 ml/min. The samples were read off of a curvilinear standard regression line as described for plasma tocopherol then corrected for weight differences and reported in µg/g of milk.

Milk Se values were determined by the procedure described earlier for plasma Se determination (Whetter and Ullrey, 1978), with a few minor revisions to compensate for the high fat content and viscous consistency of sows milk. Approximately 1 ml of milk was weighed into the digestion flask and the weight (g) recorded. The final concentrations were then recorded as µg of Se/g of sample to correct for

inconsistencies in allotting the thick samples. To eliminate problems associated with the high fat content the samples were allowed to digest slowly, overnight, in the acids (3 ml, HNO_3 and 2 ml, $HClO_4$, 70%) before heat was applied to complete the digestion process. The rest of the analysis was carried out as described for the plasma Se analysis.

Total fat was determined on duplicate milk and colostrum samples by a procedure modified by Loudenslager and Whetter (1984) from the Roese-Gottlieb method (1980).

Approximately one gram of milk (colostrum) was weighed into a test tube and the sample weight recorded. To allow for a more complete fat extraction the fat micelles were broken down by adding 0.2 ml concentrated ammonium hydroxide (NH_4OH) and vortexing (10 sec), and the protein was precipitated as a fine white powder by adding 3 ml absolute ethanol and vortexing (1 min). The fat was extracted with diethyl ether (3 ml), vortexing (1 min) and petroleum ether (3 ml) and vortexing (1 min) followed by 15 min. of centrifugation at 1440 x g. The ether layer was decanted into a pre-weighed 25 ml Erlenmeyer flask, using the indentation on the test tube to hold back debris. The substances remaining in the test tube were re-extracted as described above, and the second ether layer was added to the flask with the first. The ether was allowed to evaporate under the hood overnight. The flasks containing the fat

were dried in an evaporating oven, removed and weighed. Percent fat was calculated as follows:

$$\frac{\text{Sample flask wt. - initial flask wt.}}{\text{milk sample wt.}} \times 100 = \% \text{ fat}$$

Statistical Analyses

The sow data were analyzed in a split-plot design, testing the effects of diet and time on each variable measured. In the event of a significant ($P<.05$) interaction Scheffe's test was used to determine the significance of the diet effect at each time and the time differences for each diet.

The pig data for birth and twenty-one days of age were analyzed using a nested, one-way analysis of variance design. In this design pigs were nested within litters, to eliminate a bias associated with non-random allotment of pigs to treatment groups.

The measures analyzed for two-day old pigs required a split-plot design to test for diet and iron treatment effects. Once again, a nested analysis was used. When a significant diet X iron interaction occurred Scheffe's test was used to test the differences between means.

In this study a difference was considered highly significant if $P<.01$; significant if $P<.05$ and nearly significant if $P<.10$. Probability estimates of .25 were

reported to indicate trends, as this study is preliminary and a relatively small number of sows were used.

Homogeneous variance is one of the main assumptions underlying statistical analyses. In the analysis for this study homogeneity was tested by Bartlett's and Cochran's tests. If the probability values for the tests were less than 0.05 heterogeneous variance was considered to be present.

All statistical analyses were made by using the multiple analysis of variance program which is part of the SPSS[1] program package.

[1]SPSS-Statistical Programs for the Social Sciences, Update 7-9.

Results and Discussion

I. Sows

One of the sows on the basal diet went down during the second parity gestation. She delivered her pigs but she had to be sacrificed after her second week of lactation. A necropsy of the sow found E + Se-deficiency lessions in her muscle, however it was a fractured vertebra that crippled her. This sow and her pigs were not included in the analyses for this study.

There was no significant effect of dietary treatment on the hematology of the second and third parity sows (Tables 2 and 3). There was a tendency for second parity sow's hemoglobin and hematocrit to drop at farrowing and remain at a depressed level throughout lactation. This trend however was not seen in the third parity sows. The mean corpuscular hemoglobin concentration decreased significantly in the second parity sows from farrowing to the end of the lactation period. Again, this change was not seen in the third parity sows. The inconsistencies of the hematology findings between parities may be due to the small number of sows represented in the third parity, rendering small changes in blood values undetectable, or the heterogeneous variance seen in the second parity blood values may have created false differences.

Table 2. Effects of Dietary E & Se Supplementation on 2nd Parity Sow's Hematology

Diet	Time: Late Gestation	P[a]	Farrowing	P[a]	21 Days	MSE
			Hemoglobin (g/dl)[c]			
Supplemented	12.7	<.25	11.8	NS	11.5	1.1
Basal	12.9	<.25	11.8	NS	12.1	1.1
P-Value[b]	NS		NS		NS	
MSE	2.4		2.4		2.4	
			Hematocrit (%)[c]			
Supplemented	36.8	<.10	33.4	NS	34.6	8.7
Basal	37.1	<.10	33.3	NS	34.9	8.7
P-Value[b]	NS		NS		NS	
MSE	18.8		18.8		18.8	
			Mean Corpuscular Hemoglobin Concentration (%)[c]			
Supplemented	34.5	NS	35.4	<.05	33.2	2.2
Basal	34.7	NS	35.5	<.05	34.6	2.2
P-Value[b]	NS		NS		NS	
MSE	2.6		2.6		2.6	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sows diet.
[c]Heterogeneous variance existed, therefore the significant may be incorrect.

Table 3. Effects of Dietary E & Se Supplementation on 3rd Parity Sow's Hematology

Diet	Time					
	Late Gestation	P[a]	Farrowing	P[a]	21 Days	MSE
			Hemoglobin (g/dl)			
Supplemented	11.8	NS	10.8	NS	11.2	0.5
Basal	12.6	NS	11.3	NS	11.2	0.5
P-Value[b]	NS		NS		NS	
MSE	2.3		2.3		2.3	
			Hematocrit (%)			
Supplemented	33.7	NS	30.3	NS	31.5	3.0
Basal	33.6	NS	33.0	NS	33.0	3.0
P-Value[b]	NS		NS		NS	
MSE	19.0		19.0		19.0	
			Mean Corpuscular Hemoglobin Concentration (%)			
Supplemented	35.2	NS	34.6	NS	35.4	2.1
Basal	34.9	NS	34.2	NS	33.9	2.1
P-Value[b]	NS		NS		<.10	
MSE	0.8		0.8		0.8	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sows diet.

Table 4. Effects of Dietary E & Se Supplementation on 2nd Parity Sow's Plasma

Diet	Time					
	Late Gestation	P[a]	Farrowing	P[a]	21 Days	MSE
			Plasma Selenium (µg/ml)			
Supplemented	0.22	NS	0.22	NS	0.20	0.005
Basal	0.15	NS	0.12	<.10	0.09	0.005
P-Value[b]	<.001		<.001		<.001	
MSE	0.001		0.001		0.001	
			Plasma Vitamin E (µg/ml)			
Supplemented	2.35	<.001	1.52	NS	1.42	0.118
Basal	1.31	<.001	0.69	NS	1.04	0.118
P-Value[b]	<.001		<.001		<.001	
MSE	0.186		0.186		0.186	
			Plasma Glutathione Peroxidase (EU/ml)			
Supplemented	1.18	<.01	0.89	<.10	1.06	0.023
Basal	0.97	<.01	0.78	<.10	0.67	0.023
P-Value[b]	<.10		<.10		<.10	
MSE	0.140		0.140		0.140	
			Plasma Iron (µg/dl)			
Supplemented	119.4	<.10	129.3	<.01	147.0	816.5
Basal	120.7	<.10	103.6	<.01	143.9	816.5
P-Value[b]	NS		NS		NS	
MSE	1954.4		1954.4		1954.4	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sows diet.

Table 5. Effects of Dietary E & Se Supplementation on 3rd Parity Sow's Plasma

Diet	Time					
	Late Gestation	P[a]	Farrowing	P[a]	21 Days	MSE
			Plasma Selenium (µg/ml)			
Supplemented	0.15	NS	0.13	<.01	0.26	0.001
Basal	0.08	<.25	0.14	<.10	0.07	0.001
P-Value[b]	<.01		NS		<.001	
MSE	0.0003		0.0003		0.0003	
			Plasma Vitamin E (µg/ml)			
Supplemented	1.86	<.06	0.88	NS	0.87	0.28
Basal	0.80	<.06	0.41	NS	0.24	0.28
P-Value[b]	<.01		<.01		<.01	
MSE	0.03		0.03		0.03	
			Plasma Glutathione Peroxidase (EU/ml)			
Supplemented	1.05	<.02	0.62	NS	0.79	0.02
Basal	0.70	<.02	0.59	NS	0.50	0.02
P-Value[b]	NS		NS		NS	
MSE	0.10		0.10		0.10	
			Plasma Iron (µg/dl)			
Supplemented	132.2	NS	136.1	NS	136.1	781.8
Basal	115.6	NS	118.5	NS	162.3	781.8
P-Value[b]	NS		NS		NS	
MSE	165.1		165.1		165.1	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sows diet.

Plasma values for second and third parity sows are presented in tables 4 and 5. Plasma Se analyses on samples from second parity sows showed a highly significant ($P<.001$) response to dietary supplementation of vitamin E and Se. The supplemented sows had a much higher level of plasma Se during gestation and maintained this level throughout lactation, whereas the sows on the basal diet showed a highly significant decrease in plasma selenium by the end of the lactation period. The sows who remained on the basal diet into their third parity gestation did not appear to recover their initial plasma selenium level. A nearly significant ($P<.10$) decrease in plasma selenium occurred during the third parity lactation.

Mahan et al. (1975) reported that sows on a Se-supplemented diet maintained their serum selenium value while sows on a non-supplemented diet containing 0.03 and 0.05 ppm natural selenium during gestation and lactation showed a decrease in serum selenium by the end of lactation and did not return to their initial serum selenium value during the subsequent gestation. The trend was similar to that seen in the sows on this study. Mahan et al. (1975) suggested that this trend may indicate an increased requirement for selenium during lactation, which can be effectively met by supplementing the sow's diet with 0.1 ppm selenium.

Sows fed the basal diet in both parities had significantly ($P<.001$, $P<.01$) lower plasma vitamin E levels than the supplemented sows. There was a drop in plasma vitamin E levels ($P<.001$) in sows on both dietary treatments at the second parity farrowing. This same trend was seen in the third parity sows, however, due to the low number of sows represented in this parity the confidence level was only 94%. The rapid decline in plasma vitamin E at parturition may be a result of the very high level of vitamin E secreted into colostrum (Tables 7 and 8). There was no significant change in the sow's plasma vitamin E level throughout lactation. This may also be indicative of the levels of total-tocopherols the sow passes into the milk. After the initial secretive burst of colostral vitamin E resulting in a depletion of the sow's plasma vitamin E, the milk tocopherol level immediately decreases. The level of tocopherol in milk continues to decline at a much slower rate from the second day post-farrowing to the end of the lactation period, thereby allowing the sow to maintain her plasma vitamin E level.

Malm et al. (1976) reported serum vitamin E values for sows on a vitamin E and lard supplemented diet similar to those reported for the sow in this study. However, the sows on the basal and lard diet had much lower serum selenium values than were indicated here. The drop in tocopherol at parturition and the relatively constant circulating levels

of serum tocopherol throughout lactation appeared to be consistent with findings in this study.

There was a nearly significant ($P<.10$) diet effect on plasma glutathione peroxidase (GSH-Px) activity in second parity sows, with the higher levels associated with sows on supplemented diets. The same diet to GSH-Px relationships appeared to exist in the third parity sows, however, the differences were non-significant.

Since GSH-Px is a selenium dependent enzyme, it is appropriate to look at the relationship between selenium and GSH-Px over time. In Figure 2 it is clear that plasma selenium levels remain consistently high throughout the reproductive cycle in the supplemented sows. It is also apparent that GSH-Px activity is not highly correlated with the plasma selenium levels in these sows. Plasma GSH-Px decreased significantly ($P<.01$, $P<.02$) in both parities at parturition and remained low throughout lactation.

Lane et al. (1984) found that in mice under certain stressful conditions, such as maintaining a pregnancy while in the growth stage and during lactation, that basal levels of dietary selenium (0.03 ppm) were not sufficient to maintain an effective level of GSH-Px activity in the mammary gland. Plasma GSH-Px values were not measured. They also suggested that the decline in GSH-Px activity during lactation was due to a partitioning of selenium into the milk. The graph in Figure 2 indicates a highly

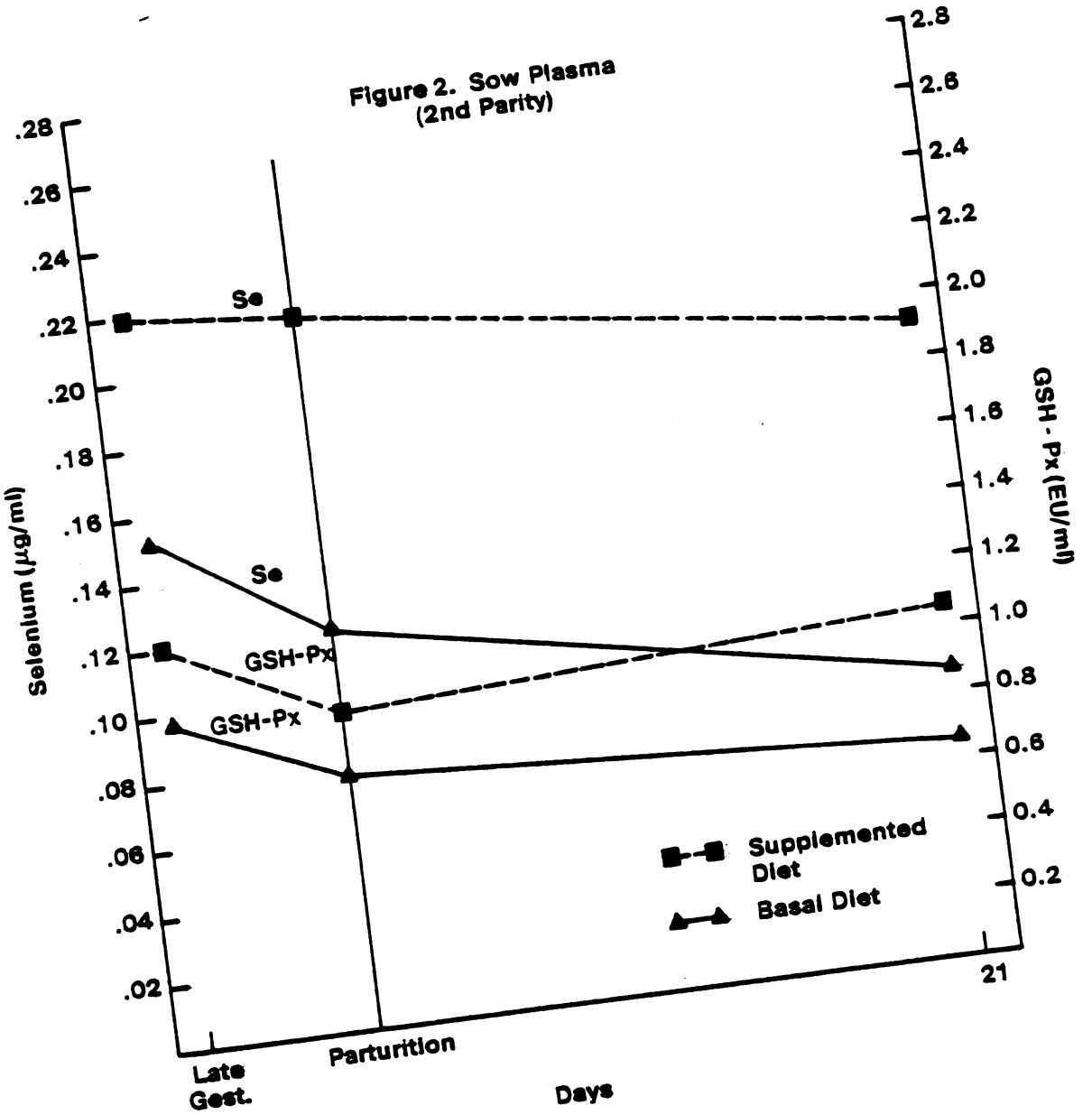


Figure 2. Sow Plasma
(2nd Parity)
Selenium (µg/ml)
.28
.26
.24
.22
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
GSH - Px (EU/ml)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Se
Se
GSH-Px
GSH-Px
Supplemented Diet
Basal Diet
Late Gest.
Parturition
21
Days

significant ($P<.001$) correlation ($r=.68$) between the plasma selenium level and plasma GSH-Px activity in the sows on the unsupplemented diet. A significant correlation did not exist between selenium levels and GSH-Px activity in the plasma of the supplemented sows.

This was in agreement with work cited in Selenium in Nutrition (NRC, 1983), which states that in humans a strong correlation between blood Se and GSH-Px activity has been observed in individuals consuming low levels of Se (Thompson et al., 1977; McKenzie et al., 1978). However, others have reported a lack of correlation between blood GSH-Px activity and blood selenium level when adequate or high Se levels are consumed (Schmidt and Heller, 1976; Schrauzer and White; 1978). This difference is thought to be due to the non-specific incorporation of selenomethionine into blood proteins (NRC, 1983).

A significant correlation did not exist between plasma Se level and GSH-Px activities in the third parity sows on either treatment. The small sample size (3 sows/treatment) may have resulted in inconsistencies in the values reported for the third parity sows.

Plasma iron concentration in the sows were analyzed. The results indicate no significant change in plasma iron level in either parity due to the dietary treatment. Plasma iron significantly ($P<.01$) increased from parturition to weaning in the second parity sows, however, these differences were not detected in third parity sows.

Table 6. Effects of Dietary E & Se Supplementation of Sows on Milk Selenium

Diet	Time					
	Colostrum	P[a]	2 Day	P[a]	21 Days	MSE
	Second Parity Selenium (μg/g milk)					
Supplemented	0.15	<.001	0.06	<.05	0.03	0.0002
Basal	0.10	<.001	0.04	<.05	0.02	0.0002
P-Value[b]	<.001		<.001		<.05	
MSE	0.0003		0.0003		0.0003	
	Third Parity Selenium (μg/g milk)					
Supplemented	0.17	<.001	0.07	<.10	0.03	0.0003
Basal	0.09	<.10	0.05	<.25	0.02	0.0003
P-Value[b]	<.05		NS		NS	
MSE	0.0007		0.0007		0.0007	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sow's diet.

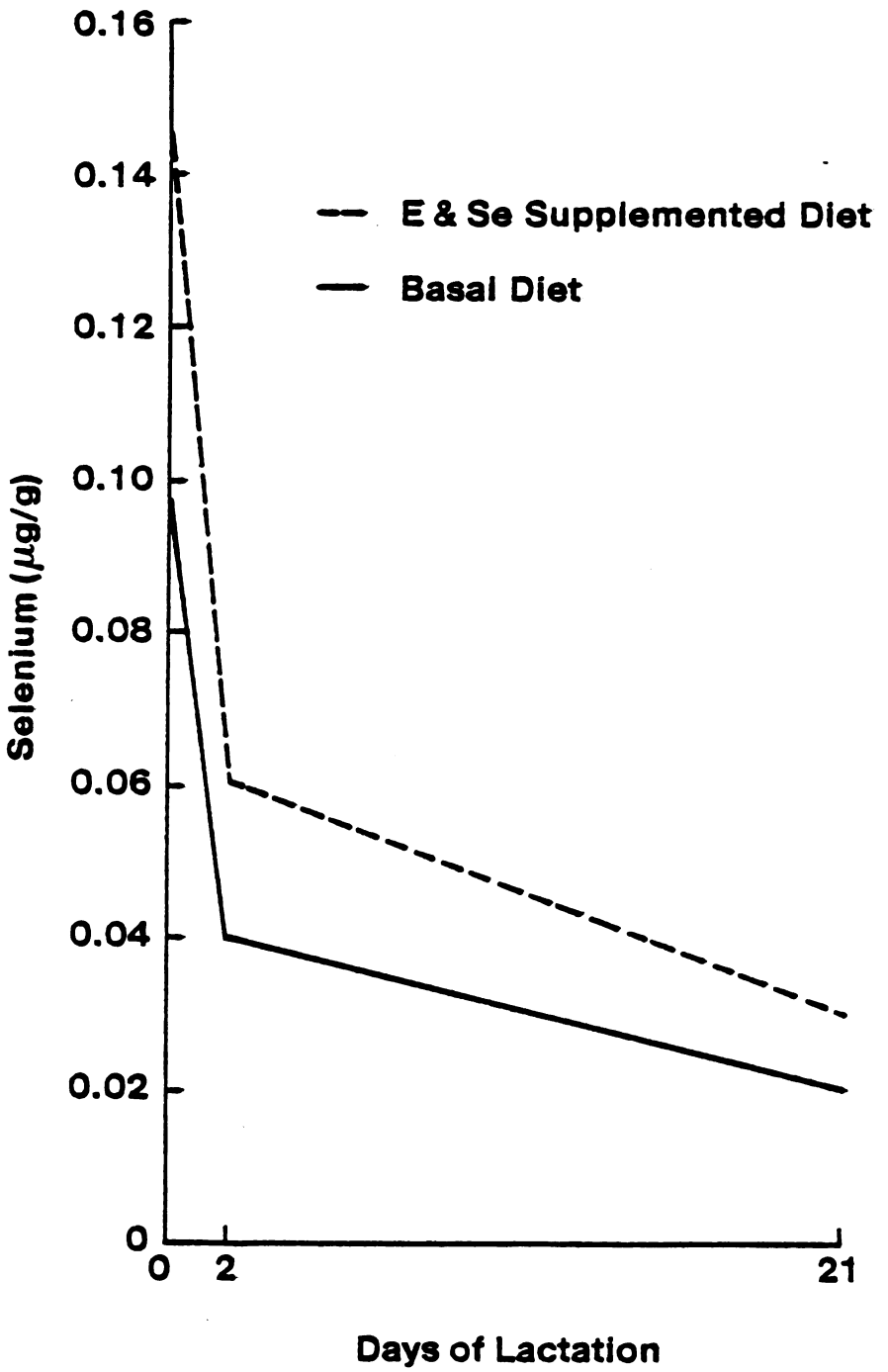
II. Sow Milk

Sow's milk was analyzed for selenium concentration at three stages: colostrum, two days and 21 days post-partum (Table 6). The second parity sows had significantly higher ($P<.001$) milk selenium concentration as a result of dietary supplementation with vitamin E and selenium, in colostrum and two day milk. Towards the end of lactation milk selenium levels became more nearly similar, however, supplemented sow's milk was still higher ($P<.05$) than that of the sows on the basal diet. The third parity sows showed a similar difference but this difference was not statistically significant.

Mahan et al. (1975) found a significantly greater dietary effect on milk selenium levels throughout lactation when sows were fed a diet supplemented with 0.1 ppm selenium. This is in agreement with the findings of this study.

There was a highly significant decrease in the milk selenium level from colostrum to the two day milk sample in second parity sows regardless of dietary treatment. A less dramatic ($P<.05$) decline was detected from two to twenty-one days post-farrowing (Figure 3). The third parity sows on the supplemented diet showed a similar pattern of selenium depletion in the milk through lactation. This is in agreement with studies which found colostrum levels of selenium significantly higher than later milk (Mahan et al.,

Figure 3. Milk Selenium



1975). This rapid decline may be a consequence of the declining milk protein level as colostrum changes to milk, as several workers have found that the selenium present in milk is primarily associated with the protein fraction, (Jones and Godwin, 1963; Jones and Godwin, 1969; McConnell and Roth, 1964).

Alpha- and β+γ-tocopherols were measured in the colostrum and milk of the sows. The sketches shown in figures 4 and 5 represent typical tracings from the recorder of the HPLC for colostrum and later milk, respectively. The first peak is the β+γ-tocopherol peak. Separation of these two tocopherols was not possible. The second peak represents the α-tocopherol present in the colostrum. In colostrum the β+γ-tocopherol concentration was very low. This was typical of all colostrum samples. However, by two days the β+γ-tocopherol peak was much larger (Figure 5) and the α-tocopherol peak, which was very large in colostrum, was much smaller.

The values for the α- and β+γ-tocopherol peaks were corrected to μg of tocopherol per gram of milk and summed to obtain the value for total tocopherol. All of these values are reported in tables 7 and 8 and are graphically presented in figure 6.

There was a highly significant dietary effect reflected in higher levels of total and α-tocopherol and lower concentrations of β+γ-tocopherol in the colostrum and 2 day

Figure 4. HPLC Trace For Tocopherols in Colostrum

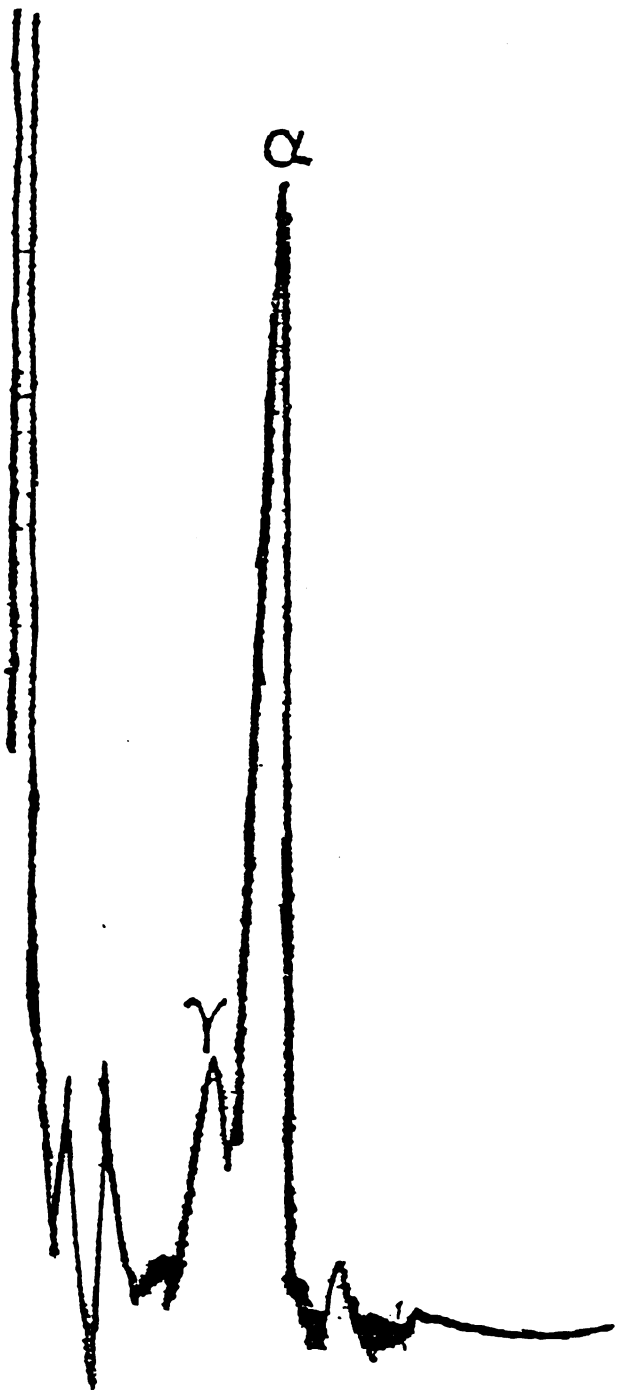


Figure 5. HPLC Traces For Tocopherols In Milk

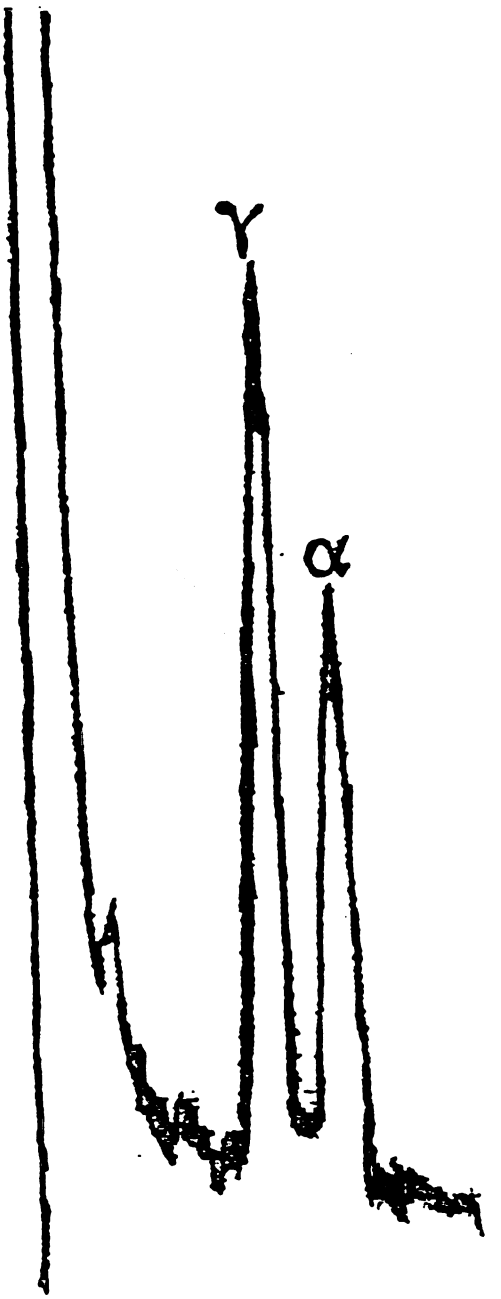


Table 7. Effects of Dietary E & Se Supplementation of 2nd Parity Sows on Milk Tocopherol.

Diet	Time					
	Colostrum	P[a]	2 Day	P[a]	21 Days	MSE
			Total Tocopherol (µg/g of milk)			
Supplemented	10.0	<.001	4.7	<.05	3.0	0.9
Basal	6.1	<.001	4.5	<.10	3.3	0.9
P-Value[b]	<.001		<.01		<.10	
MSE	0.9		0.9		0.9	
			Alpha-Tocopherol (µg/g of milk)			
Supplemented	8.7	<.001	2.9	<.05	1.2	0.5
Basal	4.6	<.001	1.8	<.05	0.8	0.5
P-Value[b]	<.001		<.001		<.10	
MSE	0.5		0.5		0.5	
			Beta + Gamma-Tocopherol (µg/g of milk)			
Supplemented	1.3	<.001	1.8	NS	1.8	0.3
Basal	1.5	<.001	2.7	NS	2.5	0.3
P-Value[b]	<.01		<.01		<.01	
MSE	0.4		0.4		0.4	

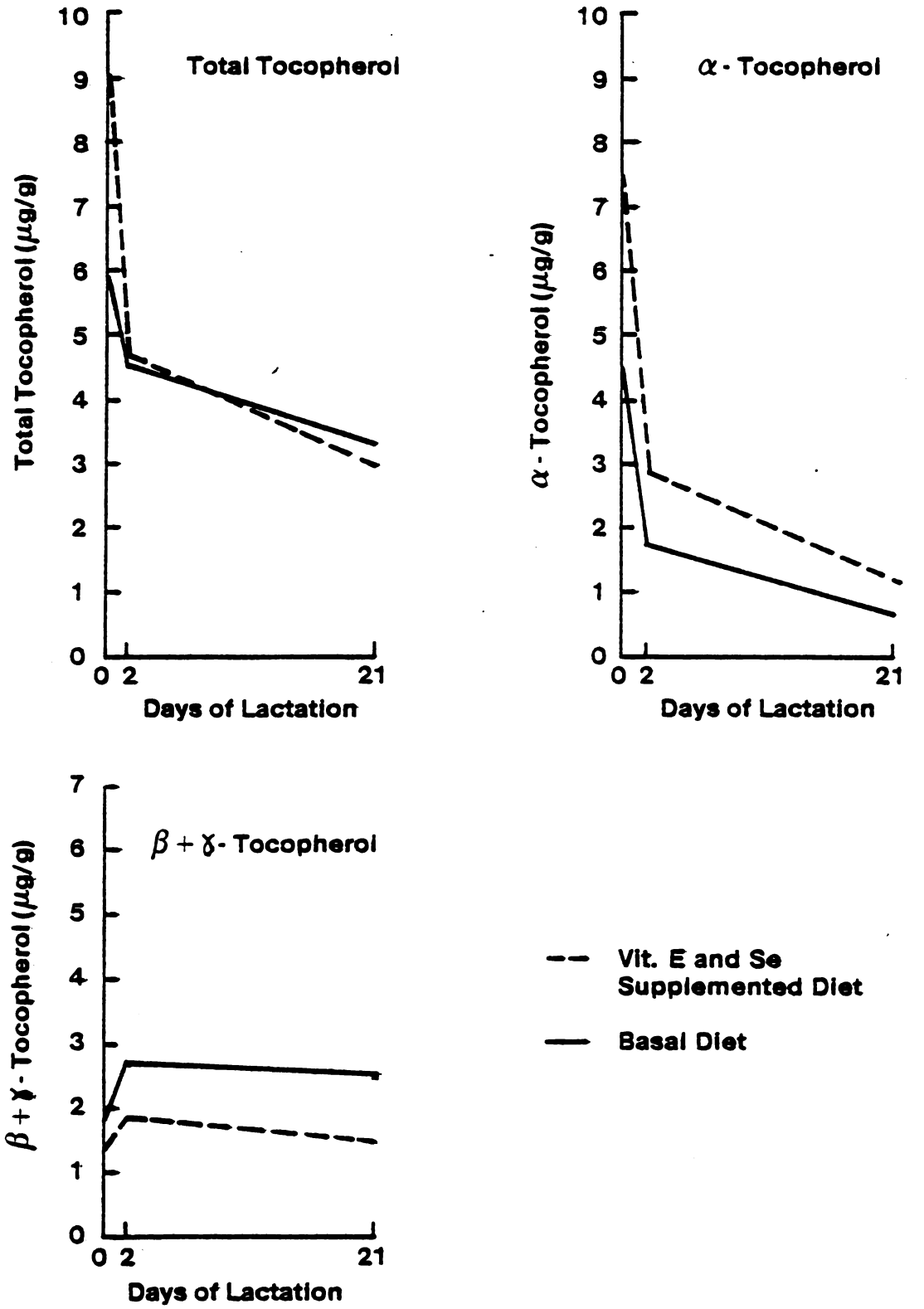
[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sow's diet.

Table 8. Effects of Dietary E & Se Supplementation of 3rd Parity Sows on Milk Tocopherol

Diet	Time					
	Colostrum	P[a]	2 Day	P[a]	21 Days	MSE
			Total Tocopherol (µg/g of milk)			
Supplemented	10.2	<.01	6.4	<.01	2.9	0.8
Basal	3.7	NS	5.9	<.05	3.0	0.8
P-Value[b]	<.001		NS		NS	
MSE	0.4		0.4		0.4	
			Alpha-Tocopherol (µg/g of milk)			
Supplemented	9.8	<.001	4.5	<.001	1.3	0.6
Basal	3.1	<.001	2.7	<.001	0.9	0.6
P-Value[b]	<.001		<.05		NS	
MSE	0.1		0.1		0.1	
			Beta + Gamma-Tocopherol (µg/g of milk)			
Supplemented	0.4	<.001	1.9	NS	1.6	0.3
Basal	0.6	<.001	3.2	NS	2.1	0.3
P-Value[b]	<.01		<.01		<.01	
MSE	0.1		0.1		0.1	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sow's diet.

Figure 6. Milk Tocopherols Per Gram of Milk (2nd Parity Sows)



milk of E-Se supplemented sows. However, the tocopherol level of sows on both dietary treatments drop by the second day of lactation. The differences due to dietary treatment diminished by the end of the lactation period for total and α-tocopherol but the $\beta+\gamma$-tocopherol continued to be significantly lower in the supplemented sows throughout lactation. Literature was not found pertaining to the effects of dietary supplementation of E and Se on the specific tocopherols present in milk, however, Malm et al. (1976) showed an increase in colostral and three week milk total tocopherol levels due to supplementation of vitamin E in the sow's diet.

Total tocopherol was high in colostrum and dropped significantly ($P<.001$) by the second day of lactation in both second parity, dietary groups. The variation in total tocopherol content of milk was similar to the findings reported for human milk (Jagadeesan and Prema, 1980; Jansson et al., 1981), bovine milk (Herting and Drury, 1969), and in swine milk (Malm et al., 1976; Nielsen et al., 1973). After the initial drop in tocopherol there was a lesser decline in tocopherol content over the remainder of the lactation period and it appeared that the total milk tocopherol in the supplemented sows may decline at a faster rate than the total tocopherol of the sows on the basal diet (figure 6). The $\beta+\gamma$-tocopherol was very low in colostrum and shows a significant ($P<.001$) increase by the second day of lactation

and then was maintained at a constant concentration throughout lactation. The third parity sows showed a similar pattern in total and β+γ-tocopherol changes over the lactation period, however the α-tocopherol content of milk declined significantly ($P<.001$) throughout lactation. The weather was very hot during the time the third parity sows were in the farrowing house resulting in a stressful environment and possibly exerting an additional drain on their circulating α-tocopherol. Ullrey et al. (1970) suggested stress may induce vitamin E and selenium deficiency signs.

It has been recommended by Bieri and Everts (1975) that plasma α-tocopherol levels be expressed in relation to plasma lipid levels. Since sow's milk has a relatively high fat content it is also important to express the colostrum and milk tocopherol on a total lipid basis. The percentages of total lipids at the three stages of lactation are reported in table 9 and the second parity results are graphically presented in figure 7. No dietary effect was seen for percent total lipids in second parity sows, however, third parity sows on the basal diet exhibited a significantly ($P<.05$) greater concentration of milk lipids than sows on the supplemented diet.

Total milk lipids were low in colostrum, increased ($P<.001$) by two days, and remained constant throughout lactation. This pattern is similar to the pattern seen

Table 9. Effects of Dietary E & Se Supplementation of Sows on Percent Milk Fat

Diet	Time					
	Colostrum	P[a]	2 Day	P[a]	21 Days	MSE
			Second Parity Milk Fat (%)			
Supplemented	6.2	<.001	11.1	NS	10.8	2.8
Basal	6.3	<.001	11.9	NS	11.2	2.8
P-Value[b]	NS		NS		NS	
MSE	3.7		3.7		3.7	
			Third Parity Milk Fat (%)			
Supplemented	4.9	<.001	12.7	NS	11.3	1.6
Basal	4.6	<.001	17.7	<.001	11.3	1.6
P-Value[b]	NS		<.05		NS	
MSE	2.9		2.9		2.9	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sow's diet.

Figure 7. Total Milk Lipids (2nd Parity)

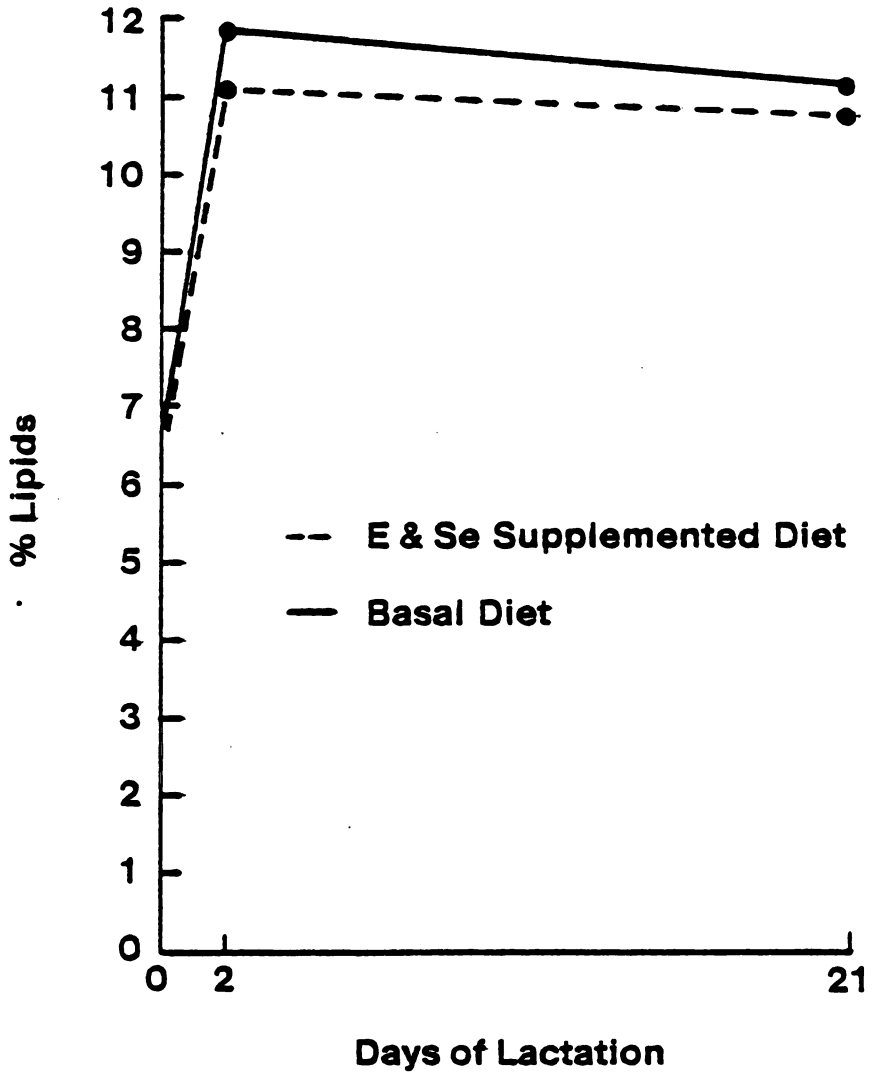


Table 10. Dietary E & Se Supplementation of 2nd Parity Sows on Milk Fat Tocopherol

Diet	Time					
	Colostrum	P[a]	2 Day	P[a]	21 Days	MSE
	Total Tocopherol (µg/g of fat)					
Supplemented	163.9	<.001	43.1	NS	28.5	265.8
Basal	98.7	<.001	37.5	NS	29.8	265.8
P-Value[b]	<.001		<.01		<.10	
MSE	170.4		170.4		170.4	
	Alpha-Tocopherol (µg/g of fat)					
Supplemented	143.3	<.001	26.1	<.25	11.6	158.0
Basal	74.5	<.001	15.1	NS	6.9	158.0
P-Value[b]	<.001		<.01		<.01	
MSE	137.3		137.3		137.3	
	Beta + Gamma-Tocopherol (µg/g of fat)					
Supplemented	20.6	NS	17.0	NS	16.9	39.6
Basal	24.2	NS	22.4	NS	22.9	39.6
P-Value[b]	<.01		<.01		<.01	
MSE	24.3		24.3		24.3	

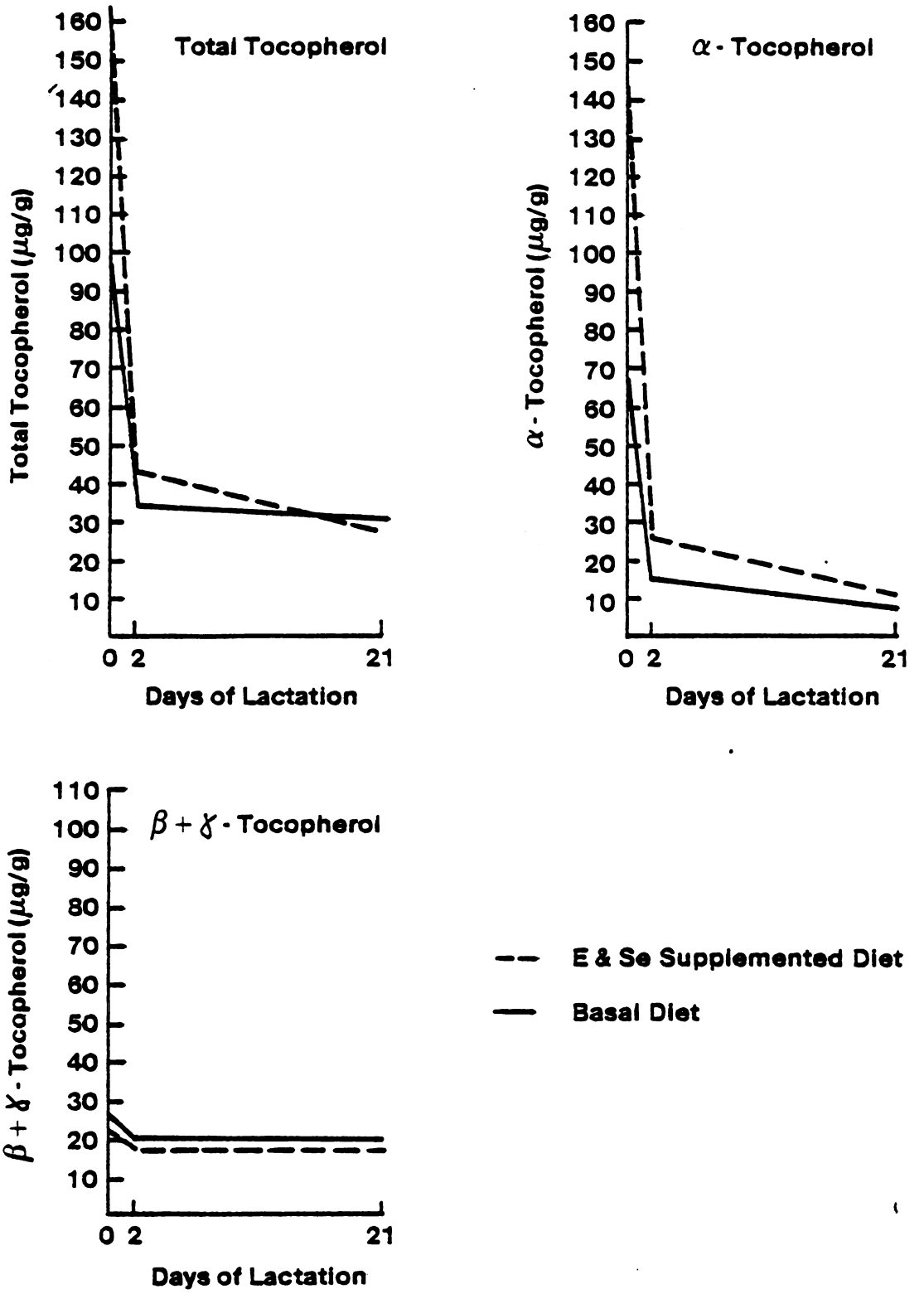
[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sow's diet.

Table 11. Effects of E & Se Supplementation of 3rd Parity Milk Fat Tocopherol

Diet	Time					
	Colostrum	P[a]	2 Day	P[a]	21 Days	MSE
	Total Tocopherol (µg/g of fat)					
Supplemented	211.2	<.001	51.1	NS	26.3	513.3
Basal	72.7	<.05	33.3	NS	25.9	513.3
P-Value[b]	<.05		NS		NS	
MSE	381.9		381.9		381.9	
	Alpha-Tocopherol (µg/g of fat)					
Supplemented	203.7	<.001	35.7	NS	12.0	481.8
Basal	68.9	<.10	15.4	NS	7.5	481.8
P-Value[b]	<.001		<.25		NS	
MSE	300.3		300.3		300.3	
	Beta + Gamma-Tocopherol (µg/g of fat)					
Supplemented	7.5	<.10	15.4	NS	14.3	15.0
Basal	11.1	<.10	17.9	NS	18.4	15.0
P-Value[b]	<.04		<.04		<.04	
MSE	6.2		6.2		6.2	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sow's diet.

Figure 8. Milk Tocopherols Per Gram of Fat (2nd Parity Sows)



previously for β+γ-tocopherol changes in milk over the lactation period (Figure 6). This may indicate that the β+γ-tocopherols vary with the amount of fat in the milk.

Total, α- and β+γ-tocopherol values are reported as micrograms of tocopherol per gram of lipid in Tables 10 and 11 and are graphically presented in Figure 8. From these figures it is clear that the β+γ-tocopherol concentration in the milk lipid does not change significantly over the lactation period. A nearly significant ($P<.10$) increase was detected in the third parity sows. A correlation analysis over the entire lactation period demonstrated that β+γ-tocopherol in milk was highly correlated with the percent total lipid in milk in second ($r=.6505$, $P<.001$) and third ($r = .9061$, $P<.001$) parity sows, further proof that β+γ-tocopherols vary with percent lipids.

Total and α-tocopherol were also correlated with total lipids. This calculation indicated that total and α-tocopherol were negatively correlated with percent lipid in both second ($r=-.64$, $P<.001$) parity sows. The graphic presentation in Figure 6 illustrate the relationship between milk and tocopherol. Milk total and α-tocopherols decline as colostrum changes to milk while over the same time frame total lipids are increasing. In human milk no correlation was seen between total lipids and total tocopherol (Jansson et al., 1981).

Referring to Tables 10 and 11 it is clear that α-tocopherol per gram of lipid is very high in colostrum,

but by the second day of lactation this level has significantly dropped ($P<.001$) and remains constant per gram of fat throughout lactation. This is in accordance with Herting and Drury's (1969) findings in bovine milk and the findings in human's milk by Jansson et al. (1981) which suggest that this increased level of α-tocopherol in colostrum may be due to an increased transport capacity for vitamin E.

As would be expected, similar dietary effects are present when the tocopherols are expressed per gram of lipid as when reported earlier as tocopherols per gram of milk. The colostrum and milk values for total tocopherol per gram of fat for the E-Se supplemented sows were in agreement with values reported by Malm et al. (1976) for vitamin E supplemented sows. The values which Malm et al. (1976) reported for non-supplemented sows were much lower than those reported in this study. This difference was most likely due to the semi-purified diets supplemented with either lard or corn oil which were fed to their sows.

As total tocopherol changes in concentration from colostrum to milk, so also does the composition of this tocopherol. Table 12 lists the levels of α- and β+γ-tocopherol as a percentage of total-tocopherol. A graphic presentation of these value are shown in Figure 9. As colostrum changed to milk there was a great increase in the percentage of β+γ-tocopherol as a percentage of total-tocopherol, and this percentage continued to rise

Table 12. Effect of Dietary E+Se Supplementation on the Composition of Milk Tocopherol

Diet	Second Parity Sow Milk					
	Colostrum		2 Days		21 Days	
	Tocopherols[a]		Tocopherols[a]		Tocopherols[a]	
	α-	β+γ-	α-	β+γ-	α-	β+γ-
Supplemented	87.0	13.0	61.7	38.3	40.0	60.0
Basal	75.4	24.6	40.0	60.0	24.2	75.8
			Third Parity Sow Milk			
Supplemented	96.1	3.9	70.3	29.7	44.8	55.2
Basal	83.8	16.2	45.8	54.2	30.0	70.0

[a]Tocopherols expressed as a percentage of total tocopherol.

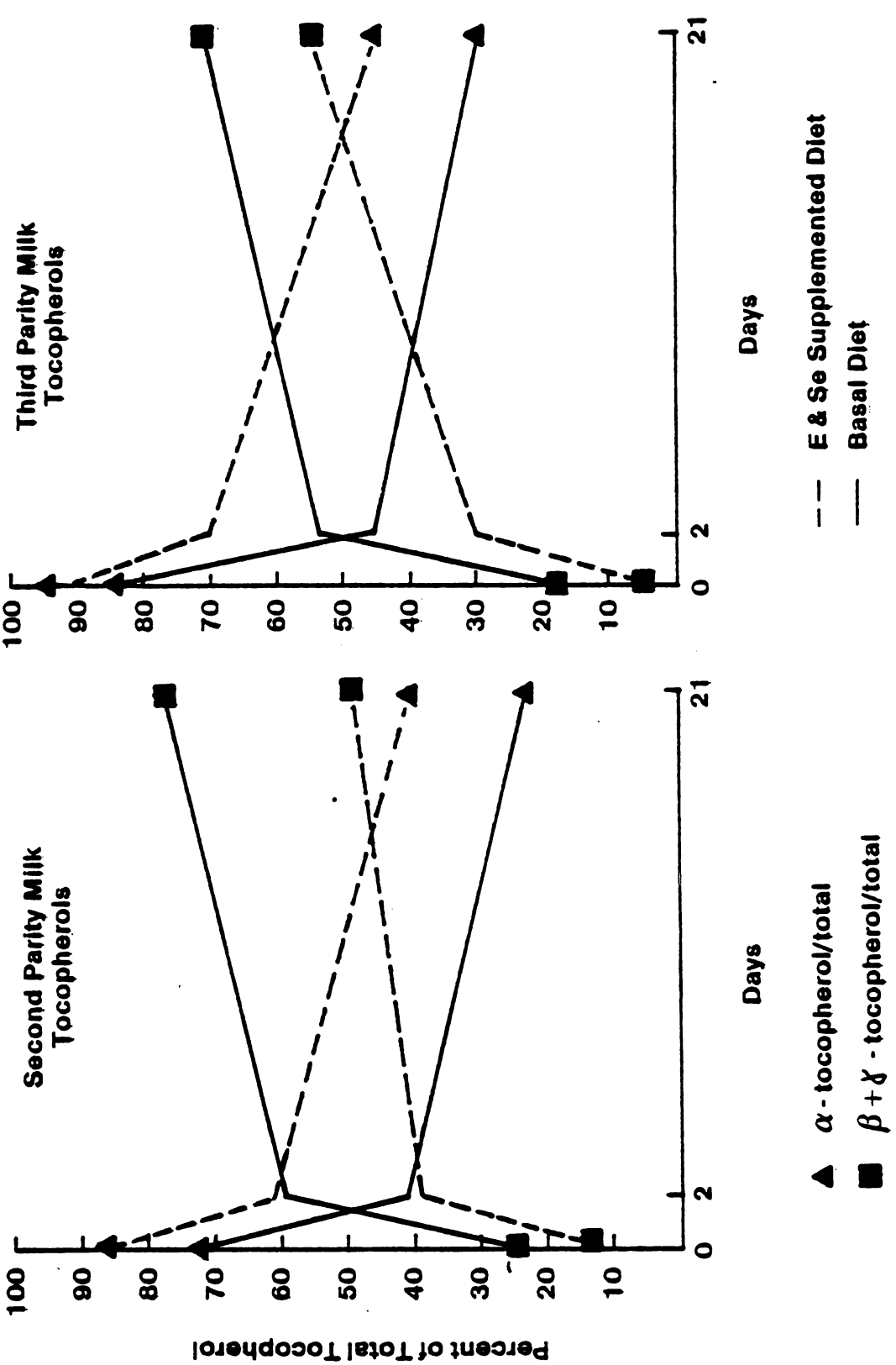


Figure 9. Change in Milk Tocopherol Composition

throughout lactation. It was clear that β+γ-tocopherol made up the majority (55-70%) of the tocopherol found in the milk of sows on the basal diet. With no supplemental vitamin E the vitamin E compounds that were present in the ration came from the corn and soybean meal, which are both higher in γ-tocopherol than in α-tocopherol (Bauernfeind and Cort, 1974), possibly explaining the higher level of β+γ-tocopherol found in colostrum and milk of the sows on basal diets. The practical importance of this finding is obvious. The vitamin E biopotencies of β- and γ-tocopherol in relation to α-tocopherol are only 40% and 10% respectively (Bieri and McKenna, 1981). Therefore, not only was the pig nursing the sow on the basal diet receiving significantly less total-tocopherol in the milk, but the majority of the tocopherol was in a less potent form. Ullrey (1981) stated that absorption of γ-tocopherol was about as efficient as α-tocopherol, but the turnover rate of the gamma form was much faster. Pigs continually nursing may be able to maintain a relatively high level of total tocopherol in their plasma. However, when they are weaned this level may drop more rapidly if the pigs are nursing a sow on the basal diet rather than the supplemented diet. This may explain why Ullrey et al. (1970) reported that frequently one of the first signs of vitamin E and selenium deficiency in swine is the occurrence of sudden death in weaned pigs.

III. Pigs

Heterogeneous variance was detected for many of the variables measured on the pigs throughout the nursing period. The areas where heterogeneous variance occurred are marked in the tables. Probability values of .01 or .001 can still be considered significant when heterogeneous variance is present, however values greater than 0.01 may not be reliable.

The analyses of hematological measures of the pigs at zero, two and twenty-one days are reported in Table 13. There were no significant differences due to the dietary or the iron treatment in the second parity pigs at any stage throughout the nursing period.

Hematocrit values were not significantly different due to diet over the nursing period with the exception of the second parity pigs. Pigs which nursed supplemented sows and were given an iron injection prior to colostrum consumption had a higher ($P<.10$) hematocrit value at 2 days of age than pigs which did not receive iron and pigs which received iron but which were nursing sows on the basal diet. Third parity pigs follow the same trend, however significance levels were low. It would be interesting to know if the dam's diet effect was real, and if supplementation of vitamin E and selenium of the sow's diet might result in neonatal, pre-colostral pigs with cells that are more resistant to oxidative stress.

Table 13. Effects of E & Se Supplementation of the Sow's Diet and Time of Iron Injection on Hematology of Offspring

Parity	Days of Age	Iron Injection[a]	+(E+Se)	Basal	MSE	P value[c]
Hemoglobin (g/dl)						
II	0		11.4	11.1	15.3	NS[d]
	2	+	8.1	7.9	1.0[e]	NS
	2	-	7.7	7.8	5.9	NS
	21		11.7	11.9	3.0	NS
III	0		10.7	10.8	8.9	NS
	2	+	7.7[T]	7.3[T]	1.6[e]	NS
	2	-	6.8[T]	7.1[T]	10.7	NS
	21		10.7	11.6	3.5	<.25
Hematocrit (%)						
II	0		35.9	35.4	126.2	NS
	2	+	26.1[N]	23.7	8.3[e]	<.25
	2	-	23.4[N]	23.9	59.2	NS
	21		36.6	37.0	27.6	NS
III	0		33.8	34.7	116.9	NS
	2	+	23.6	21.5	13.8[c]	NS
	2	-	21.0	21.3	102.3	NS
	21		32.8	36.7	49.3	<.25
Mean Corpuscular Hemoglobin Concentration (%)						
II	0		31.6	31.2	8.1	NS
	2*	+	31.4	33.4	4.4[e]	<.25
	2	-	32.9	33.1	10.7	NS
	21		32.1	32.2	4.0	NS
III	0		31.9	31.5	10.0	NS
	2	+	33.1	33.7	3.4[e]	<.25
	2	-	32.8	33.6	2.3	<.25
	21		32.7	31.8	3.8	<.25

[a]+ indicates pig which received 200 mg of iron in the form of gleptoferron prior to colostrum consumption.
[b]Means squared error, diet.
[c]Significance of difference due to diet.
[d]NS = Not significant (P>.25).
[e]Mean squared error, iron.
[N]Means within column with same superscript differ significantly (P<.10) due to iron treatment.
[T]Means within columns with same superscript differ significantly (P<.25) due to iron treatment.
*Heterogeneous variance.

The decline in both hemoglobin and hematocrit from birth to two days can be explained by the 10 ml blood sample taken from each pig at birth and the extensive protein influx into the blood of the pig as a result of colostrum consumption, thereby causing the vascular system to take up water.

The effect of supplementing the sow's diet with vitamin E and Se on the biological antixoidant status of the neonatal and the nursing pig is reported in Table 14. Plasma Se in the neonate of sows fed the vitamin E-Se supplemented diet is approximately 0.015 µg/ml greater than for pigs born to sows on the basal diet. This is in agreement with finding of Young et al. (1976) for pigs and Pazak (1983) for rats. Therefore, it appears that placental transfer of vitamin E occurs and this transfer may be increased by supplementing Se in the dam's diet (Pazak, 1983). However, the neonate's plasma selenium levels are relatively low in relation to the plasma level in the dam (~25% supplemented and ~30%, basal). Hyroner-Dabek et al. (1984) monitored the plasma Se levels of women in early and late gestation and at parturition they obtained a sample of umbilical cord blood. Plasma from umbilical cord blood contained approximately 57% less Se than that of plasma from maternal blood samples. They suggested that the decline in maternal plasma Se was due to placental transfer of Se to the young with greater incorporation of the Se into tissue.

Table 14. Effects of E & Se Supplementation of the Sow's Diet and Time of Iron Injection on Plasma Antioxidant Levels

Parity	Days of Age	Iron Injection[a]	+(E+Se)	Basal	MSE[b]	P value[c]
Selenium (g/ml)						
II	0		0.053	0.038	0.002	<.25
	2*	+	0.071[S]	0.044[S]	0.00008[e]	<.001
	2*	-	0.065[S]	0.040[S]	0.0004	<.001
	21*		0.076	0.054	0.001	<.01
III	0		0.046	0.064	0.0002	<.05
	2	+	0.058	0.067	0.0001[e]	NS[d]
	2	-	0.057	0.066	0.0007	NS
	21*		0.120	0.052	0.01	<.10
Vitamin E (g/ml)						
II	0*		0.27	0.13	0.19	<.25
	2*	+	4.36[II]	2.24[II]	0.82[e]	<.001
	2*	-	5.22[II]	2.45[II]	7.92	<.001
	21		2.19	1.58	3.56	<.25
III	0		0.41	0.06	1.02	NS
	2	+	3.66	1.30	0.17[e]	<.05
	2	-	3.37	1.29	4.66	<.05
	21		1.28	2.14	5.47	NS
Glutathione Peroxidase (EU/ml)						
II	0*		0.18	0.13	0.02	<.10
	2*	+	0.27[S]	0.19[S]	0.006[e]	<.05
	2*	-	0.24[S]	0.17[S]	0.02	<.05
	21*		0.50	0.40	0.06	<.10
III	0		0.12	0.12	0.004	NS
	2	+	0.28	0.19	0.0002[e]	NS
	2	-	0.26	0.18	0.06	NS
	21		0.44	0.27	0.02	<.05

[a]+ Indicates pig which received 200 mg of iron in the form of gleptoferron prior to colostrum consumption.
[b]Standard Deviation of the mean.
[c]Means squared error, diet.
[d]NS = Not significant (P>.25).
[e]Mean squared error, iron.
[II]Means within columns with same superscript differ significantly (P<.01) due to iron treatment.
[S]Means within columns with same superscript differ significantly (P<.05).
*Heterogeneous variance.

A similar decline in maternal plasma Se was discussed earlier for the sows on this study which were on the basal diet. In this case, it was suggested that the drop in plasma Se was a result of a partitioning of the nutrient to the milk, however, it is conceivable that the decline could be a result of both placental and mammary transfer.

In reference to Figure 10 it appears that plasma selenium values for the pigs nursing supplemented sows greatly increased by the second day of life, reflecting the high selenium content of the sow's colostrum. The pigs nursing sows on the basal diet do not have this distinct increase in plasma selenium. By the second day of life pigs nursing supplemented sows had significantly higher plasma Se levels than pigs nursing sows on the basal diet. The significantly higher plasma Se level is maintained in the supplemented pig's plasma throughout the nursing period.

Glutathione peroxidase activity in the neonate's plasma was very low in relationship to the levels found in the plasma of their dams. However, even at very low levels there was a trend toward greater activity in the plasma of pigs from vitamin E and Se supplemented sows. Pazak (1983) found similar differences in pre-colostral rats for blood GSH-Px due to supplementation of the dam's diet with vitamin E and selenium. Plasma GSH-Px activity in the pigs tended to reflect dietary E & Se supplementation of the sows diet throughout the nursing period. The plasma GSH-Px activity

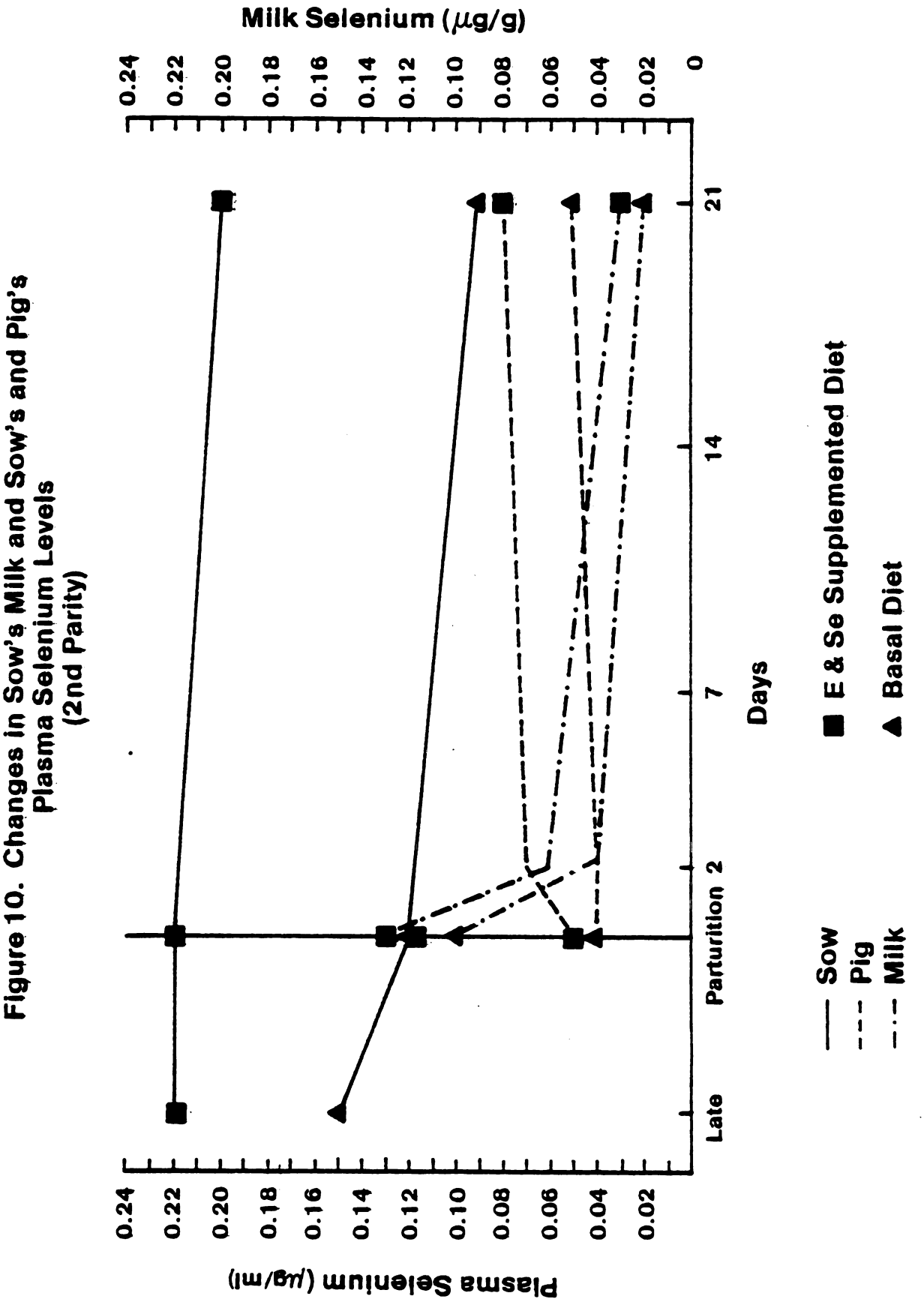
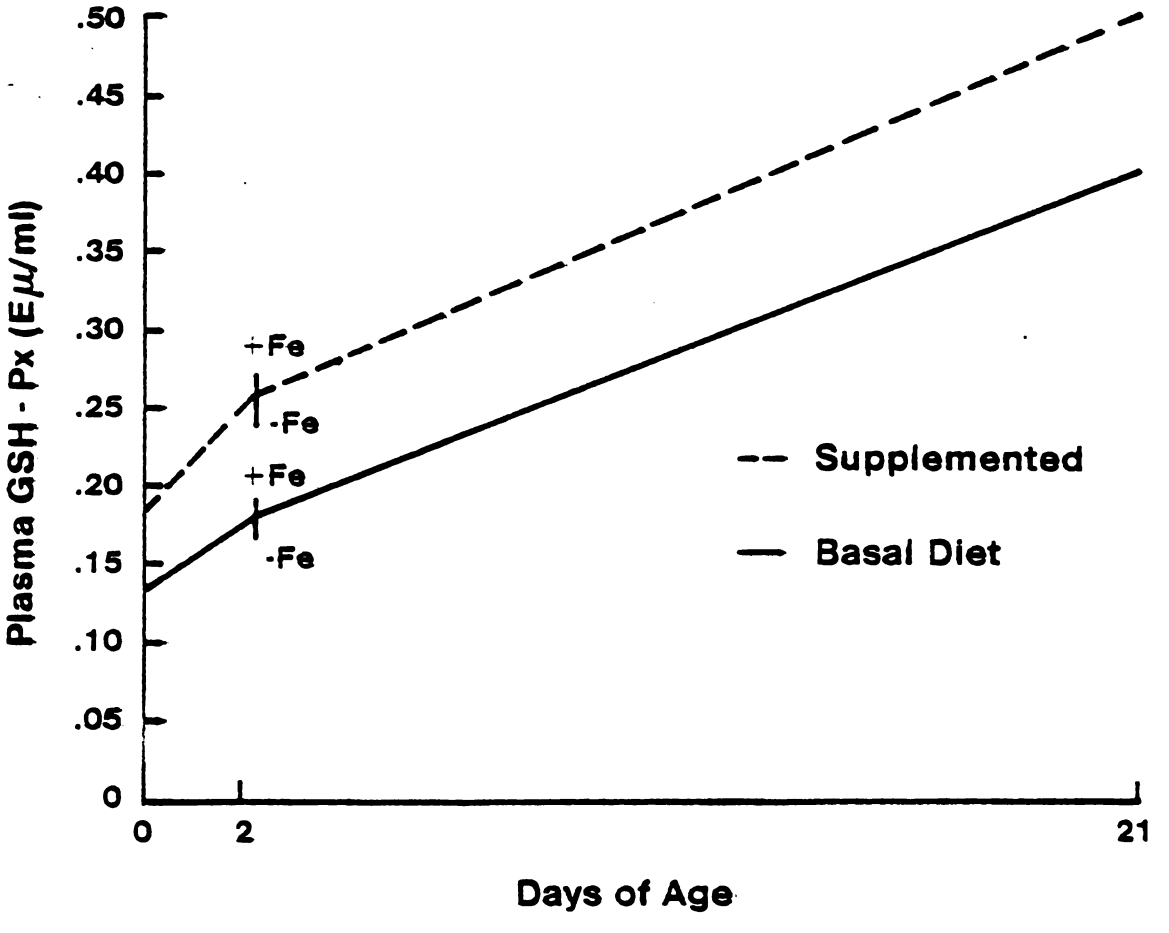


Figure 10. Changes in Sow's Milk and Sow's and Pig's Plasma Selenium Levels (2nd Parity)

Figure 11. Changes in Plasma GSH - Px Activity in Pigs (2nd Parity)



change over the nursing period showed a trend similar to that seen for plasma selenium (Figure 11). This may indicate that as selenium becomes available to the pig, through colostrum and milk consumption, glutathione peroxidase activity increases.

Vitamin E levels in plasma were very low at birth and in many samples were non-detectable, possibly indicating inefficient placental transfer. Malm et al. (1976) reported that sows have efficient placental transfer of vitamin E resulting in 2.2 to 19.3 fold higher serum vitamin E levels in neonatal pigs than that of their dam. This report is inconsistent with findings in pigs (Young et al., 1977), rats (Pazak, 1983) and human (Martinez et al., 1981) where circulating levels of vitamin E in neonates are low. The conclusions on the efficiency of placental transfer of vitamin E vary. Pazak (1983) found low circulating levels of serum vitamin E in the pre-colostral rat pup, but indicated a preferential incorporation of the vitamin in lung and liver tissue. Therefore, efficient placental transfer was suggested. Martinez et al. (1981) on the other hand suggested that there was a barrier present between placental and fetal blood making the transfer of vitamin E inefficient.

Plasma vitamin E increased about 18 fold in both dietary groups of pigs by 2 days of age, with a greater ($P<.001$) concentration found in the pigs nursing

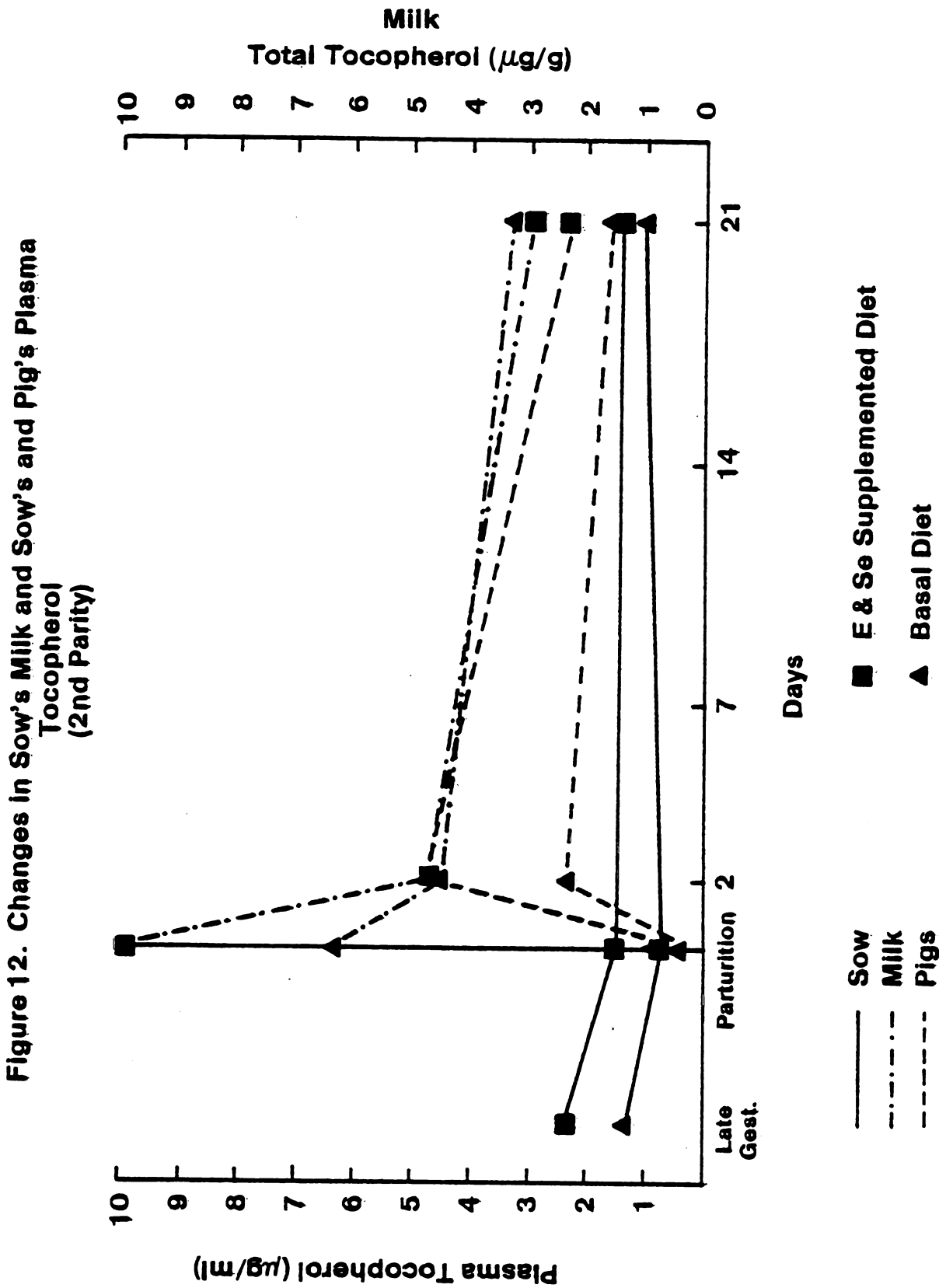


Figure 12. Changes in Sow's Milk and Sow's and Pig's Plasma Tocopherol (2nd Parity)

supplemented sows. The increase in plasma tocopherol at 2 days of age was most likely due to the very high level of tocopherol in colostrum. Figure 12 graphically depicts the relationship between sow and pig plasma and milk tocopherol levels through the end of the nursing period. The decline in colostrum total tocopherol was nearly the same as the increases in plasma vitamin E level in the pig by 2 days. The decrease in colostrum tocopherol from parturition to the second day of lactation equaled 5.3 µg/g of milk for sows supplemented with vitamin E and 1.6 µg/g of milk for sows on the basal diet and the increase in plasma vitamin E concentration between parturition and the second day of lactation were 4.5 µg/ml and 2.2 µg/ml for pigs nursing dams fed supplemented and basal diets, respectively.

The plasma vitamin E level of the pig declined from 2 to 21 days of age, however, there still tended to be differences present at weaning due to the sow's diet. This is not consistent with the results of Young et al. (1977) who reported increases in plasma tocopherol of pigs throughout the nursing period for sows on diets containing 40.6 and 56.1 µgE/g DM of and no increase at all in pigs nursing sows on a diet containing 0.5 µg/g DM of vitamin E. The lactation diets fed in this study contained calculated values of 4.7 and 14.9 µgE/g DM, in the basal and supplemented diets, respectively. The levels which Young et al. (1977) reported for the three-week milk from the

supplemented sows appear to be lower than the three-week values found in this study, however, from the results of pig plasma in their study it would appear that higher vitamin E supplementation of the sow's diet during lactation may prevent a decline in plasma tocopherol levels by weaning.

Second parity pigs which received 200 mg of iron in the form of gleptoferron, IM, prior to colostrum consumption tended to have higher plasma selenium ($P<.05$), higher plasma GSH-Px activity ($P<.05$), and significantly lower ($P<.01$) plasma vitamin E levels at two days of age than pigs that did not receive iron. The decrease in plasma vitamin E may be a result of its interaction with peroxy radicals generated in the process of lipid peroxidation which is catalyzed by the iron. This series of reactions has been shown to occur in vitro (Fukuzawa et al., 1980). The increase in plasma GSH-Px activity may be a result of the higher plasma selenium level present in the iron treated pigs, or it may be a response to an increased requirement of the pig for biological antioxidants. Sklan et al. (1981) reported that chicks on vitamin E-deficient diets had higher GSH-Px activity.

Plasma iron was measured in pigs through the nursing period (Table 15). There was a great deal of variation in plasma levels at two days after the iron injection. This variation can be explained by inconsistent time intervals between the time of the iron injection and the two-day blood

Table 15. Effects of E & Se Supplementation of the Sow's Diet and Time of Iron Injection on Iron Level and Weight of the Offspring

Parity	Days of Age	Iron Injection[a]	+(E+Se)	Basal	MSE[b]	P value[c]
Plasma Iron (g/dl)						
II	0		110.9	94.3	5×10^3	NS[d]
	2*	+	955.5^H	2189.5^H	5×10^5	<.25
	2*	-	85.4^H	95.3^H	6×10^6	NS
	21		126.8	129.7	4×10^3	NS
III	0		92.6	85.8	6×10^3	NS
	2*	+	178.1^S	2018.9^S	2×10^{6e}	<.25
	2*	-	95.7^S	96.3^S	3×10^6	NS
	21		106.5	153.6	6×10^3	<.25
Weight (kg)						
II	0		1.5	1.5	0.3	NS
	2	+	1.9^S	1.8^S	0.1^e	NS
	2	-	1.7^S	1.7^S	0.3	NS
	21		5.7	5.6	11.2	NS
III	0		1.7	1.5	0.8	NS
	2	+	2.0	1.7	0.2^e	NS
	2	-	2.1	1.6	1.1	NS
	21*		6.9	4.6	13.9	<.25

[a] + indicates pig which received 200 mg of iron in the form of gleptoferron prior to colostrum consumption.
[b] Mean squared error, diet.
[c] Significance of difference due to diet.
[d] NS = Not significant (P>.25).
[e] Mean squared error, iron.
[H] Means within column with same superscript differ significantly (P<.001) due to iron treatment.
[S] Means within columns with same superscript differ significantly (P<.05) due to iron treatment.
* Heterogeneous variance.

sampling. By the end of the nursing period there were no differences in plasma iron levels, between the sow's dietary treatment.

Analyses of individual pig weights are reported in Table 15, there appears to be no difference in weight due to the sow's dietary treatment throughout the nursing period. This is in agreement with Malm et al. (1976) who found no difference in birth or weaning weights of pigs from sows fed two levels of vitamin E (0 or 100 IU/kg). Pre-colostral iron injection did not appear to affect the pigs performance as pigs from sows on both dietary treatments had significantly ($P<.05$) higher two day weights than pigs which did not receive iron.

The parameters for reproductive performance are reported in Tables 16 and 17. There was no dietary effect on average litter size at birth, two or twenty-one days of age. Average pig and sow weights were not affected by the sow's dietary treatment.

Conclusions

Supplementation of the gestation (vitamin E 50 IU/kg, selenium, 0.1 ppm) and lactation (vitamin E 17.5 IU/kg, selenium 0.1 ppm) diets of sows increased the plasma vitamin E and selenium level of the sows, but did not increase the sow's glutathione peroxidase activity. The sows fed diets supplemented with vitamin E and selenium had higher levels

Table 16. Effect of Dietary E & Se Supplementation on 2nd Parity Sow's Performance

Diet	Time					
	Farrowing	P[a]	2 Days	P[a]	21 Days	MSE
			Average Litter Size			
Supplemented	9.2	<.10	8.3	<.10	7.6	0.86
Basal	9.0	<.10	8.3	<.10	7.6	0.86
P-Value[b]	NS		NS		NS	
MSE	26.0		26.0		26.0	
			Average Pig Weight (kg)			
Supplemented	1.5	NS	1.8	<.001	6.0	0.41
Basal	1.5	NS	1.8	<.001	6.0	0.41
P-Value[b]	NS		NS		NS	
MSE	0.81		0.81		0.81	
	Late Gestation		Farrowing		21 Days	
			Sow Weight (kg)			
Supplemented	210.3	<.10	194.2	<.25	206.6	197.3
Basal	204.7	<.10	189.4	<.25	195.1	197.3
P-Value[b]	NS		NS		NS	
MSE	782.3		782.3		782.3	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sows diet.

Table 17. Effect of Dietary E & Se Supplementation on 3rd Parity Sow's Performance

Diet	Time					
	Farrowing	P[a]	2 Days	P[a]	21 Days	MSE
			Average Litter Size			
Supplemented	6.7	NS	6.7	NS	6.3	1.3
Basal	11.0	NS	10.3	NS	8.0	1.3
P-Value[b]	NS		NS		NS	
MSE	37.8		37.8		37.8	
			Average Pig Weight (kg)			
Supplemented	1.8	NS	1.8	<.001	7.3	0.4
Basal	1.6	NS	1.8	<.001	4.9	0.4
P-Value[b]	NS		NS		<.10	
MSE	1.5		1.5		1.5	
	Late Gestation		Farrowing		21 Days	
			Sow Weight (kg)			
Supplemented	237.3	NS	232.0	NS	225.7	38.3
Basal	228.0	NS	220.0	<.01	196.3	38.3
P-Value[b]	NS		NS		<.25	
MSE	326.1		326.1		326.1	

[a]Significance of mean difference over time.
[b]Significance of mean difference due to E, Se supplementation of sows diet.

of selenium, total and α-tocopherol in their colostrum and lower levels of β+γ-tocopherol throughout lactation than sows fed the basal diet. The biological antioxidants were concentrated in the colostrum of all sows and decreased as colostrum changed to milk. Total and α-tocopherols do not vary with the level of fat in colostrum. However, β+γ-tocopherol concentration is highly correlated with milk fat concentration throughout lactation.

Pigs are born with relatively low circulating levels of biological antioxidants. The level of antioxidants increases by two days of age, primarily due to colostrum consumption. This increase is not as great if pigs are nursing sows whose diets are not supplemented with vitamin E and selenium and their biological antioxidant status remains lower throughout the nursing period than pigs nursing sows whose diet is supplemented with vitamin E and selenium.

Iron injection (200 mg of Fe from gleptoferron) given prior to colostrum consumption did not affect the livability or performance of the pigs from either dam dietary group. However, there was a decrease in plasma vitamin E and a trend towards an increase in plasma selenium and GSH-Px activity in pigs from both dietary groups which received iron prior to colostrum consumption.

LIST OF REFERENCES

LIST OF REFERENCES

Ames, S.R. 1979. Biopotencies in Rats of several forms of Alpha-tocopherol. J. Nutr. 109:2198.

A.O.A.C. 1980. Official Methods of Analysis (13th ed.). Association of Official Agricultural Chemists. Washington, DC.

ARC. 1981. The nutrient requirements of pigs. p. 141-153. In Commonwealth Agricultural Bureaux, Farnham Royal, Slough, England.

Arpi, T. and G. Tollerz. 1965. Iron poisoning in piglets, autopsy findings in experimental and spontaneous cases. Acta Vet. Scandinavica 6:360.

Asplund, J.M., R.H. Grummer and P.H. Phillips. 1962. Absorption and colostral gamma-globulins and insulin by the newborn pig. J. Anim. Sci. 21:412.

Bauernfiend, J.C. and W.M. Cort. 1974. Tocopherols. p. 891-99. In A.H. Johnson and M.S. Peterson (Ed.) Encyclopedia of Food Technology. The AVI Publishing Co., Inc., Westport, CT.

Bauernfeind, J. 1980. Tocopherols in Foods. P. 99-167. In W.P.T. James, R.H. Herman and G.A. Bray (Ed.) Vitamin E: A Comprehensive Treatise. Marcel Dekker Inc., New York and Basel.

Bell, E.F., E.J. Brown, R. Milner, J.C. Sinclair and H. Lipursky. 1979. Vitamin E absorption in small premature infants. Pediat. 63(6):830.

Bieri, J.G. and M.C. McKenna. 1981. Expressing dietary values for fat soluble vitamins; change in concepts and terminology. Amer. J. Clin. Nutr. 34:289.

Blecha, F. and K.W. Kelly. 1981. Cold stress reduces the acquisition of colostral immunoglobulin in piglets. J. Anim. Sci. 52:594.

Bowland, J.P. 1966. Swine milk composition, a summary. P. 97. In L.K. Bustad, R.O. McClellan and M.P. Burns (Ed.). Swine in Biochemical Research. Pacific Northwest Laboratory, Richland, WA.

Brady, P.S., J.J. Brady, M.J. Parsons, D.E. Ullrey and E.R. Miller. 1979. Effects of riboflavin deficiency on growth and glutathione peroxidase system enzymes in the baby pig. J. Nutr. 109:1615.

Braude, R., M.E. Coates, R.M. Henery, S.K. Kon, S.J. Rowland, S.Y. Thompson and D.M. Walker. 1947. A study of the composition of sow's milk. Br. J. Nutr. 1:64.

Brown, H., V.C. Speer, L.Y. Quinn, V.W. Hays and D.V. Catron. 1961. Studies on colostrum-acquired immunity and active antibody production in baby pigs. J. Anim. Sci. 20:323.

Burk, R.F. 1983. Biological activity of selenium. Ann. Rev. Nutr. 3:53.

Chow, C.K. and H.H. Draper. 1969. Effect of artificial drying on tocopherols and fatty acids of corn. J. Agr. Food Chem. 17:1316.

Chow, C.K. and A.L. Tappel. 1974. Response of glutathione peroxidase to dietary selenium in rats. J. Nutr. 104:444.

Cook, R.W., E.R. Miller, C.K. Whitehair, P.K. Ku and R. Michel. 1981. The tolerance to iron injections in vitamin E-selenium deficient pigs. MSU Swine Research Report. 437:94.

Committee on Nutrition. 1976. American Academy of Pediatrics, Commentary on breast-feeding and infant formulas including proposed standards for formulas. Pediatrics. 57:278.

Dam, H. 1962. Interrelations between vitamin E and polyunsaturated fatty acids in animals. Vitamins and Hormones. 20:527.

Desai, I.D. 1966. Effect of kidney beans (Phaseolus vulgaris) on plasma tocopherol-level and its relation to nutritional muscular dystrophy in the chick. Nature (London) 209:810.

Dillard, C.J., V.C. Gavino and A.L. Tappel. 1983. Relative antioxidant effectiveness of α-tocopherol and γ-tocopherol in iron-loaded rats. J. Nutr. 113:2266.

Diplock, A.T. 1973. The interaction between vitamin E and selenium. Acta Agric. Scand. Sup. 19:113.

Diplock, A.T. 1981. The role of vitamin E and selenium in the prevention of oxygen induced tissue damage. p. 303-316. In J.E. Spallhalz, J.L. Martin and H.E. Ganther (Ed.). Selenium in Biology and Medicine. AVI Publishing Company, Inc., Westport, CT.

Diplock, A.T. and J.A. Lucy. 1973. The biochemical modes of action of vitamin E and selenium: A hypothesis. FEBS Lett. 29(3):205.

Dunkley, W.L., M. Conning, A.A. Franke and J. Robb. 1967. Supplementing rations with tocopherol and ethoxyquin to increase oxidative stability of milk. J. Dairy Sci. 50:492.

Evans, D.E. 1959. Milk composition of mammals whose milk is not normally used for human consumption. Dairy Sci. Abstr. 21:278.

Fridovich, I. 1978. The biology of oxygen radicals, the superoxide radical as an agent of toxicity; superoxide dismutase provides an important defense. Science. 201:875.

Fukuzawa, K., H. Chida, A. Tokumura and H. Tsukatani. 1981. Antioxidative effect of α-tocopherol incorporation into lecithin liposomes on ascorbic acid -Fe^{2+} induced oxidation. Arch. Biochem. Biophys. 206:173.

Gardner, R.W. and D.E. Hogue. 1967. Milk levels of selenium and vitamin E related to muscular dystrophy in the suckling lamb. J. Nutr. 99:418.

Goff, J.P., R.L. Horst and E.T. Littledike. 1984. Effect of sow vitamin D status at parturition on the vitamin D status of neonatal piglets. J. Nutr. 114:163.

Goihl, J.H. 1984. Selenium in weanling pig diets studied. Feedstuffs 56(15):30.

Golberg, L., L.E. Martin and J.P. Smith. 1960. Iron overloading phenomena in animals. Toxicol. and Appl. Pharm. 2:883.

Groce, A.W., E.R. Miller, K.K. Keahey, D.E. Ullrey and D.J. Ellis. 1971. Selenium supplementation of practical diets for growing-finishing swine. J. Anim. Sci. 32:905.

Groce, A.W. 1972. Selenium and/or vitamin E supplementation of practical swine diets. Ph.D. Thesis. Michigan State University, East Lansing.

Groce, A.W., E.R. Miller, J.P. Hitchcock, D.E. Ullrey and W.T. Magee. 1973a. Selenium balance in the pig as affected by selenium source and vitamin E. J. Anim. Sci. 37:942.

Groce, A.W., E.R. Miller, D.E. Ullrey, P.K. Ku, K.K. Keahey and D.J. Ellis. 1973b. Selenium requirements in corn-soy diets for growing-finishing swine. J. Anim. Sci. 37:948.

Hallwell, B. 1978. Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. FEBS Lett. 92:321.

Harris, P.H., M.L. Quaife, P.O'Grady. 1952. Tocopherol content of human milk and cow milk products used for infant feeding. J. Nutr. 46:459.

Harrison, J.H., D.D. Hancock and H.R. Conrad. 1984. Vitamin E and selenium for reproduction of the dairy cow. J. Dairy Sci. 67:123.

Hassan, H., S.A. Hashim, T.B. Van Itallie and W.H. Sebrell. 1966. Syndrome in premature infants associated with the low plasma vitamin E levels and high polyunsaturated fatty acid diet. Am. J. Clin. Nutr. 11:147.

Herting, D.C. and E-JE Drury. 1969. Vitamin E content of milk, milk products and simulated milks. Relevance to infant nutrition. Am. J. Clin. Nutr. 22:147.

Hicks, M. and J.M. Gebicki. 1981. Inhibition of peroxidation in linoleic acid membranes by nitroxide radicals, butylated hydroxytoluene, and α-tocopherol. Arch. Biochem. and Biophys. 210(1):56.

Hintz, H.F. and D.E. Hogue. 1964. Effect of selenium and sulfur amino acids on nutritional muscular dystrophy in the lamb. J. Nutr. 84:3.

Hjarde, W., A. Neimann-SØrensen, B. Palludan, and P. Havskov Sorensen. 1961. Investigations concerning vitamin A requirement, utilization and deficiency symptoms in pigs. Acta. Agri. Scand. 11:13.

Jagadeesan, V. and K. Prema. 1980. Lactation and vitamin E status: Relationship between plasma and milk levels at different lactational periods. Nutr. Rep. Int. 23:135.

Jansson, L., M.D., B. AKesson, M.D., and L. Holmberg, M.D. 1981. Vitamin E and fatty acid composition of human milk. Amer. J. Clin. Nutr. 34:8.

Jansson, L., L. Halmberg, B. Nilsson and B. Johansson. 1978. Vitamin E requirements of preterm infants. Acta. Paediat. Scand., 67:459.

Jensen, P.T., K.B. Pedersen. 1979. Studies on immunoglobulins and trypsin inhibitor in colostrum and milk from sows and in serum of their piglets. Acta Vet. Scand. 20:60.

Jones, G.B. and K.O. Godwin. 1963. Studies on the nutritional role of selenium. I. The distribution of radioactive selenium in mice. Australian J. Agr. Res. 14:716.

Kellogg, E.W. and I. Fridovich. 1975. Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by xanthine oxidase system. J. Biol. Chem. 250:8812.

Kerner, J., J.A. Froseth, E.R. Miller and L.L. Bieber. 1983. Acylcarnitine content of sows colostrum, milk and serum and neonatal pig serum and tissues. M.S.U. Swine Research Report. 456:19.

Ku, P.K., W.T. Ely, A.W. Groce and D.E. Ullrey. 1972. Natural dietary selenium, α-tocopherol and effect on tissue selenium. J. Anim. Sci. 34:208.

Ku, P.K., E.R. Miller, R.C. Wahlstrom, A.W. Groce, J.P. Hitchcock and D.E. Ullrey. 1973. Selenium supplementation of naturally high selenium diets for swine. J. Anim. Sci. 37:502.

Lane, H.W., C.K. Tracey and D. Medina. 1984. Growth, reproduction rates and mammary gland selenium concentration and glutathione peroxidase activity of BALB/c female mice fed two dietary levels of selenium. J. Nutr. 114:323.

Lannek, N. 1973. The importance of vitamin E for domestic animals in sickness and in health. Acta. Agri. Scandi. Suppl. 19:13.

Lannek, N., P. Lundberg and G. Tollerz. 1962. Lowered resistance to iron in vitamin E-deficient piglets and mice. Nature. 195:1006.

Lawrence, R.A. and R.F. Burk. 1976. Glutathione peroxidase activity in selenium deficient rat livers. Biochem. Biophys. Res. Commun. 71:952.

Lawrence, R.A., R.A. Sunde, G.L. Schwartz. 1974. Glutathione peroxidase activity in rat lens and other tissues in relation to dietary selenium intake. Exp. Eye. Res. 18:563.

Leece, J.G. 1966. Absorption of macromolecules by neonatal intestine. Dev. of Met. as Rel. to Nutr., Symp. Biol. Neonate. 9:50.

Lee, Y.H., D.K. Layman and R.R. Bell. 1981. Glutathione peroxidase activity in iron deficient rats. J. Nutr. 111:194.

Loosli, J.K. 1949. Vitamin E requirement and economy in farm animals. Ann. New York Acad. Sci. 52:243.

Loudenslager, M.J. and P.A. Whetter. 1984. A modified procedure for total fat analysis in milk and colostrum. Dept. of Anim. Sci., Michigan State University, East Lansing.

Mahan, D.C., A.L. Moxon and J.H. Cline. 1975. Efficacy of supplemented selenium in reproductive diets on sow and progeny serum and tissue selenium values. J. Anim. Sci. 40:624.

Malm, A., W.G. Pond, E.F. Walker, Jr., M. Homan, A. Aydin and D. Kirtland. 1976. Effect of polyunsaturated fatty acids and vitamin E level of the sow gestation diet on reproductive performance and on level of alpha-tocopherol in colostrum and milk and dam and progeny blood serum. J. Anim. Sci. 42:393.

Martinez, F.E., A.L. Goncalves, S.M. Jorge and I.D. Desai. 1981. Vitamin E in placental blood and its interrelationship to the maternal and newborn levels of vitamin E. J. Ped. 99:298.

McConnell, P.K. and D.M. Roth. 1964. Passage of selenium across the placenta and also into the milk of the dog. J. Nutr. 84:340.

McCord, J.M., B.B. Keele, Jr., and L. Fridovich, 1971. An enzyme-based theory of obligate anaerobiosis: The physiological function of superoxide dismutase. Proc. Natl. Acad. Sci. U.S.A. 68:1024.

McGovern, J.J., A.R. Jones, A.G. Steinberg. 1955. The hematocrit of capillary blood. New Eng. J. Med. 253:308.

McKenzie, R.L., H.M. Rea, C.D. Thomson and M.F. Robinson. 1978. Selenium concentrations and glutathione peroxidase activity in blood of New Zealand infants and children. Am. J. Clin. Nutr. 31:1413.

Miller, E.R. 1982. Nutritional factors affecting milk production and immunity. NPPC. Production Symp. P. 10.

Miller, E.R., J.P. Hitchcock, K.K. Kuan, P.K. Ku, D.E. Ullrey and K.K Keahey. 1973. Iron tolerance and E-Se status of young swine. J. Anim. Sci. 37:287.

Miller, E.R., D.E. Ullrey, B.G. Harmon, D.A. Schmidt, R.W. Luecke and J.A. Hoefer. 1962. Antibody absorption, retention and production by the baby pig. J. Anim. Sci. 21:309.

Miller, G.M., J.H. Conrad and R.B. Harrington. 1971. Effect of dietary unsaturated fatty acids and stage of lactation on milk composition and adipose tissue in swine. J. Anim. Sci. 32:79.

Neilsen, H.E., N.J. Hojgaard-Olsen, W. Hjarde and E. Leerbeck. 1965. Vitamin A content in colostrum and milk from sows given vitamin A orally as cod liver oil or in a dry synthetic form. Acta. Agric. Scand. 15:235.

Neilsen, H.E., N.J. Hojgaard-Olsen, W. Hjarde and E. Leerbeck. 1973. Vitamin E content in colostrum and sow's milk and milk yield at two levels of dietary fats. Acta. Agri. Scand., Suppl. 19:35.

Nockels, C.F. 1980. The biological immune response - The effect of dietary vitamin E. Feedstuffs 52(24):22.

NRC. 1968. Nutrient Requirements of Domestic Animals, No. 2. Nutrient requirements for swine. 6th Revised Ed. National Academy of Sciences - National Research Council, Washington, D.C.

NRC. 1979. Nutrient Requirements of Domestic Animals, No. 2. Nutrient Requirements of Swine. 8th Revised Ed. National Academy of Sciences - National Research Council, Washington, D.C.

NRC. 1983. Selenium in Nutrition, Revised Ed. National Academy of Sciences - National Research Council, Washington, D.C. p. 22.

Olson, A.D. and W.B. Hamlin. 1969. A new method for serum iron and total iron binding capacity by atomic absorption spectrophotometry. Clin. Chem. 15:438.

Oski, F.A. and L.A. Barnes. 1967. Vitamin E deficiency: A previously unrecognized cause of haemalytic anemia in the premature infants. J. Pediatr. 70:211.

Paglia, D.E. and W.N. Valentine. 1967. Studies on the quantitative and qualitative characteristics of erythrocyte glutathione peroxidase. J. Lab and Clin. Med. 70:158.

Patterson, D.S.P., W.M. Allen, D.C. Thurley and J.T. Done. 1967. The role of tissue peroxidation in iron-induced myodegeneration of piglets. Biochem. J. 104:2.

Pazak, H.E. 1983. Effect of maternal vitamin E and selenium nutrition on the antioxidant status of the off-spring. Thesis, Pennsylvania State University. University Park, PA.

Perrin, D.R. 1955. The chemical composition of the colostrum and milk of the sow. J. Dairy Res. 22:103.

Perry, G.C. and J.H. Watson. 1967. Variation in the absorption of the colostrally secreted antibody in piglets. Anim. Prod. 9:385.

Pond, W.G., R.H. Allaway, E.F. Walker, Jr. and L. Krook. 1971. Effects of corn selenium content and drying temperature and of supplemental vitamin E on growth, liver, selenium and blood vitamin E content in chicks. J. Anim. Sci. 33:996.

Pond, W.G. and K.H. Houpt. 1978. Milk composition. p. 185-191. In the Biology of the Pig. Cornell University Press, Ithaca, NY.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoelstra. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 179:588.

Ruth, G.R. and J.F. Van Vleet. 1974. Experimentally induced selenium-vitamin E deficiency in growing swine. Selective destruction of type 1 skeletal muscle fibers. Am. J. Vet. Res. 35:237.

Schmidt, K. and W. Heller. 1976. Selenkonzentration und activitat der glutachion peroxydase in lyst menschlicher erythrozcyten. Blut. 33:247.

Schrauzer, G.N. and D.A. White. 1978. Selenium in human nutrition; dietary intakes and effects of supplementation. Bioinorg. Chem. 8:303.

Schwarz, K. 1976. Essentiality and metabolic functions of selenium. Symposium on trace elements. Medical Clinics of North America. 60(4):745.

Scott, M.L. 1973. New information on the function of selenium and vitamin E in metabolism and animal nutrition. Distill. Feed Res. Counc. Proc. 28:15.

Sklan, D., H.D. Robinowitch and S. Donoghue. 1981. Superoxide dismutase: Effects of vitamins A and E. Nutr. Rep. Int. 24(3):551.

Taylor, S.L. 1976. Sensitive method for tissue tocopherol analysis. Lipids. 11:530.

Thomas, M.J., K.S. Mehl and W.H. Pryor. 1978. The role of the superoxide anion in the xanthine oxidase-induced autoxidation of linoleic acid. Biochem. Biophys. Res. Commun. 23:927.

Thomson, D.C., H.M. Rea, M.F. Robinson and O.W. Chapman. 1977. Low blood selenium concentrations and glutathione peroxidase activities in elderly people. Proc. Univ. Otago Med. Sch. 55:18.

Tinberg, H.M. and A.A. Barber. 1970. Studies on vitamin E action: Peroxidation inhibition in structural protein-lipid micelle complexes derived from rat liver microsomal membranes. J. Nutr. 100:413.

Tollerz, G. 1973. Vitamin E, selenium (and some related compounds) and tolerance towards iron in piglets. Acta. Agric. Scand., Suppl. 19, 184.

Trapp, A.L., K.K. Keahey, D.L. Whiteneck and C.K. Whitehair. 1970. Vitamin E-selenium deficiency in swine: Differential diagnosis and nature of field problem. J. Amer. Vet. Med. Assoc. 157:289.

Ullrey, D.E. 1973. Selenium deficiency in swine production. Feedstuffs. 45(47):30.

Ullrey, D.E. 1981. Vitamin E for swine. J. Anim. Sci. 53:1039.

Ullrey, D.E., A.W. Groce, E.R. Miller, D.J. Ellis and K.K. Keahey. 1970. Vitamin E and/or selenium for swine. Feedstuffs. 42(9):26.

Ullrey, D.E., E.R. Miller, D.J. Ellis, D.E. Orr, J.P. Hitchcock, K.K. Keahey and A.L. Trapp. 1971. Vitamin E (selenium and choline), reproduction and MMA. Michigan State University Swine Research Report. 148:48.

Uri, N. 1961. p. 55-106. In Autoxidation and antioxidants, Vol. 1. (W.O. Lundberg, ed.), Wiley (Interscience), New York.

Vale, Oswaldo, E. 1983. Mastitis, Metritis, Agalactia in swine: Role of vitamin E and selenium. Thesis. Michigan State University, East Lansing.

Vos, G., I. Molenaar, M. Searle-Van Leeuwen and F.A. Hommes. 1973. Cellular membranes in vitamin E-deficiency: an ultrastructural and biochemical study of isolated outer and inner mitochondrial membranes. Acta Agric. Scandi., Supple. 19:192.

Whetter, P.A. and P.K. Ku. 1982. Procedure for plasma/serum α-tocopherol determinations. Dept. of Anim. Sci., Michigan State University, East Lansing, MI.

Whetter, P.A. and M.J. Loudenslager. 1984. Procedure for milk α-γ-tocopherol by HPLC on Bondopak C18 reverse phase column. Dept. Anim. Sci., Michigan State University, East Lansing, MI.

Whetter, P.A. and D.E. Ullrey. 1978. Improved fluorometric method for determining selenium. J. Assoc. Off. Anal. Chem. 61:927.

Williams, D.M., R.E. Lynch, F.G. Lee and G.E. Cartwright. 1975. Superoxide dismutase activity in copper-deficient swine. Proc. Soc. Exp. Biol. Med. 149:534.

Wilson, M.R. 1974. Immunologic development of the neonatal pig. J. Anim. Sci. 38:1018.

Wintrobe, M.M. 1980. Blood Pure and Eloquent. Nat. Acad. Sci, McGraw Book Co., New York.

Witting, L.A. 1980. Vitamin E and lipid antioxidants in free radical - initiated reaction. p. 295-315. In Free Radicals in Biology. Vol. IV (Pryor, W.A., ed.) Academic Press, New York.

Young, L.G., A. Lun, J. Pos, R.P. Forshaw and D. Edmeades. 1975. Vitamin E stability in corn and mixed feeds. J. Anim. Sci. 40:495.

Young, L.G., R.B. Miller, D.E. Edmeades, A. Lun, G.C. Smith and G.J. King. 1977. Selenium and vitamin E supplementation of high moisture corn diets for swine reproduction. J. Anim. Sci. 45:1051.

Young, L.G., H.E. Nielsen and P.T. Jensen. 1980. Vitamin E and selenium for pigs. Commonwealth Agri. Bureaux. 1(2):73.