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EFFECTS OF VITAMIN E AND SELENIUM
ON THE NUMBER AND IMMUNORESPONSIVENESS
OF CELLULAR COMPONENTS
OF SOW PERIPHERAL BLOOD, COLOSTRUM AND MILK

presented by

HASTARI WURYASTUTI

has been accepted towards fulfillment
of the requirements for

Ph.D degree in Large Animal Clin. Sci.

Major professor

Dr. H. D. Stowe

Date November 10, 1989

EFFECTS OF VITAMIN E AND SELENIUM ON THE NUMBER AND IMMUNORESPONSIVENESS OF CELLULAR COMPONENTS OF SOW PERIPHERAL BLOOD, COLOSTRUM AND MILK

By

Hastari Wuryastuti

A Dissertation

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

Doctor of Philosophy

Department of Large Animal Clinical Sciences

1989

ABSTRACT

EFFECTS OF VITAMIN E AND SELENIUM ON THE NUMBER AND IMMUNORESPONSIVENESS OF CELLULAR COMPONENTS OF SOW PERIPHERAL BLOOD, COLOSTRUM AND MILK

By

Hastari Wuryastuti

Twenty four multiparous sows were bred as they came into postweaning estrus, and assigned to one of the following dried high moisture-corn / soybean meal-based gestation diets: unsupplemented (-E-Se); plus 0.3 mg selenium/kg (-E+Se); plus 60 IU vitamin E/kg (+E-Se) and plus 0.3 mg selenium/kg and 60 IU vitamin E/kg (+E+Se/ control). Blood samples for cell isolation and for vitamin E, selenium, glutathione peroxidase and cholesterol analyses were collected at 0, 30, 60 and 90 days of gestation and at parturition. Colostrum and milk samples were obtained at parturition and on postpartum day 4 , respectively, for cell isolation. Immunoresponsiveness of cellular components of peripheral blood, colostrum and milk were determined by measuring the lymphocyte proliferation response to phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM), and the phagocytic and microbicidal activities of polymorphonuclear (PMN) cells. At 90 days of gestation and at parturition, vitamin E-deficiency reduced ($p<0.05$) the response of peripheral blood lymphocytes (PBL) to PHA and PWM stimulation. Reduction in the response of PBL to PHA and PWM was also observed in sows fed the -E-Se

diet, as early as 60 days of pregnancy. The -E+Se and -E-Se diets were associated with reduced ($p<0.05$) responses of colostral lymphocytes to PHA whereas only the -E-Se diet reduced ($p<0.05$) the response of colostral lymphocytes to PWM. Phagocytic activity of sow blood PMN cells was reduced ($p<0.05$) by 90 days of gestation and at parturition for sows fed -E+Se and +E-Se diets and by 60 days of gestation in sows fed -E-Se diet. Only sows fed the -E-Se diet had lower phagocytic activity of colostral PMN cells. Vitamin E-deficiency, selenium-deficiency and a combined vitamin E and selenium-deficiency reduced ($p<0.05$) the microbicidal activity of blood and colostral PMN cells. A significant decrease in microbicidal activity of milk PMN cells was only observed in sows fed -E-Se diet.

This study indicates that vitamin E deficiency significantly depressed functions of both B and T lymphocytes and PMN cells in gestating sows. Selenium deficiency, however, only depressed the PMN cell function.

Dedicated with love to

my mother, my father

and my husband, R. Wasito

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. H. D. Stowe, my major professor, for his encouragement, patience and valuable advice during my course of study.

I am also grateful to my committee members, Dr. R. W. Bull, Dr. E. R. Miller, Dr. S. D. Sleight and Dr. B. J. Thacker for their help in reviewing this manuscript and willingness to share their professional expertise.

Special thanks go to the Government of the Republic of Indonesia, the MUCIA-Indonesia educational project and the College of Veterinary Medicine, Michigan State University for their financial support.

I am indebted to Dr. R. W. Bull and Peggy Coffman for allowing me to use the immunology laboratory facilities for portions of the assays, and their professional assistance given in the laboratory.

My appreciation also to Paul Matzat, Ray Kromer, Steve Mussell and DuWain Simon for their assistance with the research animals; to Anne House, Tonie Thiel, Beta Borer, Faith Manning and Kathy O'hare for their assistance with the laboratory work, their warmth and their friendship; and to Mary Ellen Shea for her help in preparing the figures.

Finally, my deepest appreciation to my husband, Dr. R. Wasito who has tolerated and endured my mental and/or physical absence and most of all for his continous love and support making the completion of this degree a reality.

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INTRODUCTION

It is generally accepted that severe multinutrient deficiencies lead to immune system dysfunction. However, deficiencies, excesses or imbalances of a single nutrient can also adversely influence host resistance. For example, vitamin A deficiency in rats caused a significant reduction in their response to T- and B-cell mitogens, a situation reversible upon supplementation with vitamin A (Beisel, 1982). Iron deficiency was associated with an impaired lymphocyte stimulation response to mitogens and decreased neutrophil bacterial capacity (Krantman et al., 1982; Kuvibidila et al., 1983). However, excess iron can also disrupt the host's ability to defend itself against infectious disease (Sherman, 1984).

During the last decade, it has been demonstrated that vitamin E exerts a significant influence on immune response. Because of the close nutritional and bichemical relatonship between selenium and vitamin E , the involvement of dietary selenium in immunity has also been studied in both animals and humans. Decreased bactericidal activity of polymorphonuclear cells has been observed in vitamin E and/or selenium-deficient rabbits (Lafuze et al., 1983), man (Arvilommi et al., 1983), goats (Aziz et al., 1984),

cattle (Gyang et al., 1984) and mice (Boyne and Arthur, 1986). Depressed proliferative response of lymphocytes to various mitogens has been demonstrated in vitamin E and/or selenium-deficient mice (Corwin and Shloss, 1980), pigs (Larsen and Tollersrud, 1981; Jensen et al., 1988) and dogs (Langweiler et al., 1981). Vitamin E and/or selenium deficiency has also been associated with the reduction of antibody production against foreign pathogens (Reffet et al., 1988a,b; Marsh et al., 1981). Some studies have indicated that vitamin E and/or selenium-deficient animals are more susceptible to infectious diseases than vitamin E and/or selenium-adequate animals. Teige et al. (1982) reported that pigs fed a vitamin E- and selenium-deficeint diet had decreased resistance to experimentally induced Treponema hyodysenteriae. The metritis-mastitis-agalactia syndrome of sows has been associated with inadequate dietary vitamin E and selenium (Whitehair et al., 1983). If the infectious aspects (metritis and mastitis) of this complex were manifestations of impaired immunity, the sow could serve as an animal model for investigations of immunocompetence.

It is known that ingestion of mammary secretions by neonates serves two major purposes: to transfer immunity from mother to the offspring before they can produce their own antibodies and to sustain the young nutritionally during the early weeks of life.

Recent research interest in the immunobiology of mammary secretions has focused not only upon the secretory immunoglobulin components and their contribution to local intestinal mucosa immunity in the neonate, but also upon the significant immunologic role of those viable leukocytes present in the mammary secretions (Schollenberger et al., 1986).

Drew et al. (1983) have reported that human colostral lymphocytes can respond in vitro to various nonspecific mitogens such as phytohemagglutinin (PHA) and concanavalin A (Con A). Megs and Beer (1979) have demonstrated that the phagocytic cells in colostrum and milk of humans have the capacity to phagocytize and kill both E. coli and C. albicans. Erskine et al. (1987) suggested that supplementation of 1000 IU vitamin E and 6 mg selenium per cow per day during the dry period reduced somatic cell counts and incidence of mastitis in subsequent lactations; however, the immunoresponsiveness of colostral and/or milk leukocytes was not assessed.

We therefore, decided to study the influence of vitamin E and/or selenium on the immunoresponsiveness of cellular components of peripheral blood, colostrum and milk of sows.

The specific objectives of this study were: (1) to determine the effects of dietary vitamin E and/or selenium on immunoresponsiveness of cellular components of peripheral blood of gestating and peripartum sows, (2) to characterize the cellular components of porcine colostrum and

milk and (3) to determine the effects of dietary vitamin E and/or selenium on the immunoresponsiveness of the cellular components of colostrum and milk of these sows.

LITERATURE REVIEW

Historical

Vitamin E: Vitamin E was discovered in 1920 by Matill and Conklin. They observed that rats fed basal semi-purified diets, supplemented with all vitamins known at that time, were able to grow normally but were unable to reproduce. Two years later, Evans and Bishop (1922) found that an unknown dietary factor, first called factor X, in fresh green lettuce leaves and wheat germ prevented sterility in rats. The factor X was then known as fat-soluble vitamin E. The findings of Evans and Bishop were supported by independent work of Sure (1924) and Matill *et al*. (1924).

Further investigations by Evans and co-workers (1936) led to the isolation from wheat germ oil of an alcohol substance having vitamin E activity which was named alpha tocopherol. The name tocopherol was from the Greek *tokos* (childbirth) and *phero* (to bear) and *ol* for the alcohol form. Subsequently, 2 other tocopherols were isolated from vegetable oil and were designated as β and γ-tocopherol. These 2 substances have lesser biological activity than α tocopherol (Emerson *et al*., 1937).

In the following years, some vitamin E-responsive diseases, such as nutritional muscular dystrophy in

suckling rats (Olcott, 1938) and rabbits (Mackenzie et al., 1940), exudative diathesis in chicks (Dam and Glavind, 1939) and nutritional encephalomalacia (Pappenheimer and Goettsch, 1931) were described.

Selenium: Selenium was first recognized in 1934 as a result of its association with the poisoning of farm animals (Franke, 1934). Interest in this element increased considerably following the discovery by Schwarz and Foltz (1957) that selenium was an integral part of the "Factor 3" that protected against dietary liver degeneration occurring in rats fed Torula yeast diets. This finding was supported by Patterson et al. (1957) who found the beneficial effect of selenium in preventing exudative diathesis in chicks. Subsequently, selenium has been found to alleviate several vitamin E-responsive animal diseases of practical agricultural importance, such as white muscle disease in lambs and calves and hepatosis dietetica in young pigs.

Extensive work had been reported concerning the biological activity of selenium compounds, both inorganic and organic. Sodium selenite was active whereas elemental selenium was inactive. Organic selenium compounds varied widely in their bio-potencies, some being inactive and others more active than sodium selenite against dietary liver necrosis in rats (Schwarz and Foltz, 1958; Schwarz and Fredga, 1969; Schwarz, 1961).

Glutathione peroxidase: In the 1950s, the enzyme glutathione peroxidase (GSH-Px) was discovered by Mills (1957) and Mills and Randall (1958). They reported that GSH-Px was primarily located in cell cytosol and mitochondrial matrix where it could protect hemoglobin from ascorbic acid-induced oxidation but only in the presence of glucose. This finding was confirmed by Cohen and Hochstein (1963) who also discovered the existence of GSH-Px in erythrocytes and considered it to be an important enzyme for elimination of hydrogen peroxide (H_2O_2). Glutathione peroxidase was also reported to be present in other organs such as liver and kidney (Mills, 1959).

In 1973, Flohe *et al*. studied extensively the enzymology and biological importance of glutathione peroxidase and found that, unlike other peroxidases, GSH-Px did not contain heme. The same year, Rotruck *et al*. (1973) studied this enzyme from rat erythrocytes and discovered that selenium was a component of GSH-Px. Oh *et al*. (1974) reported that GSH-Px in most species contained 4 gram-atoms of selenium per mole. These discoveries have explained the nutritional function of selenium and have improved the understanding of the complex interrelationships between selenium and vitamin E.

Sources

Vitamin E: Tocopherols (tocols) and tocotrienols (trienols) are two classes of chemically distinct compounds

with vitamin E activity. Each class has four isomers known as α , β , γ , and δ tocols or α , β , γ , and δtrienols which occur naturally in variable amounts. Plants are generally better sources of vitamin E than foods of animal origin. This is because of the inability of animals to synthesize vitamin E in their body. Therefore, the vitamin E concentrations in animal tissues or products are usually low and reflect of dietary vitamin E intake (Machlin, 1984; Pike and Brown, 1984). In plants, the highest concentrations of vitamin E are found in many of the seed oils like soybean, corn, cottonseed, sunflower and wheat germ oil. Some leafy vegetables such as kale and spinach also contain considerable amounts of vitamin E (Bauernfeind, 1980).

Selenium: Many studies point to the fact that the selenium contents in feedstuffs from plant origin tend to be greatly influenced by the selenium concentration in the soil. According to Gissel-Nielsen et al. (1984), however, a combination of climate and soil conditiom has a more important effect on the availability of selenium from soil than the selenium concentration in the soil.

Selenium that is released from alkaline and well-aerated soils usually is present in the form of selenate. Selenates are highly soluble in water and therefore are easily transported by ground water. In contrast, selenium that is released from acid and poorly-aerated soils is

present in relatively reduced insoluble forms such as selenides and selenites. These types of selenium can form stable adsorption complexes with ferric hydroxide and become unavailable to plants (Allaway, 1972; Allaway, 1973). Soil clay content also significantly influences the utilization of selenium by plants. Selenium uptake by plants is generally better from sandy soil than from loamy soil (Sharma and Singh, 1983). As a consequence of the factors previously mentioned, selenium concentration in plants can vary from 0.005 ppm to 5000 ppm (Nigam and McConnell, 1976; Lorenz, 1978; Tan et al., 1986).

The selenium content of foods of animal origin depends on the selenium intakes of livestock. Animals that are raised in selenium deficient regions will deposit relatively low concentrations of this mineral in their edible tissues and products; animals raised in relatively high selenium nutriture, on the other hand, will have much higher selenium concentrations (Hazell, 1985). According to Burk (1984), however, the variations in selenium contents of animal tissues are less than in plant tissues because animals can conserve selenium when it is in short supply and excrete excesses.

METABOLISM

Absorption

Vitamin E: Studies with ruminants and monogastrics have indicated that vitamin E is absorbed in the mid region of the small intestine and only 20 to 40% of ingested vitamin E and/or its derivatives are absorbed (Gallo-Torres, 1980). Blomstrand and Forsgren (1968) suggested that unesterified vitamin E is absorbed less efficiently than any of its derivatives. Absorption of vitamin E is also significantly reduced when a large dose of vitamin E is consumed orally (Schmandke et al., 1969). Efficiency of vitamin E absorption from the intestine is facilitated by dietary lipids, especially medium chain triglycerides and phospholipids, whereas, polyunsaturated fatty acids (PUFA) reduce the absorption of vitamin E (Schmandke and Schmidt, 1965; Mathias et al., 1981).

Like other fat-soluble vitamins, the intestinal absorption process of vitamin E closely follows the pathways of lipid absorption. During the digestion process, the dietary vitamin E is released from associated foods either as a result of acidity in the stomach or by the activity of proteolytic enzymes. In the next step, vitamin E is dissolved in small fat globules which are formed from lipids of the ingested food during mechanical mixing of the chyme. The fat droplets are then emulsified within the lumen of the small intestine by the interaction of bile acid and

pancreatic juice (Mathias et al., 1981).

The pancreatic enzymes, especially lipase, efficiently hydrolyze tocopherol esters. Bile salts are responsible for solubilizing tocopherols by the formation of mixed micelles together with monoglycerides, long-chain fatty acids, phospholipids and cholesterol (Gallo-Torres, 1980). The absolute requirement for bile acids for tocopherol absorption is clearly demonstrated in children who do not secrete bile acids into the intestinal lumen. These individuals do not absorb vitamin E, and as a result, will develop peripheral neurologic abnormalities, primarily areflexia and ataxia. The progression of these disorders can be controlled by parenteral administration of vitamin E (Rosenblum et al., 1981; Guggenheim et al., 1982; Sokol et al., 1983).

The formation of mixed micelles is an important step which allows vitamin E to traverse to the unstirred water layer covering enterocytes, and, thereby, reaching the absorptive surface in the intestinal mucosa (Gallo-Torres, 1970; Mathias et al., 1981). The mechanism by which tocopherol penetrates into the mucosal cells is still unclear, however, it may involve diffusion processes (Gallo-Torres, 1980; Machlin, 1984).

Selenium: Absorption of selenium occurs in all segments of the small intestine. In a study on rats by Whanger et al. (1976), using ^{75}Se, it was demonstrated

that the greatest absorption of selenite or selenomethionine occurred from the duodenum with slightly less absorption from jejunum or ileum. Maximum absorption of selenate, on the other hand, occurred from the ileum (Wolffram et al., 1985). Selenium is essentially not absorbed from the stomach of monogastrics or the rumen or abomasum of ruminants (Whanger et al., 1976; Wright and Bell, 1966).

The intestinal absorption of soluble selenium compounds by rats appears to be more efficient than by humans. It has been shown that rats absorbed 92, 91 and 81% of doses of selenite, selenomethionine and selenocystine, respectively (Thomson and Stewart, 1973; Thomson et al., 1975). In humans, the absorption of soluble selenium was found to range from 44 to 70% (Thomson and Stewart, 1974).

The inorganic form of selenium is absorbed from the gastrointestinal tract to a greater extent by monogastric animals than by ruminants. This difference may be due to the reduction of the ingested selenite to insoluble or unavailable forms by rumen microorganisms (Wright and Bell, 1966).

Little is known regarding the physiological processes regulating the absorption of selenium compounds. It has been shown in hamsters that selenomethionine is actively absorbed from the mucosal to the serosal side of small intestine and is inhibited by L-methionine, whereas, selenite and selenocystine are neither transported against

a concentration gradient nor inhibited by L-methionine (McConnell and Cho, 1965; Spencer and Blau, 1961). Wolffram et al. (1985) have found that selenate is absorbed by a carrier-mediated process and that the absorption mechanism is not inhibited by selenite but is inhibited by sulfate.

According to Li and Vallee (1973), the absorption of selenium is dependent upon the solubility of the selenium compound ingested and also on the dietary ratio of selenium to sulfur.

Transport

Vitamin E: Following absorption, vitamin E is carried away from the intestine by the chylomicrons. These then enter the lacteals and the intestinal lymphatics through the lamina propria and finally reach the systemic circulation via the thoracic duct. Movement of chylomicrons across the lymphatic endothelium occurs both by passive diffussion and active transport within pinocytotic vesicles (Kayden and Traber, 1986).

The necessity of chylomicrons in vitamin E metabolism is demonstrated in patients with abetalipoproteinemia. This is a condition in which β-apolipoprotein is not synthesized, therefore, the chylomicrons, the very low density ipoprotein (VLDL) or the low density lipoprotein (LDL) are not secreted and vitamin E can't be transported. Pigmentary retinopathy and ataxic neuropathy which develop in this disease are due to vitamin E deficiency (Brin et al., 1986;

Runge et al., 1986).

In the circulation, tocopherols are incorporated into lipoproteins, primarily the LDL fractions (Machlin, 1984). There are indications that a small amount of vitamin E is also associated with VLDL, high density lipoprotein (HDL) and erythrocytes (Bjornson et al., 1976; Kayden and Bjornson, 1972; Kayden, 1978).

Selenium: Once absorbed, selenium is transported in the blood where it is apparently associated with the plasma proteins. In dogs, selenium is mainly transported by alpha-2 and beta-1-globulins (Schwarz and Foltz, 1957), whereas, albumin seems to be the selenium-carrier protein in mice (Sandholm, 1975). Lee et al. (1969) reported that, besides gamma-globulin, selenium is also carried by plasma lipoprotein. In more recent studies, Motsenbocker and Tappel (1982) have proposed that, in rats, a particular selenocysteine-containing protein, selenoprotein P, serves as a selenium transport protein. From the plasma proteins, selenium is rapidly distributed to tissues, including hair and bones, and to erythrocytes and leukocytes (Cousins and Cairney, 1961).

Tissue distribution

Vitamin E: Studies in animals and humans have confirmed that vitamin E is distributed throughout the body.

Some tissues like testis, adipose tissue, adrenal glands, pituitary glands and platelets have relatively high concentrations of vitamin E (Gallo-Torres, 1980; Machlin, 1984). Tissue uptake of tocopherols has a positive correlation with the logarithm of the dose administered. This linear relationship has been shown in lung, heart,testis and plasma (Behrens et al., 1982). According to Igarashi and his colleagues (1986), concentrations of tocopherols in kidney, lung and heart are influenced by some nutritional factors such as dietary fats, proteins and status of other vitamins.

Selenium: Results of animal studies indicated that kidneys have by far the highest concentration of selenium and lesser amounts are in the liver and pancreas (Jones and Godwin, 1962; Schamberger, 1983). Similar results were also reported by Schroeder et al. (1970) who examined the selenium concentration in various human internal organs from autopsy samples and found the following descending order for selenium concentration in tissues: kidney>liver> spleen>pancreas>testis>heart>muscles>lung>brain. The distribution of selenium among tissues appears to depend on the form of selenium ingested and the selenium status of the animals at the time of administration but is relatively independent of the route of administration.

Studies in chicks (Jensen et al., 1963), rats (Burk et al., 1968) and sheep (Lopez et al., 1968; Wright and Bell,

1964) have shown that selenium was more efficiently retained in selenium-deficient animals than in selenium-adequate animals. This increased retention is probably due to greater tissue demand for selenium. Selenomethionine is preferentially taken up in the pancreas and selenite is mostly taken up in the liver, regardless of whether selenomethionine or selenite is given orally or parenterally (Osman and Latshaw, 1976).

Metabolic conversion

Vitamin E: Metabolism of vitamin E in animal tissue is relatively minimal. The absorbed alpha tocopherol remains in an unesterified form in tissue membranes where its biochemical functions occur (Machlin, 1984). However, there is some evidence for the occurrance of tocopheryl quinone (TQ) in animal tissues (Gallo-Torres, 1980; Hughes and Tove, 1980).

Studies with chickens (Scott and Desai, 1964) and rats (Mackenzie and Mackenzie, 1959) indicated that d-alpha-tocopheryl quinone can protect against experimental muscular dystrophy due to vitamin E deficiency. Chow et al. (1967) found that, in rat liver, TQ was reduced to tocopheryl hydroquinone which was then excreted as a conjugate of hydroquinone in feces and tocophrenoic acid in urine.

Two other vitamin E metabolites, designated as tocopheronolactone and tocopheronic acid have been isolated

from the urine of rabbits and humans after consuming large doses of alpha-tocopherol. These compounds are also referred to as Simon's metabolites (Simon et al., 1956a,b; Green et al., 1961). Even though the mechanism for the formation of Simon's metabolites is not clear, it has been postulated that the quinone is an intermediate in their formation (Simon et al., 1956a,b).

Selenium: The main pathway of selenium metabolism in animals involves a reduction process (Levander, 1976). Selenite first reacts with glutathione or protein sulfhydryls to form selenotrisulfides (Jenkins and Hidiroglou, 1971). By enzymatic action, selenotrisulfide is reduced to selenide, a common intermediate metabolite. Under normal intake of selenium, selenide is methylated to trimethylselenonium which is then excreted through the urinary tract (Burk et al., 1973; Hsieh and Ganther, 1977; Foster and Ganther, 1984).

In cases of excessive selenium intake, the pathway described above is overloaded. As a result, dimethylselenide is formed instead of trimethylselenonium. Dimethylselenide is a detoxified form of selenium which is excreted via the lungs, and is responsible for the typical "garlicky odor" in animals consuming toxic amounts of selenium (Schamberger, 1983).

Excretion

Vitamin E: Three different routes of vitamin E excretion have been identified. The primary route is via the feces, even though there is a great deal of variation, ranging from 10 to 75% of an administered dose. Approximately 1% of vitamin E in the form of Simon's metabolites is excreted via urine (Hidiroglou *et al.*, 1970). It has also been reported that up to 8% of vitamin E administered is excreted in the bile as an unidentified metabolite (MacMahon *et al.*, 1975).

Selenium: Excretion of selenium from the body can occur through three different routes: the urinary tract, the intestinal tract and the respiratory tract. The amounts and proportions of selenium excreted are affected by many factors such as dietary selenium level, the route of administration, the form of selenium ingested and the composition of the diet (Schamberger, 1983).

It has been demonstrated in rats (Burk *et al.*, 1973) and in humans (Griffiths *et al.*, 1976) that the urinary tract is the main route of selenium excretion when organic or inorganic selenium intake is limited, whereas equal proportions of selenium were excreted in the urine and feces when adequate amounts of selenium were consumed.

The effect of route of administration of selenium on selenium excretion was investigated by Lopez *et al.* (1968). In ruminant and some nonruminant species, the urinary tract

was the major pathway of excretion when selenium was given orally. The increased fecal excretion by ruminants has been associated with poor selenium absorption rather than elevated endogenous excretion. Excretion via the lungs only occurs significantly under condition of very high selenium exposure (Olson et al., 1963).

Nahapetian et al. (1983) administered three different forms of selenium to rats to study the effect of the form of selenium on mode of selenium excretion. They found that more selenocystine or selenite than selenomethionine was excreted in the urine.

Urinary excretion in humans also was significantly higher when they consumed a combination of high-protein, low-phosphorus and high-protein, high phosphorus diets than a combination of low-protein, low-phosphorus and low-protein, high-phosphorus diets (Greger and Marcus, 1981).

Function

Vitamin E: The principal function of vitamin E *in vivo* is as an effective antioxidant. This role is very important to protect the intracellular membrane against lipid peroxidation. Lipid peroxidation is a process that may be induced by either endogenously produced metabolic by products such as free radicals and other oxidants, or by exposure to such environmental agents as photochemical air pollutants, pesticides and tobacco smoke. (Freeman and Crapo, 1982; Mason and Chignell, 1982).

To carry out its function, vitamin E may have several actions. Firstly, vitamin E both quenches and reacts with singlet oxygen (Foote, 1968). Secondly, vitamin E reacts with superoxide radicals (O_2^-). This reaction, however, is not considered to be importence since the reaction is very slow and O_2^- does not initiate lipid peroxidation. Except in the present of iron (Fe^{+3}), it can interact with peroxide to form the very reactive hydroxyl radical (Fong et al., 1973). Thirdly and probably the most important action is the effectiveness of vitamin E as chain terminator. In this situation, vitamin E will react with lipid peroxy radicals to form vitamin E radicals and, eventually, tocopheryl quinone in order to remove H· from the membrane lipid. The vitamin E radicals produced are fairly stable because the unpaired electron on the oxygen atom can be delocalized into the aromatic ring structure of the quinone (Burton and Ingold, 1981; Burton et al., 1983). It was first suggested that the formation of tocopheryl quinone is irreversible so that molecules of tocopherol were thereby lost from the system. According to Tappel (1962) and Diliberto et al. (1982), however, the vitamin E radical can be regenerated back to vitamin E with ascorbic acid, glutathione and NADPH. This may be a further mechanism by which high concentrations of vitamin C protect against peroxidation.

In addition to its role as an antioxidant, vitamin E has also been implicated in the modulation of arachidonic

acid and prostaglandin (PG) metabolism (Hope et al., 1975). A vitamin E deficiency in the rat has been known to cause both elevation of serum PGE_2 and $PGF_{2\alpha}$ concentrations (Hope et al., 1975) and enhancement of platelet aggregation in response to collagen (Machlin et al.1975). The synthesis of PG in testis or muscle of vitamin E-deficient animals is also increased (Carpenter, 1981; Chan et al., 1980).

The mechanism by which vitamin E alters the serum PG level has not been established. Several early studies suggested that vitamin E modulated serum PG level through enhanced cyclooxygenase activity (Lands et al., 1972; Nugteren et al., 1966). On the other hand, Karpen et al. (1981) has reported that platelets from vitamin E-deficient rats produced more thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) than did platelets from vitamin E-supplemented rats. Therefore, Karpen et al. (1981) suggested that vitamin E deficiency raised serum PG level via enhanced activity of phospholipase A_2. Inhibition of PG synthesis by vitamin E might account for its involvement in the immune system (Tengerdy et al., 1978) and in the inflammatory response (Vane, 1971; Jarrat, 1976).

Selenium: Since a number of investigators found that selenium could prevent some of the symptoms of vitamin E deficiency, the function of selenium has always been associated with that of vitamin E and, hence, with anti-

oxidant property. However, the mechanism of action of selenium was not established until Rotruck et al. (1973) discovered that selenium is an essential constituent of the enzyme GSH-Px.

In the tissues, GSH-Px catalyses the reduction of hydrogen peroxide and of fatty acid hydroperoxides by using reduced glutathione (GSH) as a hydrogen donor to produce water and hydroxy acid and oxidized glutathione (GSSG) (Ganther et al., 1976). It is clear that both vitamin E and selenium play separate but related roles in the cellular defense mechanism against oxidative damage (see Figure 1).

Bryant et al. (1982) reported that GSH-Px also catalyses the conversion of 12 hydroperoxy eicosatetraenoic acid (HPETE) to 12 hydroxy eicosatetraenoic acid (HETE) in platelets. They later found that platelets from selenium-depleted rats accumulated more HPETE than did platelets from selenium-supplemented rats (Bryant et al., 1983). Accumulation of HPETE in platelets causes an accumulation of PGI_2 and TXA_2 and eventually increases platelet aggregation and vasoconstriction. Similar results were reported by Schiavon et al. (1984) who used cultured endothelial cells from pig thoracic aorta and by Levander et al. (1985) who used rat aortas and found a significant reduction in the production of platelet aggregation when GSH-Px was added to the incubation medium. From the foregoing evidence, it is likely that vitamin E and selenium, as an essential part of GSH-px, modulate the biosynthesis

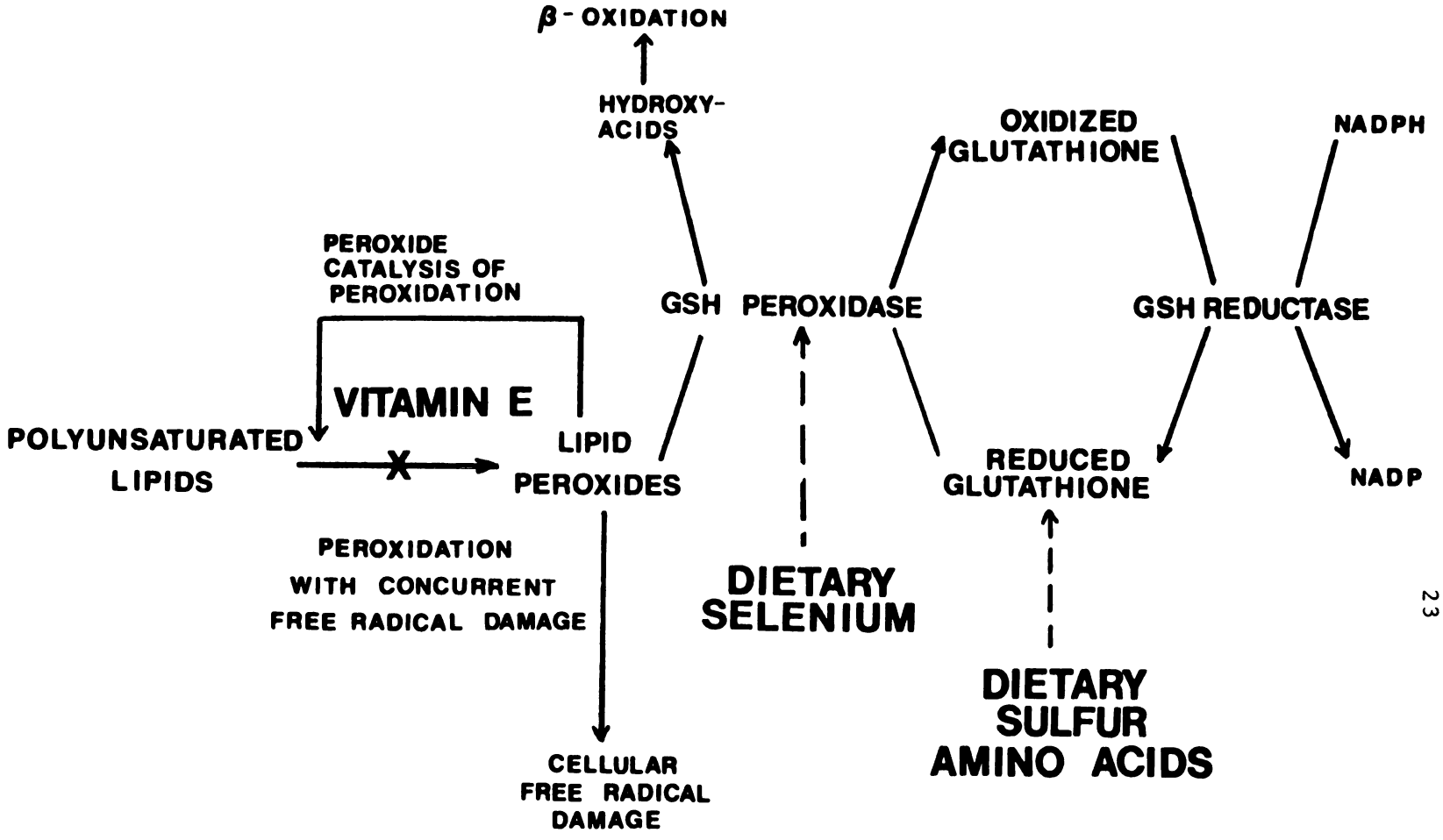

Figure 1. Function of selenium and its relatioship to the antioxidant function of vitamin E. Modified from Hoekstra (1975).

synthesis pathways in arachidonic acid and PG metabolism at a number of different points (see Figure 2).

Another metabolic role of selenium that has been reviewed is its role on reproduction. Studies in rat (McCoy and Weswig, 1969), mouse (Wallace et al., 1981), boar (Liu et al., 1982) and bull (Palleni and Bacci, 1979) have indicated that selenium is important for normal spermatogenesis. Wu et al. (1973) have reported that male rats born to dams fed selenium-deficient diets have abnormal sperm morphology and impaired sperm motility. They also found that supplementation of the diet with a high level of vitamin E and other antioxidants could not prevent these selenium deficiency effects.

It is known that selenium can't prevent fetal resorption in vitamin E-deficient rats (Harris et al., 1958) nor can it improve the reproductive performance in vitamin E-deficient chickens or turkeys (Creger et al., 1960; Jensen and McGinnis, 1960). However, selenium supplementation had beneficial effects on both egg production and hatchability of fertile eggs in chickens (Cantor and Scott, 1974) and in turkeys (Cantor et al., 1978). The selenium role in hatchability could involve integrity of the special muscles necessary for the chick or poultry to break out of the shells. The mechanism by which dietary selenium could maintain the integrity of sperm morphology in the rat remains unexplained.

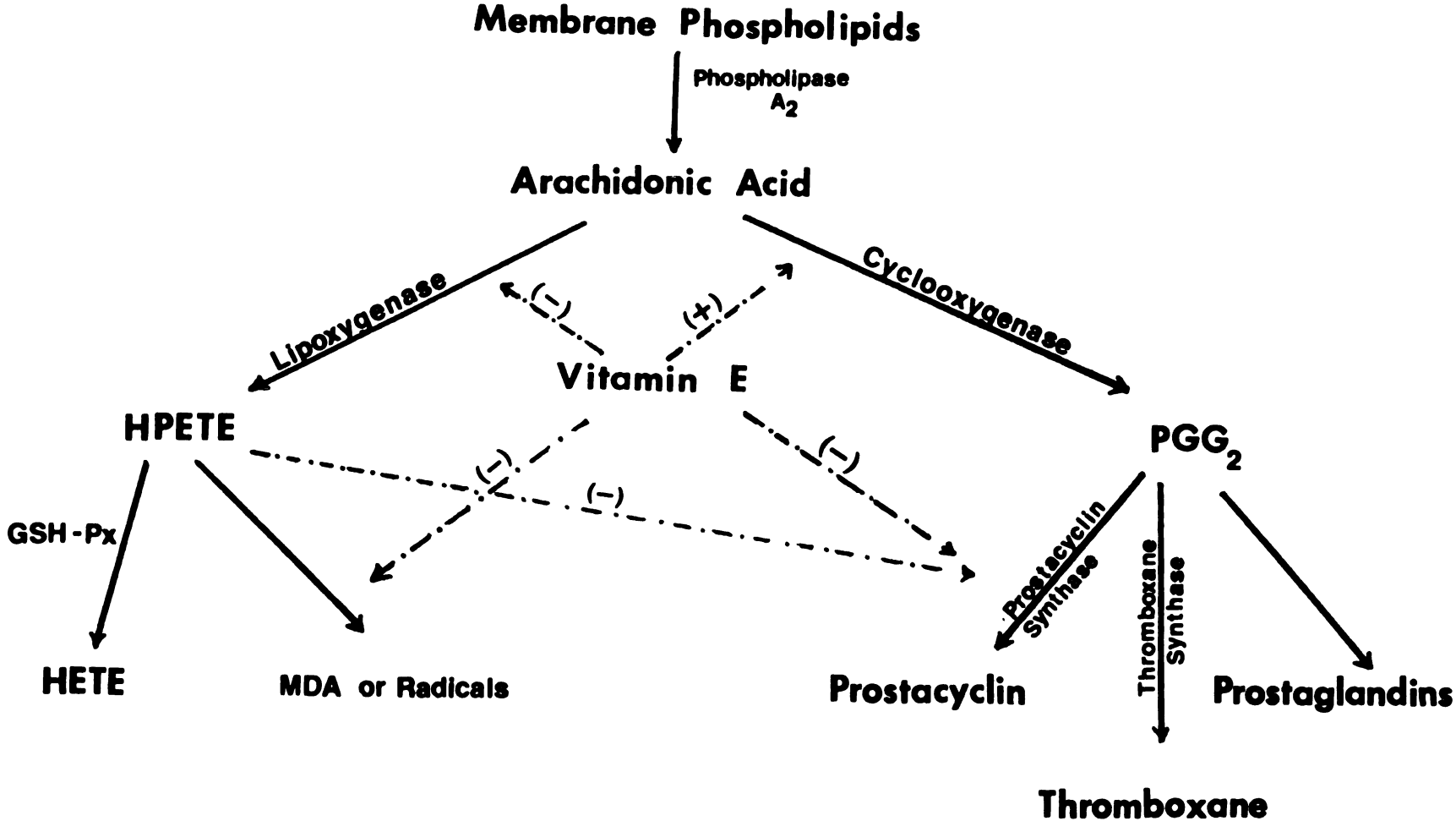

Figure 2. The effects of vitamin E and selenium dependent GSH-Px on the metabolic pathway of arachidonic acid. HPETE= 12 hydroperoxy eicosatetraenoic acid; HETE= 12 hydroxy eicosatetraenoic acid; MDA= malonaldehyde; PG= prostaglandin. Modified from Panganamala and Cornwell (1982).

Deficiency

Vitamin E: The vitamin E deficiency diseases in most species of animals are now well established. Unlike other vitamins, vitamin E deficiency can result in a wide diversity of clinical signs and pathological changes in different species of animals. The lesions can occur in the reproductive tract, nervous tissues, muscle tissues (skeletal, cardiac and smooth), liver, alimentary tract, fat depots or even outer integument. The lesions of deficiency usually are degenerative in nature due to instability of biological membranes.

According to Horwitt (1965) and Machlin (1984) the tissues affected appear to depend on the species and other nutrients, especially PUFA and selenium. For example, young bullocks develop nutritional degenerative myopathy, whereas, young pigs develop microangiopathy when they are fed a low vitamin E diet. Gershoff and Norkin (1962) showed experimentally that in cats, vitamin E deficiency, concurrent with a low level of dietary PUFA, produced chronic degenerative myositis. In addition, vitamin E deficiency with normal or excess dietary PUFA caused steatitis which is the commonly recognized clinical symptom often seen in the overweight cats on inappropriate diets. Similarly, in chickens, a combination of vitamin E-deficiency and high PUFA caused encephalomalacia. Selenium can't prevent this disorder. A combination of vitamin E and selenium deficiency diet, on the other hand, produced exudative diathesis,

which was preventable by either vitamin E or selenium supplementation (Machlin, 1984; Scott, 1980).

Selenium: The vitamin E-related, selenium-responsive deficiency diseases in several domestic animals have been well documented for years. A summary of those conditions is given in Table 1.

A specific selenium deficiency disease was first produced experimentally in chicks by Thompson and Scott (1969) and in rats by McCoy and Wesarch et al., 1973). The most significant abnormalities during selenium deficiency in the chicks were poor growth, poor feathering and pancreatic degeneration followed by mortality. Attempts to diminish the disorders by supplemneting the diet with a high dose of vitamin E failed (Thompson and Scott, 1969). Subsequent investigations by Thompson and Scott (1970) showed that prolonged depletion of selenium in chicks caused significant impairment of fat hydrolysis and a reduction of vitamin E level. They also reported that pancreatic fibrosis occurred before the chicks became deficient in vitamin E. Similarly, rats fed a low selenium ration containing Torula yeast and adequate vitamin E, had a selenium-responsive disease with the characteristic signs: poor growth and coat, sterility and depigmentation of the iris. These signs of deficiency were different from those of the liver necrosis syndrome in rats caused by a deficiency of both vitamin E and selenium (McCoy and Weswig, 1969). Selenium-responsive disease also

Table 1. Vitamin E and/or selenium responsive diseases in animals.

Disease	Tissue/ organ affected	Animals	Responsive to Vit. E	Se	PUFA influence
Abortion	fetus/ placenta	cattle, pig,horse, sheep	x	x	-
Embryonic degeneration	vascular syatem of embryo	pig,rat, mouse	x	?	x
Encephalo-malacia	cerebellum	chicken	x	-	x
Exudative diathesis	vascular system	chicken, turkey	x	x	-
Gastric ulcers	stomach	pig	x	-	-
Hepatic necrosis	liver	rat	x	x	-
Hepatosis dietetica	liver	pig	x	x	-
Impaired spermato-genesis	spermatozoa	rat,pig, mouse	?	x	-
Kidney degeneration	kidney tubules	rat,mouse, monkey	x	-	-
Nutritional muscular dystrophy	skeletal &smooth muscles	all species	x	x	-
Pancreatic fibrosis	pancreas	chicken	-	x	?
Poor hatchability	egg embryo	turkey, chicken	x	x	x
Retained placenta	uterus/ placenta	cattle	?	x	-
Steatitis	adipose tissue	cat,mink, horse	x	?	x
Testicular degeneration	testis	calf,pig, mouse	-	x	-

Modified from Machlin (1984).

occurs naturally. Multiple myocardial necrosis and low selenium status are observed in pigs fed grain and vegetables grown in the areas in which Keshan disease is endemic in China (Zhu and Lu, 1981).

Toxicity

Vitamin E: Vitamin E is considered to be relatively nontoxic in both animals and human beings (Anonymous, 1975; Hillman, 1957). Hypervitaminosis E in chicks can be induced by feeding 2200 IU of vitamin E/kg of diet (March *et al.*, 1973). According to Martin and Hurley (1977) the LD_{50} for all-rac-alpha-tocopherol or all-rac-alpha-tocopheryl acetate is 2000 mg/kg body weight for rats, mice and rabbits.

Selenium: Historically, cases of selenium poisoning of animals had been recognized long before the causative agents were even defined (Franke, 1934; Schwarz and Foltz, 1957). The clinical manifestations of selenium toxicosis in domestic animals were described by Rosenfeld and Beath (1964) who classified the syndromes into 3 different catagories: (1) acute; (2) subacute, blind staggers type; (3) chronic, alkali disease type.

Acute selenosis occurs under condition in which large quantities of selenium accumulator (seleniferous) plants are consumed by animals within a short period of time. Acute selenosis can also be produced experimentally by

giving a lethal dose of selenite or selenate to animals by any route (Morrow, 1968; MacDonald et al , 1980; Carvaggi et al., 1970). It is generally known that acute lethality of selenium compounds is greater when given parenterally than orally. The average oral LD_{50} is about 5.9 mg selenium/kg of body weight, whereas, the average parenteral LD_{50} value is about 2.0 mg selenium/kg of body weight (Ammar and Couri, 1981; Palmer et al., 1973; Ostadalova et al., 1978). The clinical signs of selenium toxicity include anorexia, elevated body temperature and pulse rate , abnormal movement and posture, diarrhea, and "garlicky" breath odor. Labored breathing and death from respiratory failure often follows within a few hours. The incidence of acute selenium toxicity among livestock under field conditions is low, since grazing animals usually avoid seleniferous plants, except in times of pasture shortage (Underwood, 1981; Hopper et al., 1985).

Subacute selenosis or the blind staggers syndrome usually occurs as a result of ingestion of limited amounts of seleniferous plants over a period of weeks to months. The affected animals have ataxia, disorientation, impaired vision and respiratory distress (Rosenfeld and Beath, 1964). This type of selenium toxicity can be produced experimentally by administration of water extracts of selenium accumulator plants. However, it has been reported to be almost impossible to reproduce the syndromes by the administration of pure selenium. Factors other than

selenium are thought to contribute to the development of blind staggers (Maag and Glenn, 1967; Van Kampen and James, 1978).

Alkali disease is the chronic type of selenium toxicity associated with prolonged ingestion of feeds containing 5 to 40 mg selenium/kg. Most common signs are emaciation, rough hair coat, hair loss, hoof malformations and lameness. Death usually results from starvation, since affected animals are unable to graze (Rosenfeld and Beath, 1964).

Toxicity of selenium in animals is influenced by many factors. Among these are the chemical form of selenium, type of diet, duration of intake, species of animals and individual acclimation (Jaffe and Mondragon, 1975; Halverson and Monty, 1960; Palmer and Olson, 1974).

MATERIALS AND METHODS

Animals and diets

Twenty four multiparous sows, consisting of purebred Yorkshires, Duroc/Yorkshire crossbreds and Landrace/ Yorkshire crossbreds, averaging approximately 225 kg in body weight, served as experimental animals. These sows were serviced at the first postweaning estrus, equalized for parity and allotted to 4 groups. In actuality, because the analytical aspects of the planned experiment were labor intensive and could not be conducted on stored samples, 4 sows (2 controls and 2 treated) were assigned to experiment each month (Table 2), creating a split plot design. Assignment to diet was by random numbers.

The experimental diets (Table 3) were corn/soybean meal based in which the corn was previously ensiled, high-moisture corn (for destruction of vitamin E) dried before use to approximately 13% moisture. This diet, without supplemental Se, constituted the -vitamin E-Se treatment; with 0.3 mg Se/kg, constituted the -vitamin E+Se diet; with 60 IU vitamin E/kg and without supplemental Se, constituted the +vitamin E-Se diet; and with both 0.3 mg Se/kg and 60 IU vitamin E/kg, constituted the +vitamin E +Se or control diet. By analyses, the basal diet contained 0.29 IU vitamin

Table 2. Experimental periods

Treatment group	Diet	n/group	Observation period									
			Jun'88	Jul'88	Aug'88	Sep'88	Oct'88	Nov'88	Dec'88	Jan'89	Feb'89	Mch'89
1	-E +Se	2	B	B	B	B	B,C,M					
2	+E +Se	2	B	B	B	B	B,C,M					
1	-E +Se	2		B	B	B	B	B,C,M				
2	+E +Se	2		B	B	B	B	B,C,M				
3	+E -Se	2			B	B	B	B	B,C,M			
4	+E +Se	2			B	B	B	B	B,C,M			
3	+E -Se	2				B	B	B	B	B,C,M		
4	+E +Se	2				B	B	B	B	B,C,M		
5	-E -Se	2					B	B	B	B	B,C,M	
6	+E +Se	2					B	B	B	B	B,C,M	
5	-E -Se	2						B	B	B	B	B,C,M
6	+E +Se	2						B	B	B	B	B,C,M

B = Blood sample
C = Colostrum sample
M = Milk sample

Table 3. Composition of diets

Ingredient	-E -Se	-E +Se	+E -Se	+E +Se
Dried high moisture corn	860.00	858.50	859.88	858.38
Soybean meal (44%)	100.00	100.00	100.00	100.00
Calcium carbonate	12.50	12.50	12.50	12.50
Mono-dicalcium phosphate	17.50	17.50	17.50	17.50
Regular salt	5.00	5.00	5.00	5.00
MSU VTM premix[a]	2.50	2.50	2.50	2.50
Choline chloride (60%)	2.50	2.50	2.50	2.50
Vitamin E 50% Rovimix[b]	0.00	0.00	0.12	0.12
Selenium 90[c]	0.00	1.50	0.00	1.50
	1000.00	1000.00	1000.00	1000.00

a Supplied the following per kg of diet: vitamin A, 3960 IU; vitamin D_3, 792 IU; riboflavin, 3.96 mg; d-pantothenic acid, 15.84 mg; niacin, 21.12 mg; vitamin B_{12}, 23 µg; vitamin K, 2.64 mg; choline chloride, 152.1 mg; zinc, 90 mg; iron, 71.3 mg; manganese, 4.5 mg; copper, 12 mg; iodine, 0.53 mg.

b Premix contained 500,000 IU vit. E/kg (supplemented to supply 60 IU vit. E/kg diet).

c Premix contained 200 mg Se/kg (supplemented to supply 0.3 mg Se/kg diet).

E/kg and 0.089 mg selenium/kg whereas, the supplemented diets contained 55.31 IU vitamin E/kg and/or 0.35 mg selenium/kg.

The bred sows were fed 2.5 kg of diet/day and tap water was available ad libitum. At approximately 7 days before farrowing the sows were moved into a facility equipped with metal farrowing stalls.

Sample collections

Blood samples from the jugular vein of the sows were collected into heparinized-vacutainer[1] tubes for cell isolation and into red top vacutainer[1] tubes for vitamin E, selenium, glutathione peroxidase and cholesterol analyses at the beginning of experiment, at the end of each trimester of pregnancy and at parturition.

Colostrum samples were obtained during parturition and milk samples were obtained on day 4 postpartum by administering 40 USP of oxytocin intramuscularly. Subsequently, the udder was washed with warm water, disinfected with 70% ethanol, dried with paper towelling and hand-milked. Approximately 50 ml of colostrum or milk were collected into sterile polypropylene centrifuge tubes[2] at each sampling.

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[1]Beckton Dickinson, Rutherford, New Jersey.

[2]Corning Glass Works, Corning, New York.

Tubes were capped and immediately placed in an ice-bath and transported to the laboratory.

Blood samples were allowed to clot at room temperature for at least 3 hours and were centrifuged at 400xg for 15 minutes. Serum from each sample was withdrawn with a Pasteur pipette, placed into a 5 ml plastic vial and frozen until assays were conducted.

Isolation of cells

Peripheral blood lymphocytes: Approximately 20 ml of heparinized blood were diluted 1:4 with sterile 0.9% NaCl solution. Ten milliliters of diluted blood were then layered carefully onto 3 ml Ficoll-hypaque (1.3570) density gradient in a 17x100 mm sterile plastic tube with cap[1] and centrifuged at 400xg for 20 min. After centrifugation, the lymphocyte-rich layer was withdrawn from the buffy inter- face with a sterile Pasteur pipette, placed into another sterile 17x100 mm plastic tube, diluted with 10 ml of sterile EDTA phosphate buffered saline (EPS) solution and centrifuged at 275xg for 6 min. The supernatant was then discarded and the cell pellet was examined for red blood cell (RBC) contamination. If RBC were present, the cell pellet was subjected to RBC decontamination (described below) before continuing. After RBC decontamination, the cell pellet was washed with sterile saline two times; the first followed by centrifugation at 225xg for 6 min and the

second followed by centrifugation at 275xg for 6 min. Washed lymphocytes were suspended in 2 ml of Rosewell Park Memorial Institute (RPMI) 1640 culture media[3] which was supplemented with fungizone (6 ul/ml), Hepes (12 ul/ml), $NaHCO_3$ (20 ul/ml), gentamycin sulfate (0.32 ul/ml) and heat-inactivated porcine serum (0.1 ml/ml). A Neubauer hemacytometer was used for counting the lymphocytes under light microscope and viability was assessed by trypan blue exclusion. Dilution with RPMI media was done to obtain a final concentration of 1 to $2x10^6$ viable cells per ml.

RBC decontamination: Cell pellets with evidence of RBC contamination were resuspended in 5 ml sterile, doubly distilled water (dd H_2O) and mixed gently for 15 seconds to lyse the RBC. An equal volume of sterile, double isotonic strength EDTA phosphate-buffered saline (2xEPS) (1.8% NaCl, 0.7% EDTA-Na_3, 0.2% KH_2PO_4, ph 7.4) was added to the cell suspension to prevent lymphocyte lysis. The mixture was then centrifuged at 150xg for 8 min.

Polymorphonuclear (PMN) cells: After the lymphocyte layer had been removed for lymphocyte isolation, the plasma layer and ficoll layer were withdrawn with a Pasteur pipette. The RBC-granulocyte pellet was then suspended in

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[3]Gibco Laboratories, Grand Island, New York.

5 ml of dd H_2O and mixed vigorously for 5 seconds to lyse RBC. Five milliliters of 2xEPS were immediately added, mixed well and centrifuged at 150xg for 8 min. After centrifugation, the supernatant was carefully removed and discarded. The RBC lysis steps were repeated until the cell pellet was clean and slightly greenish in color. The granulocytes were counted on a Neubauer hemacytometer and the cell count was adjusted to $1x10^6$ cells/ml in Hanks' balanced salt solution[3] (HBSS) without phenol red.

Colostrum and milk cell isolation: The 50 ml colostrum and milk samples were diluted 1:5 and 1:2, respectively with cold, sterile, phosphate-buffered saline (PBS). The diluted colostrum or milk was then defatted by centrifugation at 400 xg for 20 min. The supernatant with the fat layer was discarded and the cell pellet was washed twice in cold PBS by centrifugation at 215xg for 15 min.

For differential cell counts, the washed cells were suspended in 1 ml PBS containing 50% inactivated bovine serum and smeared on 3x1 inch glass slides for staining and counting.

For lymphocyte and granulocyte isolation, the washed cells were reconstituted with EPS. Ten milliliters of the cell suspension were then layered onto 3 ml 1.3570 specific gravity Ficoll-hypaque and centrifuged at 400xg for 40 min. After centrifugation, the lymphocyte layer was isolated from the buffy interface and the granulocytes were isolated

from the cell pellet lying at the bottom of the tubes. The number of lymphocytes and granulocytes was adjusted to $1x10^6$ cells per ml in media.

Lymphocyte blastogenesis assay

One hundred microliters of cell suspension containing 1 to $2x10^5$ cells isolated from colostrum, milk or peripheral blood were added to each of 12 wells in a 96 well, round-bottom, tissue culture plate[4]. The first three wells were unstimulated cells. To each of the three remaining wells, 10 ul of the appropriate mitogen were added: Phytohemaglutinin (50 µg/ml), Concanavalin A[5] (50 µg/ml) and Pokeweed Mitogen[5] (10 µg/ml). A dose response curve was previously established to determine the right concentration of mitogen which provided optimal stimulation of swine lymphocytes in this experiment. The plates were then covered with mylar sealers[6] and incubated for 72 hours at 37°C in a 5% CO_2 incubator. After 72 hours, 1 uCi ^{3}H-thymidine[7] in 20 ul of sterile saline was added to each well and the plates were then incubated for an

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[4]Flow Laboratories, Inc., McLean, Virginia.

[5]Sigma Chemical Co., St. Louis, Missouri.

[6]Dynatech Laboratories Inc., Chantilly, Virginia.

[7]DuPont, Boston, Massachusetts.

additional 18 to 24 hours. After incubation, the cells were harvested, by using a semiautomatic harvester[8]. This process included aspirating the lymphocytes onto filter paper and washing with distilled water and 5% trichloro acetic acid (TCA). Each individual filter disc was then punched out into a scintillation vial by using forceps. One hundred milliliters of Soluene-350[9] were then pipetted into each vial and each vial allowed to stand at room temperature for 30 min. Following this step, 5 ml of scintillation fluid were added to each vial, and each vial was capped and labelled properly. Radioactivity was counted in a Beta Trac 6895[10] counter. Data were expressed as mean counts per minute.

Yeast phagocytic assay

The phagocytic and microbicidal activities of the PMN cells were quantified by using the method described by Simpson et al. (1979).

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[8]Titertek Cell Harvester 550, Flow Laboratories Ltd., Irvine, Scotland.

[9]Packard Instrument Co., Downer's Grove, Illinois.

[10]TM Analytic Inc., Elk Grove Village, Illinois.

Preparation of yeast solution: Dried baker's yeast[11] was used in this study. Two grams of yeast dissolved in 100 ml of saline created a stock solution of approximately 5-7 x 10^8 yeast particles/ml. An evenly suspended solution was obtained by stirring for at least 30 min on a magnetic mixer. The yeast cell-concentration was then adjusted to 1x10^6 per ml.

Test procedure: Equal volumes (200 µl) of yeast suspension (1x10^6 cells/ml), PMN cell suspension (1x10^6 cells/ml), autologous blood plasma and HBSS were pipetted into a sterile tube[1] with cap. The mixture was then incubated at 37°C on a shaking water bath for 60 min. After the incubation, 1 ml of 0.01% methylene blue was added into the mixture which was then centrifuged at 400xg for 10 min. After centrifugation, the supernatant was discarded and the cell pellet was resuspended in 20 µl of HBSS and counted using the Neubauer hemacytometer.

To quantify the phagocytic activity, the number of PMN cells containing 2 or more yeast particles per 100 cells were counted. The killing ability of PMN cells was determined by counting the number of PMN cells containing 2 or more dead yeast particles per 100 PMN cells.

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[11]Fleischmann's Yeast Inc., Oakland, California.

Vitamin E analyses

Serum and feeds were assayed for vitamin E by the high pressure liquid chromatography (HPLC) methods modified from that of Bieri *et al*. (1979) and used in the Clinical Nutrition section of the Animal Health Diagnostic Laboratory.

Serum: One ml of each serum sample was pipetted into a labelled 16x100 mm disposable culture tube. An equal volume of absolute ethanol was added and the mixture was vortexed for 5 seconds on a maximum power to denature the protein in the serum. Two milliliters of ultra violet (U.V) grade hexane were repipetted to the mixture, vortexed for 2 min and then centrifuged at 550xg for 10 min. After centrifugation, the hexane layer was withdrawn with a disposable Pasteur pipet and passed through a 0.45 micron cellulose filter[12] in a Swinney-type filter holder. One hundred microliters of the hexane extract were injected into the chromatographic system automatically[13]. Separation was isocratic in a Waters microporasil[13] column (3.9x15 cm long) with a 85:15 mixture of degassed hexane: chloroform. The mixture was pumped through the HPLC unit at

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[12]Millipore Corp., Bedford, Massachusetts.

[13]Waters Intelligent Sample Processor (WISP), Waters Associated Inc., Milford, Massachusetts.

1.1 ml/min at an intended pressure of 1500 psi and detection was with U.V. spectrometry[14] at wavelength of 280 nm. In this system the peaks of alpha-tocopheryl acetate and alpha-tocopherol occurred at 2.45 and 4.59 min, respectively, after sample injection.

Feed: Approximately 500 mg of milled feed were transferred into a labelled 16x125 mm disposable culture tube. Two milliliters of milliporre water were added to each tube and allowed to stand in the dark for at least 1 hour. Two milliliters of absolute ethanol were repipetted and vortexed for 10 min on a maximum power. Two milliliters of U.V. grade hexane were then added to the mixture, vortexed for 10 min and centrifuged at 550xg for 10 min. After centrifugation, the hexane layer was removed and the subsequent steps were as described above for serum samples.

The final concentration of vitamin E in feed was quantified on a dry weight basis. To determine dry weight of feed, approximately 2 g of milled feed were placed in an aluminium pan and dried in an oven at 56°C. After 24 hours the dried feed was weighed and the wet weight to dry weight ratio was calculated.

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[14]Waters Associates Model 440 Absorbance Detector, Waters Associated Inc., Milford, Massachusetts.

Cholesterol analysis

Serum cholesterol was determined spectrophotometrically by using a commercially available kit from Sigma[5]. Ten microliters of standards and serum samples were pipetted into labelled 12x75 mm disposable borosilicate tubes. Cholesterol standards used in this analysis had concentrations of 0, 0.5, 1.0 and 2.0 mg/ml. One milliliter of cholesterol reagent was then added to each standard and sample and the resulting mixtures were vortexed and then incubated in a water bath at 37°C for 10 min. Absorbance of the standards and samples was read in a spectrophotometer[15] set at a wavelength of 500 nm. The cholesterol values were calculated using a curvilinear standard regression line.

Selenium analyses

Serum: Selenium concentration in serum was assayed by using a modification of the phosphoric/nitric acid digestion process (Reamer and Veillon, 1983) followed by fluorometric detection procedure described by Whetter and Ullrey (1978).

One milliliter of serum sample was digested in 4 ml HNO_3 and 3 ml H_3PO_4 in 50 ml Erlenmeyer flasks on a

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[15]Model 920, Gilford Instruments, Oberlin, Ohio.

hot plate (230°C) until brown fumes disappeared. The samples was cooled down to approximately 150°C and 3 ml of 30% hydrogen peroxide (H_2O_2) were added to each flask and samples were reheated again at 230°C until bubbles were fine and flask content reached the critical weight (5.2-6.5 g). The samples were cooled down and the remaining HNO_3 was removed by adding 6 ml of 50% hydrochloric acid (HCl) and 2 ml of formic acid. The mixture was reheated for 10 min and then cooled down by turning the hot plate to low.

Five milliliters of masking agent, prepared by dissolving 10 g of EDTA and 25 g hydroxylamine HCl in 1 liter of distilled water, were added and the pH of the mixture was adjusted to between 2.3-2.8 as indicated by a golden yellow amber color with cresol red.

Five milliliters of 2,3-diaminonaphthaline (DAN) were then added to each sample to form diazoselenol, a light sensitive complex. This complex was then extracted with 5 ml of cyclohexane on a rotary shaker[16] for 5 minutes. The cyclohexane extract was floated to the neck of the Erlenmeyer flask with H_2O and then transferred into a cuvette. The fluorescence was measured with a spectro-fluorometer[17] at excitation and emission wavelengths of

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[16]Lab-line Instruments, Inc., Melrose Park, Illinois.

[17]Model LS-3B, Perkin-Elmer, Norwalk, Connecticut.

376 and 510 nm, respectively. Selenium concentration was calculated by using a curvilinear regression.

Feed: Feed selenium concentration was determined by using a modification of the microwave digestion technique (Kingston and Jassie, 1986) followed by the fluorometric detection procedure described by Whetter and Ullrey (1978).

Five hundred milligrams of milled feed were put in a 200 ml teflon digestion vessel and 5 ml HNO_3 and 3 ml H_3PO_4 were added. A relief valve and cap were put on each vessel and a venting tube was put into each cap. Twelve vessels were torqued closed and positioned in the microwave carousel (turntable). The samples were then digested simultaneously in a microwave oven[18] using 100% power for 1 min followed by 35% power for 13 min. The power was then reduced to 0% for 5 min and the vessels were vented. The second phase of digestion was done at 100% power for 1 min and 45% power for 13 min. The power was reduced to 0% for 5 min and the vessels were again vented. The last phase of digestion was done on 100% power for 6 min and 0% power for 5 min. The microwave was turned off, vessels were vented and the samples were cooled down for 60 min. The cooled samples were transferred into a 50 ml Erlenmeyer flask and 6 ml of 50% HCl and 2 ml of formic

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[18]CEM Corporation, Indian Trail, North Carolina.

acid were added. The subsequent steps were done in the same manner as described for serum samples.

Glutathione peroxidase activity

Serum glutathione peroxidase activity was determined by the coupled assay described by Paglia and Valentine (1967) and Lawrence et al. (1974).

Forty microliters of serum were pipetted into a cuvette to which was added 50 µl of reduced glutathione[5] (GSH) and 0.9 ml of reagent mixture containing 100 mM potassium phosphate buffer (pH 7), 3 mM EDTA, 1 mM NaN_3 (sodium azide), 0.1 mM nicotinamide adenine dinucleotide phosphate (NADPH)[5] and 1 enzyme unit (EU) of glutathione reductase[5]. Ten microliters of 30% H_2O_2 were then added for initiating the reaction. Rate of conversion of NADPH to NADH over time was monitored at 340 nm in a spectrophotometer[15] equipped with an automatic cuvette changer for enzyme kinetics and recorded on a Varian chart recorder[19].

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[19]Varian model 9176, Gilford Instruments Laboratories Inc., Honeywell Ft.Washington, Philladelphia.

Statistical analyses

The data were analyzed by split-plot analysis of variance with the main effect of dietary vitamin E and/or selenium and time over treatment. The linear model is as follows:

$$Y_{ijk} = M + T_i + A_{(i)j} + P_k + (TP)_{ik} + E_{ijk},$$

$(i = \cdots t,\ j = 1 \cdots n \text{ per } i,\ k = 1 \cdots p)$

where

Y - the individual variable

M - the overall (grand) mean

T - effect of treatment

A - effect of animal within treatment

P - effect of period

TP - interaction between treatment and period

E - residual error

Source of variation		Degree of freedom
T -	-E+Se vs +E+Se (Exp. 1) +E-Se vs +E+Se (Exp. 2) -E-Se vs +E+Se (Exp. 3)	(t-1)= (2-1)= 1
E(1)=	animals/T (A_{ijk})	t(n-1)= 2(4-1)= 6
P -	observation period	(p-1)= (5-1)= 4
TP -	dietary treatment x period	(t-1)(p-1)= (2-1)(5-1)= 4
E(2)=	residual error	t(n-1)(p-1)= 2(2-1)(5-1)= 24

Significant difference between treatments (within period) was determined by using Student T test. Pearson correlation coefficients were used to determine the correlation between serum selenium concentration and glutathione peroxidase activity. In this study a difference was considered significant at the level of $P<0.05$ (Gill, 1978). All statistical analyses were performed by an IBM 4381 computer using the SAS[20] program.

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[20]Statistical Analytical System, Cary, North Carolina.

RESULTS

Serum vitamin E

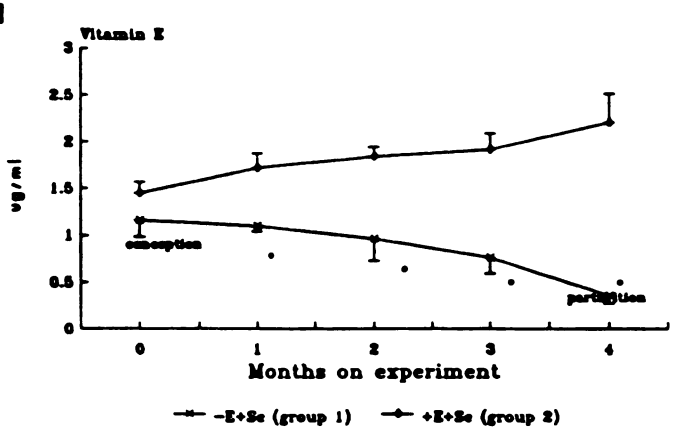
The effects of vitamin E and/or selenium depletion on sow serum vitamin E are presented in figures 3a, b and c and appendix table 10. Starting at 30 days of experiment and continuing through parturition, sows fed the vitamin E-deficient diet (group 1) and sows fed the diet deficient in both vitamin E and selenium (group 5) had significantly lower ($p<0.05$) serum vitamin E concentrations than sows fed the control diet. Serum vitamin E concentrations of the control sows increased gradually during gestation.

Serum selenium

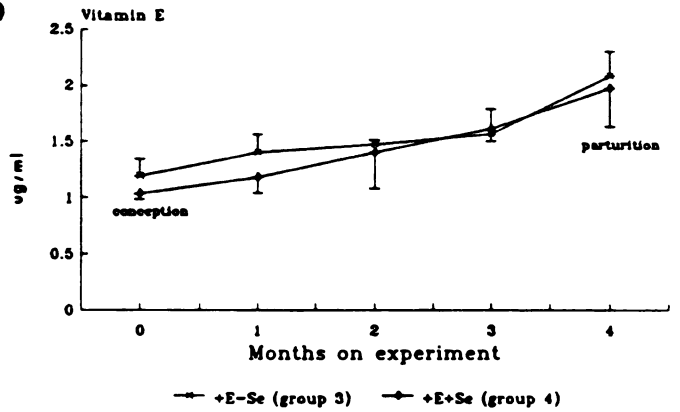
The effects of vitamin E and/or selenium depletion on sow serum selenium are presented in figures 4a, b and c and appendix table 11. Within 30 days of initiating the experimental diets, serum selenium concentrations in sows fed the +E-Se diet (group 3) and the -E-Se diet (group 5) were significantly lower ($p<0.05$) than serum selenium concentrations in sows fed the control diet. These differences continued for the remainder of the experiment. With the exception of control group 6, serum selenium concentrations of the control sows increased gradually during gestation.

Figure 3. Serum vitamin E concentrations in sows fed: (a) -E+Se (-x-) and +E+Se (◇) diets; (b) +E-Se (-x-) and +E+Se (◇) diets; (c) -E-Se (-x-) and +E+Se (◇) diets from conception to parturition. Each point represents a mean ± SD. *Significantly different from +E+Se ($p<0.05$).

a


Vitamin E
ug/ml
3
2.5
2
1.5
1
0.5
0
conception
parturition
0
1
2
3
4
Months on experiment
-E+Se (group 1)
+E+Se (group 2)

b


Vitamin E
ug/ml
2.5
2
1.5
1
0.5
0
conception
parturition
0
1
2
3
4
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

c

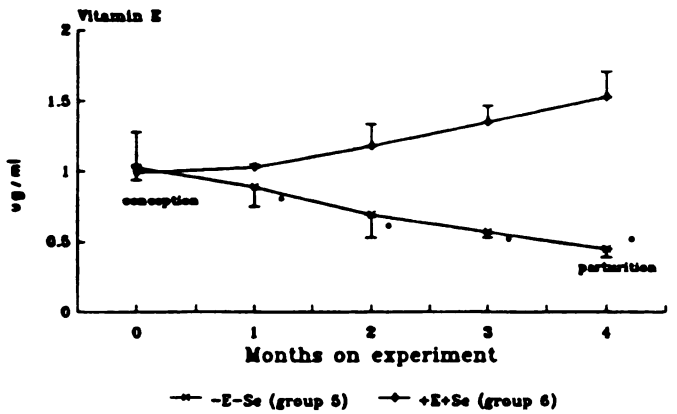
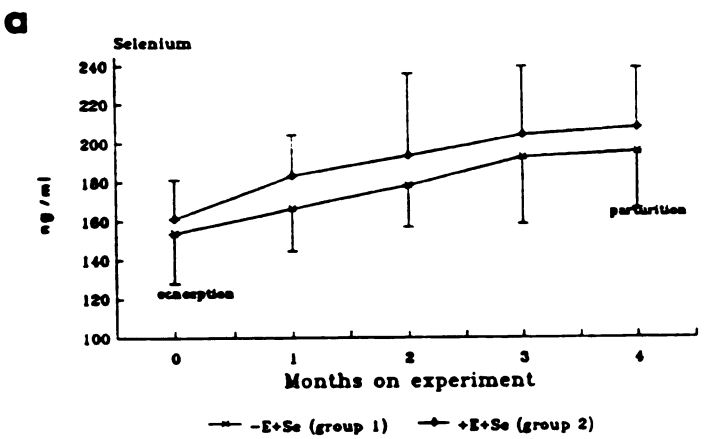
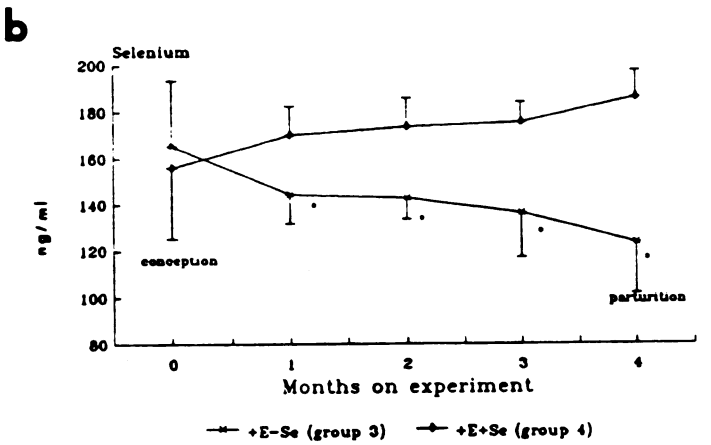

Vitamin E
ug/ml
2
1.5
1
0.5
0
conception
parturition
0
1
2
3
4
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

Fig.3

Figure 4. Serum selenium concentrations in sows fed: (a) -E+Se (-x-) and +E+Se (◇) diets; (b) +E-Se (-x-) and +E+Se (◇) diets; (c) -E-Se (-x-) and +E+Se (◇) diets from conception to parturition. Each point represents a mean ± SD. *Significantly different from +E+Se ($p<0.05$).


a
Selenium
240
220
200
180
160
140
120
100
ng/ml
conception
parturition
0
1
2
3
4
Months on experiment
-E+Se (group 1)
+E+Se (group 2)


b
Selenium
200
180
160
140
120
100
80
ng/ml
conception
parturition
0
1
2
3
4
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

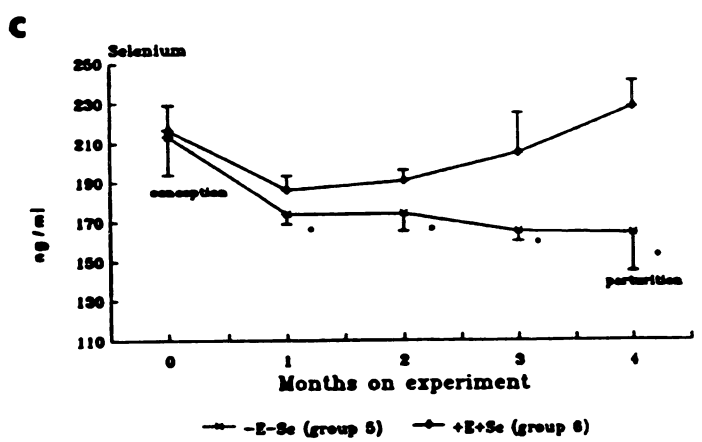

c
Selenium
250
230
210
190
170
150
130
110
ng/ml
conception
parturition
0
1
2
3
4
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

Fig.4

Serum glutathione peroxidase

The effects of vitamin E and/or selenium depletion on sow serum GSH-Px are presented in and figures 5a, b and c and appendix table 12. By 60 days on experimental diet, and continuing through parturition, serum GSH-Px concentrations of selenium-deficient sows (group 3) were significantly lower ($p<0.05$) than the selenium-adequate sows (group 4). Serum GSH-Px in sows fed diets deficient in both vitamin E and selenium (group 5) was significantly lower ($p<0.05$) than in sows fed the vitamin E and selenium-adequate diet (group 6). These differences were observed from 30 days on trial through parturition.

The mean serum GSH-Px concentrations were positively correlated with serum selenium concentrations in sows fed the vitamin E-deficient diet ($r=0.62$; $p<0.05$); in sows fed the selenium-deficient diet ($r=0.68$; $p<0.05$);and in sows fed the vitamin E and selenium-deficient diet ($r=0.83$; $p<0.05$).

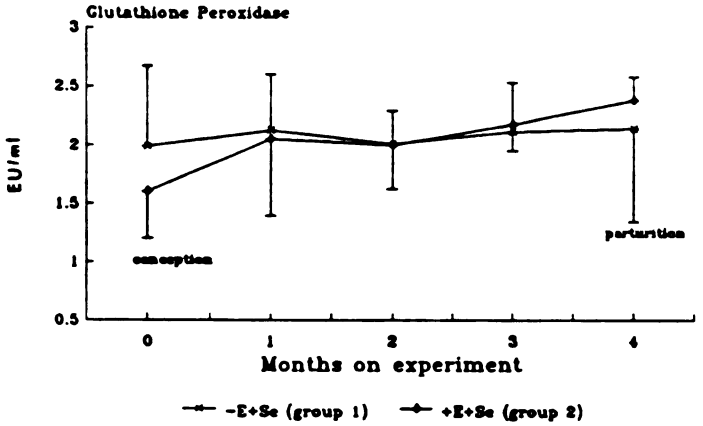
Serum cholesterol

The effects of vitamin E and/or selenium depletion on sow serum cholesterol are presented in figures 6a, b and c and appendix table 13.

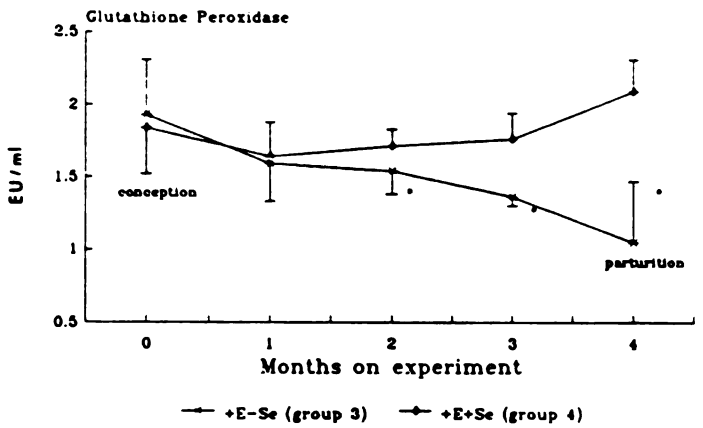
Serum cholesterol values for sows in this study were quite variable. No significant difference ($p>0.05$) was observed between the mean serum cholesterol concentrations of any groups of the sows or at any of the sampling periods during feeding.

Figure 5. Serum glutathione peroxidase activity in sows fed: (a) -E+Se (-x-) and +E+Se (◇) diets; (b) +E-Se (-x-) and +E+Se (◇) diets; (c) -E-Se (-x-) and +E+Se (◇) diets from conception to parturition. Each point represents a mean ± SD. Significantly different from +E+Se ($p<0.05$). EU= Enzyme Unit.

a


Glutathione Peroxidase
EU/ml
3
2.5
2
1.5
1
0.5
conception
parturition
0
1
2
3
4
Months on experiment
-E+Se (group 1)
+E+Se (group 2)

b


Glutathione Peroxidase
EU/ml
2.5
2
1.5
1
0.5
conception
parturition
0
1
2
3
4
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

c

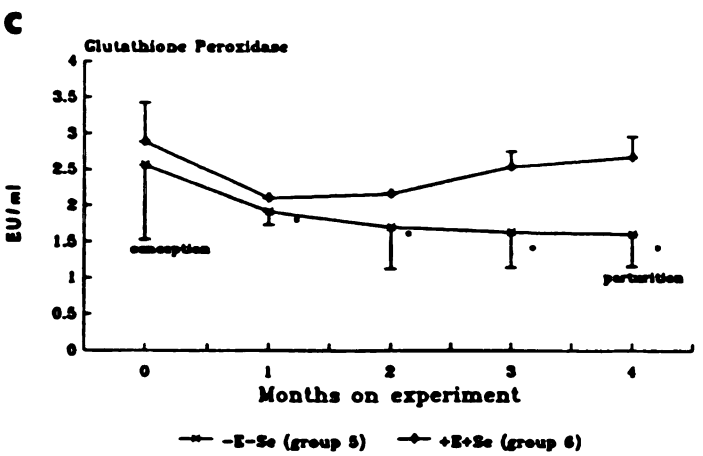
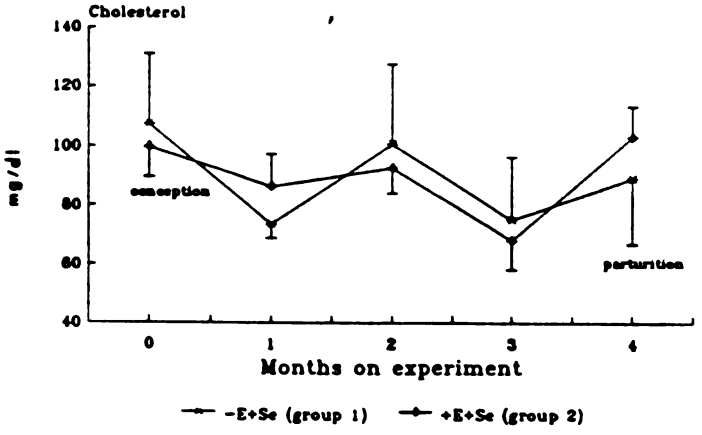

Glutathione Peroxidase
EU/ml
4
3.5
3
2.5
2
1.5
1
0.5
0
conception
parturition
0
1
2
3
4
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

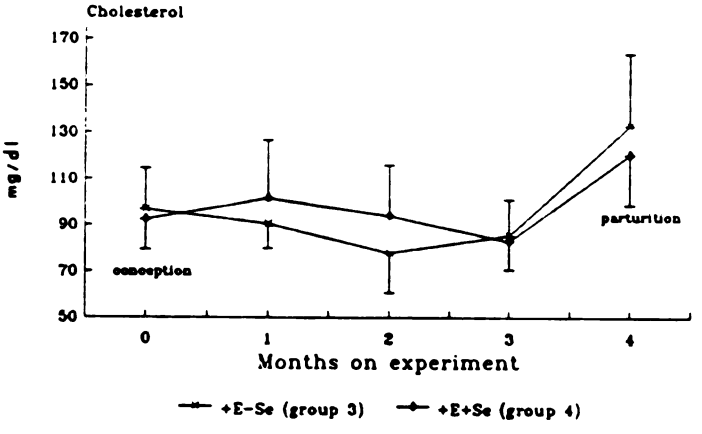
Fig.5

Figure 6. Serum cholesterol concentrations in sows fed: (a) -E+Se (-x-) and +E+Se (◇) diets; (b) +E-Se (-x-) and +E+Se (◇) diets; (c) -E-Se (-x-) and +E+Se (◇) diets from conception to parturition. Each point represents a mean ± SD.

a


Cholesterol
140
120
100
80
60
40
mg/dl
conception
parturition
0
1
2
3
4
Months on experiment
-E+Se (group 1)
+E+Se (group 2)

b


Cholesterol
170
150
130
110
90
70
50
mg/dl
conception
parturition
0
1
2
3
4
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

c

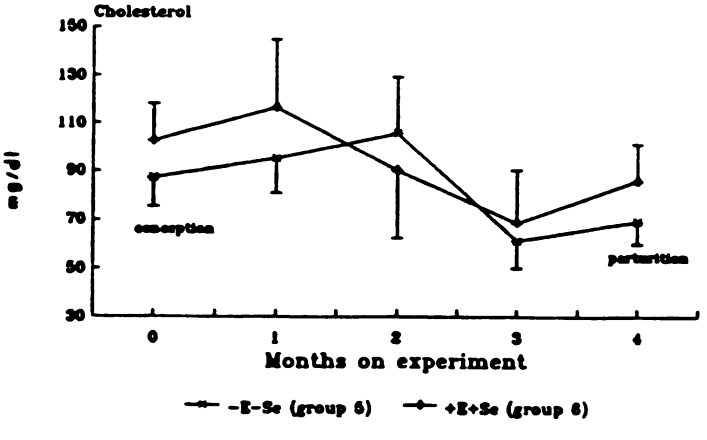

Cholesterol
150
130
110
90
70
50
30
mg/dl
conception
parturition
0
1
2
3
4
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

Fig. 6

Immunoresponsiveness of peripheral blood components

Lymphocyte Stimulation

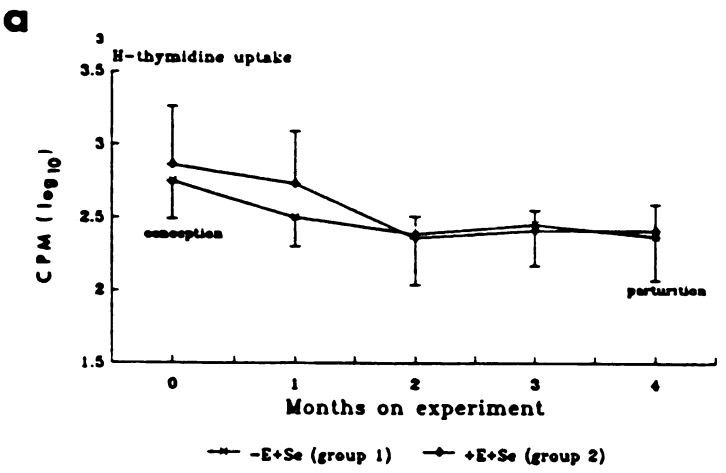
The effects of vitamin E and/or selenium depletion on ^{3}H-thymidine uptake of unstimulated lymphocyte are presented in figures 7a, b and c and appendix table 14. No significant differences in ^{3}H-thymidine uptake of unstimulated lymphocyte were observed between treatments.

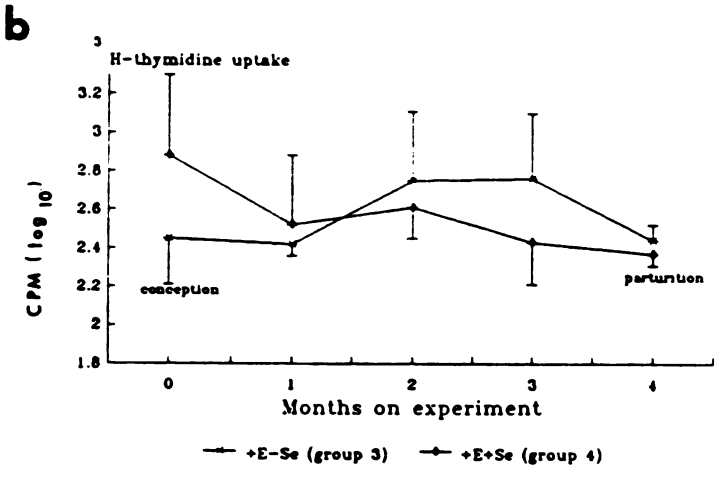
PHA mitogen: The effects of vitamin E and/or selenium depletion diet for sows on the PHA stimulation of their PBL are presented in figures 8a, b and c and appendix table 15.

At 90 days on experimental diets and at parturition, the response of the PBL to PHA stimulation was significantly lower ($p<0.05$) for sows fed the vitamin E-deficient diet (group 1) than for sows fed the control diet (group 2). A significant reduction in the response of PBL to PHA was also observed for sows fed the -E-Se diet (group 5) compared to the response of PBL to PHA stimulation in sows fed the +E+Se diet (group 6), as early as 60 days on experiment.

PW mitogen: The effects of vitamin E and/or selenium depletion diet for sows on the PWM stimulation of their PBL are presented in figures 9a, b and c and appendix table 16. Pokeweed mitogen stimulation of PBL from sows fed the -E+Se diet was significantly lower ($p<0.05$) compared to PBL from sows fed the +E+Se diet, at 90 days on experimental diet and at parturition. The PWM stimulation of PBL from sows

Figure 7. ^{3}H uptakes of unstimulated peripheral blood lymphocytes from sows fed: (a) -E+Se (-x-) and +E+Se (◇) diets; (b) +E-Se (-x-) and +E+Se (◇) diets; (c) -E-Se (-x-) and +E+Se (◇) diets from conception to parturition. Each point represents a mean ± SD.


a
H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
-E+Se (group 1)
+E+Se (group 2)


b
H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

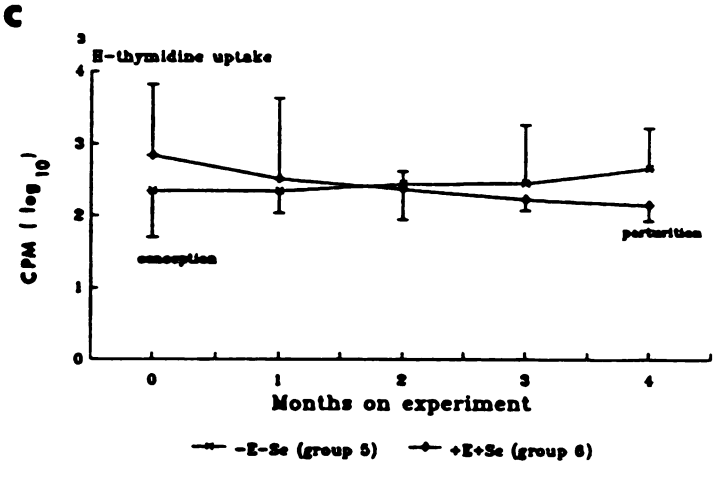
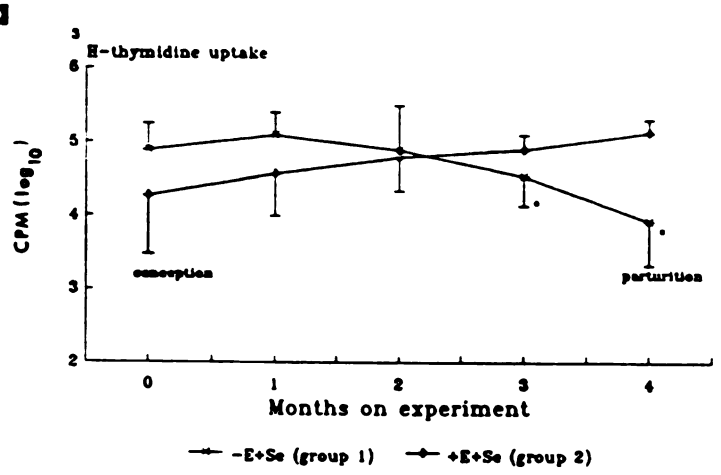

c
H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

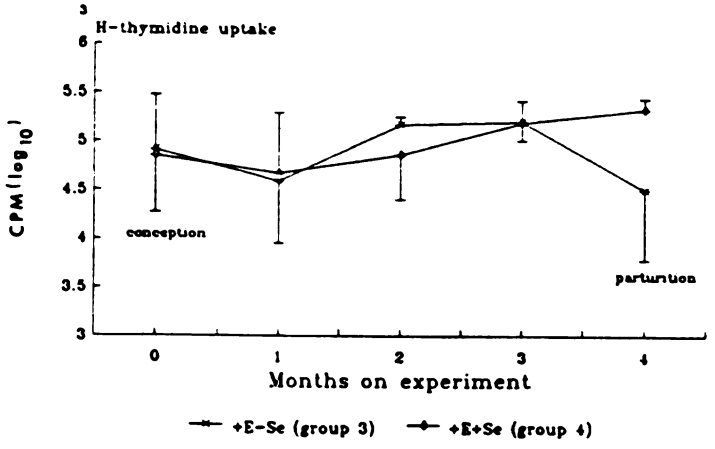
Fig.7

Figure 8. PHA stimulatory responses of peripheral blood lymphocytes from sows fed: (a) -E+Se (-x-) and +E+Se (-◇-) diets; (b) +E-Se (-x-) and +E+Se (-◇-) diets; (c) -E-Se (-x-) and +E+Se (-◇-) diets from conception to parturition. Each point represents a mean ± SD. *Significantly different from +E+Se ($p<0.05$).

a


H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
-E+Se (group 1)
+E+Se (group 2)

b


H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

c

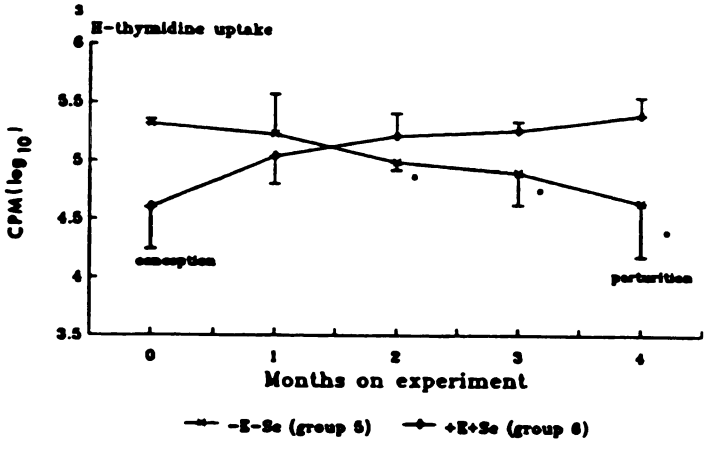
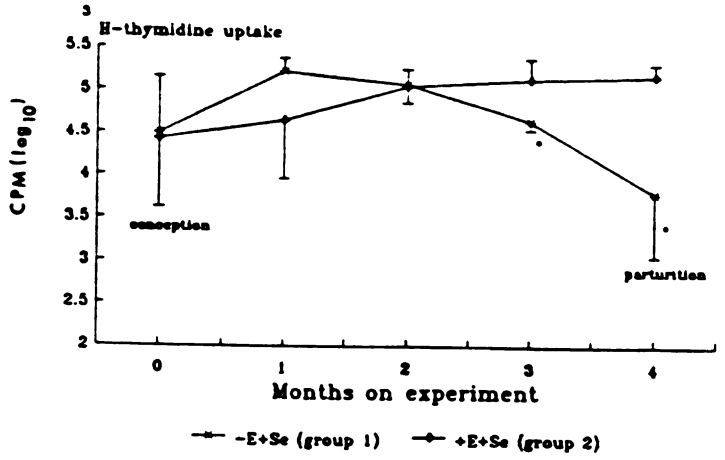

H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

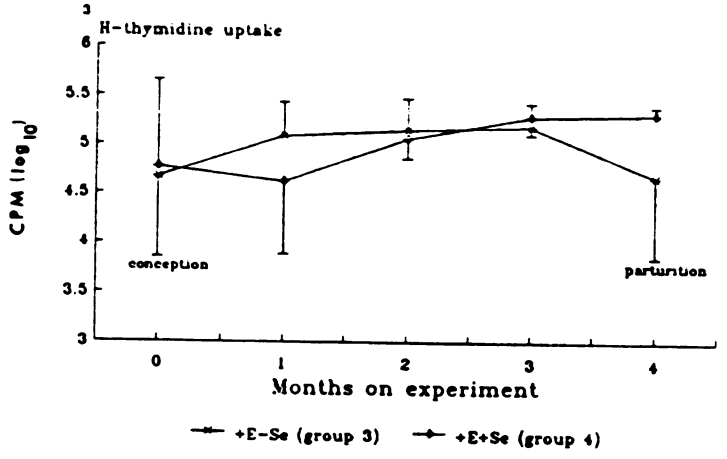
Fig.8

Figure 9. PWM stimulatory responses of peripheral blood lymphocytes from sows fed: (a) -E+Se (-x-) and +E+Se (◇) diets; (b) +E-Se (-x-) and +E+Se (◇) diets; (c) -E-Se (-x-) and +E+Se (◇) diets from conception to parturition. Each point represents a mean ± SD. *Significantly different from +E+Se ($p<0.05$).

a


H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
-E+Se (group 1)
+E+Se (group 2)

b


H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

c

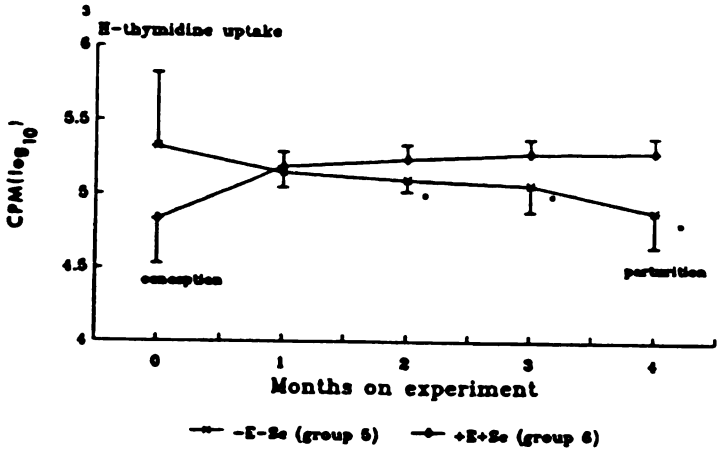

H-thymidine uptake
CPM (log10)
conception
parturition
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

Fig.9

fed the -E-Se diet, was significantly lower ($p<0.05$) than for PBL from sows fed the +E+Se diet (group 6), at 60 and 90 days of gestation and parturition.

Concanavalin A mitogen: Dietary treatments caused no significant difference in the responses of PBL to Con A (Figures 10a, b and c and Appendix Table 17).

Phagocytic activity by polymorphonuclear cells

The phagocytic activities of polymorphonuclear cells of peripheral blood are summarized in appendix table 18. The ability of PMN cells from -E+Se (group 1) and +E-Se (group 3) sows to phagocytize yeast cells was significantly decreased ($p<0.05$) at 90 days on experiment and at parturition (Figures 11a, b). The phagocytic ability of PMN from sows fed the -E-Se diet, however, was significantly decreased ($p<0.05$) by 60 days on experiment (Figure 11c).

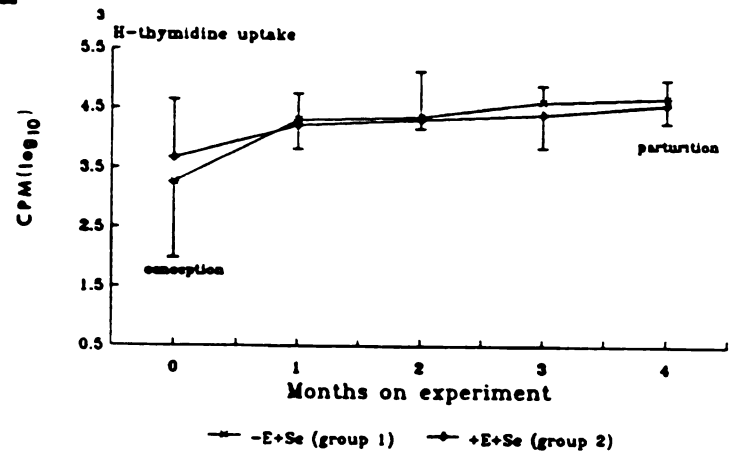
Microbicidal activity by polymorphonuclear cells

The effects of vitamin E and/or selenium depletion of sows on the microbicidal activities of peripheral blood polymorphonuclear cells are presented in figures 12a, b and c and appendix table 19.

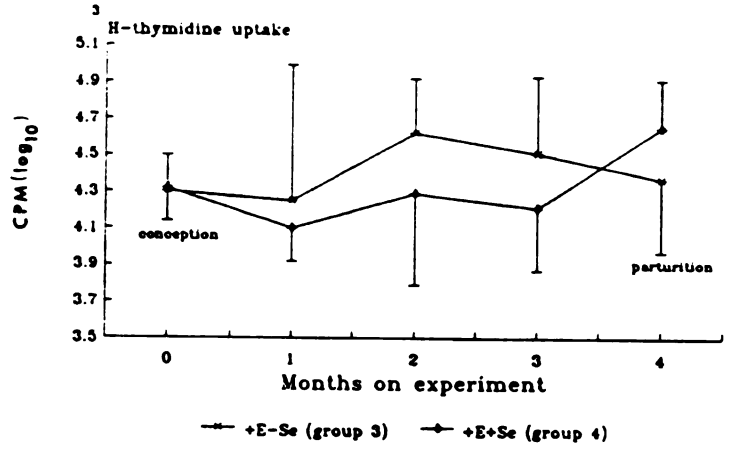
At 90 days and at parturition, vitamin E deficiency (group 1), selenium deficiency (group 3) and the combined vitamin E and selenium deficiency (group 5) resulted in decreased ability of polymorphonuclear cells to kill the

Figure 10. Con A stimulatory responses of peripheral blood lymphocytes from sows fed: (a) -E+Se (-x-) and +E+Se (◇) diets; (b) +E-Se (-x-) and +E+Se (◇) diets; (c) -E-Se (-x-) and +E+Se (◇) diets from conception and parturition. Each point represents a mean ± SD.

a


3
H-thymidine uptake
CPM(log10)
5.5
4.5
3.5
2.5
1.5
0.5
conception
parturition
0
1
2
3
4
Months on experiment
-E+Se (group 1)
+E+Se (group 2)

b


3
H-thymidine uptake
CPM(log10)
5.1
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
conception
parturition
0
1
2
3
4
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

c

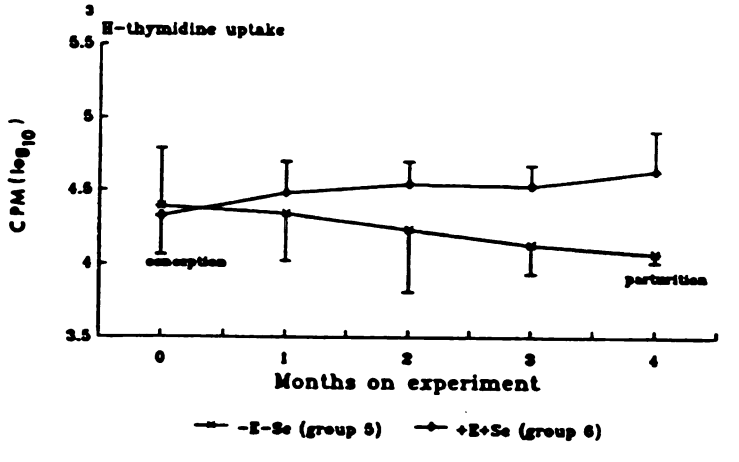
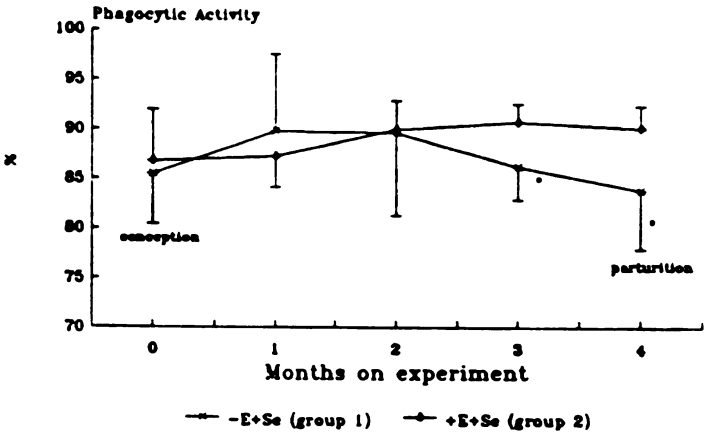

3
H-thymidine uptake
CPM(log10)
5.5
5
4.5
4
3.5
conception
parturition
0
1
2
3
4
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

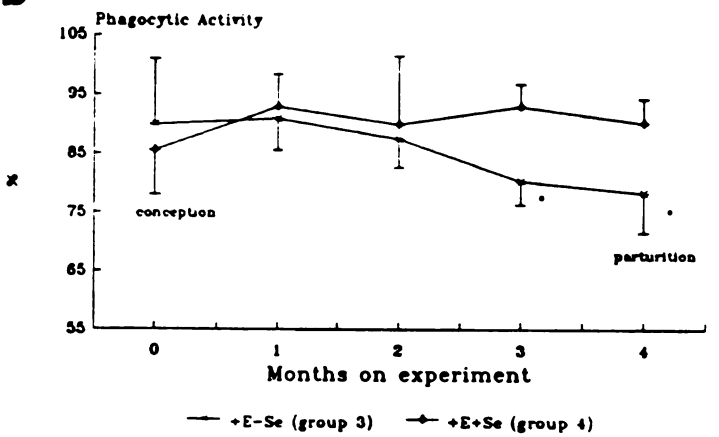
Fig. 10

Figure 11. Phagocytic activity of blood polymorphonuclear cells from sows fed: (a) -E+Se (-x-) and +E+Se (◇); (b) +E-Se (-x-) and +E+Se (◇); (c) -E-Se (-x-) and +E+Se (◇) diets from conception to parturition. Each point represents a mean ± SD. *Significantly different from +E+Se ($p<0.05$).

a


Phagocytic Activity
100
95
90
85
80
75
70
%
conception
parturition
0
1
2
3
4
Months on experiment
-E+Se (group 1)
+E+Se (group 2)

b


Phagocytic Activity
105
95
85
75
65
55
%
conception
parturition
0
1
2
3
4
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

c

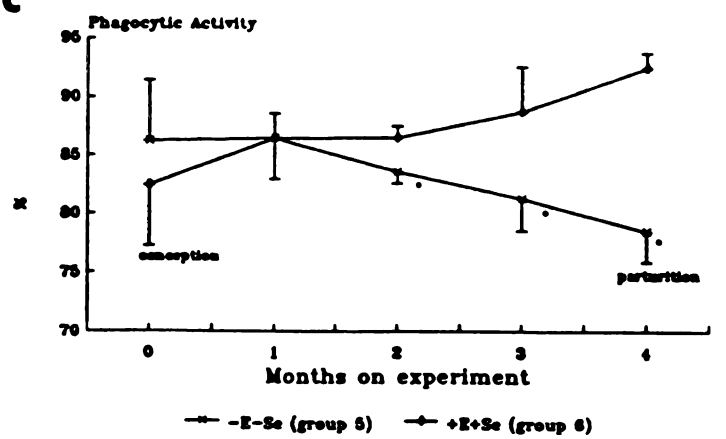
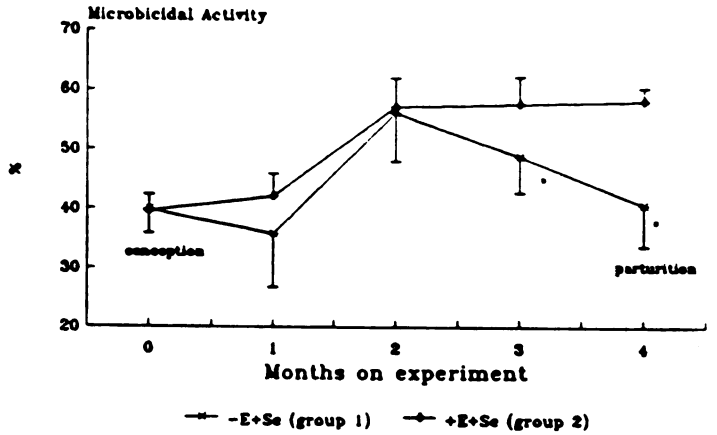

Phagocytic Activity
95
90
85
80
75
70
%
conception
parturition
0
1
2
3
4
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

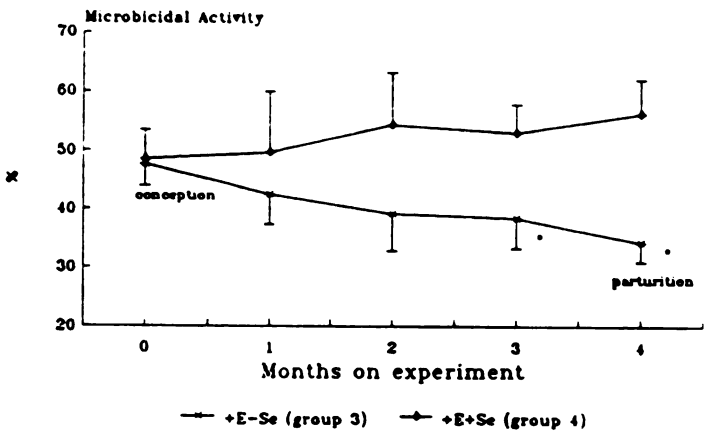
Fig.11

Figure 12. Microbicidal activity of blood polymorphonuclear cells from sows fed: (a) -E+Se (-x-) and +E+Se (◇); (b) +E-Se (-x-) and +E+Se (◇); (c) -E-Se (-x-) and +E+Se (◇) diets from conception to parturition. Each point represents a mean ± SD. *Significantly different from +E+Se ($p<0.05$).

a


Microbicidal Activity
70
60
50
40
30
20
%
conception
parturition
0
1
2
3
4
Months on experiment
-E+Se (group 1)
+E+Se (group 2)

b


Microbicidal Activity
70
60
50
40
30
20
%
conception
parturition
0
1
2
3
4
Months on experiment
+E-Se (group 3)
+E+Se (group 4)

c

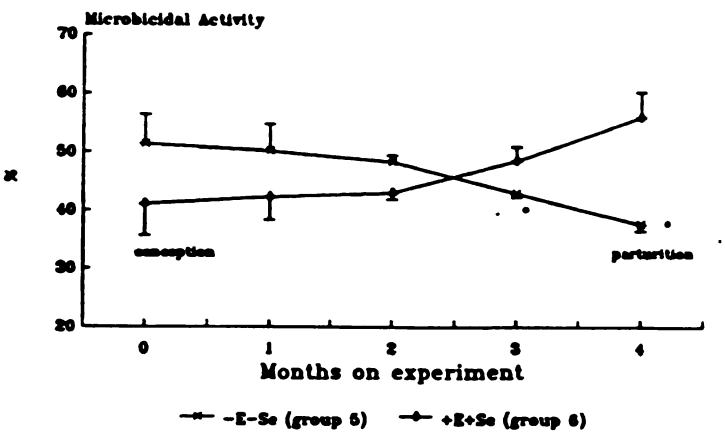

Microbicidal Activity
70
60
50
40
30
20
%
conception
parturition
0
1
2
3
4
Months on experiment
-E-Se (group 5)
+E+Se (group 6)

Fig.12

engulfed yeast cells.

Differential cell counts

The differential cell counts for sow colostrum and for sow milk are presented in table 4 and table 5, respectively.

There was no significant treatment effect on the differential cell counts for the mammary secretions (colostrum and milk) of sows. The polymorphonuclear cell was the predominant cell type (68 %) in colostrum. Polymorphonuclear cells remained prominent (44 %) in milk even though their percentage was lower than in colostrum. Epithelial cells were higher in sow milk than in sow colostrum.

Immunoresponsiveness of colostral components

Lymphocyte stimulation

The effects of vitamin E and/or selenium depletion of sows on the response of their colostral lymphocytes to mitogens are presented in table 6.

Dietary treatments caused significantly lower ($p<0.05$) responses of colostral lymphocytes to PHA stimulation in sows fed the -E+Se diet (group 1) and sows fed the -E-Se diet (group 5) compared to sows fed the control diets, groups 2 and 6.

The pokeweed mitogenic response of colostral lymphocytes from sows fed the -E-Se diet was also significantly

Table 4. Differential cell counts of colostrum from sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Cell Types				
			Neutrophils	Macrophages	Lymphocytes	Eosinophils	Epith. cells
			%				
1	-E +Se	4	64.20±4.22[b]	5.08±0.66	28.10±4.17	1.50±0.50	1.13±0.20
2	+E +Se	4	64.30±5.66	5.55±1.49	27.40±3.67	0.70±0.40	1.83±0.38
3	+E -Se	4	65.40±3.75	4.98±1.54	27.58±3.63	0.63±0.33	1.43±0.62
4	+E +Se	4	67.98±4.01	4.68±0.96	26.00±4.53	0.28±0.09	1.10±0.65
5	-E -Se	4	59.88±3.89	7.70±1.01	30.23±2.38	0.53±0.27	1.68±0.25
6	+E +Se	4	62.43±4.65	5.85±1.27	29.60±3.85	0.65±0.35	1.48±0.41

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

Table 5. Differential cell counts of milk from sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Cell Types				
			Neutrophils	Macrophages	Lymphocytes	Eosinophils	Epith. cells
			%				
1	-E +Se	4	38.95±3.69[b]	13.20±1.92	23.53±2.72	0.45±0.18	23.87±2.57
2	+E +Se	4	37.53±6.01	17.55±2.83	18.50±2.85	0.55±0.18	25.87±1.82
3	+E -Se	4	37.58±3.61	14.88±3.00	22.00±1.66	0.48±0.22	25.18±2.32
4	+E +Se	4	44.15±4.63	13.40±2.20	20.63±3.21	0.13±0.08	21.70±2.65
5	-E -Se	4	37.12±7.74	17.90±1.16	23.73±8.62	0.25±0.15	21.00±1.17
6	+E +Se	4	43.30±2.82	17.10±2.97	15.25±2.78	0.48±0.23	23.88±1.44

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

Table 6. ^{3}H-thymidine uptake of mitogen-stimulated colostral lymphocytes of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Unstimulated cells	Mitogen-stimulated cells		
				Phytohemagglutinin	Concanavalin A	Pokeweed
			CPM (log_{10})			
1	-E +Se	4	2.45±0.23[b]	3.09±0.08[c]	3.31±0.23	2.40±0.17
2	+E +Se	4	2.27±0.11	3.42±0.11	2.87±0.17	3.36±0.41
3	+E -Se	4	2.45±0.14	3.67±0.17	3.50±0.29	3.83±0.20
4	+E +Se	4	2.59±0.09	3.99±0.15	3.33±0.18	3.97±0.18
5	-E -Se	4	2.37±0.11	3.21±0.35[d]	3.15±0.33	3.19±0.39[d]
6	+E +Se	4	2.35±0.07	3.99±0.08	3.85±0.08	4.31±0.12

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

[c]Significantly different from group 2 ($p < 0.05$).

[d]Significantly different from group 6 ($p < 0.05$).

lower than for colostral lymphocytes from sows fed the +E+Se diet. No colostrum lymphocyte stimulation by Con. A was observed.

Phagocytic activity by polymorphonucelar cells

The effects of vitamin E and/or selenium depletion on the phagocytic activity of colostral and milk polymorphonuclear cells is summarized in table 7.

No significant difference in the phagocytic activity of colostral polymorphonuclear cells was attributable to either vitamin E or selenium depletion of the sows. However, in the vitamin E- and selenium-deficient sows, the phagocytic activity was significantly lower ($p<0.05$) than in the vitamin E- and selenium-supplemented sows.

Microbicidal activity by polymorphonuclear cells

The vitamin E and/or selenium-depletion effects on the microbicidal activity of colostral and milk polymorphonuclear cells of sows are presented in table 8.

The abilities of colostral polymorphonuclear cells, from sows deprived of vitamin E or selenium or both, to kill engulfed yeast cells were significantly lower ($p<0.05$) than colostral polymorphonuclear cells from sows supplemented with vitamin E and selenium.

Table 7. ^{3}H-thymidine uptake of mitogen-stimulated milk lymphocytes of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Unstimulated cells	Mitogen-stimulated cells		
				Phytohemagglutinin	Concanavalin A	Pokeweed
			CPM (log_{10})			
1	-E +Se	4	2.31±0.13[b]	2.68±0.27	2.60±0.25	2.40±0.17
2	+E +Se	4	2.12±0.10	2.66±0.39	2.66±0.37	2.66±0.21
3	+E -Se	4	2.32±0.16	2.79±0.14	2.77±0.20	2.87±0.20
4	+E +Se	4	2.45±0.03	2.99±0.17	2.73±0.13	2.77±0.12
5	-E -Se	4	2.36±0.13	2.42±0.11	2.41±0.12	2.43±0.12
6	+E +Se	4	2.31±0.13	2.82±0.30	2.88±0.29	2.64±0.26

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

Table 8. Phagocytic activity of colostral and milk polymorphonuclear cells of sows fed vitamin E and/or selenium diets between conception and parturition.

Treatment group[a]	Diet	n/group	Colostrum	Milk
			---------- %[b] ----------	
1	-E +Se	4	43.64±3.21[b]	23.73±1.46
2	+E +Se	4	50.20±5.41	27.50±3.02
3	+E -Se	4	33.42±5.14	19.90±3.40
4	+E +Se	4	44.59±8.33	30.17±5.05
5	-E -Se	4	35.20±0.91[d]	21.25±2.55
6	+E +Se	4	53.55±2.49	23.25±2.17

[a]See table 2 for experimental periods.

[b]The % phagocytic activity is defined as the number of PMN cells containing 2 or more yeast particles per 100 PMN cells.

[c]Values are means ± SEM.

[d]Significantly different from group 6 ($p < 0.05$).

Immunoresponsiveness of milk components

Lymphocyte stimulation

Lymphocytes isolated from sow milk were not stimulated by any mitogen used (Table 9).

Phagocytic activity by polymorphonuclear cells

No significant effects of diet on the phagocytic activity of milk polymorphonuclear cells were detected (Table 7).

Microbicidal activity of polymorphonuclear cells

A significant decrease in the ability of milk polymorphonuclear cells to kill the engulfed yeast cells was associated only with the combined depletion of vitamin E and selenium (group 5 vs group 6) (Table 8).

Table 9. Microbicidal activity of colostral and milk polymorphonuclear cells of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Colostrum	Milk
			---------- %[b] ----------	
1	-E +Se	4	3.65±0.56[c,d]	2.20±0.49
2	+E +Se	4	6.18±0.83	2.73±0.18
3	+E -Se	4	2.60±0.60[e]	1.65±0.40
4	+E +Se	4	6.22±1.03	2.38±0.11
5	-E -Se	4	3.17±2.68[f]	1.23±0.13[f]
6	+E +Se	4	11.04±1.78	2.23±0.27

[a]See table 2 for experimental periods.

[b]The % microbicidal activity was calculated as the number of PMN cells contained 2 or more dead yeast particles per 100 PMN cells.

[c]Values are means ± SEM.

[d]Significantly different from group 2 ($p < 0.05$).

[e]Significantly different from group 4 ($p < 0.05$).

[f]Significantly different from group 6 ($p < 0.05$).

DISCUSSION

Results of this research indicate that the vitamin E-deficient diet with or without supplemental selenium significantly reduced the response of sow PBL to PHA. Phytohemagglutinin is known to be a T-lymphocyte mitogen for several animals including pigs (Shimizu and Shimizu, 1979) and T-lymphocytes are primarily involved in cell-mediated immunity. According to Nichols et al. (1979), the effect of diminished vitamin E in this situation may relate to its metabolic function as an antioxidant. Vitamin E deficiency enhances lipid peroxidation of cellular membranes and eventually increases membrane fluidity. These changes could alter receptor movement on the lymphocyte membranes and thereby affect the initiation of lymphocyte blastogenesis.

The selenium-depletion diet, in comparison to the +E+Se diet, resulted in no significant differences in sow lymphocyte proliferation responses to PHA mitogen during gestation but tended to have a significant effect ($p<0.1$) by parturition when serum selenium concentration had declined to about 123 ng/ml. Perhaps if dietary selenium could have been reduced to less than 0.089 mg/kg in the present study, a significant difference in the lymphocyte proliferative

response to PHA in sows fed +E-Se diet would have been detected. The level of dietary selenium in this study was similar to the previously recommended level of selenium (0.1 mg/kg) for gestating sows by the NRC (1979). On the other hand, it is possible that the certain lymphocyte function tested by PHA stimulation is less affected by selenium status than by vitamin E and that vitamin E deficiency decreases the lymphocyte blastogenesis irrespective of the selenium status.

In this study, pokeweed mitogen stimulation of PBL from sows fed both the -E+Se and -E-Se diets was significantly lower than from sows fed the control diet. There is still a controversy regarding the target cell for PWM; some investigators indicate that PWM stimulates predominantly B-cells (Tizard, 1987) which are believed to be involved primarily in humoral immunity. Other investigators believe PWM stimulates both T and B cells (Greaves et al., 1974; Renshaw et al., 1977). While the target cell for PWM among swine lymphocytes was not established in this study, an effect of vitamin E on humoral immunity has been demonstrated in other species. In chickens and mice, vitamin E supplementation stimulated humoral immunity by increasing production of antibody producing cells (Tengerdy et al., 1978).

Dietary treatments caused no significant difference in response of PBL to Con A, a mitogen considered to stimulate T cells or to be indicative of cellular immunity. This is consistent with the work of Corwin and Shloss (1980) who found vitamin E ineffective in enhancing lymphocyte

proliferation in the presence of optimal concentrations of Con A. The difference between PHA and Con A stimulation of PBL may indicate that Con A affects a different subset of T-lymphocyte than PHA, as suggested by Stobo and Paul (1973).

The results of this study indicated a decrease in the abilities of blood PMN cells of sows fed the -E+Se, +E-Se and -E-Se diets to phagocytize yeast particles and to kill the engulfed yeast. This demonstrated that both vitamin E and selenium are important in phagocytic, as well as microbicidal, activities of sow polymorphonuclear cells. Comparable studies in mice and cattle fed selenium-deficient diets revealed no impairment in the ability of PMN to ingest C. albicans, although microbicidal activity was significantly impaired (Boyne and Arthur, 1986; Gyang et al., 1984). According to Aziz et al. (1984), reduction in bactericidal activity by PMN from selenium-deficient goats is associated with a reduction in PMN GSH-Px activity.

It is known that GSH-Px catalyses the reduction of hydroperoxide and superoxide produced by neutrophils during phagocytosis. The hydrogen peroxide can act through myeloperoxidase to help destroy ingested particles in neutrophils (Klebanoff, 1975). Decreased GSH-Px activity, and possible subsequent damage to the neutrophils by toxic oxygen metabolites, could therefore contribute to the decrease in PMN microbicidal activity. The data indicate that GSH-Px activity of approximately 1.5 EU/ml and above would be

adequate to maintain normal microbicidal activity of PMNs in gestating sows.

Selenium intake significantly affected the activity of GSH-Px in sow serum in the current study. The positive correlation between serum selenium and serum GSH-Px observed in this study (r=0.62 to 0.83) is in agreement with previous reports (Chavez, 1979; Hakkarainen et al., 1978; Stowe and Miller, 1985). The initial values for GSH-Px, as well as for selenium, for sows in group 5 (-E-Se) and 6 (+E+Se) were higher than those of sows in group 1, 2, 3 and 4, even though all sows had been similarly fed prior to assignment to this study. It is possible this difference was due to different genetic pools among the sows and the reported genetic influences on selenium metabolism (Atroshi et al., 1981; Jorgensen et al., 1977; Stowe and Miller, 1985).

The interrelationship between plasma GSH-Px activity and vitamin E has also been studied. Yang and Desai (1978) provided evidence that there was a negative linear relationship between the logarithm of vitamin E intake and GSH-Px activity in liver and plasma of rats. However, large amounts of dietary vitamin E were required to show its repressive effect on the activity of GSH-Px. Even though such a relationship was not evaluated in this study, the closest relationship between GSH-Px and selenium was observed in sows fed -E-Se diet; this suggests both selenium and vitamin E have a role in GSH-Px homeostasis.

It has been suggested that the mechanism of action by which vitamin E alters the phagocytic action of PMN relates to its ability to maintain the integrity of cellular membrane by preventing auto-oxidative damage during phagocytosis (Baehner et al., 1977). The increased formation of malonaldehyde in the vitamin E-deficient rat PMN (Harris et al., 1980) is evidence of membrane lipid peroxidation and auto-oxidative damage. Malonaldehyde is a peroxidation product that is used as an index of lipid peroxidation of PUFA (Stossel et al., 1979).

Results of this study indicated that there is no significant difference in the lymphocyte function between sows fed +E-Se and +E+Se diets. Other investigators have reported a significant enhancing effect of selenium on lymphocyte function when the selenium is given at a rate considerably above the NRC recommendation (Corwin and Shloss, 1980). Therefore, it is necessary to re-evaluate the established recommended intakes for nutrients like vitamin E and selenium that could be classified as "immuno stimulatory". Increased intakes of vitamin E and/or selenium could be beneficial during periods of stress such as weaning, gestation and parturition.

It is important to point out that no other clinical evidence of vitamin E-deficiency disease, such as muscular dystrophy or hepatosis dietetica, accompanied the diminished immune response. However, in the present study, a smaller litter size was noted in sows fed the -E+Se diet

($\bar{x}$=8.25) and sows fed -E-Se diet ($\bar{x}$=9.75) compared to the litter size for sows fed the +E+Se diet ($\bar{x}$=12.5) (see Table 20). Since only small numbers of sows were used and the data were recorded only over one gestation and farrowing period, the information associated with the reproductive performance in this study is likely to be biased. The possible effect of vitamin E and selenium on litter size is consistent with the work of Ullrey et al. (1971) and Vale (1983) which indicated that supplementation of vitamin E and selenium in sow diets throughout gestation and lactation could, compared to nonsupplemented sows, produce a larger litter size at birth and increase the livability of the pigs at three weeks.

Five types of cells, namely neutrophils, macrophages, lymphocytes, eosinophils and epithelial cells were identified in both colostrum and milk of the sows in this study. Similar results were reported by Schollenberger and coworkers (1986), however, they indicated the presence of a sixth cell type (anucleate cell) in sows' milk.

As in cows (Lee et al., 1980) and sheep (Lee and Outteridge, 1981), PMN cells were the predominant cell type in sow colostrum varying from 55.99 to 71.99 % and remained prominent (range 29.39 to 48.78 %) in sow milk even though their percentage was lower than in colostrum. The decline in the proportion of PMN cells was reported to occur in cow and sheep milk (Lee et al., 1980; Lee and Outteridge, 1981).

The concentrations of lymphocytes were similar for both colostrum and milk in this study with ranges of 21.47 to 33.16% for colostrum and 12.47 to 32.35% for milk. These concentrations are, however, higher than the concentrations of lymphocytes reported for other species (see Table 21). It is possible that the relatively higher lymphocyte concentrations in sow colostrum and milk provide special protection of the sow mammary gland against invading micro organisms, either by enhanced local production of antibody or by enhanced cell-mediated immunity in the gland itself. According to Bourne (1973), 60% of Ig A in colostrum or 90% of Ig A and 70% of Ig G in milk are produced locally in mammary tissues of sows.

The persistence of viable leukocytes in the mammary secretions throughout the observed lactation period (4 days) suggests that these cells may play a crucial role in defending the mammary gland, as well as the gut of the suckling neonate, from infections.

The proportion of epithelial cells in sow milk (19.83 to 27.69%) was much higher than in sow colostrum (0.55 to 2.21 %) and apparently higher than in cows' milk (7%) (Lee et al., 1980). This may be due to the more frequent and aggressive suckling habit of piglets.

Few studies involving mitogenic stimulation of colostral or milk lymphocytes in animals have been published. Results of the present study indicate a low proliferative response of colostral lymphocytes to mitogens relative to

the response of PBL to mitogens. No mitogen-induced proliferative activity was observed in milk lymphocytes of sows. Similar results have also been reported with human colostral lymphocytes (Parmely et al., 1976) and with bovine and canine milk lymphocytes (Smith and Schultz, 1977). Thus, hyporesponsiveness of mammary gland lymphocytes (MGL) to mitogen appears to be a general phenomenon.

The cause for the diminished functional capacity shown by MGL, however, is not yet clear. It has been suggested that hyporesponsiveness of MGL to the mitogen is due to a relative absence of a certain lymphocyte population in the colostrum and milk but which is prominent in PBL (Parmely et al., 1976). According to Brock and Mainou-Fowler (1983), the hyporeactivity of MGL is associated with the action of soluble milk components which may depress lymphocyte proliferation by masking surface receptors involved in the mitogen-induced blastogenesis or by affecting nutritional requirements for blastogenesis. While vitamin E and/or selenium supplementation certainly did not improve the responsiveness of sow milk lymphocytes to mitogens in the present study, the -E+Se and -E-Se diets did significantly reduce the response of colostral lymphocytes to PHA and PWM. This is further evidence of the influence of vitamin E on cell-mediated immunity.

Cytostatic and cytotoxic factors, which were reported to be present in milk but not in colostrum (Drew et al., 1984), may also contribute to the hyporesponsiveness of

MGL. This hyporesponsiveness of MGL could have an important role in preventing an allogeneic reaction in the suckling neonate or in preventing the self-destructive effects of these cells on the mammary gland.

Phagocytic capacity of PMN cells of mammary secretions from nonporcine species has been observed *in vitro* by several investigators. All the results have pointed to the fact that the phagocytic activity of PMN cells isolated from mammary secretions was lower compared to that of PMN cells isolated from the blood (Wisniowski *et al.*, 1975; Ho and Lawton, 1979). The reason for this disparity was investigated by Paape *et al.* (1975) who indicated that the fat globules and casein in milk may exert an inhibitory effect on phagocytosis. Paape *et al.* (1975) also found that preincubation of peritoneal macrophages with fatty acids significantly reduces their phagocytic activity. This was attributed to alterations in membrane viscosity brought about by incorporation of fatty acids into the cell membrane phospholipids.

In addition to reducing phagocytosis, fat globules can also reduce the ability of the PMN leukocytes to kill engulfed bacteria. This reduction may result from disruption of phagolysosomes by the ingested milk fat and from a subsequent depletion of phagolysosome enzymes (Paape and Guidry, 1977). The mechanism by which casein reduces phagocytic and killing ability of milk PMN leukocytes was reported to involve binding of casein to the surface membrane

and degranulation after ingestion of casein by PMN (Russel and Reiter, 1975; Russel et al., 1976).

Naidu and Newbould (1973) found that PMN cells isolated from milk contained 38% less glycogen than PMN cells isolated from blood and suggested that the glycogen content could be a factor affecting phagocytosis by milk PMN cells. Addition of glucose to media containing PMN cells of milk can increase the ability of those cells to ingest bacteria.

The reduced phagocytic and microbicidal activity of PMN cells from mammary secretions of sows noted in this study is consistent with the above earlier studies. In addition, the present study provides information that both phagocytic and microbicidal activities of PMN cells of colostrum and milk were significantly affected by nutrition, specifically vitamin E and/or selenium concentrations in the diet. This information could be expected to have application in mastitis control programs among farm animals.

Although vitamin E and/or selenium deficiency in this study significantly depressed both functions of T-lymphocytes and PMN cells, further investigations still need to be done to establish the practical effect of vitamin E and/or selenium on the susceptibility or resistance to diseases.

SUMMARY AND CONCLUSIONS

Twenty-four multiparous sows were assigned at conception to a split plot experiment to determine the influence of vitamin E and/or selenium deficiencies on the immunoresponsiveness of cellular components of peripheral blood, colostrum and milk. The sows were fed corn/soybean meal-based diets in which dried, high moisture corn was used because of its vitamin E deficient nature. Four groups of sows were established and fed the basal vitamin E and Se-deficient diet (.29 IU vit. E and 0.089 mg Se/kg); a vitamin E deficient-diet (basal supplemented with .3 mg Se/kg); a Se- deficient diet (basal supplemented with 60 IU vit. E/kg); and a control diet (basal supplemented with both 60 IU vit. E and .3 mg Se/kg).

Cell-mediated and humoral immunity was monitored by mitogen-stimulation studies with lymphocytes obtained from peripheral blood at the onset of, and at monthly intervals during, gestation and at parturition; from colostrum obtained at parturition; and from milk obtained at day 4 of lactation. The abilities of polymorphonuclear cells from peripheral blood, colostrum and milk to phagocytize and kill yeast cells were also monitored simultaneously with the lymphocyte stimulation studies.

The vitamin E depletion diet significantly decreased serum vitamin E (range of .27 to .48 ug/ml) and depressed both cell-mediated and humoral immunity as evidenced by decreased ^{3}H-thymidine uptake of the phytohemagglutinin and pokeweed mitogen-stimulated lymphocytes, respectively, from peripheral blood and colostrum. In addition, the vitamin E depletion diet significantly decreased the abilities of PMN cells from both peripheral blood and colostrum to phagocytize and kill yeast cells. No other clinical signs of vitamin E deficiency, such as myopathy, accompanied these manifestations of immunodepression.

The selenium depletion diet resulted in a modest depression in serum selenium (range 123 to 144 ng/ml). While this depression was not sufficient to alter ^{3}H-thymidine uptake by mitogen-stimulated lymphocytes of any source at any sampling time, it was asssociated with a decreased ability of blood and colostral PMN cells to phagocytize and kill yeast cells.

The diet deficient in both vitamin E and selenium significantly reduced the ^{3}H-thymidine uptake responses of both blood and colostral lymphocytes to PHA and PWM stimulation. In addition, this diet was associated with significant reductions in the phagocytic and microbicidal activities of PMNs from blood and colostrum as well as in the microbicidal activity of PMN cells from milk.

It can be concluded that sow diets which maintain serum vitamin E concentrations above 1.4 ug/ml and serum selenium concentrations above 160 ng/ml during late gestation and

the peripartum period should be associated with optimal cell-mediated and humoral immune response, other things being equal. However, to attain optimal phagocytic and microbicidal activity of PMN cells (nonspecific immunity) in addition to optimal cell-mediated and humoral immunity, diets are required which will maintain serum selenium concentrations in the 180 to 220 ng/ml range, as well as serum vitamin E higher than 1.4 ug/ml, for sows in late gestation and the peripartum period. These observations on selenium provide justification of the current, and rather recently approved, selenium supplementation rate of .3 mg Se/kg diet for swine.

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APPENDIX

Table 10. Serum vitamin E concentrations of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			μg/ml				
1	-E +Se	4	1.16±0.18[b]	1.10±0.06[c]	0.96±0.23[c]	0.76±0.17[c]	0.36±0.09[c]
2	+E +Se	4	1.45±0.12	1.72±0.15	1.84±0.10	1.91±0.18	2.20±0.31
3	+E -Se	4	1.19±0.08	1.47±0.08	1.47±0.02	1.56±0.03	2.08±0.11
4	+E +Se	4	1.03±0.03	1.18±0.07	1.40±0.16	1.61±0.09	1.97±0.17
5	-E -Se	4	1.03±0.25	0.89±0.07[d]	0.69±0.08[d]	0.57±0.02[d]	0.45±0.03[d]
6	+E +Se	4	0.99±0.05	1.03±0.01	1.18±0.08	1.35±0.06	1.53±0.09

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

[c]Significantly different from group 2 ($p < 0.05$).

[d]Significantly different from group 6 ($p < 0.05$).

Table 11. Serum selenium concentrations of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			ng/ml				
1	-E +Se	4	153.75±12.84[b]	166.50±11.02	178.00±10.72	192.50±17.11	195.25±14.44
2	+E +Se	4	161.25±10.05	183.25±10.64	193.00±21.33	203.75±17.73	207.75±15.50
3	+E -Se	4	165.50±13.97	144.25±6.32[c]	142.75±4.55[c]	136.25±9.53[c]	123.25±10.8[c]
4	+E +Se	4	156.00±15.37	170.00±6.21	175.59±6.22	175.25±4.40	185.75±5.86
5	-E -Se	4	213.50±9.87	174.00±2.50[d]	174.00±4.37[d]	165.30±5.14[d]	164.00±4.53[d]
6	+E +Se	4	216.50±6.29	186.50±3.53	190.80±2.62	205.00±9.94	228.00±6.43

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

[c]Significantly different from group 4 ($p < 0.05$).

[d]Significantly different from group 6 ($p < 0.05$).

Table 12. Serum glutathione peroxidase activity of sows fed vitamin E and/or selenium depletion diets between coneption and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			Enzym Units/ml				
1	-E +Se	4	1.99±0.34[b]	2.12±0.24	2.01±0.14	2.11±0.21	2.14±0.40
2	+E +Se	4	1.60±0.20	2.05±0.33	2.00±0.19	2.17±0.11	2.38±0.10
3	+E -Se	4	1.93±0.19	1.59±0.13	1.54±0.08[c]	1.36±0.03[c]	1.05±0.21[c]
4	+E +Se	4	1.84±0.16	1.64±0.12	1.71±0.06	1.76±0.09	2.09±0.11
5	-E -Se	4	2.56±0.51	1.91±0.09[d]	1.71±0.20[d]	1.63±0.24[d]	1.61±0.22[d]
6	+E +Se	4	2.88±0.27	2.11±0.03	2.16±0.01	2.54±0.11	2.68±0.14

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

[c]Significantly different from group 4 ($p < 0.05$).

[d]Significantly different from group 6 ($p < 0.05$).

Table 13. Serum cholesterol concentrations of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			mg/dl				
1	-E +Se	4	107.3±11.8[b]	73.5± 2.4	100.5±13.5	75.0±10.7	89.3±11.2
2	+E +Se	4	99.8± 5.0	86.3± 5.5	92.3± 4.3	68.0± 4.9	103.0± 5.2
3	+E -Se	4	97.0± 8.7	90.8± 5.4	78.0± 8.6	85.5± 7.8	132.8±15.5
4	+E +Se	4	92.3± 6.5	101.5±12.5	94.0±11.0	83.3± 6.3	120.0±10.8
5	-E -Se	4	87.0± 5.8	95.3± 7.2	105.7±11.8	61.5± 5.6	69.3± 4.6
6	+E +Se	4	102.5± 7.8	116.5±14.1	90.3±13.8	68.8±10.9	86.0± 7.7

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

Table 14. ^{3}H-thymidine uptake of unstimulated peripheral blood lymphocytes of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			CPM (log_{10})				
1	-E +Se	4	2.75±0.13[b]	2.50±0.10	2.39±0.06	2.45±0.05	2.37±0.15
2	+E +Se	4	2.86±0.20	2.73±0.18	2.36±0.16	2.41±0.12	2.41±0.09
3	+E -Se	4	2.45±0.12	2.42±0.03	2.75±0.18	2.76±0.17	2.44±0.04
4	+E +Se	4	2.88±0.21	2.52±0.18	2.61±0.08	2.43±0.11	2.37±0.03
5	-E -Se	4	2.34±0.32	2.34±0.15	2.44±0.09	2.45±0.41	2.66±0.28
6	+E +Se	4	2.84±0.49	2.51±0.56	2.37±0.21	2.23±0.08	2.15±0.11

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

Table 15. ^{3}H-thymidine uptake of PHA-stimulated peripheral blood lymphocytes of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			CPM (log_{10})				
1	-E +Se	4	4.89±0.18[b]	5.09±0.15	4.89±0.30	4.54±0.20[c]	3.93±0.30[c]
2	+E +Se	4	4.27±0.40	4.57±0.29	4.79±0.23	4.90±0.10	5.13±0.09
3	+E -Se	4	4.91±0.28	4.59±0.32	5.17±0.04	5.20±0.11	4.50±0.36
4	+E +Se	4	4.85±0.29	4.67±0.31	4.86±0.23	5.19±0.09	5.32±0.06
5	-E -Se	4	5.32±0.02	5.23±0.17	4.94±0.01[d]	4.90±0.14[d]	4.64±0.23[d]
6	+E +Se	4	4.60±0.18	5.04±0.12	5.21±0.10	5.26±0.07	5.39±0.08

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

[c]Significantly different from group 2 ($p < 0.05$).

[d]Significantly different from group 6 ($p < 0.05$).

Table 16. ^{3}H-thymidine uptake of PWM-stimulated peripheral blood lymphocytes of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			CPM (log_{10})				
1	-E +Se	4	4.49±0.33[b]	5.21±0.08	5.07±0.09	4.64±0.05[c]	3.79±0.37[c]
2	+E +Se	4	4.42±0.40	4.64±0.34	5.05±0.10	5.19±0.10	5.17±0.07
3	+E -Se	4	4.67±0.41	5.09±0.17	5.15±0.16	5.18±0.04	4.67±0.41
4	+E +Se	4	4.77±0.44	4.62±0.37	5.06±0.10	5.28±0.07	5.31±0.04
5	-E -Se	4	5.32±0.25	5.15±0.05	5.10±0.04[d]	5.07±0.09[d]	4.89±0.12[d]
6	+E +Se	4	4.83±0.15	5.19±0.05	5.24±0.03	5.28±0.05	5.29±0.05

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

[c]Significantly different from group 2 ($p < 0.05$).

[d]Significantly different from group 6 ($p < 0.05$).

Table 17. ^{3}H-thymidine uptake of Con.A-stimulated peripheral blood lymphocytes of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			CPM (log_{10})				
1	-E +Se	4	3.26±0.64[b]	4.31±0.22	4.35±0.39	4.61±0.14	4.67±0.16
2	+E +Se	4	3.66±0.49	4.22±0.20	4.30±0.07	4.40±0.28	4.56±0.15
3	+E -Se	4	4.30±0.08	4.25±0.37	4.62±0.15	4.51±0.21	4.37±0.20
4	+E +Se	4	4.32±0.09	4.10±0.09	4.29±0.25	4.21±0.17	4.65±0.13
5	-E -Se	4	4.39±0.20	4.34±0.16	4.23±0.21	4.13±0.10	4.07±0.04
6	+E +Se	4	4.32±0.13	4.14±0.11	4.54±0.08	4.53±0.07	4.63±0.14

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

Table 18. Phagocytic activity of blood polymorphonuclear cells of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			---------- %[b] ----------				
1	-E +Se	4	85.51±2.52[c]	89.73±3.92	89.63±4.20	86.16±1.64[d]	83.80±2.95[d]
2	+E +Se	4	86.79±2.57	87.18±1.56	89.93±1.45	90.70±0.91	90.10±1.08
3	+E -Se	4	89.86±5.59	90.94±2.70	87.38±2.37	80.52±2.02[e]	72.24±3.37[e]
4	+E +Se	4	85.49±3.73	92.88±2.76	89.90±5.79	92.91±1.90	90.18±2.06
5	-E -Se	4	86.23±2.60	86.43±1.08	83.60±0.72[f]	81.26±1.36[f]	78.48±1.79[f]
6	+E +Se	4	82.40±2.57	86.42±1.75	86.48±0.56	88.72±1.94	92.52±0.64

[a]See table 2 for experimental periods.

[b]The % phagocytic activity is defined as the number of PMN cells containing 2 or more yeast per 100 PMN cells.

[c]Values are means ± SEM.

[d]Significantly different from group 2 ($p < 0.05$).

[e]Significantly different from group 4 ($p < 0.05$).

[f]Significantly different from group 6 ($p < 0.05$).

Table 19. Microbicidal activity of blood polymorphonuclear cells of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Day(s) on feed 0	30	60	90	120
			---------- %[b] ----------				
1	-E +Se	4	39.70±1.32[c]	35.70±4.49	56.16±4.09	48.75±3.04[d]	40.60±3.44[d]
2	+E +Se	4	39.50±1.85	41.95±1.96	57.13±2.70	57.63±2.29	58.12±1.16
3	+E -Se	4	47.62±1.86	42.40±2.55	39.19±3.19	38.45±2.59[e]	34.27±1.65[e]
4	+E +Se	4	48.48±2.49	49.59±5.17	54.32±4.42	53.07±2.40	56.29±2.91
5	-E -Se	4	51.35±2.49	50.13±2.26	48.12±0.61	42.94±0.38[f]	37.63±0.55[f]
6	+E +Se	4	40.94±2.66	42.27±2.01	42.87±2.53	48.37±1.75	55.88±2.21

[a] See table 2 for experimental periods.

[b] The % microbicidal activity was calculated as the number of PMN cells contained 2 or more dead yeast particles per 100 PMN cells.

[c] Values are means ± SEM.

[d] Significantly different from group 2 ($p < 0.05$).

[e] Significantly different from group 4 ($p < 0.05$).

[f] Significantly different from group 6 ($p < 0.05$).

Table 20. Reproductive performance of sows fed vitamin E and/or selenium depletion diets between conception and parturition.

Treatment group[a]	Diet	n/group	Total pigs born/litter	Individual pig weight (kg)
1	-E +Se	4	8.25±1.49[b,c]	1.59±0.14
2	+E +Se	4	11.00±0.91	1.32±0.15
3	+E -Se	4	11.75±1.65	1.31±0.16
4	+E +Se	4	8.75±0.95	1.29±0.15
5	-E -Se	4	9.75±1.03[d]	1.58±0.15
6	+E +Se	4	12.50±0.87	1.42±0.15

[a]See table 2 for experimental periods.

[b]Values are means ± SEM.

[c]Significantly different from group 2 ($p < 0.05$).

[d]Significantly different from group 6 ($p < 0.05$).

Table 21. Normal cellular components of peripheral blood, colostrum and milk of sows, cows and ewes.

Cell Types	Sows			Cows			Ewes		
	B[a]	C[b]	M[b]	B[a]	C[c]	M[c]	B[a]	C[d]	M[d]
	%								
Neutrophils	43.60	62.20	50.91	28.00	61.50	11.00	30.00	66.25	2.00
Macrophages	4.40	6.56	8.77	4.00	25.25	62.00	2.50	24.00	84.00
Lymphocytes	49.00	10.58	11.41	58.00	9.75	10.00	62.00	8.50	13.00
Eosinophils	2.60	1.02	2.59	9.00	3.50	10.00	5.00	1.00	-
Basophils	0.40	-	-	0.50	-	-	0.50	-	-
Epithelial cells	-	19.64	26.32	-	-	7.00	-	0.25	1.00

[a]Data from the Clinical Pathology Laboratory, Michigan State University.

[b]Data from Schollenberger et al. (1986).

[c]Data from Lee et al. (1980).

[d]Data from Lee and Outteridge (1981).