# THE EFFECT OF DIETARY VITAMIN E ON THE HUMORAL AND CELL-MEDIATED IMMUNE RESPONSE OF PIGS HOUSED AT DIFFERENT ENVIRONMENTAL TEMPERATURES OR WEANED AT VARIOUS AGES

by

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# THE EFFECT OF DIETARY VITAMIN E ON THE HUMORAL AND CELL-MEDIATED IMMUNE RESPONSE OF PIGS HOUSED AT DIFFERENT ENVIRONMENTAL TEMPERATURES OR WEANED AT VARIOUS AGES

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(ABSTRACT)

A set of experiments were conducted to evaluate the antibody response, serum vitamin E level, cortisol concentration and performance of pigs weaned at three ages (21, 28 or 35 d) and fed diet containing either 11 or 220 IU/kg diet recommended level of vitamin E. Supplemental dietary vitamin E (220 IU) increased the concentration of serum vitamin E but, did not affect performance, cortisol concentrations or the antibody response. As weaning age increased, weekly performance increased linearly as did cortisol levels. Animals weaned at 35 d age had the largest primary antibody response, but this difference was not observed for the secondary response.

A second set of experiments evaluated effects of four dietary vitamin E levels (11, 110, 220 and 550 IU/kg feed) on the humoral and cell-mediated immune response and performance of 4 wk old weanling pigs housed at one of two nursery temperatures (19 or 30°C). Performance was greater for pigs housed at 19°C compared with pigs housed

at 30°C, but mitogen stimulation indices of white blood cells, plasma cortisol levels, and antibody titers were similar. Serum and liver vitamin E levels linearly increased with increasing dietary vitamin E level, but performance, cortisol, antibody levels and mitogen induced stimulation indexes were not affected by supplemental vitamin E levels.

In the third experiment, sows fed a NRC level of vitamin E demonstrated little fluctuation in serum vitamin E concentration during a 5 wk lactation period. There was a high concentration of vitamin E in colostrum, followed by a sharp decrease in milk vitamin E concentration after the first week of lactation and remained constant for the next four weeks. Piglet serum was initially low in vitamin E at 1 d of age but increased with time peaking about week 3.

These experiments suggest that supplementing dietary vitamin E above the levels recommended by the NRC to baby pigs (which nursed sows fed NRC recommended levels of vitamin E) will not influence cell-mediated or humoral immune response, performance parameters or cortisol levels when pigs were weaned at various ages or exposed to environmental temperature changes.

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# CHAPTER I

# INTRODUCTION

The baby pig acquires its first immunity (passive) from the colostrum of the sow; these antibodies have a half-life of about seven days. At approximately 10 d of age, the baby pig begins to produce its own antibodies (Wilson, 1974). Without adequate nutrition, the immune response may not develop properly, resulting in decreased resistance to disease and a greater chance of morbidity which could lead to a revenue loss for producers.

In early research, combined nutritional deficiencies as well as specific nutrient deficiencies (proteins, mineral and vitamin) were found in several species. More recent research has dealt with the influence of individual minerals and vitamins on the immune response of several species when fed at levels above National Research Council (NRC) recommendations for normal growth. Vitamin E has received considerable attention.

Several reports have suggested that humoral and cell-mediated immunity can be improved with the addition of vitamin E above NRC recommended levels (Heinzerling et al., 1974 [for chicks]; Colnago et al., 1984 [for chicks]; Ellis and Vorhies, 1976 [for pigs]; Sheffy and Schultz, 1979 [for dogs]; VanVleet and Wilson, 1984 [for lab

animals]). Many of these studies suggest that the NRC recommended vitamin E levels are adequate to prevent the onset of any deficiency symptoms, but they are inadequate for a maximum immunological response. It has been suggested that a level 3 to 6 times higher than recommended by the NRC will give an optimal immune response (Lim et al., 1981). Therefore, the objectives of this experiment were to: 1) determine if stress that might be caused by weaning or changes in housing temperature is detrimental to humoral and cellular immunity in young pigs, 2) determine whether supplemental dietary vitamin E will alleviate the potential problems caused by these stresses and 3) to characterize vitamin E in the milk and serum of lactating sows and in the serum of their nursing pigs.

# CHAPTER II

# REVIEW OF LITERATURE

## Vitamin E

Background. Early research showed that vitamin E deficiency in rats fed rancid fat caused reproductive failure (Evan and Bishop, 1922). This nutrient was originally called the anti-sterility vitamin, but lost this title after these symptoms were not seen in all animals. Several other diseases and disorders have been associated with vitamin E deficiencies including exudative diathesis which affects the capillary walls in chicks, and muscular dystrophy, which affects the skeletal and heart muscles in lambs, calves, pigs, chicks, and rats (McDonald et al., 1978). A deficiency of vitamin E in swine may also cause mulberry heart disease, white muscle disease, and sudden death syndrome (Church and Pond, 1982). Usually the most rapid growing pigs between 6 and 16 wk of age are affected.

Other problems associated with a vitamin E deficiency include poor manifestation of estrus, impaired fertility, mastitis, agalactia, and metritis (Mortimer, 1983). Harris et al. (1980) found that red blood cells deficient in vitamin E were vulnerable to hemolysis by hydrogen peroxide. Vitamin E can also influence the synthesis of proteins. While in a vitamin E deficient state, about

thirty enzymes have been found to decrease in concentration while approximately ten enzymes are increased. This phenomenon is a result of the involvement of vitamin E with the regulation of gene transcription (Church and Pond, 1982).

Vitamin E can be found in most of the body tissues, especially in the phospholipid bilayer of cell membranes (Urano et al., 1987). The structure of vitamin E is a methyl substituted 6-hydroxy chromium derivative with a 16 carbon aliphatic side chain (figure 1). This structure allows it to become a hydrogen donor within the cell membrane (McDonald et al., 1978). Its primary function is to donate two electrons to a free radical (made by peroxides formed or $H_2O_2$). Vitamin E can be oxidized easier than the fatty acids in the cell membrane. Therefore, the protection is afforded while allowing the phospholipids in the cell to stay intact. However, vitamin E has several other functions such as aiding in tissue respiration (through electron transport system), phosphorylation reactions, nucleic acid metabolism, synthesis of vitamin C, and amino acid metabolism.

Absorption. The form of vitamin E and the route of supplementation are important in determining the amount of vitamin E available for body utilization (Nockels, 1979). There are eight naturally occurring forms of vitamin E.

These are divided into two groups depending upon the structural composition of the side chains. If the carbon bonds are saturated, vitamin E will be found in an alpha, beta, gamma or delta tocopherol form. If the carbon bonds are unsaturated, then the vitamin E will be found in an alpha, beta, gamma or delta tocotrienol form. In the saturated group, the alpha form is the most abundant and easily absorbed at the mucosal surface of the small intestine with a 20 to 30 percent efficiency of absorption (NRC, 1987). Beta, gamma and delta forms are absorbed at 25%, 10% and 1% of the alpha form, respectively. In the unsaturated group, only the alpha form is absorbed and at only 25% of the saturated alpha form (McDonald et al., 1978). The intestinal uptake, plasma transport, and liver uptake of vitamin E is specific for alpha tocopherol. However, if the level of alpha-tocopherol is low, the body will absorb gamma-tocopherol (Behrens and Madere, 1983). The uptake of vitamin E by the liver and other tissues is limited by a carrier and/or a binding site but not by the amount of lipid in the tissues (Behrens and Madere, 1983). The site of greatest absorption of vitamin E is in the jejunum. The tocopherols are absorbed by micelle formation in the presence of bile salts. Micelles are absorbed into the lymphatic system and are then transported with the lipoproteins. It is thought that

some tocopherols are also carried in the red blood cells. Both d- and l- isomers are absorbed but the d isomer is absorbed at a slightly faster rate. It is believed that humans can absorb about 10 to 36% of the ingested tocopherol and animals can absorb approximately the same amount (Church and Pond, 1982).

Interactions with other nutrients. Several nutritional factors can also affect the vitamin E requirements. Increasing the level of sulfur-amino acids may cause a sparing effect on vitamin E content. This may be due to the reducing properties of the sulfhydryl groups of the sulfur amino acids performing as vitamin E (Church and Pond, 1982). On the other hand, supplementing iron may cause a vitamin E deficient anemia if the vitamin is already present at a low concentration. It was also found that vitamin E can spare vitamin A in a deficiency. However, it was found that a high level of vitamin A will increase the vitamin E requirements, which was the principle behind the practice of supplementing vitamin E to help alleviate some of the vitamin A toxicity problems. Vitamin C has been shown to alleviate some problems caused by a vitamin E deficiency, but the mechanisms for this relationship are not clear. A choline deficiency, which may cause lipid peroxidation, can be prevented by the addition of vitamin E. Another role of vitamin E is

thought to be involved in the conversion of vitamin $B_{12}$ to its cobalamide coenzyme. A deficiency in zinc or vitamin E can cause the degeneration of the testes as well as an increase in the arachidonic acid in the lipids of the testes. Zinc is also believed to act similar to vitamin E in stabilizing the cell membrane (Church and Pond, 1982).

Placental and milk sources. The transfer of vitamin E to the fetus is very limited because it cannot cross the placenta readily (Church and Pond, 1982; Horwitt, 1986). Church and Pond (1982) reported that the amount of vitamin E that the neonate obtains from milk is variable dependent upon the amount of vitamin E in the diet of the sow. Therefore, if the diet of the sow is limited in vitamin E or if the ability of the sow to absorb vitamin E is limited, the amount of vitamin E available for the piglet to absorb is limited. Nielsen and coworkers (1973) reported that colostral milk had higher levels of vitamin E than post-colostral milk. They also reported that the levels of vitamin E in colostrum and milk increased when vitamin E was supplemented in the diet.

Dietary sources. Vitamin E is widely distributed in foods, especially in cereal grains, but it is low in animal byproducts (depending upon how much was fed in the diet). Modern grain storage practices and conditions (i.e. high moisture, added fat, aflatoxins from long time

storage) can decrease levels of vitamin E. Vitamin E is very unstable, especially in the presence of certain minerals and polyunsaturated fatty acids (Church and Pond, 1982). It has been reported that the storage of high moisture grains leads to the rapid destruction of naturally occurring vitamin E. However, it was discovered that the esterification of vitamin E to an acetate form will help decrease its naturally occurring oxidation (Church and Pond, 1982). Furthermore, mycotoxins in storage bins can cause even more vitamin E losses (Mortimer, 1983). In addition, the use of fat in the diets to increase the energy content and the increased use of cereal protein sources instead of animal by-products (e.g. fish meal) has also lead to the decreased amount of vitamin E found in the diet (Mortimer, 1983).

Selenium interactions. Selenium (Se) is involved very closely with vitamin E, and it can help spare vitamin E in several ways. First, Se can spare vitamin E requirements by maintaining the integrity of the pancreas, allowing the secretion of pancreatic lipase, thus assuring normal absorption of lipids and tocopherols in the gastro-intestinal tract (Church and Pond, 1982). Secondly, and most importantly, Se is a cofactor for the enzyme glutathione peroxidase, which helps prevent cell wall damage caused by free radicals that are made outside the

cell. When superoxide dismutase reacts with a superoxide ion and two hydrogen ions, a hydrogen peroxide molecule is formed. Glutathione peroxidase will reduce the $H_2O_2$ to $H_2O$ and the oxygen is removed by oxidizing NADPH to NADP. Also, in a vitamin E deficiency, glutathione peroxidase can prevent damage due to the release of lipid peroxides from the cell wall.

The effects of vitamin E and Se on the immune response are difficult to define because of the close relationship of vitamin E and Se in cell wall protection. For example, a vitamin E deficiency will decrease the killing capacity of phagocytized bacteria before a Se deficiency (Gyang et al., 1984). Also, increasing the vitamin E levels is believed to primarily increase IgG production (Tanaka et al., 1979; Larsen and Tollersrud, 1981).

It has also been suggested that a single time point measurement of vitamin E does not indicate the rate of uptake (Cipriano et al., 1982; Gabriel et al., 1984). Many researchers have suggested several parameters to evaluate the performance response of the animal to either a vitamin E deficiency or supplemented diet. These parameters include blood and liver Se concentrations and plasma vitamin E levels for the deficiency but some believe glutathione peroxidase and lipid peroxidation

might give better results (Simesen et al., 1982). Another group suggested that SGOT (serum glutamic oxaloacetic transaminase) would be a better indicator of the vitamin E status of an animal than plasma tocopherol levels (Cipriano et al., 1982).

**Immune response**

Background. The term immunity refers to all mechanisms [physical barriers (i.e. skin), chemicals (i.e. lysozyme in the tears), fever, or white blood cells] used by the body as protection against environmental agents which are "non-self" and are recognized as foreign to the body (Benjamini and Leskowitz, 1988). These agents may include feeds, chemicals, or drugs as well as bacteria and parasites. A substance which can induce an immune response is called an antigen (Ag). Most antigens have several common characteristics which include: 1) a molecular weight greater than 5,000, and 2) a distinctive molecular structure of at least four or five amino acids or monosaccharides which together form an "antigenic determinant" (Carpenter, 1977).

The immune response combines several of these mechanisms, all working to protect an organism from a bacterial, viral or "foreign" invasion. Although these mechanisms interact to afford protection, the immune

response is divided into two groups for convenience: a) the cellular aspect and b) the humoral aspect.

Cellular immunity. Once an antigen passes the first line of defense such as intact skin, secretions of mucous membranes, and pH of the skin, the body further tries to protect itself through cellular immunity. Cellular immunity involves polymorphonuclear neutrophils (PMN), eosinophils, and basophils. This group of cells make up 70% of all white blood cells (WBC) and are vital for the roles that they play in antigen trapping and destruction as well as clot removal. However, another group of WBC called lymphocytes, primarily the thymus cells (T-cells), are involved in eliciting an immune response.

WBC function to eliminate the antigen through several different courses of action: 1) ameboid movement, which allows the WBC to move in and out of the blood vessels freely; 2) chemotaxic response, which is the movement of WBC toward a chemical released by other cells (usually another WBC); 3) phagocytosis, which allows the WBC to surround and engulf a target; and 4) cytopenia, which kills the engulfed target via enzymes (lysozyme) or by-products such as $H_2O_2$, myeloperoxide and halide ($Cl^-$, $I^-$).

T-cells are the primary white blood cells that are involved in the immune response. The ontogeny of the T-cells begins in embryonic development with the stem cell

(from the yolk sac) migrating from the area of the gills to the thymus, which is a primary lymphoid organ (Figure 2). Several thymic hormones, including thymosin, Interleukin I and II, affect the immature cells when they migrate to the spleen and lymph nodes (called the secondary lymphoid organs) where further maturation occurs converting the immature cells to active T-cells. These active T-cells will further differentiate into several types of cells such as T-helper, T-suppressor, and T-cytotoxic cells, each performing a specific function. T-helper cells are involved with macrophages and B-cells in the production of an antibody. T-suppressor cells help keep the immune response from continuing to make antibodies after the challenge has been eliminated. T-suppressors also help to prevent the development of autoimmunity. T-cytotoxic cells (also called killer cells) have the ability to quickly determine the "self" from "non-self" cells as well as removing the foreign cells from the body.

Another type of cell that integrates cellular and humoral immunity is the macrophage. The ontogeny of the macrophage starts with the same stem cell as B- and T-cells, but the macrophage develops before other lymphocytes. Macrophages go through several divisions in the bone marrow and leave in the serum as monocytes. As

the monocyte enters the tissue, it becomes a macrophage. A macrophage will engulf and destroy antigens much like a PMN, but the macrophage can manipulate a larger number of antigens. Macrophages are also involved in the production of antibodies in two mechanisms. First, the macrophage can present determinant sites of an engulfed antigen to a B-and T-cell for the production of a specific antibody. Secondly, macrophages will produce a variety of soluble factors (interleukin I, II, and IV) which facilitate the communication among the T cell, B cell and macrophage to coordinate the production of antibodies.

Humoral immunity. The main function of humoral immunity is the production of antibodies (also called immunoglobulins) which promote the destruction and removal of specific antigens. For the production of an antibody to occur, B-cells must first develop into plasma cells. Communication between the cellular (T-cells and macrophages) and the humoral (B-cells) immunity is required for the transformation. This communication will result in the production of lymphokines (chemical messengers) which change the B-cell into the antibody producing plasma cell.

The humoral response involves the subpopulation of WBC called B-cells. In the embryo, B-cells start from the same stem cell as the T-cells, but instead of migrating to

the thymus, they migrate to the bursa in the chicken or the bursa-equivalent in mammals. This bursa-equivalent was once hypothesized to be in the GALT (gut associated lymphoid tissue) but is now thought to be in the bone marrow. After parturition and throughout life, these cells leave the appropriate organ after differentiation and then migrate to the spleen for further maturation. Once the B-cell has matured, it is called a plasma cell. After a series of steps that involve the T-helper cell and the macrophage, a plasma cell gains the ability to produce a specific antibody. An antibody (Ab) is specific for the particular antigen that initiated its formation. As a general rule, B-cells tend to be sessile (immobile) and are involved with the surface immunity (mucus membranes, lining of the gut) as well as intracellular immunity. In contrast, T-cells are thought to be continuously circulating via the blood system throughout the body.

Antibody structure. Immunoglobulins are glycoproteins that have molecular weights ranging from 145,000 to 950,000 daltons and contain 3 to 12% carbohydrate. Immunoglobulins confer immunity by several mechanisms, which include: 1) opsonization, which occurs when the antibody attaches to the bacteria or virus, allowing them to be readily engulfed by phagocytes; 2) complement fixation, which can lyse gram negative bacteria

and certain parasites; and 3) inactivation of viruses by occupying host receptor sites on the viral surface (Nisonoff, 1982).

Immunoglobulins (Ig) are found in the gamma globulin fraction of the blood serum. A basic immunoglobulin unit is a four chain structure with two heavy (H) chains and two light (L) chains (Figure 3). Antigen binding sites are made of amino acids from one H chain and one L chain thus providing two binding sites per Ig.

Each antibody can be divided for clarification into two halves, one half is called the variable region and the other half is called the constant region. Variable regions are responsible for the diversity of the Ig and involve both the H and L chain. Constant regions are identical for the type of Ig involved and involve only the H chain. Within the constant region, the amino acid sequence determines the class of Ig, which includes IgG, IgA, IgM, IgE, or IgD. IgG is the basic bivalent immunological unit which was described above. IgM is a pentamer (5 member) chain of the basic immunological unit arranged circularly. IgA is a dimer with two of the basic units bound together. IgA has a secretory piece around the hinge area where the two basic units are joined, which functions to protect the hinge area against proteolytic enzymes. The secretory piece provides enough protection

of the IgA so that, relative to other Ig, it can remain in the gut for a longer period of time without rapid degradation. IgD is a monomer and believed to be involved as an antigen receptor on the cell. IgE is also a monomer involved in allergic reactions.

Van der Waals and Coulombic forces enable antibodies to bind with the antigens. Van der Waals forces will allow the antibody and antigen to bond if there is a close spatial fit between the two. Coulombic forces will bind the two if the electrical charges between the spatial fit are complementary (Hyde and Patnode, 1978).

Most of the carbohydrates of an immunoglobulin are in the constant region of the H chain and are covalently bound to asparagine, serine or threonine. Carbohydrates may increase the solubility of the immunoglobulins, facilitate secretions from antibody producing cells and have some roles concerning complement (Nisonoff, 1982).

Passive immunity of piglets. The immune response of the fetal pig is functional by midgestation. However, the pig is immunologically naive. The immune system is not actively producing any protection until about ten days postpartum (Wilson, 1974), yet protection of the neonate from infection is a necessity. The neonate is protected by the passive immunity which is received by the

absorption of immunoglobulins from the colostrum of the mother.

Baby pigs are good models for studying the immune response because of the epitheliochorial placenta in the sow (Kim et al., 1966). This type of placenta does not allow immunoglobulins to pass through it causing the piglets to be born hypogammaglobulinemic. Without colostrum, the pig may die unless it is kept in a sterile environment. Colostrum not only contains an excellent source of nutrients necessary for the maintenance and growth of the neonate, but also provides a variety of Ig including IgG, IgM and IgA. These nutrients and Ig must be absorbed by the piglet through the gastrointestinal tract. Unfortunately, antigens (i.e. foreign proteins or bacteria) can also be absorbed at the same time. One way to prevent the absorption of these unwanted antigens is the development of specialized cells on the lining of the intestines called enterocytes. Certain enterocytes are developed at birth to absorb only Ig. Electron microscopy shows tubovesicular canals in the microvilli which carry the Ig to the basolateral membrane and ultimately to the circulation (Moran, 1982). Termination of Ig absorption may be due to the shedding and subsequent replacement of the enterocytes on the villi at birth. Enterocytes are continuously migrating from the crypts of the villi to the

top of the villi, but these new cells do not have the capacity to absorb Ig (Smith and Jarvis, 1978).

Most of the IgG fraction of the colostrum is absorbed via pinocytosis into the blood (establishing passive immunity), whereas the IgA fraction coats the lining of the gastro-intestinal tract, providing protection against antigens in the intestines. Most of the Ig are absorbed in the duodenum rather than in the jejunum or ileum (Allen and Porter, 1973). Approximately two-thirds of the IgG is absorbed, with the remainder of the IgG remaining in the gut (along with the IgA and IgM) where it can bind and neutralize antigens.

The ability of the newborn pig to absorb antibodies lasts for only a short time. Under normal conditions, the complete cessation of Ig absorption occurs about 36 hr postpartum (Lecce, 1973). Duration of absorption can be influenced slightly by giving either saline or water to the piglet, which slows down absorption. Conversely, absorption rate can be accelerated if feeding frequency is increased or diarrhea occurs (Lecce and Morgan, 1962).

Once absorbed, the Ig have a half life of about 10 to 20 d (Curtis and Bourne, 1973; Frenyo et al., 1981). Also as the baby pig grows older, there is an increase in body size and the amount of Ig in the blood becomes "diluted". Thus, a piglet must develop its own active immune response

to ward off an antigen invasion when the passive immunity begins to fail.

In colostrum deprived piglets, IgM and IgA are the first Ig to be produced. They are measurable at d 9 and 16 respectively, with IgM maintaining a greater concentration than IgA until d 23 (Yabkik et al., 1979). After one month, IgG has a greater concentration than either IgA or IgM.

Birth order also has some influence on immunity and subsequent survival rate. Hartsock and Graves (1976) found a positive correlation between birth order and percent survival. The piglets that were born first were allowed more time to ingest colostrum, were heavier at birth, and were the strongest aggressors (Hartsock and Graves, 1976).

## Vitamin E and the immune response

Diets supplementing vitamin E. The mechanism by which vitamin E influences the immune response is not yet fully understood. One of the factors hampering this understanding is the complex interrelationships of vitamin E with other nutrients, including selenium and polyunsaturated fat. Several hypotheses have been advanced to explain the mechanisms by which vitamin E may affect the immune response (Nockels, 1986). Vitamin E was

reported to increase the activity of T-cells (Tanaka et al., 1979; Larsen and Tollersrud, 1981). These researchers discovered that vitamin E stimulates helper T-cells more than the other T-cell populations, which in turn increases the cooperation between B- and T-cells. This increased cooperation will result in an increased humoral response. It is also thought that vitamin E accelerates the maturation of cytotoxic immunity (Lim et al., 1981). Campbell et al. (1974) reported that vitamin E stimulates the non-adherent cells (T-and B-cells) without the help of adherent cells (macrophage).

Barber et al. (1972) found an increase in antibody titer to an attenuated live virus vaccine when supplementing vitamin E in the diets of guinea pigs. They concluded that the stimulation of the immune response among different species is more closely related to the dose requirements, method of administration and form of vitamin E than it is to the nature of the antigen stimulus.

Additionally, supplemental vitamin E can help stimulate the transformation of WBC against mitogens. In agreement, Bendich et al. (1984) found that there was an increase in blastogenic response of splenocytes from guinea pigs to T- and B-cell mitogens due to supplemental vitamin E. Bendich et al. (1986) found that the immune

system is sensitive to changes in vitamin status. They reported that the mitogen response was highly correlated with plasma vitamin E levels ranging from .04 to 18 ug per ml in the rat. Bendich et al. (1983) also found that an increase in vitamin E increased the antibody production by the stimulation of T-helper cells.

Large doses of vitamin E were found to decrease the bactericidal activity of leukocytes by decreasing the $H_2O_2$ production and increasing the stability of the cell membrane (Prasad, 1980). These decreases are thought to be caused by vitamin E interference in the electron transport system in the phagocytic vesicles, which is required for the NADPH to form $H_2O_2$ via the hexose monophosphate shunt in the phagocytizing granulocytes (Baehner et al., 1982; Butterick et al., 1983; Gyang et al., 1984). However, Combs (1981) reported the opposite to be true. He suggested that the levels of vitamin E that prevented problems of exudative diathesis did not affect the enzymatic component of the oxidant defense system.

After feeding or injecting vitamin E into calves, Reddy et al. (1986) found that lymphocyte stimulation indices were higher in supplemented vitamin E animals than in unsupplemented animals. However, adding vitamin E directly to lymphocyte culture did not increase PHA

induced stimulation. These authors also found that animals given supplemental vitamin E from birth to 12 wk of age (orally or injected) had higher serum IgM levels until 6 wk of age. As IgM levels decreased, the animal increased $IgG_2$ production which remained at high concentrations.

A higher antibody response will be observed when vitamin E is supplemented through an injection containing an adjuvant (used to enhance the effectiveness of a medical treatment) than if vitamin E is supplemented in the diet (Tengerdy et al., 1983). Other groups determined that tocopherol acetate is more effective in changing the immune response than tocopherol nicotinate (Tanaka et al., 1979; Larsen and Tollersrud, 1981).

Supplemental vitamin E has not been found to affect blood chemistry or other hematological parameters with the exception of serum vitamin E levels (Prasad, 1980; Horwitt, 1986).

Diets deficient in vitamin E. Hamilton et al. (1977) found a decrease in the number of peritoneal cells in rabbits fed a vitamin E deficient diet compared with controls after an injection of mineral oil, but there was no evidence of depletion of WBC. They also found that PMN from deficient and control rabbits responded equally well to a chemotactic stimulus of E. coli toxins. They

concluded that a reduction in the inflammation response in an animal with a vitamin E deficiency was not due to a depletion of response cells or to a reduction in the capacity of PMN.

Rats fed a vitamin E deficient diet have been noted to have an increased mitochondria size (Bendich et al., 1983). This may explain the differences in energy production of the vitamin E deficient cells that are stimulated by mitogens. Lehmann and McGill (1982) also reported that swollen and deformed mitochondria were the problems of reticulocytes and lymphocytes in animals with a vitamin E deficiency, but they found that platelets were not effected. They also found an increase in the number of PMN in the blood of pigs and monkeys fed a vitamin E deficient diet. According to Nockels (1979), the deficiency also affects the primary response before the secondary response.

Campbell et al. (1974) found that vitamin E as well as some reducing agents including 2-mercaptoethanol(ME), can stimulate the non-adherent cells (T- and B-cells) without an accessory cell (macrophage). Eskew et al. (1985) also found similar results, in that blastogenic responses were decreased with vitamin E (and Se) deficient diets. This relationship may be explained due to the ability of vitamin E and 2 ME to replace the lymphocyte

activating factor (LAF or IL-2) which is usually provided by the macrophage (Corwin et al., 1981; Hoffeld, 1981; Gebremichael et al., 1984).

Harris et al. (1980) and Baehner et al. (1982) discovered that the PMN from vitamin E deficient animals had a decreased level of $H_2O_2$ but normal $O_2$-. PMN did not respond normally to chemotactic or phagocytic responses, but recovered quickly if vitamin E was added (5 and 18 hours respectively) (Baehner et al., 1982). Heinzerling et al. (1974) also found that vitamin E increased the phagocytic activity. They believed that the reason for the different results was that the other researcher used much lower levels of vitamin E in the treatments. Other researchers found similar results and concluded that vitamin E normally protects the lymphocytes from the products of phagocytosis which includes $H_2O_2$ and $O_2^-$ especially from the macrophage (Harris et al., 1980; Eskew et al., 1985).

A vitamin E deficiency has also been shown to decrease the catalyst (which removes $H_2O_2$ from the cell) activity of rat erythrocytes (Combs, 1981).

Lymphatic organ. A vitamin E and Se deficiency has been shown to decrease the bursa weight in chickens. This results in a decrease in the overall number of lymphocytes, especially in the primary immune organs

(Colnago et al., 1984; Marsh et al., 1986). Heinzerling et al. (1974), Bendich et al. (1983) and Carlomagno et al. (1983) suggested another way vitamin E may enhance antibody production is by increasing the size or amount of lymphopoietic tissue. The larger antibody titer was thought to be caused by increased cell proliferation within these tissues. These increases in antibody titers have been shown to protect chickens which had decreased bursa and spleen weights from an infection of Escherichia coli.

Additional interactions. Increasing levels of dietary vitamin E levels fed to 6 month old sheep produced a non-linear dose response in antibody production with the largest peak due to the highest level of vitamin E (476 mg per kg of feed). The authors also fed vitamin A in conjunction with vitamin E and found no effect of increasing levels of vitamin A on the immune response except when vitamin A was used as a carrier for the antigen (Ritacco et al., 1986).

It is also thought that vitamin E overcomes a soluble suppressor factor which is about 10,000 daltons in size (Corwin et al., 1981). This splenic suppressor factor is called a chalone which is chemically similar to spermine (Combs, 1981). Spermine interferes with $Ca^{++}$ binding. Eskew et al. (1985) believes this suppressor factor could

affect mitogen stimulation unless lymphocytes are extensively washed prior to culture.

The use of synthetic antioxidants such as ethoxyquin, butylated hydroxytoluene (BHT) and diphenyl-p-phenylenediamine (DPPD) may have similar action on the immune response as vitamin E. However, Tengerdy et al. (1972), after feeding an antioxidant to chickens, discovered a similar effect of increased phagocytes due to supplemental vitamin E, but the effects were less pronounced.

The ultimate effect of vitamin E depends on the ability of macrophage and neighboring cells to protect themselves from their environment (high $pO_2$) and from the products of the macrophage ($O_2^-$, $OH^-$, $H_2O_2$) by the endogenous protective mechanism that includes GSH, catalase, SOD and vitamin E (Hoffeld, 1981).

Toxic effects. There have been several papers which suggested either no effect or detrimental effects of supplemental vitamin E. Kurek and Corwin (1982) reported that a 10-fold increase in the amount of vitamin E did not result in a significant change in the number of sarcoma cells between mice fed a vitamin E supplemented or a vitamin E deficient diet. To the contrary, Lim et al. (1981) found that rats consuming a high vitamin E diet (20 times the control levels) for long periods of time

decreased lymphocyte response to PHA. In a review paper, Briggs (1976) found that prolonged high levels of vitamin E (120 to 200 X requirement) may induce sarcoma in rats, interfere with normal blood coagulation, and (some evidence) act as a teratogen (agent which causes fetal defects). Church and Pond (1982) cited a few published reports of vitamin E toxicosis in humans and animals. Several of these articles described the symptoms of large doses of vitamin E, which included hemorrhagic syndromes, nervous disorders, edema, and changes in the endocrine glands.

Findings of several studies reported in NRC (1987) demonstrated adverse effects of very high levels of vitamin E in animals and humans. The toxicity symptoms in chickens ranged from decreased growth rate to decreased calcium and phosphorus in plasma and dry fat-free bone ash. Rats also experienced problems ranging from a decreased in femur ash content to a significant decrease in the weight of the adrenal glands. Toxic effects of vitamin E in humans include minor symptoms like nausea, generalized dermatitis and fatigue. Studies also found a decrease in $T_3$ and $T_4$ in human females and males and an increase in serum triglycerides in females.

Vitamin E and prostaglandins. A group of hormone-like compounds called prostaglandins (especially the

family of $E_2$) may also have an influence on the immune response. Marsh et al. (1981) found that feeding vitamin E has several effects including influencing T helper cells and phagocytes, mitogen responsiveness and the level of prostaglandin synthesis. Corwin and Shloss (1980) also suggested that vitamin E will help in regulation of prostaglandins.

Because of its importance in the regulation of the immune response, research in prostaglandins has been rapidly growing. One group of investigators found that adding vitamin E can decrease the prostaglandin in lymphopoietic organs and thus enhance the immune response (Colnago et al., 1984; VanVleet and Wilson, 1984). Another group found that vitamin E directly affects the incidence and rate of tumor cells (Kurek and Corwin, 1982; VanVleet and Wilson, 1984). This action is believed to be a result of controlled prostaglandin synthesis and not the immune response. Stress also can make lymphocytes more sensitive to inhibition by $PGE_2$ (Goodwin et al., 1981). The addition of an inhibitor like indomethacin can restore the response, therefore suggesting that PG (an immunomodulator) is responsible for the decrease in the cellular immunity after physical stress.

Nutrition also can be involved; the basic precursor of prostaglandin is the fatty acid, arachidonic acid. The

dietary consumption of several essential fatty acids including arachidonic acid and linoleic acid (Sheffy and Schultz, 1979) and cholesterol, saturated fatty acids and certain lipoprotein fractions (Carlomagno et al., 1983) can regulate the immune response. This regulation can range from the suppression of the primary antibody response to reduced phagocytosis. Vitamin E may indirectly be involved as an antagonist of the precursor (arachidonic acid), which enters into the prostaglandin synthesis cascade. Vitamin E is thought to have a direct effect because of its influence on the plasma membrane fluidity. Baehner et al. (1982) found that arachidonic acid is released from PMN supplemented with vitamin E during phagocytosis. It is also thought that prostaglandins and oxygen intermediates work together in the regulation of the cytotoxic activity causing the suppression of lymphocyte proliferation (Hoffeld, 1981; Eskew et al., 1985). With the decrease in prostaglandin $E_2$, there is a decrease in the suppressive effect on the immune response which is due to the increase in Ia gene (accessary cell surface recognition marker e.g. macrophage) expression (Gebremichael et al., 1984). But the same group does not believe that PG is involved with the effect of vitamin E on lymphocytes stimulated with mitogens.

**Stress**

Causes. Stresses such as weaning, temperature extremes, crowding, mixing, limit feeding, noise, and restraint are sometimes placed upon the baby pig and may affect its immune response (Kelley, 1980). Stress is thought to cause immunosuppression depending upon the nature of the agent used. These immunosuppressors usually act as inhibitors of cell division.

Several compounds (histamine, hydrocortisone, prostaglandin and interferon) have been noted to decrease cell proliferation as seen by the decrease in $^{3}H$ thymidine uptake into the WBC (Goodwin et al., 1981).

Mechanisms on regulators of stress and immune response. Blalack (1984) in a review of the interaction of stress and the immune response, concluded that it is not a simple anatomical relationship between the immune and neuroendocrine systems. In fact it is very difficult to distinguish receptor signals within and between the neuroendocrine system and the immune response. Based upon several observations, he suggested that peptide hormones and lymphokines work by similar mechanisms. The first proof of this close relationship was the demonstration of innervation of the thymus, bone marrow, spleen and lymph nodes. Next, the cell-mediated immune response was proven to be significantly impaired in hypopituitary animals,

possibly due to the lack of growth hormone. Blalack (1984) suggested that changes in electrical impulses were found in the hypothalamus after a stimulation with an antigen. He also reported a series of experiments which proved that vasopressin and oxytocin could replace interleukin-2 requirements for interferon production.

Corticotrophin (ACTH), alpha-endorphin and enkephalin have also been suggested to be involved in the regulation of antibody production (Blalack, 1984; Johnson et al., 1984). Johnson et al. (1984) demonstrated that treating lymphocytes with inducers of interferon resulted in the cells releasing a ACTH protein which has endorphin-like activity . Interferon is produced by the T-cell and regulates several functions including 1) antibody production, 2) Ia antigen expression macrophage, 3) priming macrophage for tumor cell killing, and 4) activation of T-cells for expression of Il-2 receptors. However, Johnson and coworkers (1984) suggested that the activities of interferon are suppressed by the presence of ACTH, which may be important for compounds that cause the simultaneous release of interferon and lymphocyte derived ACTH. The ACTH produced may be acting as a regulator to keep the actions of interferon under control.

Effect of cortisol on the immune response. One group of immunosuppressors called lympholytic agents

includes X-rays and cortisol (also called glucocorticoid and corticosteroid). These immunosuppressors are thought to cause the destruction of lymphocytes. Their action can also cause damage to the chromosomes which impairs the capacity of surviving lymphocytes to be stimulated and undergo mitosis (Eisen, 1980).

The involvement of cortisol in the regulation of the immune response has been a controversial subject. Some scientists question research dealing with cortisol for they are not sure if the level of cortisol is a result of handling the animal to obtain a blood sample or if it is of real physiological value.

Several researchers have reported detrimental effects of cortisol upon the animal and its immune response (Eisen, 1980). Lim et al. (1981) have reported that increased levels of glucocorticoids in the serum will destroy immature T-cells. Young et al. (1975) found that levels of cortisol above physiological concentration will not affect the *in vivo* reactivity of chicken anti-SRBC hemagglutination. However, the addition of exogenous sources of the steroid preparation will result in different suppressive effects on the cellular and humoral response (Sato and Glick, 1970). Gwazdauskas et al. (1978) suggested that high cortisol levels (from stress) depressed the immune response. These researchers

concluded this effect occurs from experiments in which the differences in the antibody titers among test animals was related to the plasma glucocorticoid differences.

Another effect of glucocorticoids is to cause leucocytopenia especially of the macrophage as well as decreasing leukocyte diapedesis (thus creating a redistribution of the cells) (Parrillo and Fauci, 1979). Cortisol also causes a change in the ratio of the different subpopulations of T-cells. Lim et al. (1981) found that glucocorticoids decreased the number of T-helper cells. Furthermore, they found that cytotoxic activity differs at different sites (spleen and intestinal), possibly relating to the site of the vitamin E absorption. They also reported that animals fed high levels of vitamin E consistently had decreased cortisol levels. The authors concluded that vitamin E affected the adrenal glands. Another hypothesis suggested cortisol decreased the size of the lymphoid tissue (thymus and lymph nodes) resulting in less tissue. In turn, the number of lymphocytes decrease and therefore retard the formation of antibodies (Ganong, 1981). The increased cortisol levels have also been shown to decrease the response of T-lymphocytes to PHA and Con A (Watson, 1984).

Similarly, Quinn (1968) stated that the general agreement of other researchers is that cortisone decreases

antibody formation through the reduction of the size of lymphoid tissue (thymus and lymph nodes). Thus, smaller tissue sizes decreased the number of lymphocytes, and therefore, retarded formation of antibodies (Ganong, 1981).

While reviewing the literature on the effects of hormones on the immune response, a question must be considered: were pharmacological concentrations used instead of physiological doses of hormones? Westly and Kelley, (1984) looked at the difference in pharmacological and physiological concentration of cortisol. They found that physiological levels decreased mitogen-induced proliferation of lymphoid cells, but pharmacologic levels caused a significant but minimal cell death. They stated that it is unlikely that the lytic effect of cortisol will explain the decrease in the blastogenic assay.

Another inhibition mechanism of glucocorticoids was reported by Gordon and Nouri (1981). They suggested that glucocorticosterone inhibits T-cell proliferation by inhibiting a T-cell growth factor (interleukin). Interleukin is thought to be needed as a secondary signal that is required for mitogen activation.

Weaning. Weaning is a critical point in the life of a piglet. The environment of the piglet as well as the type of feed (animal or plant protein) is changed. Modern pig

producers must find the best time to wean in order to reduce stress thus optimizing the growth rate of the piglet.

Early weaning (3 to 4 wk) has adverse effects upon the piglet. One aspect that is affected is growth. Stanton and Mueller (1976) found that weaning at 21 d of age caused a stress which was expressed through increased anxiety, increased gastrointestinal dysfunction, and decreased body weight gain.

Blecha et al. (1983) found that weaning before 5 wk of age caused physiological changes that were detrimental to cellular immune reactivity. This was based on the observation of a decrease in an _in vivo_ PHA skin test reactivity and a decrease in an _in vitro_ blastogenic response of mitogen-stimulated lymphocyte reactivity in piglets weaned at 2, 3 and 4 wk of age, but not at 5 wk of age.

It has been suggested that the detrimental effects from early weaning will persist for at least 18 d (Stanton and Mueller, 1976). McCauley and Hartman (1984) found that the number of lymphocytes peaked (double the adult level) about 12 d after weaning.

Gwazdauskas et al. (1978) found that antibody production to porcine and equine red blood cells in beef calves (6 months old) was greater when the antigen was

given one week before weaning than when the antigen was given on the day of or one day after weaning. These researchers concluded that stress resulting from weaning may impair the function of the immune response.

Several environmental factors are interrelated with the problems of weaning. Blecha and Kelley (1981) found that weaning at 21 compared with 35 d of age reduced the antibody titers regardless of temperature exposure. Peplowski et al. (1981) suggested that a decrease in antibody at weaning may be due to a depletion in stores of both Se and vitamin E in piglets. These researchers also found this effect was accelerated by stress.

Early weaning can affect behavior of the piglets. Worsaae and Schmidt (1980) found that early weaning (three weeks) was a stress factor. They based this on their discovery of a positive correlation between plasma cortisol concentration and both aggressive and non-nutritive oral behavior, but a negative correlation between cortisol concentration and playing behavior. How ever, McCauley and Hartman (1984) suggested that cortisol levels may not be a good indicator of stress because of the experiments which reported that weaning gave a slight non-significant rise in the plasma cortisol levels.

Another problem at weaning is the mixing of different litters into large groups. This is thought to place more

stress upon the piglet and must be considered when designing a management scheme. Upon mixing, the piglets fight to establish a social order. This development of a pecking order increases stress and so it would be expected to decrease the immune response. Hartsock and Graves (1976) found that after farrowing, there was aggressive behavior between littermates at a rate of eight occurrences per hour at two hours of age. Kochleiser et al. (1979) observed this problem and suggested that intermingling of litters before they are weaned may help to alleviate some of the stress. It was emphasized that care must be taken to mix litters that are about the same size instead of mixing at the same age.

Temperature. Another stress that a baby pig encounters early in life is temperature extremes. Maintaining comfortable temperatures for the piglets may be costly due to the variability in heating and cooling the surroundings. Temperature is important because the baby pig has a very small store of body fat. This small amount of fat affords little insulation against the cold and provides little energy storage. The pig requires energy for nursing and growth which may be hampered if the energy is spent on thermoregulation. While the pig is in a thermoneutral condition, heat production rate depends

mostly on the amount of feed intake and metabolic body size. Physical activity is only a small factor.

Performance. Researchers (NRC, 1981) have suggested the average lower critical temperature of pigs in a group of nine on a well insulated floor is 13 to 14°C for grower size and 10 to 11°C for finishers. If the feed intake rate remains constant, the average daily gain is depressed by 15 g per °C decrease. However, if feed is ad libitum, pigs weighing 6 to 35 kg will eat more in a cold environment and so the daily gain will increase.

Heat stress will influence an animal differently than a cold stress. Most of the work (NRC, 1981) involving temperature is based on experiments around 32-38°C but these are daily maximums and not the mean of daily temperatures. Other research (Holmes and Close, 1977) has found that an increase in relative humidity by 18% at 30°C is equivalent to raising the temperature 1°C. Also, the effects of air speed, thermal radiation, housing and equipment factors and group size should be considered. Morrison and Mount (1971) noted that as the environmental temperature increases, the feed consumption decreases in pigs. Caution must be observed when feeding animals based upon daily intakes and not percentages (NRC, 1981).

Morrison and Mount (1971) found that a cold temperature will increase the rate of gain due to increase

feed consumption, but they found no differences in the feed to gain ratio.

Effect of temperature on immunity. Henken and coworkers (1982) suggested that stress from increased temperature would increase protein catabolism. This would in turn decrease the weight of the lymphoid organs. The authors suggested that any experiment which did not observe a decrease in lymphoid tissue weight due to a temperature treatment was still in the thermoneutral zone.

Aberle and coworkers (1974) defined some physiological effects from stress. They found that increasing the environmental heat of a pig to 35°C will increase the pH of the serum in heat-susceptible pigs, whereas blood $pCO_2$ and $pO_2$ decreased in both heat sensitive and insensitive animals. These changes may be due to the increase in body temperature, and thus cause an increase in respiration rate. They also found increased plasma protein levels which may help increase the buffering capacity of the animal to the blood parameter changes. In addition, they found that the level of cortisol increased with the first exposure to a temperature but not for the secondary exposure. The authors suggested that the stress-susceptible pigs had a less responsive adrenal cortex to exogenous ACTH.

Kelley et al. (1982b) investigated the effect of heat on 3 wk old calves. They showed that placing an animal in a heat stress (35°C) decreases the delayed-type hypersensitivity after 24 hr and after two wk. Exposing the same calves to a cold stress (-5°C) had more complex results. The delayed-type hypersensitivity increased in the first week but decreased in the second week. PHA skin sensitivity decreased after two weeks. This would suggest that the animals can tolerate a cold environment for a limited time before the immune response is impaired. Exposure of calves to cold temperature (which differed as much as 25°C from the controls) increased antibody titers (Blecha and Kelley, 1981) and serum gamma-globulins whereas weaning decreased gamma-globulin (Blecha et al., 1983).

Kelley et al. (1982a) found that if the environment is cold enough to induce hypothermia, piglets would still absorb colostral macromolecules. However, hypothermia increased the incidence and severity of diarrhea. It has been suggested by Parker et al. (1980) that cold exposed neonatal pigs spend more time trying to control body temperature than suckling, thus consuming less colostrum. This would decrease the amount of Ig and nutrients ingested.

Blecha and Kelley (1981) studied the effects of a cold environment on the antibody mediated response. They found that weaning reduced antibody titers regardless of thermal exposure. They also found that cold stress (0°C) increased antibody titer regardless of weaning. However, the cold stress elevated antibody titers more than weaning depressed it.

The effect of different kinds of stress on the immune response of weanling pigs is not well understood. Therefore more work is needed in quantifying these effects using more controlled experiments, including multiple stresses (Kelley, 1980; Dantzer and Mormede, 1983; Blecha et al., 1983; McCauley and Hartman, 1984).

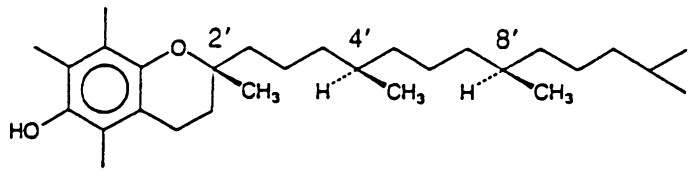
Summary

The relationship of how nutrition and the environment affect the immune response still poses many questions. It has been demonstrated that levels of vitamin E above those recommended by NRC is helpful in developing a functional immune response. At a higher level, vitamin E may be very useful in helping a neonate successfully fight for survival during times of various stress.

Therefore, the objectives of these experiments were to: 1) determine if stress that might be due to weaning or change in housing temperature is detrimental to humoral

and cellular immunity in young pigs, 2) determine whether supplemental dietary vitamin E will alleviate the problem caused by these stresses and 3) to characterize vitamin E in the milk and serum of lactating sows and in the serum of their nursing pigs.

**d-alpha-Tocopherol**




| | |
|---|---|
| Common names: | d-α-Tocopherol, Natural vitamin E |
| Trivial name: | RRR-α-tocopherol |
| IUPAC name: | 2R, 4'R, 8'R-α-tocopherol |
| Chemical name: | 2,5,7,8-Tetramethyl-2'-(4',8',12'-trimethyltridecyl)-6-chromanol |
| Molecular weight: | 430.69 |
| Empirical formula: | $C_{29}H_{50}O_2$ |

Source: Horwitt, 1988

Figure 1. Structure of vitamin E.

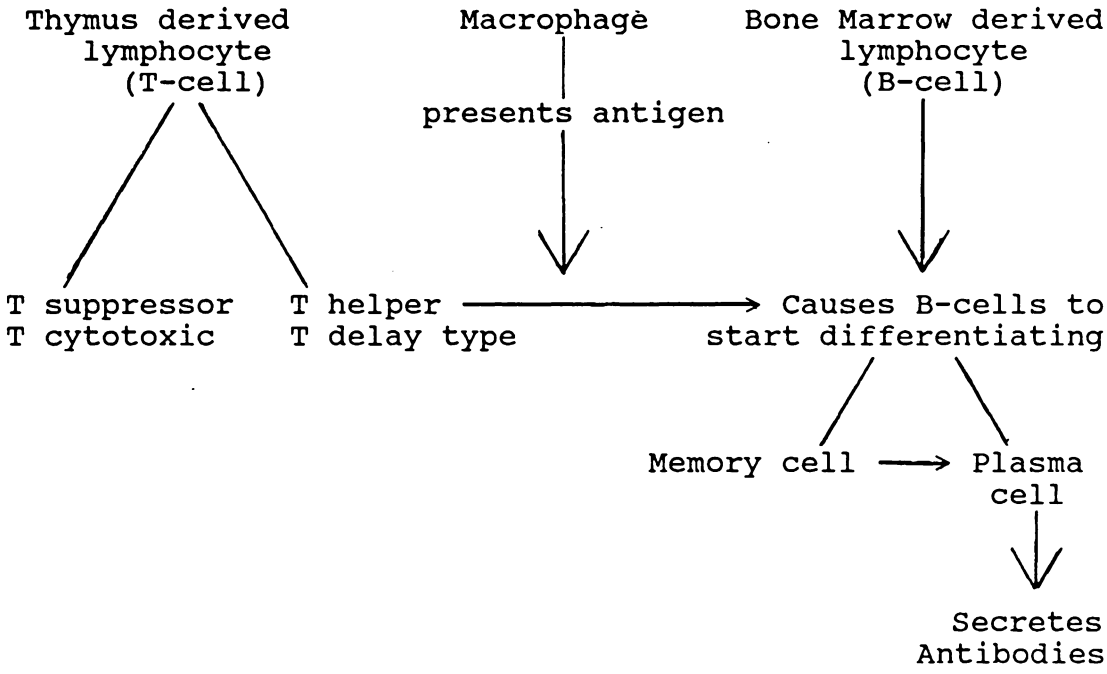
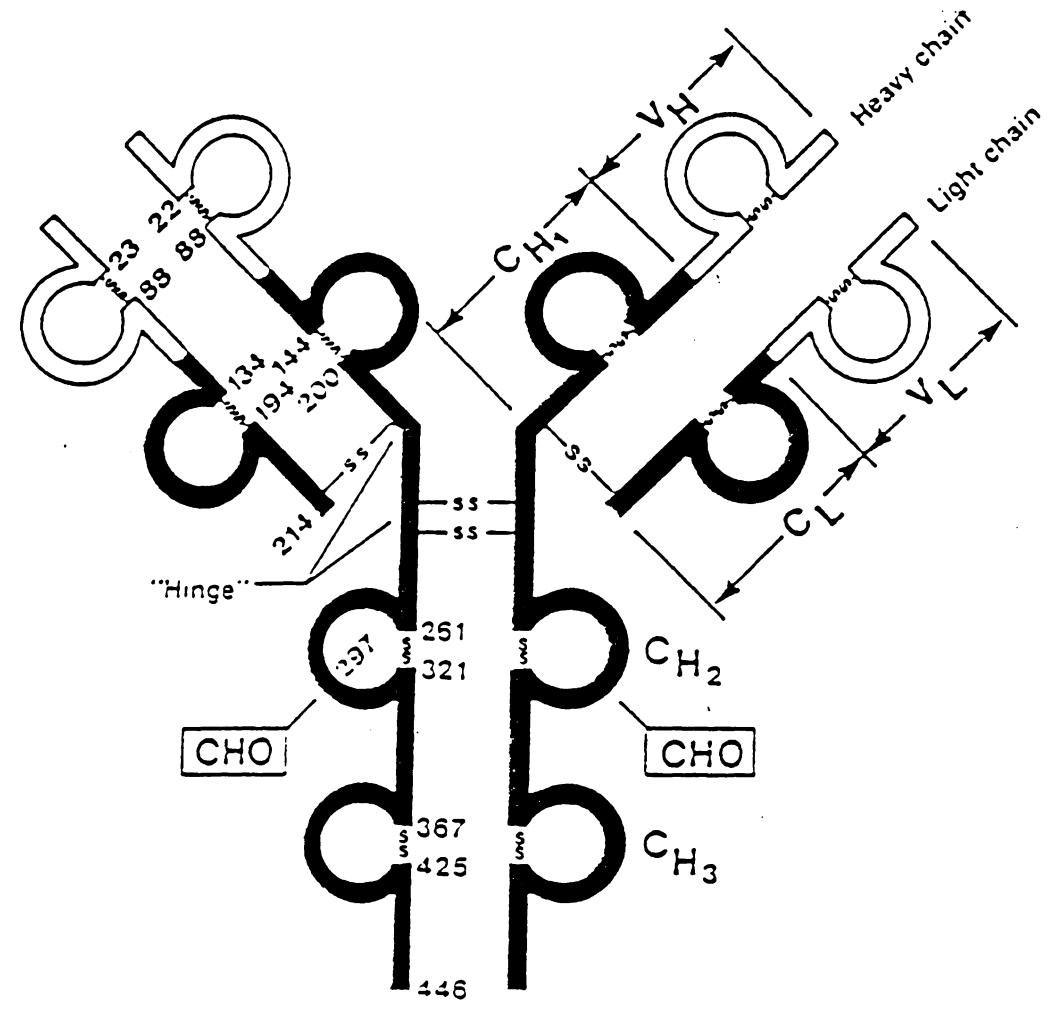



Figure 2. Cellular interactions of the Immune Response




From: Eisen, 1980.

Figure 3. Structure of an antibody.

CHAPTER III

Running Head: Vitamin E and Weaning Age Effect on Pigs

# INFLUENCE OF SUPPLEMENTAL VITAMIN E AND WEANING AGES ON PERFORMANCE, HUMORAL ANTIBODY PRODUCTION AND SERUM CORTISOL LEVELS OF PIGS

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## ABSTRACT

In three trials, 156 Yorkshire X Hampshire X Duroc crossbred pigs (average initial weight, 7.9 kg) were conducted to evaluate the effects of supplemental dietary vitamin E (11 vs 220 IU/kg diet) and weaning age (21, 28 or 35 d) on the performance and immunocompetence of pigs. Supplemental (220 IU per kg diet) vitamin E increased serum concentration of vitamin E in all animals. However, supplemental vitamin E did not affect performance, serum cortisol concentration or the primary and secondary antibody response to sheep red blood cells. As weaning age increased, weekly ADG and avg daily feed intake increased linearly; also, cortisol levels linearly increased over time for pigs weaned at 21 and 28 d, with

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the pigs weaned at 35 d of age having higher values. Pigs weaned at 35 d had a higher primary response to sheep red blood cells than pigs weaned at 21 and 28 d of age, but this effect did not carry over to the secondary response. There were no interactive effects of vitamin E level and weaning age. In summary, supplementing vitamin E increased the serum vitamin E concentration but did not affect performance, cortisol levels or antibody titers. However, pigs weaned at an older age (35 d) showed an increase in weekly ADG and avg daily feed intake and gave a greater primary immune response.

(Key words: Pigs, Vitamin E, Weaning Age, Immune Response.)

## Introduction

In many modern production systems, pigs are weaned at 21 to 28 d of age to increase the number of farrowings per sow per year and to more efficiently use high investment confinement facilities and equipment. While early weaning often increases sow productivity, it is also often associated with poor postweaning performance. Suboptimal performance of pigs following weaning may be due to poor nutrient utilization (Leibbrandt et al., 1975) as the pig is transferred from a liquid diet (i.e. milk) to a solid nursery diet. Other factors involved in poor postweaning

performance include low disease resistance (Inoue et al., 1978) and environmental stress (Kelley, 1980). The administration of a high level of vitamin E may be one method that could be used to improve a depressed immune response of a stressed pig (Ellis and Vorhies, 1976; Reddy et al., 1986). The National Research Council (NRC, 1979) recommended amount of vitamin E in a diet appears to be adequate to prevent the onset of disease symptoms, but some recent research (Heinzerling et al., 1974 [in chickens]; Peplowski et al., 1981 [in pigs]) suggests that a vitamin E level 6 to 20 times higher than that previously recommended is needed for an optimal immune response.

The objectives of this study were to determine if differences in weaning ages could have an antagonistic effect on the humoral immune response and to determine if supplemental dietary vitamin E can alleviate problems associated with weaning.

## Materials and Methods

In three trials, 156 Yorkshire X Hampshire X Duroc crossbred pigs (average initial weight, 7.9 kg) were conducted to evaluate the effects of supplemental dietary vitamin E and weaning age on the performance and immunocompetence of pigs. A 2 X 3 factorial arrangement

of treatments incorporated two dietary vitamin E treatments: 1) NRC recommended level of vitamin E - 11 IU per kg feed or 2) 20 X NRC - 220 IU vitamin E per kg feed; and three weaning ages (21, 28 and 35 d). The 20% CP basal diet (ground corn, soybean meal and dried whole whey) contained .2 ppm Se (Table 1).

At 21 d of age, six piglets within a litter were paired by weight and randomly assigned to one of three weaning ages. Dietary vitamin E treatments were randomly assigned within each pair of pigs. Sexes were equalized across diets. On the assigned weaning day, the designated pigs were removed, leaving the remaining pigs (including unassigned piglets) with the sow to nurse until their assigned weaning day. Sows were fed a complete diet with 11 IU of added vitamin E and .2 mg of added Se per kg diet. Pigs were weaned, mixed with piglets from other litters, and moved to the nursery pens before blood samples were obtained and an antigen mixture[3] was injected. Pigs were housed at 30°C in raised deck nursery pens and provided feed and water ad libitum. Weekly and cumulative ADG, avg daily feed intake and efficiency of feed utilization were calculated for five weeks.

[3]A 1 ml mixture of 10 $\mu$l packed sheep RBC, .1 ml Freund's adjuvant, .8 ml physiological saline, .1 ml AlOH and MgOH mixture.

Serum samples were collected initially and weekly for 5 wk after weaning and frozen (-20°C) for later determination of serum vitamin E, cortisol, and antibody levels. Animals were exposed to light continuously for 24 h per day, handled at the same time of day when obtaining blood samples, and in the same order to reduce variation in cortisol levels within the pigs. Humoral immune response was measured by determining the response to an antigenic challenge injection at the time of weaning and again at 17 d postweaning (Appendix Table 3).

Antibody titers to sheep red blood cells (RBC) were measured using a hemagglutination assay (Schurig et al., 1978; Appendix Table 4). Cortisol levels were analyzed using a radioimmunoassy (RIA) kit[4] (Appendix Table 5). Serum and feed vitamin E content were determined in an independent laboratory using a high performance liquid chromatography (HPLC) procedure (Bendich et al., 1984). Feed samples were analyzed for selenium by hydride generation and atomic absorption spectroscopy (Brodie, 1979).

Data were analyzed using the GLM procedure of SAS (1986). The model included all main effects (diet, weaning age, wk, trial, and individual pig), all main effect two and three way interactions and error terms

[4]Amersham Corporation, Arlington Heights, IL.

(Appendix Table 6). Pen was the experimental unit of performance parameters. for the other measured parameters, pig was the experimental unit because each litter contained each of the treatment combinations. Orthogonal polynomials were used to define the response curves for the main effects over time. Time was expressed on two different scales with the time origin at either 1) the start of the test regardless of chronological age or 2) based on pig chronological age regardless of weaning treatment. Residual correlations between cortisol concentration and animal weight for each wk were obtained.

## Results and Discussion

Diets were analyzed for vitamin E and found to contain 12 and 235 IU per kg diet for diets 1 and 2, respectively. Both diets were found to contain .2 ppm selenium.

All data related to vitamin E treatment were reported related weaning regardless of chronological age. Similar results were obtained when the data were analyzed using the chronological age (regardless of weaning age), but, data will not be shown.

Supplementation of dietary vitamin E to weanling pigs did not influence performance (Table 2). Avg daily feed, ADG and efficiency of feed utilization were not affected.

Similar values and results were noted by Peplowski et al. (1981) and Meyer et al. (1981).

Weaning treatments did, however, influence the performance parameters (Table 2). The pigs weaned at a later age had additional time to grow under less stressed conditions (still nursing) and thus were heavier (6.0, 7.6 and 10.1 kg for 21, 28 and 35 d of age, respectively ) at weaning. It follows that older animals would have higher daily gains and would consume more feed over the experiment. However, efficiency of feed utilization was not influenced by weaning age over the duration of the experiment.

Increasing the dietary level of vitamin E increased the serum concentration of vitamin E ($P < .01$; Table 3) which agrees with results of other experiments (Peplowski et al., 1981; Blodgett et al., 1988). However, the magnitude of the increase in serum vitamin E was not the same as the increase in the diet. This relationship was also reported by Rousseau et al., 1957.

Regardless of dietary vitamin E treatment, serum vitamin E levels were highest at weaning and lowest at wk 1 (Table 3); however, the magnitude of the drop was much less for pigs fed the higher dietary vitamin E level (Figure 1). Mahan and Moxon (1980) also reported that serum vitamin E concentrations of nursing animals (similar

to the values in this experiment) were high as compared with the lower levels of vitamin E noted when pigs were weaned and fed a diet deficient in vitamin E. In this experiment, high initial vitamin E levels were possibly due to the high availability of vitamin E in the milk. Serum vitamin E concentration in the following week dropped as animals were switched from milk to dry feed with the commonly observed low feed consumption.

In our experiment, the levels of serum vitamin E after 1 wk on test reflected the amount of vitamin E supplied in the diet (Table 3). Vitamin E absorption is similar to the mechanism of fat absorption such that the increased levels in the diet would be expected to increase levels in the blood (Church and Pond, 1982).

In general, regardless of weaning age, serum vitamin E levels in pigs fed the 20X NRC diet were higher ($P < .01$) than those in pigs supplied with the NRC level (Figure 1). Serum vitamin E levels of pigs weaned at 28 d of age were similar to values in other experiments (chapter 4, Figure 2, Bonnette et al., 1988) in which all pigs were weaned at 28 d of age. An interactive effect of dietary vitamin E and weaning age was observed. Pigs fed the NRC level of vitamin E had approximately the same serum vitamin E values regardless of weaning treatments. However, animals fed the 20X NRC level of vitamin E had

different levels of serum vitamin E over time, depending upon the weaning treatment (Figure 1). Pigs weaned at 21 d of age had an large increase in serum vitamin E after wk 1 on test; pigs weaned at 28 d of age had an large increase after wk 2 on test and pigs weaned at 35 d of age had an large increase after wk 1. Initial piglet serum vitamin levels may have been especially sensitive to the amount of milk (and therefore vitamin E) produced by the sow because the pigs were not creep fed.

Supplementing vitamin E in the diet did not affect the mean overall level of cortisol in the blood, or the level at any time postweaning (Table 3). However, cortisol values differed ($P < .01$) between weaning treatments over time (Table 4). Cortisol levels were high initially, decreased to the lowest value the following week, and then linearly increased until termination of the trial, finishing at approximately the same level as the initial week on test (Table 4). Pigs weaned at 21 and 28 d of age had similar cortisol values except for wk 5. Pigs weaned at 35 d of age had the highest levels until wk 3, then similar levels were observed in wk 3 and 4. At wk 5 cortisol levels were similar to wk 1 and higher than observed for pigs weaned at 21 and 28 d. Cortisol values in this experiment were similar to the normal baseline cortisol value (13 ng per ml serum) which were produced

with added adrenocorticotropic hormone (Westly and Kelley, 1984).

The large initial cortisol values are undoubtedly due to the combination of several factors, including weaning, moving, bleeding, injecting, mixing with non-littermates, and fighting associated with the development of a social hierarchy. Many of these factors have been hypothesized to cause stress (Kelley, 1980). These results are in agreement with research by Worsaae and Schmidt (1980) which suggested that weaning animals at older ages is associated with increased cortisol levels. This initial increase was also noted by Lim et al. (1981), who reported a continuous increase in cortisol levels until mice were 17 wk of age, after which there was a steady decrease.

Antibody titers to sheep RBC were not influenced by supplementing vitamin E in the feed (Table 3), but they followed a typical antibody response curve (Figure 2). No significant antibody titers to sheep RBC were noted in the initial sample, which would be expected because the animals had not previously been exposed to the antigen. After the initial blood samples were taken, the antigen mixture was injected. A primary response to the antigen was noted as shown by the large increase in antibody titers at wk 1 and the decrease at wk 2 as the antigen was being processed by the immune system. The next exposure

of antigen at d 17 resulted in a secondary response as seen by the increase in antibody titers at wk 3, 4 and 5. The response was higher because memory cells produced from the primary response would have helped produce the antibody faster (Benjamini and Leskowitz, 1988).

In contrast to our findings, Peplowski et al. (1981) reported that supplementing vitamin E (220 IU per kg diet) caused a large increase in the antibody titer of weanling pigs. The differences between studies may have been due to the initial high level of serum vitamin E (.20 $mg \cdot dl^{-1}$) of pigs in our studies as compared with the low initial serum vitamin E levels (.04 $mg \cdot dl^{-1}$) in pigs in the study reported by Peplowski et al. (1981). The low serum vitamin E concentrations in the piglets were probably due to 1) a limited capacity of the placenta to transfer vitamin E to the fetus (Nitowsky et al., 1962) and 2) the relationship between low dietary vitamin E supplied and the small concentration of vitamin E in the colostrum and milk of the lactating sow (Nielsen et al., 1973). Sows in the study by Peplowski et al. (1981) were not given any vitamin E for several generations whereas the sow in our studies were given the NRC recommended levels of vitamin E.

Differences in antibody response among various studies involving supplemental vitamin E may also be due

to the concentration of antigen, frequency of injection, and the type of adjuvant used (Blodgett et al., 1988). For example, in our study we did not note any antibody titer differences between dietary treatments when the pigs were injected IM with sheep RBC at d 0 and d 17 of the trial. However, Peplowski et al. (1981) noted an increase in antibodies with supplemental vitamin E when they injected sheep RBC weekly for 6 wk intraperitoneally without an adjuvant. Also, Ellis and Vorhies (1976) noted an increase in antibody titers when older pigs (weaned at 6 to 8 wk) were supplemented with NRC or 5X NRC levels of vitamin E and injected IM on d 14 and 35 with Escherichia coli suspended in physiological saline containing .5% formalin.

Considering that our sows were fed a NRC recommended level of vitamin E, the initial blood sample from pigs should reflect routine serum vitamin E levels. The drop in serum vitamin E levels in pigs fed the NRC diet (Figure 1) postweaning may suggest that feeding approximately 70 IU of vitamin E per pig per day (20X NRC) in the first week and 140 IU of vitamin E per pig per day in the second week after weaning (based upon feed intake) is needed to maintain serum vitamin E similar to those of nursing pigs.

In some related work, Marsh et al. (1986) suggested that the primary lymphoid tissues were the main target for

a vitamin E deficiency, and Bausted and Nafstead (1972) suggested that vitamin E was a hematopoietic factor in newborn pigs. Thus, the lack of differences in this experiment in antibody titer or cortisol may be due to the antigens being injected while the tissue stores of vitamin E were sufficient (until approximately 3 wk) and therefore lymphoid tissues were unaffected. Experiments to observe longer term exposure to an antigen may be necessary.

Antibody levels were influenced by weaning treatments over time (Table 4). Initial antibody titer in all three weaning ages were at low background levels, which was expected for pigs not exposed to the antigen. After the first injection of the antigen at d 0 postweaning, antibody titers were the highest for the 35 d weaning treatment and remained high until after the secondary injection of antigen at d 17. At wk 3, the beginning of a secondary response, differences among weaning ages were not significant ($P > .10$). Our findings generally agree with results of Blecha et al. (1983). They suggested that weaning before 5 wk of age resulted in a depression of antibody titers. After wk 5, the titers were much higher. Part of this depression may be due to the decrease in passive immunity because of natural catabolism of the immunoglobulins with slow replacement (Kelley, 1980) and due to the dilution of immunoglobulins because of

increasing body size. Also, the active immunity is developing in the pig at 3 to 4 wk of age (Peplowski et al., 1981).

Several other researchers have suggested that weaning will decrease the immune response in an animal. Gwazdauskas et al. (1978) reported a decrease in antibody titers to porcine and equine RBC when animals were injected within 24 h of weaning. Blecha and Kelley (1981) noted a decrease in titers to sheep RBC in 5 wk old pigs immunized 24 h before weaning compared with littermates weaned 2 wk before immunization. Also, Haye and Kornegay (1979) reported that 20 d old pigs had a decrease in antibody response to sheep RBC injected at weaning compared with animals injected 5 d before weaning.

A functioning immune response at weaning is necessary, especially if it is a management practice to vaccinate at the time of weaning (Blecha and Kelley, 1981). If the immune response is compromised at this time, the long term effect is a continuously low antibody response.

In summary, supplementing dietary vitamin E to weaned pigs in excess of the NRC recommendation increased serum vitamin E but had little affect on performance, cortisol concentrations or primary and secondary antibody responses. Weaning pigs at an older age caused a linear

increase in daily gain and daily feed intake but not efficiency of feed utilization. Cortisol concentrations, antibody titers, and serum vitamin E levels were influenced by weaning pigs at 21, 28 and 35 d of age. Cortisol levels were higher as the weaning age increased. Pigs weaned at 35 d had higher primary immune responses than the other pigs, but no differences were noted in secondary responses. Serum levels of vitamin E decreased in all three weaning ages from weaning to wk 1. Afterwards, serum vitamin E increased over time for pigs which were fed the 20X NRC levels of vitamin E, with a large increase noted in a 21 d weaning group beginning with wk 1 on trial, in the 35 d treatment group with 1 wk and in the 28 d weaning group after 2 wk.

TABLE 1. COMPOSITION OF BASAL DIETS[a] FOR TRIALS 1, 2 AND 3

| Ingredients | Percentage |
|---|---|
| Ground corn | 59.54 |
| Soybean meal (44% CP) | 27.73 |
| Dried whole whey | 10.00 |
| Defluorinated phosphate | 1.03 |
| Limestone | .75 |
| Salt | .30 |
| Vitamin premix[b] | .25 |
| Trace mineral premix[c] | .10 |
| Antibacterial[d] | .25 |
| Se premix[e] | .05 |

[a]Calculated to contain 20% CP, .8% Ca, .6% P and analyzed to contain .2 ppm Se.
[b]Supplied per kg of diet: 4,400 USP vitamin A, 440 IC vitamin $D_3$, 11 IU vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 23.9 mg d-calcium pantothenic acid, 22 mg niacin, 489.5 mg choline as 506.8 mg choline chloride, .022 mg vitamin $B_{12}$, 1.1 mg menadione dimethylpyrimidol, .44 mg d-biotin.
[c]Supplied per kg of diet: .02% Zn, .01% Fe, .006% Mn, .001% Cu, and .0002% I.
[d]Supplied per kg of diet: 44 g chlortetracycline, 22 g procaine penicillin and 4.4 % sulfamethazine.
[e]Calculated to supply .2 mg of Se per kg of the diet.

TABLE 2. EFFECT OF VITAMIN E SUPPLEMENTATION AND WEANING AGE ON OVERALL PEN PERFORMANCE LEAST SQUARES MEANS OF WEANLING PIGS IN TRIALS 1, 2 AND 3

| | Supplemental vitamin E[a] | | | Weaning age[b] (d) | | | |
|---|---|---|---|---|---|---|---|
| Item | X[c] | 20X | SE | 21 | 28 | 35 | SE |
| Avg weight (kg) | | | | | | | |
| Initial | 8.0 | 7.9 | .65 | 6.0 | 7.6 | 10.1 | .26[d] |
| Final | 21.8 | 21.5 | 1.26 | 17.8 | 21.4 | 25.7 | .55[d] |
| Avg daily feed intake (kg) | .74 | .73 | .04 | .64 | .73 | .82 | .03[d] |
| Avg daily gain (kg) | .40 | .39 | .07 | .34 | .39 | .45 | .05[d] |
| Feed/gain | 1.88 | 1.88 | .24 | 1.93 | 1.87 | 1.85 | .20[d] |

[a]Number of observations; thirty pens, eighty-one pigs per diet.
[b]Number of observation; twenty pens, fifty-four pigs per weaning treatment.
[c]Recommended NRC levels of vitamin E (11 IU per kg).
[d]Weaning age effect ($P < .01$).

TABLE 3. EFFECT OF SUPPLEMENTAL VITAMIN E ON LEAST SQUARES MEANS OF SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL AND ANTIBODY TITERS FOR TRIALS 1, 2 AND 3 EXPRESSED BY WEEK

| Item | Supplemental vitamin E | Across Weeks | | Week[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Means | SE | 0 | 1 | 2 | 3 | 4 | 5 | SE |
| Serum Vitamin E ($mg \cdot dl^{-1}$) | | | | | | | | | | |
| | X[b] | .09 | | .22 | .07 | .07 | .06 | .07 | .07 | |
| | 20X | .24 | .01[d] | .20 | .16 | .20 | .25 | .29 | .32 | .01[e] |
| Cortisol ($ng \cdot dl^{-1}$) | | | | | | | | | | |
| | X | 14.7 | | 18.1 | 8.6 | 10.6 | 14.5 | 17.0 | 19.4 | |
| | 20X | 13.8 | .55 | 17.6 | 9.0 | 11.1 | 12.9 | 14.8 | 17.3 | 1.16 |
| Antibody Titer[c] | | | | | | | | | | |
| | X | 2.47 | | .07 | 1.67 | 1.88 | 3.11 | 4.12 | 3.96 | |
| | 20X | 2.39 | .08 | .18 | 1.69 | 1.50 | 2.77 | 4.30 | 3.90 | .18 |

[a]Number of observations; seventy-eight pigs in each diet treatment, thirty pens; and analyzed by number of week on test.
[b]Recommended NRC levels of vitamin E (11 IU per kg).
[c]Injected with sheep red blood cells (.1 ml per pig on d 0 and d 17) and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Diet effect ($P < .01$).
[e]Diet X week interaction effect ($P < .01$).

TABLE 4. EFFECT OF WEANING AGE ON LEAST SQUARES MEANS OF SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL AND ANTIBODY TITERS FOR TRIALS 1, 2 AND 3 EXPRESSED BY WEEK

| Item[a] | Weaning age (d) | Across Weeks | | Week | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Means | SE | 0 | 1 | 2 | 3 | 4 | 5 | SE |
| Serum Vitamin E ($mg \cdot dl^{-1}$) | | | | | | | | | | |
| | 21 | .17 | | .18 | .14 | .17 | .16 | .17 | .20 | |
| | 28 | .16 | | .23 | .12 | .12 | .13 | .17 | .20 | |
| | 35 | .16 | .01 | .21 | .10 | .12 | .17 | .18 | .20 | .01[c] |
| Cortisol ($ng \cdot dl^{-1}$) | | | | | | | | | | |
| | 21 | 12.1 | | 13.2 | 6.3 | 9.4 | 14.1 | 15.1 | 14.7 | |
| | 28 | 13.6 | | 15.1 | 7.6 | 9.2 | 14.7 | 16.6 | 18.2 | |
| | 35 | 17.0 | .67[b] | 25.2 | 12.5 | 13.9 | 12.5 | 16.0 | 22.0 | 1.37[c] |
| Antibody Titer[d] | | | | | | | | | | |
| | 21 | 2.31 | | .18 | .85 | 1.38 | 3.07 | 4.22 | 4.15 | |
| | 28 | 2.24 | | .31 | 1.67 | 1.37 | 2.54 | 3.97 | 3.60 | |
| | 35 | 2.74 | .10[b] | .00 | 2.53 | 2.32 | 3.22 | 4.45 | 4.02 | .21[c] |

[a]Number of total observations; fifty-two pigs, thirty pens in each weaning treatment.
[b]Weaning age effect ($P < .01$).
[c]Weaning age X week interaction ($P < .01$).
[d]Injected with sheep red blood cells (.1 ml per pig on d 0 d 17) and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

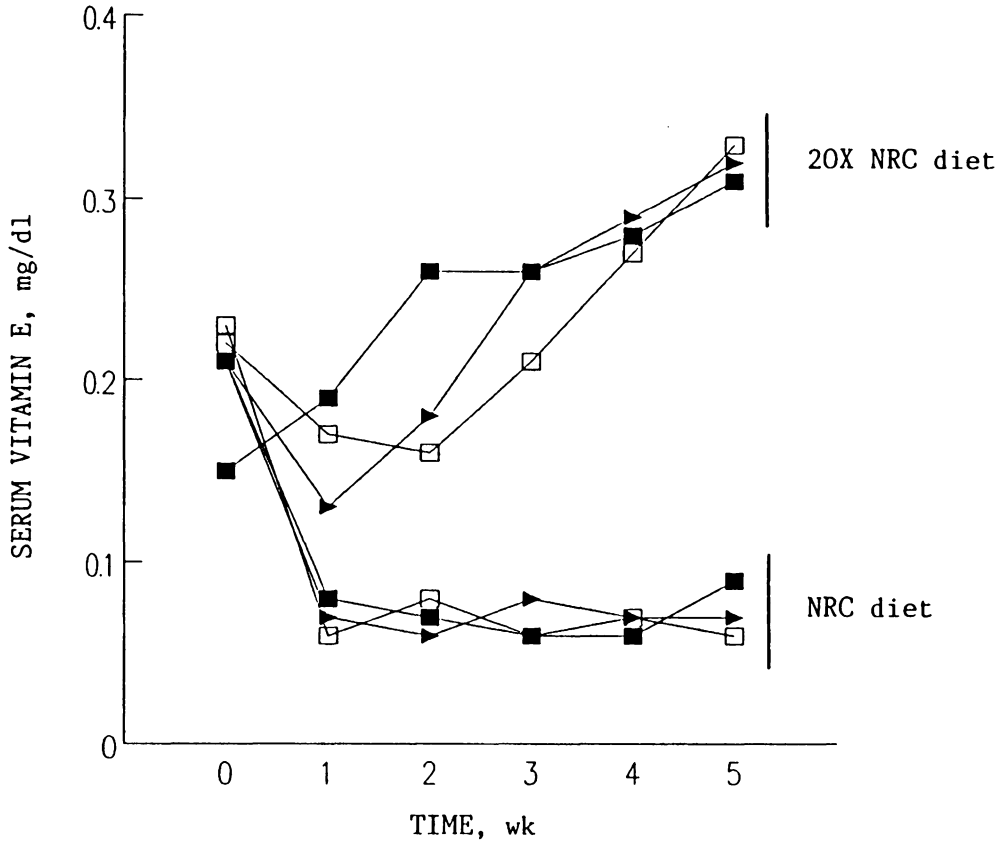


Figure 1. Effect of weaning age X diet interactions on serum vitamin E concentration expressed by wk. SEM = .02.

Weaning ages: —■— 21 d —□— 28 d —▶— 35 d

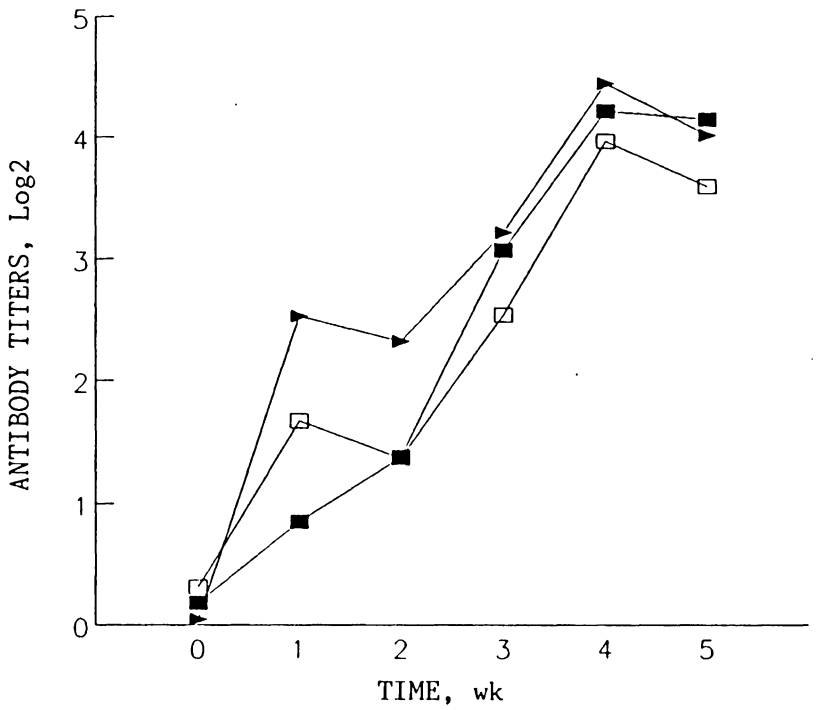


Figure 2. Effect of weaning age on the antibody titers to sheep red blood cells expressed by wk. SEM = .21

Weaning ages: —■— 21 d —□— 28 d —▶— 35 d

CHAPTER IV

Running Head: Vitamin E and Temperature Effects on Pigs

# HUMORAL AND CELL-MEDIATED IMMUNE RESPONSE AND PERFORMANCE OF WEANED PIGS FED FOUR SUPPLEMENTAL VITAMIN E LEVELS AND HOUSED AT TWO NURSERY TEMPERATURES.

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## ABSTRACT

Three trials using 80 Yorkshire X Hampshire X Duroc crossbred pigs (6.9 kg initial wt) were conducted to evaluate effects of four dietary vitamin E levels (11, 110, 220, and 550 IU per kg of feed) on the humoral and cell-mediated immune response and performance of 4 wk old weanling pigs housed at one of two nursery temperatures (19 or 30°C). ADG and avg daily feed intake were higher for pigs housed at 19°C compared with pigs housed at 30°C, but feed per gain ratios, mitogen stimulation index of white blood cells, plasma cortisol levels, and antibody titers were similar. Interactive effects of temperature and vitamin E were not observed for any measurements.

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Serum and liver vitamin E levels linearly increased with increasing level of dietary vitamin E, but performance, cortisol, antibody levels and mitogen induced stimulation indices were not affected by supplemental levels of vitamin E. Although supplemental vitamin E above NRC (1979) recommended levels increased serum and liver vitamin E concentrations, humoral and cell-mediated immune response, cortisol levels, and performance were not responsive for weanling pigs housed at 19 or 30°C temperature.

(Key words: Vitamin E, Temperature, Weanling pigs, and Immune Response)

## Introduction

The immune response of young pigs is believed to be compromised when they are subjected to a stress, such as weaning and(or) exposure to suboptimal environmental temperatures (Kelley, 1980). Blatchford et al. (1978) reported that pigs (approximately 15 kg) exposed to short term (3 hr) temperature extremes (-5 and 40°C) had elevated cortisol levels. Westly and Kelley (1984) demonstrated that elevated cortisol levels resulted in detrimental effects on the cell-mediated immunity. However, fluctuating ambient temperatures was reported not to influence the immune response of weanling pigs (Minton et al., 1988).

Recent evidence (Ellis and Vorhies, 1976; Lim et al., 1981; Reddy et al., 1986) suggests that vitamin E may also play an important role in the maintenance of optimal humoral and cell-mediated immune responses. Vitamin E is an integral part of cell membranes, including mitochondrial and nuclear membranes. Limited vitamin E may result in damage to the cell membrane, including lymphocyte membranes (Bendich et al., 1983). Eskew et al. (1985) suggested that vitamin E protects the lymphocyte from its own by-products, including singlet oxygen, hydrogen peroxide, and prostaglandins. With a decrease in by-products, lymphocytes may increase activity (e.g. phagocytosis) (Heinzerling et al., 1974).

Although the National Research Council (NRC, 1979) recommended levels of vitamin E will prevent the onset of overt deficiency symptoms, Heinzerling et al. (1974) and Peplowski et al. (1981) have suggested that levels of vitamin E that are 6 to 20 times higher than those recommended by NRC (1979) will optimize the immune response. Therefore, the objectives of this experiment were to determine if changes in housing temperature, which may be stressful, would be detrimental to humoral and cell-mediated immune responses and to determine if supplemental dietary vitamin E would enhance the immune

response and thus alleviate any problems caused by this potential stress.

## Materials and Methods

In three trials, 80 Yorkshire X Hampshire X Duroc crossbred pigs (16, 32 and 32 for Trials 4, 5 and 6, respectively) were used in a 4 X 2 factorial arrangement of treatments to evaluate the effects of dietary vitamin E and environmental temperature on performance and immune response of weanling pigs. All pigs (6.9 kg initial weight at 28 d) were fed ad libitum a 20% CP basal diet (ground corn, soybean meal, and dried whole whey) containing .2 ppm Se with one of the following four dietary treatments (Table 2): (1) 11 IU of vitamin E added per kg of feed (NRC basal diet ), (2) 110 IU/kg (10 X NRC), (3) 220 IU/kg (20 X NRC), and (4) 550 IU/kg (50 X NRC). Two environmental temperatures were superimposed upon the diets by housing pigs in separate rooms maintained at either 19 (cold) or 30°C (normal). At 28 d of age, eight pigs within a litter were paired by weight and sex and each pair was randomly assigned to a dietary vitamin E treatment. Temperature treatments were randomly assigned within each pair of pigs. Pigs were weaned, mixed, and moved to nursery pens on the same day. A blood sample was then obtained from the vena cava, and

afterwards, each pig was given an injection of the antigen mixture[8] (Appendix Table 7). Pigs were housed (two per pen) in .6 X 1.3 m raised deck nursery pens. Lights were on continuously for 24 h per day. Pigs were weighed and feed consumption was recorded weekly.

Humoral immune response was measured by determining the antibody response to antigens (sheep red blood cells and lysozyme) injected at the time of weaning and 17 d postweaning. Serum samples were collected initially and weekly for 5 wk after weaning and frozen (-20°C) for later determination of serum vitamin E, cortisol, and antibody levels. Antibody titers to sheep red blood cells (RBC) were measured using a hemagglutination assay (Schurig et al., 1978; Appendix Table 4). Antibody titers to lysozyme were determined using an enzyme-linked immunosorbent assay (ELISA; Schurig et al., 1978; Appendix Table 9). At the end of the trial, pigs were slaughtered and livers were removed for determination of vitamin E and Se concentrations. Diet, serum, and liver vitamin E contents were determined in an independent laboratory by high performance liquid chromatography (HPLC) procedure (Bendich et al., 1984). Liver and dietary Se levels were also determined in an independent laboratory by hydride

[8]A 1 ml mixture of 10 μl packed sheep red blood cells, 10 μg lysozyme (Sigma, L6876), .1 ml Freund's complete adjuvant, .8 ml physiological saline, .1 ml AlOH and MgOH mixture.

generation and atomic absorption spectroscopy (Brodie, 1979). Cortisol levels were analyzed using a commercial RIA[9] (Appendix Table 5). Cell-mediated immune response was measured using a modified cell blastogenic assay reported by Bendich et al. (1984, Appendix Table 10). White blood cells were separated using Histopaque 1077[10] diluted in RMPI 1640 media[11] (modified with Hepes buffer and 10% porcine serum[12]) to 2 x $10^6$ cells per ml. Then, the diluted cells were added to a microtiter plate[13] already prepared in triplicate wells to contain each of the following mitogens: concanavilin A[14] (diluted either 100 or 10 $\mu$g per ml of media respectively), phytohemagglutinin[15] (M form, diluted either 1:10 or 1:500 in the media), and lysozyme[4] (diluted either 1.0 or 0.1 mg per ml of media). After 48 h at 37°C, and in a 5.0% $CO_2$ environment, cells were pulsed with tritiated thymidine[16] (1 $\mu$Ci per well). After 18 h, cells were frozen, harvested on filter paper[17], and counted in a scintillation counter[18] using Ecoscint[19] scintillation

[9]Amersham Corporation, Arlington Heights, Ill.
[10]Sigma, 1077, St. Louis, MO.
[11]K.C. Biological, LM 263-5, Lenexa, KS.
[12]K.C. Biological, 3007-5, Lenexa, KS
[13]Nunc, 1-63320, Newbury Park, CA.
[14]Sigma, C5275, St. Louis, MO.
[15]Gibco, 670-0576, Grand Island, NY.
[16]Dupont, NET 027, Boston, MA.
[17]Whittaker, 23-995, Walkerville, MD.
[18]Beckman LS 1800, Fullerton, CA.
[19]National Diagnostics, Highland Park, NJ.

cocktail. Counts per minute of stimulated cells were divided by counts per minute of unstimulated cells and reported as a stimulation index.

All data were analyzed using the GLM procedure of SAS (1986) with the model including the effects of diet, temperature, litter, trial, time, and individual pig (Appendix Table 8). Pen was the experimental unit for performance parameters. For the other measured parameters, pigs were the experimental units because each litter contained each of the treatment combinations (a replicate). Litter effects were nested within trials because each litter was unique to its trial. Effects of trials were tested with the mean square for litter nested within trial. The effects of diet, temperature and the interactions of trial and diet, trial and temperature, and temperature and diet, were tested with the mean square pigs nested within litter, trial, diet, and temperature. All main effect and time interactions (two and three way) were included. No diet by temperature interactions were noted for any of the measurements; therefore, only main effect means are presented.

## Results and Discussion

Analyzed dietary vitamin E levels were: diet 1 (NRC), 12 IU/kg; diet 2 (10 X NRC), 101 IU/kg; diet 3 (20 X NRC)

235 IU/kg; and diet 4 (50 X NRC), 512 IU/kg. All diets were analyzed and found to contain .2 ppm Se. Livers from pigs fed each of the diets were analyzed and found to contain .25 ppm Se (wet weight basis) which is comparable to levels in liver from pigs fed .12 ppm Se (Meyer et al., 1981).

Daily room temperatures were recorded and the means averaged across trials are shown in Figure 1. Temperatures were generally constant for the temperature treatments except for a mechanical problem in Trial 6. The air conditioner malfunctioned and room temperatures rose (seen as the peaks in the 19° C treatments around wk 4). Portable fans and water evaporation were used to lower the temperature as soon as the increases were noted. The malfunction was repaired within 12 h. No apparent detrimental effects on the immune function or performance were noted for the exposure to high temperature, probably due to the short duration of exposure (Minton et al., 1988).

Supplementing vitamin E in the diet (Table 2) did not affect ADG, avg daily feed intake, or efficiency of feed utilization. Peplowski et al. (1981) also found little effect of dietary supplemental vitamin E on performance. Also, the large increase in serum vitamin E concentration noted (wk 2 for 10X NRC and wk 3 for 20X and 50X NRC,

Figure 2 ) did not correspond to an increase in ADG or avg daily feed intake.

On the other hand, because the environmental temperature comfort zone changes (Pork Industry Handbook, 1987) as the pig becomes larger, temperature treatment influenced performance of the pig over the experiment (Table 3). According to the Pork Industry Handbook (1987) pigs from about 7 kg to 14 kg have a comfort zone between 30 and 24°C. After 14 kg, the comfort zone gradually decreases and becomes 26 to 21°C for pigs weighing 23 kg (Figure 3).

No differences in performance were noted until after wk 1 on test between the two temperature treatments (Table 3). By wk 2, pigs had adapted to the colder (19°C) temperature by increasing feed consumption. This increase in feed consumption throughout the experiment resulted in an increase in cumulative ADG ($P < .01$). After wk 4, pigs in the 19°C room had obtained a weight where they were entering the comfort zone (Figure 3). In contrast, pigs in the 30°C room had reached a similar weight and thus were now becoming heat stressed as they had left the comfort zone. The heat stress (30°C as compare with 19°C) may have caused a depression in ADG and resulted in poorer utilization of feed which was noted in wk 5.

Crenshaw et al. (1986) noted an opposite effect when approximately 6.0 kg pigs were exposed to 18 vs 25°C rooms for 24 d. They reported that pigs in the 25°C environment gained more and had a better feed efficiency. Performance differences between the two experiments may have been influenced by other factors such as the temperature differences between the experiments (6°C in theirs as compared to 11°C in our experiment) a 5°C lower upper temperature treatment in their experiment, temperature fluctuations, differences in humidity, and differences in air velocity (Crenshaw et al., 1986).

Morrison et al. (1975) found that a decrease in temperature would increase the rate of gain and feed consumption but not affect the F/G ratio. Also, Henken et al. (1982) suggested that a temperature stress which did not cause a decrease in pig weight (performance) may indicate that the pigs were never beyond the thermoneutral zone and therefore were not stressed.

Increasing dietary vitamin E levels increased (linear, quadratic, cubic: $P < .01$) the serum concentration of vitamin E (Table 4), which is in agreement with other reports (Peplowski et al., 1981; Blodgett et al., 1988). Liver vitamin E concentration linearly increased as the levels of vitamin E increased in the diet (Table 4), with the magnitude of the increase in

liver vitamin E concentration was much greater than the increase found in the serum. Rousseau et al. (1957) reported similar results.

In agreement with Bonnette et al. (1988), serum vitamin E levels were higher initially for NRC and 10X NRC vitamin E treatments with a drop at wk 1 (Figure 2). Mahan and Moxon (1980) also reported that vitamin E serum concentrations of nursing animals (similar to the values obtained in this experiment) were greater than concentrations obtained from weaned pigs fed a diet deficient in vitamin E. Initially, serum vitamin E levels were high, possibly due to the high availability of vitamin E in the milk as compared with the feed (Meyer et al., 1981). Serum vitamin E concentrations in the following week dropped as the animals were switched from milk to a dry feed, which was associated with low feed consumption and a decrease in absorption and(or) retention of dietary vitamin E. Serum vitamin E values increased over time for pigs fed dietary vitamin E levels above NRC with the increase proportional to the levels of vitamin E fed.

Dietary vitamin E levels did not affect the level of cortisol in the blood (Table 4). Across dietary vitamin E levels, cortisol levels were high initially, but decreased to about a third of the initial value after the first wk

(Figure 4). Afterward, cortisol levels increased linearly ($P < .01$) to near the initial level at the end of the experiment. Similar results were noted by Bonnette et al. (1988). The high values initially may have been due to the combination of several factors, including weaning, moving, bleeding, injecting the antigen, mixing with non-littermates, and fighting to establish social dominance, many of which have been suggested to cause stress (Kelley, 1980).

Antibody titers, to sheep RBC or lysozyme, were not influenced by supplementing vitamin E in the feed (Table 4). Antibody titers to sheep RBC followed a typical antibody response (Figure 5) ; a primary response to the antigen was noted at wk 1 with a slight decrease at wk 2 as the antigen was being processed by the immune system. The next exposure to antigen at d 17 resulted in a secondary response as seen by the increase in antibody titers after wk 3. Bonnette et al. (1988) also noted a similar shape for an antibody titer response curve. The response was higher because memory cells produced from the primary response would have helped produce the antibody faster (Benjamini and Leskowitz, 1988).

In contrast to our results, Peplowski et al. (1981) reported a large increase in antibody titers when 20X NRC dietary level of vitamin E were fed. This difference

between experiments may have been due to the initial high serum vitamin E level (.20 $mg \cdot dl^{-1}$) of pigs in our experiment as compared with low initial serum vitamin E levels (.041 $mg \cdot dl^{-1}$) reported by Peplowski et al. (1981). The low serum vitamin E concentrations in the piglets were probably due to 1) a limited capacity of the placenta to transfer vitamin E to the fetus (Nitowsky et al., 1962) and 2) the relationship between low dietary vitamin E and the small concentration of vitamin E in the colostrum and milk of the lactating sow (Nielsen et al., 1973). Sows in the study by Peplowski et al. (1981) were not given any vitamin E for several generations whereas the sow in our studies were given the NRC recommended levels of vitamin E.

Differences in antibody response among various experiments involving supplemental vitamin E may also be due to the concentration of the antigen, frequency of injection, and the type adjuvant used (Blodgett et al., 1988). For example, in our experiment where antibody titer differences were not observed between dietary or temperature treatments, the pigs were injected IM with sheep RBC and lysozyme in complete adjuvant at d 0 and d 17 of the trial. However, in research reported by Peplowski et al. (1981), a positive antibody response to 20X NRC levels of supplemental vitamin E and .5 ppm Se was

observed when pigs were injected IP weekly for 6 wk with sheep RBC without an adjuvant. Also, Ellis and Vorhies (1976) noted an increase in antibody titers when older pigs (weaned at 6 to 8 wk of age) were supplemented with NRC or 5X NRC levels of vitamin E; and, pigs were injected IM with Escherichia coli suspended in physiological saline containing 0.5% formalin.

The immune response to lysozyme was similar to that of sheep RBC (Figure 5) except that initial values being high instead of being near zero. The assay was repeated using another ELISA procedure (O'Sullivan et al., 1979), but it too, resulted in similar values. This would suggest that the animals may have been exposed to a similar antigenic stimulation, possibly through the milk. The high initial value may have, therefore, skewed the observations of the following weeks.

Lymphocytes undergoing blastogenesis in vitro are reliable indicators of the white blood cells ability to respond to an antigenic challenge in vivo (Bendich et al., 1986). Increasing serum vitamin E levels did not affect stimulation indices in either of the two levels of concanavilin A (ConA) and lysozyme or in the two dilutions of phytohemagglutinin (PHA) as shown in Table 4). Also, no significant interactions were noted for dietary treatments over time.

The lysozyme-induced stimulation indices were used as an indicator of a B-cell specific response (Figure 6). B-cells (after stimulation and proliferation) can produce antibodies, and thus the mitogen response should be and was in fact similar to the (Figure 5) sheep RBC antibody response (showing both a primary and secondary response). Cells in both concentrations of lysozyme (1.0 $mg \cdot ml^{-1}$ and .1 $mg \cdot ml^{-1}$; Figure 5) responded in the same fashion. An increase in stimulation index would suggest more cell capability of responding to the specific antigen (lysozyme).

Concanavilin A (ConA - 100 $\mu g \cdot ml^{-1}$) induced stimulation index, which is an indication of T-suppressor cells activity (Figure 6), had a response curve similar to lysozyme induced stimulation index. As the number of cells capable of producing antibody increased, more T-suppressor cells were needed to prevent the immune response from overproducing antibodies. However, cells stimulated with the lower concentration of ConA (10 $\mu g \cdot ml^{-1}$) responded in an opposite direction during the primary response to cells stimulated with higher levels of ConA (Figure 6). But, after the second injection of antigen at d 17, the stimulation index increased, which was similar to the high concentration of ConA. These results would suggest that the lymphocyte stimulation with

the higher concentration (100 $\mu g \cdot ml^{-1}$) of ConA and the lower concentration (10 $\mu g \cdot ml^{-1}$) of ConA (in the secondary response only) could respond as expected.

The PHA induced stimulation index for the large dilution (1:10) did not have a response curve similar to the antibody response curve (Figure 6). Helper T-cells are necessary to "help" with the communication between B-cells and the antigen presenting cell (usually a macrophage). This communication (due to lymphokines e.g. interleukin 2) is necessary for the production of antibody to a specific antigen. Therefore, a high number of helper T-cells would be expected before a peak in antibody titers. Response peaks of helper T-cells were seen just before the peaks in antibody titer at wk 1 and wk 4. The lower index of helper T-cells at the secondary response in wk 5 was due in part to memory cells. Therefore, not as many helper T-cells are needed to start producing antibodies. However, the stimulation index of cells exposed to the lower dilution of PHA (1:500) did not react like the cell stimulation with the higher dilution. The response curve to the lower dilution of PHA had a increase in index numbers starting at wk 3 where the secondary antibody response begins (Figure 6). Thus, the indexes resulting from the higher dilution of PHA reacted as the

immune system would normally respond to an antigenic challenge.

In contrast to our results, several researchers have shown a positive effect of supplemental vitamin E on the cell-mediated immune response. Tanaka et al. (1979) reported that supplemental vitamin E (200 mg per kg of diet) to mice stimulates helper T-cells. Corwin et al. (1981) suggested that vitamin E (50 mg per 100 g of diet) helped the stimulation of rat lymphocytes by mitogens. Reddy et al. (1986) found that supplementing vitamin E (1400 to 2800 mg per week) to calves increased PHA-induced lymphocyte stimulation indices as compared with unsupplemented calves; however, when vitamin E was added to the cell cultures, no increases in PHA-induced lymphocyte stimulation indices were noted. This finding suggests that the increase may not be due directly to vitamin E, but to some factor influenced by vitamin E.

Considering that dams of the pigs used in our trials were fed a NRC recommended level of vitamin E, the initial blood sample of the pigs should reflect adequate vitamin E levels. The drop in serum vitamin E levels in pigs fed the NRC diet and 10X NRC (Figure 2) suggest that this level is not adequate to maintain serum vitamin E levels. The feeding of 30 to 70 IU of vitamin E per pig per day in the first week, and 60 to 140 IU of vitamin E (10X to 20X

NRC, respectively, for both weeks) per pig per day in the second week postweaning (based upon avg feed intake), is needed to maintain levels similar to serum vitamin E levels of nursing pigs.

The low initial concentration of serum vitamin E in experiments like that of Peplowski et al. (1981) may suggest an explanation for the positive effects noted when supplementing greater levels of dietary vitamin E. Marsh et al. (1986) suggested that the primary lymphoid tissues were the main target for a vitamin E deficiency, and in a related paper, Baustad and Nafstad (1972) suggested that vitamin E is a hematopoietic factor in newborn piglets. Thus, the lack of an effect on antibody titer, mitogen stimulation, or cortisol in our experiment may be due to the antigens being injected while the tissue stores of vitamin E were sufficient (until approximately 3 wk) and therefore lymphoid tissues were unaffected. Experiments to observe longer term exposure to an antigen may be necessary.

It has also been suggested that elevated cortisol values in mice can suppress the cellular immune response (Blecha et al., 1983). Westly and Kelley (1984) reported that adding various levels of cortisol to the media decreased the mitogen stimulation index of lymphocytes of adult sows. The average cortisol value was determined to

be around 13 $ng \cdot ml^{-1}$ for a normal baseline level which could be produced with added adrenocorticotropic hormone (ACTH). However, values of cortisol in our experiment did not reach the higher levels of cortisol used in experiments by Westly and Kelley (1984); and therefore, this may explain why we did not observe the dramatic effects on the immune response that they did. Also, the cortisol effects may be due to the constant levels in the in vitro experiment which would not be seen in vivo.

Decreasing the environmental temperature (19 vs 30° C) of the weanling animals did not influence the cortisol concentration, serum or liver vitamin E content, antibody titer to sheep RBC and lysozyme, or the mitogen stimulation indices (Table 5). In agreement, Crenshaw et al. (1986) reported that an 18°C compared with 25°C environmental temperature did not detrimentally influence antibody titers levels to human RBC (humoral immunity) nor skin-fold thickness due to an intra-dermal injection of PHA (cell-mediated immunity) in 4 and 8 d postweaned pigs.

However, colder temperatures (8 to 12°C) have been reported to increase susceptibility of weaned pigs to disease (Furuuchi and Shimizu, 1976). Further, support for the detrimental effect of cold temperature was reported by Blatchford et al. (1978). They indicated that temperatures between 5 and 30°C rarely caused a

significant increase in cortisol, whereas temperatures above 40°C or below -5°C increased plasma ACTH and cortisol values. Therefore, small differences in cortisol concentrations seen in this experiment (resulting from temperature treatments) may have resulted in the small differences noted in antibody titers to sheep RBC or lysozyme (Table 4). Several researchers have suggested that increased cortisol levels decreased antibody titers due to a decrease in lymphoid tissue (Quinn, 1968). The decrease in antibody titers also may be due to a decrease in the number of lymphocytes (Ganong, 1981). In contrast, Blecha and Kelley (1981) reported an increase in antibody titer when 21 and 35 d old pigs were exposed to a cold temperature (0 vs 25°C) for 4 d.

Henken et al. (1982) suggested that the temperature stress would increase protein catabolism, and would in turn decrease the weight of the lymphoid tissues. This decrease in the size of lymphoid tissue would decrease the immune response. Therefore, the lack of effect in immune function in our experiment may suggest the environmental temperature differences were not overtly stressful, and although outside the thermoneutral zone, still within a functionally acceptable physiological range for the weanling pig.

In summary, reducing environmental temperatures from 30 to 19°C for 4 wk old weanling pigs increased ADG and avg daily feed intake but did not change efficiency of feed utilization, cortisol levels, antibody titers or mitogen-stimulation indices. Supplementing dietary vitamin E (10X, 20X and 50X NRC) to weanling pigs increased the level of liver and serum vitamin E, but had little effect on cortisol, antibody titers, performance, or mitogen-stimulation indices. No significant interactions of the main effects were noted.

TABLE 1. COMPOSITION OF BASAL DIETS[a] FOR TRIALS 4, 5 AND 6

| Ingredients | Percentage |
|---|---|
| Ground corn | 59.54 |
| Soybean meal (48% CP) | 27.73 |
| Dried whole whey | 10.00 |
| Defluorinated phosphate | 1.03 |
| Limestone | .75 |
| Salt | .30 |
| Vitamin premix[b] | .25 |
| Trace mineral premix[c] | .10 |
| Antibacterial[d] | .25 |
| Se premix[e] | .05 |

[a]Calculated to contain 20% CP, .8% Ca, .6% P and analyzed to contain .2 ppm Se.
[b]Supplied per kg of diet: 4,400 USP vitamin A, 440 IC vitamin $D_3$, 11 IU vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 23.9 mg d-calcium pantothenic acid, 22 mg niacin, 489.5 mg choline as 506.8 mg choline chloride, .022 mg vitamin $B_{12}$, 1.1 mg menadione dimethylpyrimidol, .44 mg d-biotin.
[c]Supplied per kg of diet: .02% Zn, .01% Fe, .006% Mn, .001% Cu, and .0002% I.
[d]Supplied per kg of diet: 44 g chlortetracycline, 22 g procaine penicillin and 4.4 % sulfamethazine.
[e]Calculated to supply .2 mg of Se per kg of the diet.

TABLE 2. EFFECT OF SUPPLEMENTAL DIETARY VITAMIN E ON PEN PERFORMANCE LEAST SQUARES MEANS IN TRIALS 4, 5 AND 6

| | Supplemental Vitamin E | | | | | |
|---|---|---|---|---|---|---|
| Item[a] | X[b] | 10X | 20X | 50X | SE | P |
| Avg daily feed (kg) | .74 | .77 | .78 | .77 | .03 | .74 |
| Avg daily gain (kg) | .44 | .44 | .43 | .42 | .02 | .81 |
| Feed/gain | 1.71 | 1.76 | 1.85 | 1.81 | .06 | .40 |

[a]Number of observations; ten pens per diet; two pigs per pen. Initial and final weights were 7.0 kg and 22.0 kg, respectively.
[b]Recommended NRC levels of Vitamin E (11 $IU \cdot kg^{-1}$).

TABLE 3. EFFECT OF TEMPERATURE ON PEN PERFORMANCE LEAST SQUARES MEANS IN TRIALS 4, 5 AND 6

| Item[a] | Temperature (°C) 30 | 19 | SE | P |
|---|---|---|---|---|
| Avg daily feed (kg) | | | | |
| Week 1 | .30 | .35 | .02 | .15 |
| 2 | .53 | .68 | .03 | .02 |
| 3 | .73 | .88 | .05 | .03 |
| 4 | .89 | .97 | .04 | .19 |
| 5 | 1.08 | 1.23 | .05 | .04 |
| 1-5 | .71 | .82 | .02 | .01 |
| Avg daily gain (kg) | | | | |
| Week 1 | .20 | .21 | .01 | .47 |
| 2 | .36 | .40 | .02 | .11 |
| 3 | .36 | .43 | .02 | .01 |
| 4 | .52 | .57 | .04 | .38 |
| 5 | .55 | .73 | .03 | .01 |
| 1-5 | .40 | .47 | .01 | .01 |
| Feed/gain | | | | |
| 1 | 1.61 | 2.04 | .18 | .12 |
| 2 | 1.54 | 1.76 | .17 | .38 |
| 3 | 2.12 | 2.18 | .13 | .76 |
| 4 | 1.79 | 1.73 | .12 | .74 |
| 5 | 2.10 | 1.67 | .15 | .07 |
| 1-5 | 1.80 | 1.77 | .04 | .57 |

[a]Number of observations; twenty pens per temperature treatment; two pigs per pen; Initial weight was 6.9 kg. Final weight was 20.9 and 23.4 kg for 30 and 19°C temperature treatments, respectively.

TABLE 4. EFFECT OF SUPPLEMENTAL VITAMIN E ON LEAST SQUARES MEANS OF THE OVERALL SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS, AND STIMULATION INDICES FOR TRIALS 4, 5 AND 6

| | Supplemental Vitamin E | | | | | |
|---|---|---|---|---|---|---|
| Item[a] | X[b] | 10X | 20X | 50X | SE | P |
| Vitamin E | | | | | | |
| Serum ($mg \cdot dl^{-1}$)[efg] | .09 | .21 | .24 | .30 | .01 | .01 |
| Liver ($mg \cdot 100g^{-1}$)[he] | .23 | 1.72 | 2.99 | 5.32 | .39 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | 10.5 | 10.4 | 9.8 | 10.6 | .62 | .98 |
| Antibody titer[c] | | | | | | |
| SRBC[f] | 2.0 | 1.6 | 1.3 | 1.9 | .16 | .49 |
| Lysozyme | 4.7 | 4.6 | 4.8 | 4.5 | .20 | .85 |
| Stimulation indices[d] | | | | | | |
| Lysozyme | | | | | | |
| 1.0 $mg \cdot ml^{-1}$[g] | 2.1 | 2.9 | 2.9 | 1.9 | .43 | .34 |
| .1 $mg \cdot ml^{-1}$ | 2.3 | 2.9 | 2.5 | 1.9 | .34 | .28 |
| Concanavilin A | | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 131.1 | 117.4 | 145.4 | 152.6 | 14.00 | .39 |
| 10 $\mu g \cdot ml^{-1}$ | 17.7 | 17.8 | 21.7 | 21.6 | 3.23 | .79 |
| Phytohemagglutinin | | | | | | |
| 1:10 | 205.5 | 230.0 | 236.3 | 228.6 | 34.90 | .87 |
| 1:500 | 9.3 | 12.4 | 9.6 | 10.3 | 2.70 | .64 |

[a]Number of observations; twenty pigs per diet, two pigs per pen.
[b]Recommended NRC (1979) levels of vitamin E (11 $IU \cdot kg^{-1}$).
[c]Pigs were injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17; titers measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.
[e]Linear effect ($P < .01$).
[f]Cubic effect ($P < .01$).
[g]Quadratic effect ($P < .01$).
[h]Number of livers; 13, 11, 11, 12 for diets 1,2,3,4 respectively.

TABLE 5. EFFECT OF TEMPERATURE ON LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES FOR TRIALS 4, 5 AND 6

| Item[a] | Temperature (°C) 30 | 19 | SE | P |
|---|---|---|---|---|
| Vitamin E | | | | |
| Serum ($mg \cdot dl^{-1}$) | .21 | .20 | .01 | .11 |
| Liver ($mg \cdot 100g^{-1}$)[b] | 2.28 | 2.74 | .52 | .29 |
| Cortisol ($ng \cdot dl^{-1}$) | 10.3 | 10.3 | .44 | .82 |
| Antibody titer[c] | | | | |
| SRBC | 1.63 | 1.80 | .12 | .51 |
| Lysozyme | 4.65 | 4.61 | .14 | .87 |
| Stimulation indices[d] | | | | |
| Lysozyme | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 2.6 | 2.3 | .30 | .55 |
| .1 $mg \cdot ml^{-1}$ | 2.6 | 2.2 | .25 | .25 |
| Concanavilin A | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 132.6 | 140.7 | 10.00 | .47 |
| 10 $\mu g \cdot ml^{-1}$ | 19.3 | 20.2 | 2.30 | .71 |
| Phytohemagglutinin | | | | |
| 1:10 | 210.0 | 240.3 | 24.70 | .30 |
| 1:500 | 9.6 | 11.2 | 1.92 | .27 |

[a]Number of observations; forty pigs per temperature treatment; two pigs per pen; three trials.
[b]Number of livers; 22 and 25 for 30° and 19°C respectively.
[c]Pigs were injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17; titers measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.

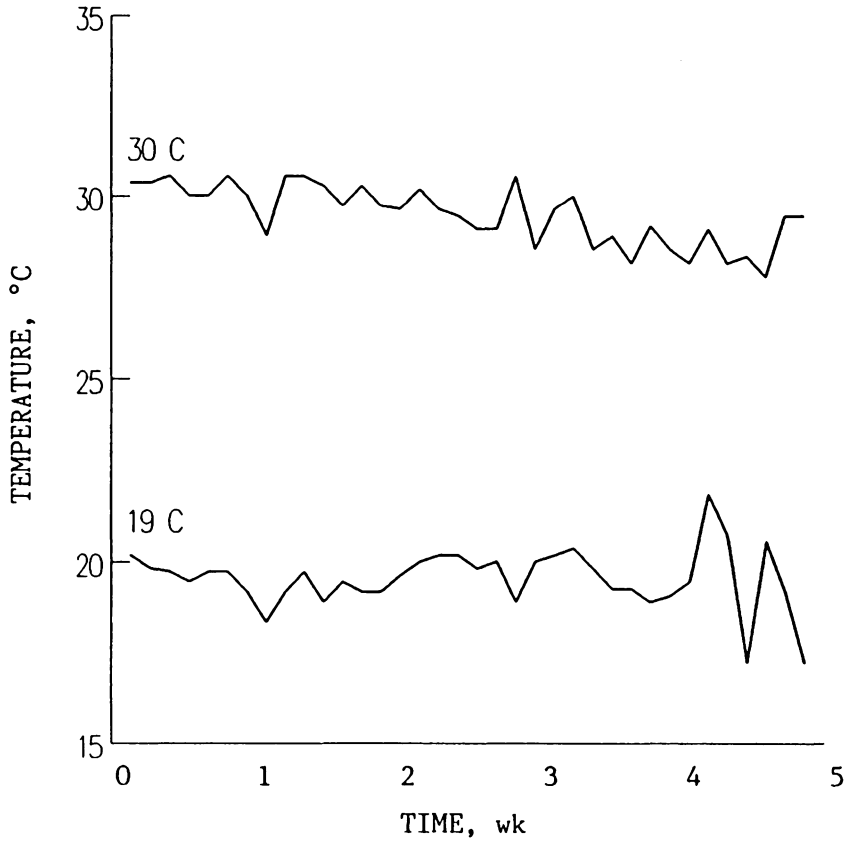


Figure 1. Mean room temperature expressed by wk for Trials 4, 5 and 6.

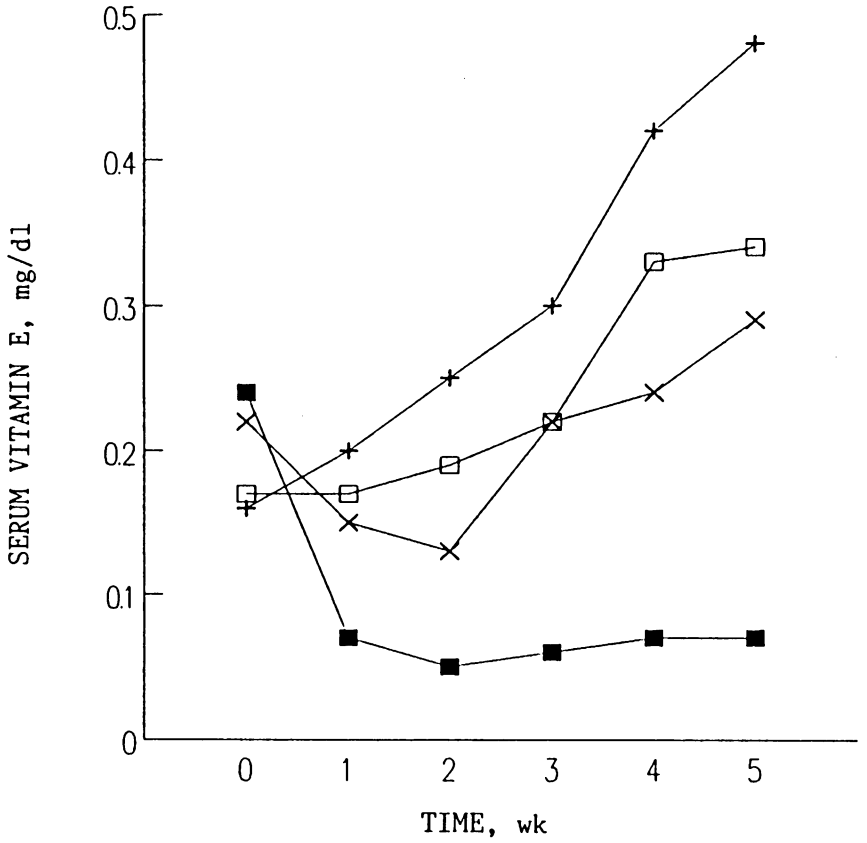


Figure 2. Mean serum vitamin E levels for dietary treatments in Trials 4, 5 and 6 expressed by wk. SEM = .02.

Diets: NRC 10X NRC 20X NRC 50X NRC

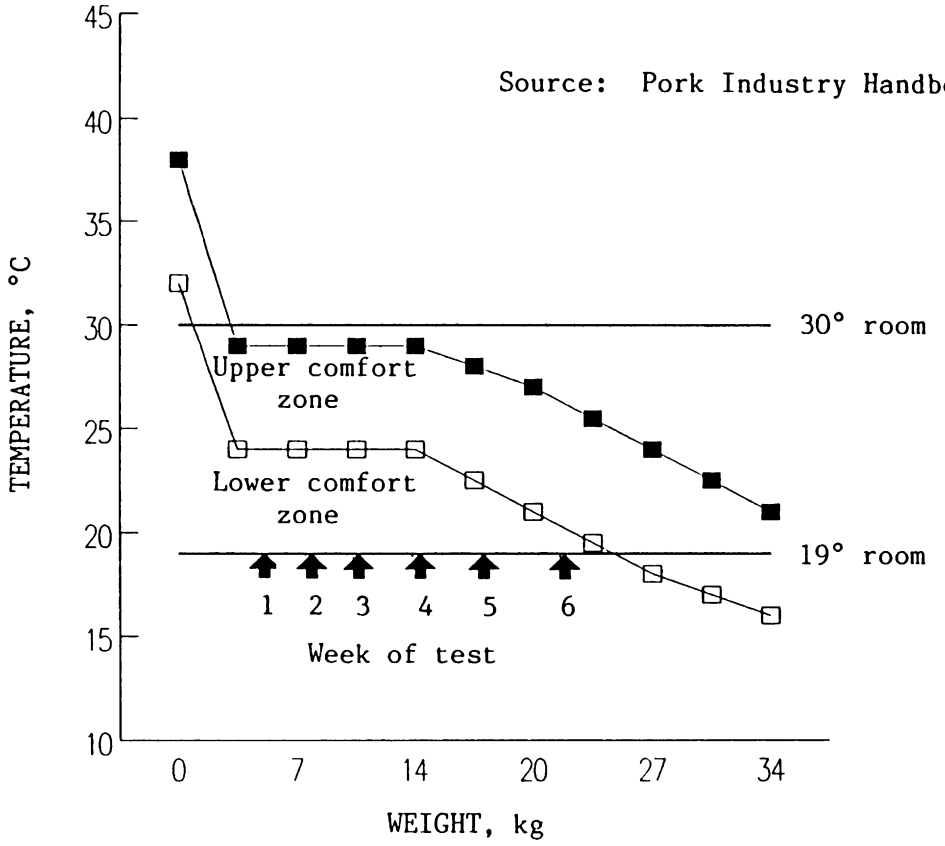


Figure 3. Room temperature and week of trial related to upper and lower comfort zone for pigs 0 to 34 kg in weight.

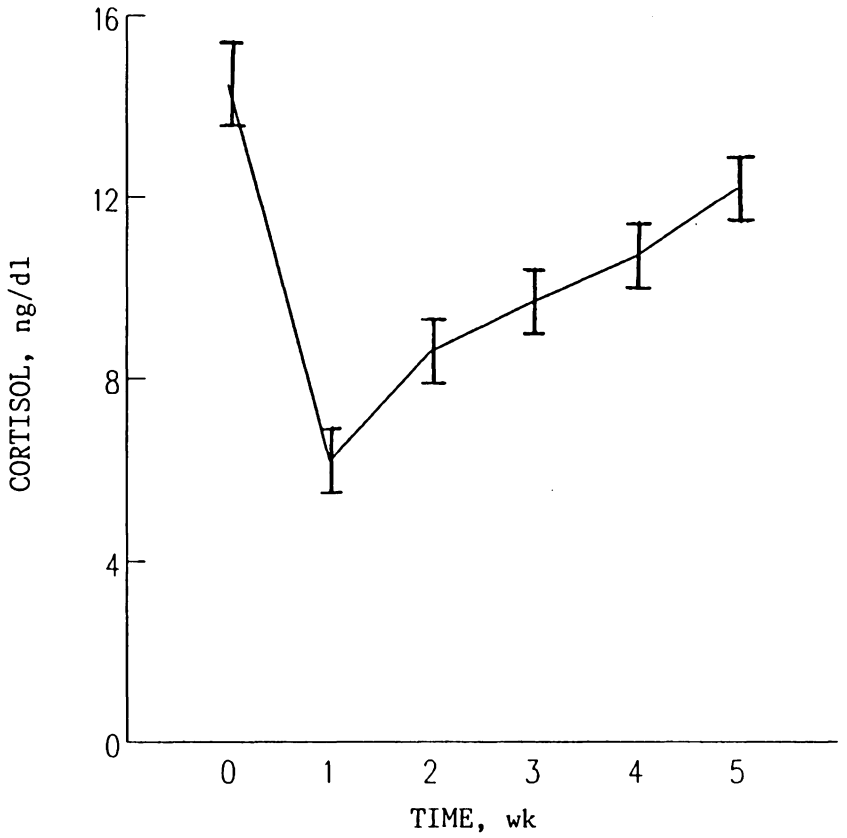


Figure 4. Mean cortisol concentrations for Trials 4, 5 and 6 expressed by wk. SEM = .74.

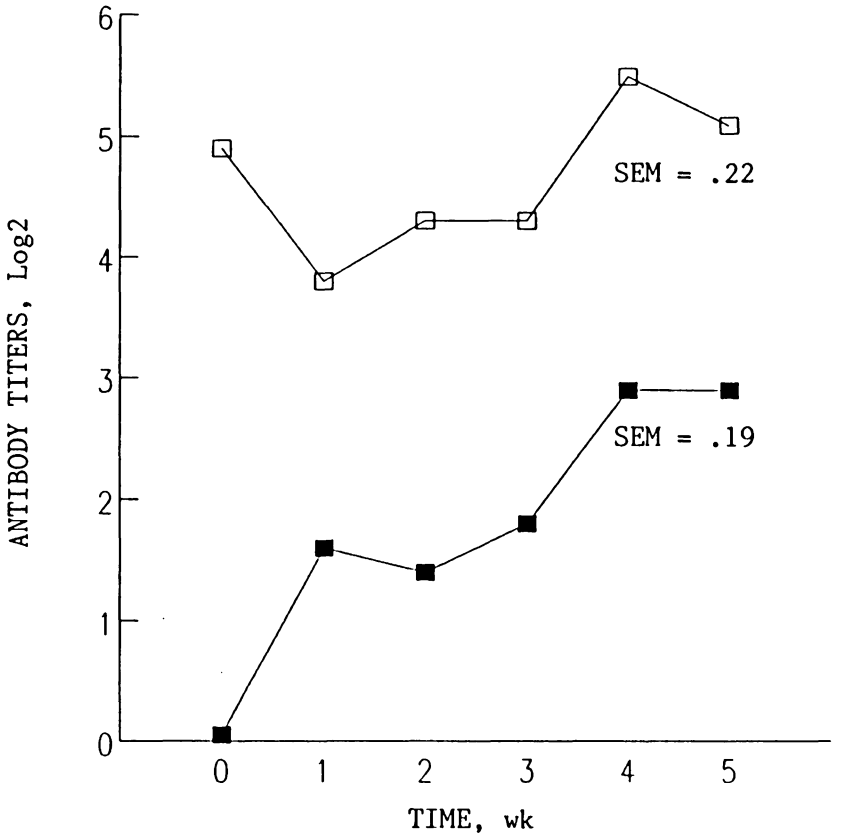


Figure 5. Mean antibody titers for sheep red blood cells and lysozyme for Trials 4, 5 and 6 expressed by wk.

Antigens: —■— Sheep RBC —⊟— Lysozyme

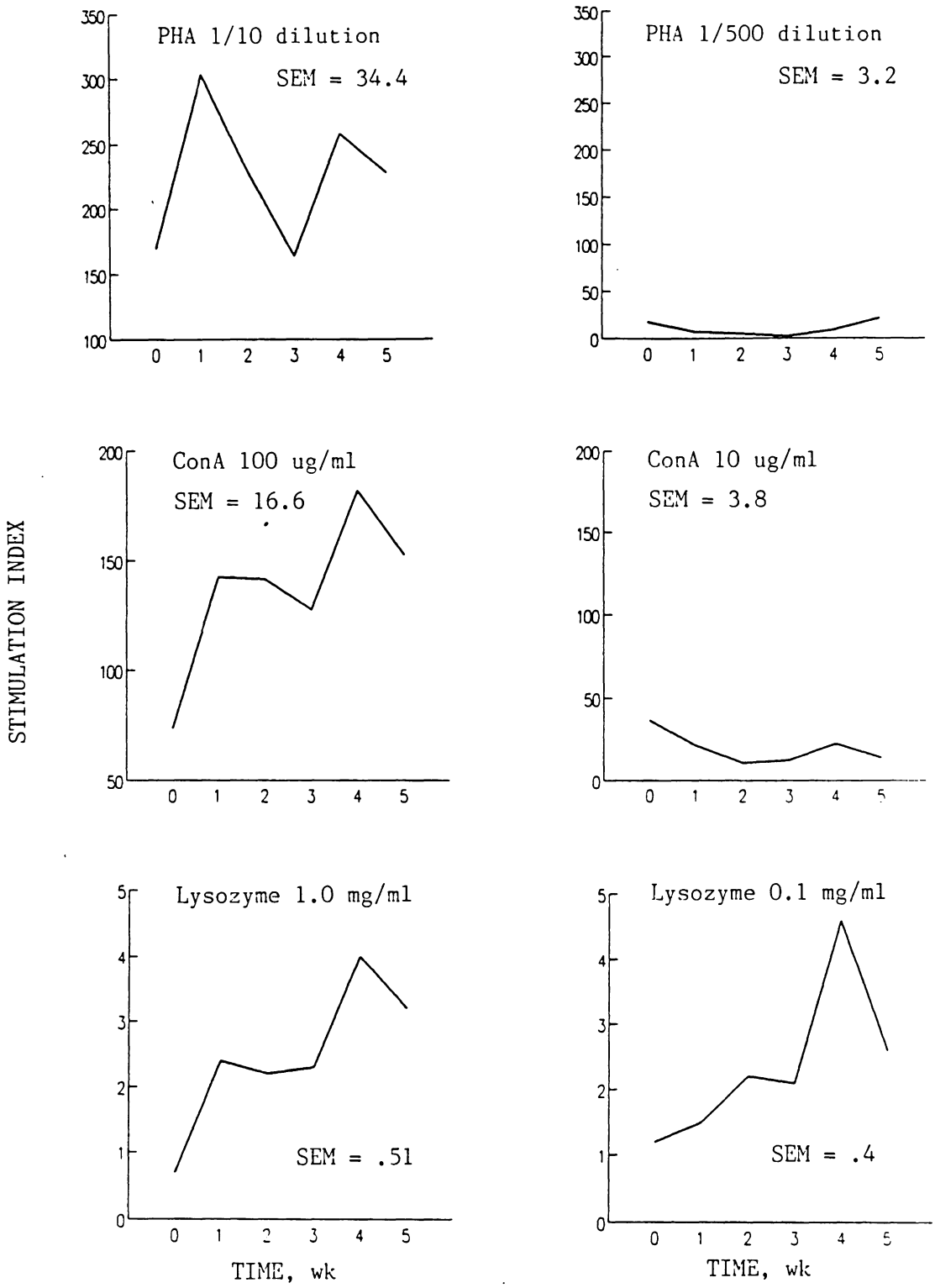


Figure 6. Stimulation indices of white blood cells induced by two levels of the mitogens PHA, ConA and lysozyme expressed by wk for Trials 4, 5 and 6.

Chapter V

Running Head: Vitamin E in Serum and Milk of Swine

# CONCENTRATIONS OF VITAMIN E IN THE SERUM AND MILK OF POSTPARTUM SOWS AND IN THE SERUM OF THEIR SUCKLING PIGS

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## ABSTRACT

A trial was conducted to characterize the concentration of vitamin E in the serum and milk of ten multiparous lactating Yorkshire X Hampshire X Duroc sows and in the serum of their progeny. Sows were fed approximately 7 kg of a 14% CP diet daily, which supplied .1 mg Se and 11 IU of vitamin E per kg of diet. Blood and milk samples were obtained weekly for 5 consecutive weeks starting within a day of farrowing. Although the concentration of vitamin E in the sow serum remained stable from farrowing through 5 wk postpartum, the concentration of vitamin E in the colostrum was very high initially. The milk vitamin E concentration then dropped sharply after 1 wk postfarrowing, with lower but a constant concentration recorded for the remainder of the trial ($P < .01$). Serum vitamin E concentrations of the

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suckling pigs were lowest ($P < .12$) at birth with a peak occurring at 2 wk of age.

(Key words: Pigs, Vitamin E, Milk)

## Introduction

The amount of vitamin E that neonate pigs obtain from milk is variable, depending upon the amount of vitamin E in the diet of the sow (Nielsen et al., 1973). Nielsen et al. (1973) and Mahan (1985) demonstrated that increasing the dietary vitamin E levels of sows will increase the concentration of vitamin E in the colostrum and milk. Both groups also found higher levels of vitamin E in colostral milk than in post-colostral milk. Additionally, Loudenslager et al. (1985) reported that the concentration of alpha-tocopherol was initially high in colostrum and decreased over time while the levels of beta- and gamma- tocopherol (both of which have lower biological values) increased over time.

Interest in the effect of vitamin E on biological functions including the immune response has increased in the last several years. Several researchers (Heinzerling et al., 1974; Tanaka et al, 1979; Peplowski et al, 1981; Nockels, 1986) have suggested that vitamin E plays an important role in cell membrane protection from damaging peroxides and is important in maintaining an optimal humoral and cell-mediated immune response. In a related experiment, Baustad and Natstal (1972) have suggested that

vitamin E is a hematopoietic factor (affects development and production of white blood cells) in a newborn pig. Because the active immune response in the pig begins development around 3 to 4 wk of age, the concentration of vitamin E before and during this time could play an important factor in the immune response development.

Therefore the objectives of this study were to characterize the concentrations of vitamin E in the milk and serum of sows over a 5 wk lactation, and to determine the relationship between the concentration of vitamin E in the serum of suckling pigs and in milk of sows.

## Materials and Methods

Ten multiparous sows (Yorkshire X Duroc X Hampshire) were randomly placed in farrowing crates (.6 m X 2.1 m) approximately 4 d prior to the estimated parturition date. Sows were given water ad libitum and were fed a 14% CP ground corn and soybean meal diet supplemented with .1 mg Se and 11 IU vitamin E per kg of diet (NRC, 1979) twice daily (approximately 7 kg per d).

Starting at farrowing and weekly thereafter for 5 wk, blood samples were obtained from the vena cava of sows which were housed in farrowing crates. After the blood sample was removed, .1 ml of oxytocin[23] was injected in the same blood vessel to facilitate milk letdown. Milk

[23]Contains 20 USP per ml, J.A. Webster, Billerica, MA.

was collected from at least three teats into a 12 ml polypropylene tube. Tubes were sealed with polypropylene caps and frozen at -20°C until analyzed. Serum was removed from whole blood by centrifugation (500 X g) and stored in 12 ml polypropylene tubes at -20°C. Both serum and milk samples of sows were analyzed for vitamin E in an independent lab using a HPLC method (Bendich et al., 1984).

Concurrently, six baby pigs in each litter (n=60) were weighed and a blood sample was obtained from the vena cava also weekly for 5 wk. Initial blood sample obtained after pigs had nursed. Serum was separated from the whole blood and frozen in a polypropylene tube at -20°C. Serum sample of the pigs were analyzed colorimetrically (Fabianek et al., 1968) for vitamin E content.

All data were analyzed using the GLM procedure of SAS (1986). All pig data were analyzed using a model that included effects of litter, pig within litter, time and litter X time interaction.

Sow data were analyzed with a model including litter and time. Partial correlation coefficients between serum vitamin E content (both for sow and for the mean of the piglets) and milk vitamin E content were also obtained.

Orthogonal comparisons were used to define the response curves of the sow milk and serum vitamin E and serum vitamin E of the suckling pigs over the time of the

experiment.

## Results and Discussion

Serum vitamin E concentration of the sow did not differ over time (Figure 1). However, a decreasing amount of vitamin E in the milk over time was noted. Concentration of vitamin E in milk was very high for the initial sample after farrowing, decreased sharply in the subsequent week and then gradually decreased thereafter (cubic response, $P < .01$). Similar results were noted by both Mahan (1985) and Loudenslager et al. (1985), who reported the vitamin E content was higher in colostral milk as compared with post-colostral milk. Milk vitamin E values were approximately the same as those reported by Mahan (1985). Serum vitamin E levels of sows (fed 11 IU vitamin E per kg diet) in our experiment are between values reported by Malm et al. (1976) for sows that were not supplemented with dietary vitamin E (approximately .03 mg per 100 ml) and the values reported by Mahan and Moxon (1980) for sows given 22 IU per kg diet (approximately .20 mg per 100 ml).

The serum content of vitamin E of the nursing pig had a different response curve than the vitamin E levels found in milk (Figure 1). The lowest value was observed in the initial sample. Vitamin E levels then gradually increased

and peaked around wk 2 before starting to decline. This initial low value may be explained by Nitowsky et al. (1962) who suggested that vitamin E transfer in utero to piglets is limited.

Until 3 wk of age, all pigs continued to nurse the sow. However, at 21 d of age, two pigs per litter were removed for a concurrent experiment. Two less pigs per litter may result in more milk for the remainder of the litter which remained. This may explain in part the small increase in serum vitamin E in piglets when analysis was repeated using only 40 pigs (wk 4, Figure 1). And again at wk 4, two pigs per litter were removed for the concurrent experiment thus the opportunity for even more milk (and therefore vitamin E) for the pigs remaining with the sow. Again, an increase was noted in serum vitamin E when the analysis was rerun using only 20 pigs (wk 5, Figure 1).

At wk 1, the concentration of serum vitamin E (.35 mg/dl) in piglets were similar to values reported by Mahan and Moxon (1980) for newly weaned pigs. Although the amount of vitamin E in the milk decreases over time, piglets would ingest larger amounts of milk as they grow. Church and Pond (1982) suggested that the amount of vitamin E that a baby pig could ingest is directly related to the amount of vitamin E provided in the milk, which in

turn, is dependent upon the amount of vitamin E contained in the diet of the sow.

No differences ($P > .10$) were noted among individual sows for the vitamin E content in the serum (Table 1). There was also a difference ($P > .01$) in piglet serum vitamin E concentrations among litters. This range of serum vitamin E concentrations may have been due to the varying amount of milk produced by individual sows or the differences in the amount of feed consumed by the sow. However, the partial correlation coefficient between the vitamin E in the milk and the vitamin E in the serum of the baby pig was not significant ($P > .10$).

Mahan (1985) suggested that the concentration of vitamin E in the milk decreased as the number of parities of the sow increased (Table 2). There were no differences among parities for the concentration of vitamin E in the milk or in the sow serum. However, the small number of sow may not have allowed detecting any differences.

Therefore, our results suggest that the concentration of vitamin E in piglet serum is not directly related to the vitamin E content in the milk or serum of a sow.

TABLE 1. LEAST SQUARES MEANS OF VITAMIN E CONCENTRATION IN THE SERUM AND MILK OF INDIVIDUAL LACTATING SOWS AND THE SERUM OF THEIR SUCKLING OFFSPRING

| | Litter[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 105 | 106 | 108 | 109 | 110 | 112 | 113 | 114 | 115 | 116 | SE | P |
| Sow serum ($mg \cdot dl^{-1}$) | .04 | .09 | .09 | .04 | .04 | .10 | .05 | .01 | .03 | .02 | .02 | .15 |
| Sow milk[b] ($mg \cdot dl^{-1}$) | .14 | .15 | .20 | .21 | .18 | .21 | .28 | .18 | .12 | .20 | .03 | .10 |
| Pig serum ($mg \cdot dl^{-1}$) | .47 | .54 | .45 | .52 | .31 | .39 | .38 | .36 | .22 | .62 | .03 | .01 |

[a]Ten sows, fed 11 IU of vitamin E per kg of diet.
[b]Obtained using .1 ml oxytocin injected intravenously.

TABLE 2. EFFECT OF PARITY ON THE LEAST SQUARES MEANS OF THE VITAMIN E CONCENTRATIONS IN THE SERUM AND MILK OF LACTATING SOWS AND SERUM OF THEIR SUCKLING OFFSPRING

| | Parity | | | | | | |
|---|---|---|---|---|---|---|---|
| Item[a] | 1 | 2 | 3 | 4 | 5 | SE | P |
| Number of sows | 2 | 1 | 5 | 1 | 1 | | |
| Sow Serum ($mg \cdot dl^{-1}$) | .06 | .10 | .05 | .03 | .02 | .02 | .16 |
| Sow Milk[b] ($mg \cdot dl^{-1}$) | .19 | .21 | .17 | .12 | .20 | .05 | .82 |

[a]Fed 11 IU of vitamin E per kg of diet.
[b]Obtained using .1 ml oxytocin injected intravenously.

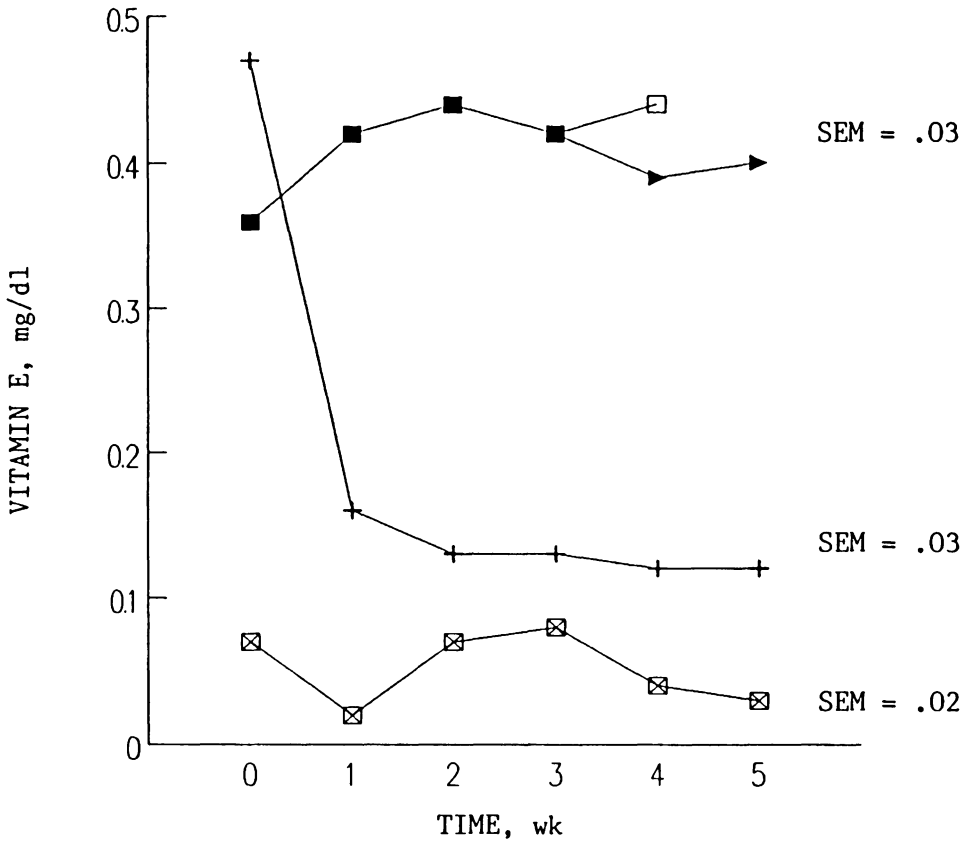


Figure 1. Vitamin E concentrations in serum and milk of lactating sows and the serum of their suckling offspring expressed by wk.

Sow milk: —+— Sow serum: —⊠—

Pig serum: —■— n=60 —□— n=40 —▶— n=20

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APPENDIX TABLE 1. COMPOSITION OF BASAL DIETS[a] FOR TRIALS 1, 2 AND 3

| | Percentage | Calculated for a 226.8 kg batch |
|---|---|---|
| Ground corn | 59.59 | 135.1 kg |
| Soybean meal (48%) | 27.73 | 62.9 kg |
| Dried whole whey | 10.00 | 22.7 kg |
| Defluorinated phosphate | 1.03 | 2338 g |
| Limestone | .75 | 1703 g |
| Salt | .30 | 750 g |
| Trace mineral premix[b] | .10 | 227 g |
| Vitamin premix[c] | .25 | 568 g |
| Antibacterial[d] | .25 | 568 g |
| Se premix[e] | .05 | 114 g |
| | 100.00 | 226.8 kg |

Mixed for 10 minute, remove 113.4 kg => NRC vit E level add 46 g vit E[f], mix, remove 113.4 kg => 20X NRC vit E level

[a]Calculated to contain 20% CP, .8% Ca, .6% P and analyzed to contain .2 ppm Se.
[b]Supplied per kg of diet: 4,400 USP vitamin A, 440 IC vitamin $D_3$, 11 I vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 23.9 mg d-calcium pantothenic acid, 22 mg niacin, 489.5 mg choline as 506.8 mg choline chloride, .022 mg vitamin $B_{12}$, 1.1 mg menadione dimethylpyrimidol, .44 mg d-biotin.
[c]Supplied per kg of diet: .02% Zn, .01% Fe, .006% Mn, .001% Cu, and .0002% I.
[d]Supplied per kg of diet: 44 g chlortetracycline, 22 g procaine penicillin and 4.4 % sulfamethazine.
[e]Calculated to supply .2 mg of Se per kg of the diet.
[f]Vitamin E contains alpha tocopherol acetate 551,876 IU/kg

APPENDIX TABLE 2. COMPOSITION OF BASAL DIETS[a] FOR TRIALS 4, 5 AND 6

| Ingredients | Percentage |
|---|---|
| Ground corn | 59.59 |
| Soybean meal (48% CP) | 27.73 |
| Dried whole whey | 10.00 |
| Defluorinated phosphate | 1.03 |
| Limestone | .75 |
| Salt | .30 |
| Vitamin premix[b] | .25 |
| Trace mineral premix[c] | .10 |
| Antibacterial[d] | .25 |
| Se premix[e] | .05 |

Diet 1: Basal diet plus .00% vitamin E[f]
Diet 2: Basal diet plus .04% vitamin E
Diet 3: Basal diet plus .08% vitamin E
Diet 4: Basal diet plus .20% vitamin E

[a]Calculated to contain 20% CP, .8% Ca, .6% P and analyzed to contain .2 ppm Se.
[b]Supplied per kg of diet: 4,400 USP vitamin A, 440 IC vitamin $D_3$, 11 I vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 23.9 mg d-calcium pantothenic acid, 22 mg niacin, 489.5 mg choline as 506.8 mg choline chloride, .022 mg vitamin $B_{12}$, 1.1 mg menadione dimethylpyrimidol, .44 mg d-biotin.
[c]Supplied per kg of diet: .02% Zn, .01% Fe, .0o6% Mn, .001% Cu, and .0002% I.
[d]Supplied per kg of diet: 44 g chlortetracycline, 22 g procaine penicillin and 4.4 % sulfamethazine.
[e]Calculated to supply .2 mg of Se per kg of the diet.
[f]Vitamin E contains alpha tocopherol acetate 551,876 IU/kg

APPENDIX TABLE 3. ANTIGEN MIXTURE FOR TRIALS 1, 2 AND 3

| | |
|---|---|
| 1) | 89.0 ml sterile physiological saline |
| 2) | 10.0 ml maldrox[a] |
| 3) | 1.0 ml packed sheep red blood cells |
| 4) | 10.0 ml Freund's adjuvant[b] |

[a]Contains 200 mg aluminum hydroxide and 250 mg magnesium hydroxide.
[b]Trials 1 and 2 used Freund's incomplete adjuvant (Gibco 660-5720) and trial 3 used Freund's complete adjuvant (Gibco 660-5721).

APPENDIX TABLE 4. HEMAGGLUTINATION ASSAY FOR THE MEASUREMENT OF ANTIBODIES TITERS TO SHEEP RED BLOOD CELLS

Reagents

1. Phosphate buffered saline (10X stock)
   a) Mix:
      1) 20.5 g $NaH_2PO_4.H_2O$
      2) 179.9 g $Na_2HPO_4.7H_2O$
      3) 4 l of distilled water
         Adjust pH to 7.2-7.4
      4) 701.3 g NaCl
      5) 4 l distilled water

   b) Dilute 1:10 with distilled water for working solution.

2. Dynatech microtiter plates (Fisher 14-245-74).

3. 2% Sheep Red Blood Cells (SRBC).

   a) Sheep red blood cells were collected in a 10 ml heparinized vacutainer tube and centrifuged for 10 minutes at 2000 X g. The clear serum layer (and the "buffy coat") was aspirated off the top, leaving the "packed" red blood cells. 9 mls of physiological saline was added to the tube, the cells were shaken to "wash" them, and then centrifuged and packed as above. This was repeated a total of three times.
   b) 2 mls of washed packed sheep red blood cells was added to 100 ml of physiological saline

## Procedures

1. Add 100 ul of heat inactivated serum (56 C for 30 minutes) in first well of each column on the 96 "U" bottom well microtiter plate. Each serum sample is placed in a different well.

2. Add 50 ul of physiological saline to each of the remaining 84 wells.

3. Serial dilutions were made along one axis of the plate using a Costar octapette making dilutions of 1:1 to 1:4,096.

4. Make all serial dilutions, then add 50 ul of 2% SRBC to each well.

5. Allow plates to set for 2 hours at room temperature.

6. Then observe plates to note a positive reaction which is indicated by a diffuse spreading of the SRBC on the bottom of the well (shield). A negative result is indicated by the formation of a tight button of the red blood cells on the bottom of the well. The reciprocal of the last dilution at which the "shield" is seen is defined as the agglutination titer of the test.

7. Make further dilutions for testing if no "buttons" are observed at the end of a serial dilution.

APPENDIX TABLE 5. RADIOIMMUNOASSAY[a] FOR DETERMINATION OF SERUM CORTISOL CONCENTRATIONS

Reagents

1. Distilled water
2. Cortisol Standards, human source (0 to 60 $\mu g \cdot dl^{-1}$)
3. Cortisol antibody suspension (on 40 mg polymer particles)
4. Cortisol labeled with $^{125}I$ derivative (10 $\mu Ci$ $^{125}I$)
5. Test tubes (Fisher 14-962-10B)

Procedures

A. Standard Curve

1. Reconstituted standards with 500 $\mu l$ distilled water.
2. Gently mix and allow to stand for 10 minutes.
3. Add 50 $\mu l$ of each standard to a tube.
4. Add 100 $\mu l$ cortisol $I^{125}$ (red) to each tube.
5. Add 100 $\mu l$ antibody (blue) to each tube.
6. Mix gently with vortex and incubate in water bath (37°C) for one hour.
7. Centrifuge at > 1500 g (2500 rpm) for 15 minutes.
8. Invert the tubes and decant fluid, place on absorbant tissue for five minutes. Blot rims to remove any drops. Do NOT re-invert!
9. Read in gamma counter (Bechman 5500 counting system).
10. Create the standard curve.

B. Samples

1. Add 100 $\mu l$ of serum to a label tube, two tubes for each sample.
2. Follow the above instruction lines 4 thru 9.
3. Obtain concentration by comparing to standard curve.

[a]Amersham Amerlex Cortisol RIA kit

APPENDIX TABLE 6. MODEL EQUATION USED IN TRIALS 1, 2 AND 3

$$Y_{ijklm} = u + V_i + A_j + W_k + T_l + P_m + V_i \cdot A_j + W_k \cdot V_i + W_k \cdot A_j + W_k \cdot V_i \cdot A_j + V_i \cdot T_l + A_j \cdot T_l + P_{m(i \cdot j \cdot l)} + E_{ijklm}.$$

| | | |
|---|---|---|
| $u$ | = unknown mean | |
| $V_i$ | = effect of diet | fixed |
| $A_j$ | = effect of weaning ages | fixed |
| $W_k$ | = effect of time | fixed |
| $T_l$ | = effect of trial | fixed |
| $P_m$ | = effect of pig | random |
| $E$ | = residual error term | |

Weaning age X trial interaction used weaning age as the error term. Pig (diet X weaning age X trial) was used as the error term for diets, diets X trial and diets X weaning age. All other main effects and interactions used the residual error.

APPENDIX TABLE 7. ANTIGEN MIXTURE FOR TRIALS 4, 5 AND 6

1) 89.0 ml sterile physiological saline
2) 10.0 ml maldrox[a]
3) 10.0 mg lysozyme (Sigma L 6876)
4) 1.0 ml packed sheep red blood cells
5) 10.0 ml Freund's adjuvant[b]

[a]Contains 200 mg aluminum hydroxide and 250 magnesium hydroxide.
[b]Trials 4, 5 and 6 used Freund's complete adjuvant (Gibco 660-5721).

APPENDIX TABLE 8. MODEL EQUATION USED IN TRIALS 4, 5 AND 6

$$Y_{ijklm} = u + V_i + T_j + S_k + W_l + L_m + P_n + V_i \cdot W_l + T_j \cdot W_l + L_{m(k)} + V_i \cdot S_k + T_j \cdot S_k + V_i \cdot T_j + V_i \cdot T_j \cdot W_l + P_{n(l \cdot j \cdot m \cdot k)} + E_{ilklm \cdot}$$

| | | |
|---|---|---|
| $u$ | = unknown mean | |
| $V_i$ | = effect of diet | fixed |
| $T_j$ | = effect of temperature | fixed |
| $S_k$ | = effect of trial | fixed |
| $W_l$ | = effect of time | fixed |
| $L_m$ | = effect of litter | random |
| $P_n$ | = effect of pig | random |
| $E$ | = residual error term | |

Litter(trial) was used as the error term for trial. Pig (diet X temperature X trial X litter) was used as the error term for diet, temperature, litter(trial), diet X trial and diet X temperature and temperature X trial. All other main effects and interactions used the residual error.

APPENDIX TABLE 9. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETERMINATION OF ANTIBODY TITERS

Reagents

1. Antigen
   mix: 1) 10 mg lysozyme (Sigma L6876)
   2) 10 ml 0.9% saline

   a) Place 0.1 ml increments in ultra-low freezer
   b) add 0.1 ml lysozyme to 19.9 coating buffer to use (sufficient for three plates)

2. PBST (phosphate buffered saline with Tween)

mix: 1) 8.0 g NaCl
2) 0.2 g $KH_2PO_4$
3) 0.9 g $Na_2HPO_4$
4) 0.2 g KCl
5) 1.0 l distilled water

a) adjust pH to 7.4,
b) add .5 ml Tween 80

3. Acid Buffer

mix: 1) 4.0 l distilled water
2) 34.0 g NaCl
3) 24.0 g Sodium acetate
4) 4.5 ml acetic acid

a) adjust pH to 5 with the acetic acid
b) 8.0 ml Tween 40

4. Coating Buffer

mix: 1) 1.59 g $Na_2CO$
2) 2.93 g $NaHCO_3$
3) 1 liter distilled $H_2O$

a) adjust pH to 9.6

5. Conjugated antibody solution (makes 8 plates)

mix: 1) 40.0 ml acid buffer
2) 0.1 ml anti-swine peroxidase, heavy and light chain (Cappel 3215-0082)
3) 0.4 g bovine serum albumin (BSA) (Sigma A-7030)
4) 2.0 g NaCl

a) sufficient for eight plates

6. Substrate

mix: 1) 10.0 mg O-phenylenediamine
2) 1.0 ml methanol
3) 100.0 ml distilled water
4) .1 ml hydrogen peroxide

a) add the hydrogen peroxide just before adding substrate to plates

7. PBST plus 2% BSA

   mix: 1) 100 ml PBST
        2) 2 g BSA

   a) add heat to help dissolve
   b) trial 5 & 6 used Knox's unflavored gelatin (0.3%) instead of BSA

8. Nunc ImmulonII plates (4-42404)

## Procedures

1. coat all wells of 96 well plate with 50 µl of antigen, incubate 2 h at 37°C
2. wash three times with PBST
3. add 50 l PBST + 2% BSA to each well incubate 37°C, 30 minutes
4. wash three times with acid buffer
5. add 50 µl of acid buffer to each well add 50 µl of serum (diluted 1:50) to the first row in plate and mix well. Then serially dilute serum by removing 50 µl from the first well and add to the second row, mix, remove 50 µl from second row and add to third row, and continue to end of plate.
6. incubate 37°C for 30 minutes
7. wash three times with acid buffer
8. add 50 µl of conjugated antibody each well
9. incubate 37°C for 30 minutes
10. wash three times with acid buffer
11. add 100 µl of substrate to each well, incubate in dark, at room temperature for 30 minutes
12. add 25 µl sulfuric acid (8N)
13. read in Multiskan MCC 340 plate reader at 492 nm, single wavelength

APPENDIX TABLE 10. MITOGEN STIMULATED CELL BLASTOGENIC ASSAY TO EVALUATE LYMPHOCYTE SUBPOPULATION PROLIFERATION

ALL WORK MUST BE PERFORMED IN A STERILE ENVIRONMENT

i.e. in a laminar air flow hood; with all tubes, pipettes, media also sterile; tubes must be capped if removed from laminar air flow hood:

Reagents

1. Hank's balanced salt solution

Mix:
a) 10.0 ml Hank's balanced salt solution (10X) (Gibco, 310-41809)
b) 0.5 ml sodium bicarbonate (Sigma S8761)
c) 0.5 ml N-2 hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Sigma H0087)
d) 0.5 ml penicillin-streptomycin (Gibco 600-5145)
e) 88.5 ml sterile distilled water

100.0 ml

2. Media

Mix:
a) 10.0 ml pig serum (K.C. Biological 3007) (heat inactivated, 56 C for 30 min)
b) 1.5 ml Amino acid-sodium pyruvate mix
c) 1.0 ml Penicillin-streptomycin
d) 1.0 ml L-glutamine (Hazelton,AA-112-01)
e) 0.5 ml HEPES
f) 0.1 ml 2-mercaptoethanol (Sigma M6250)
g) 83.9 ml RMPI 1640 (KC Biological LM263-5)

100.0 ml

3. Tris

A) Stock solution:

Mix:
1) $NH_4Cl$ stock
a) dissolve 8.3 g $NH_4Cl$ in 1 L distilled water

2) Tris stock
   a) 6 g Trizma Base
      [Tris(hydroxymethyl)-aninomethane]
      (Sigma T1503)
   b) adjust pH to 7.65

B) Working solution

Mix: 1) 90 ml $NH_4Cl$ stock
     2) 10 ml Tris stock
     ---
     100 ml

Sterile filter
(Gelman Sciences 4192, 0.2 microns)

4. 2-Mercaptoethanol (2-ME)

A) Stock solution

1) 1.0 M solution
   Mix: 1) .5 ml 2-ME
        2) 6.6 ml sterile distilled water

2) 5 X $10^{-2}$ M solution
   a) Mix: 1) 5 ml 1.0 M 2-ME
           2) 95 ml sterile distilled water
   b) Sterile filter
      (Gelman Sciences 4192, 0.2 microns)
   c) store 4°C

3) 5 X $10^{-5}$ M solution
   a) add to culture medium for final concentration of 5 X $10^{-5}$ M

5. Sodium pyruvate-amino acid mixture

a) Stock

Mix: 1) .5 g sodium pyruvate (Sigma S8636)
     2) 50 ml distilled water

Sterile filter
(Gelman sciences 4192, 0.2 microns)

b) Mixture

Mix : 1) 1ml sterile sodium pyruvate stock
      2) 2 ml Amino acid (Sigma M7145)

6. Mitogens

   a) Concanavalin A (Con A) (Sigma C5275)

      1) mix 9.0 ml RMPI 1640 with 1.0 ml reconstituted ConA final dilution in media: 100 ug ConA per ml
      2) mix 9.0 ml RMPI 1640 with 1.0 ml mixture in step 1. Final dilution in media: 10 ug ConA per ml

   b) Lysozyme, (Sigma L6876)

      1) mix 10.0 ml RMPI 1640 with 10 mg lysozyme final dilution in media: 1 mg lysozyme per ml
      2) mix 9.0 ml RMPI 1640 with 1.0 ml mixture in step 1. Final dilution in media: .1 mg lysozyme per ml

   c) Phaesolus Vulgaris agglutin [M form] (PHA) (Gibco, 670-0576)

      1) add 1.1 ml reconstituted PHA to 10 ml media final dilution: 1 to 10
      2) remove 0.2 ml of 1:10 mixture, add to 9.8 ml media, final dilution: 1 to 500

7. Plasma vacutainers (American Scientific b-2984-54)

8. Histopaque 1077 (Sigma 1077)

9. Nunc u-bottom immunoplates (1-63320)

10. Dupont,Tritiated Thymidine, 6.7 Ci/mmol (NET-027)

11. Whittaker, filter paper (#23-995)

12. Ecosint (National diagnostic, LS-271)

## Procedures

1. Collect blood samples in heparin tubes.
2. Mix a 8 ml Hank's balanced salt solution with 8 ml of blood.
3. Carefully layer the mixture over 8 ml of histopaque 1077 in a sterile plastic centrifuge tube.
4. Spin capped tubes for 30 minutes at 1800 rpm, use minimal brake.

5. After centrifugation, use a pasteur pipet to remove the layer of white blood cell which is at the two fluid interface. Place cells in 10 ml test tubes.
6. Spin tubes for 10 minutes at 1200 rpm.
7. After centrifugation, decant and discard the liquid (while working in the hood), leaving a pellet on the bottom of the tube, which must be broken (by hitting the tube with a finger).
8. If the pellet formed has red blood cells present, add 1 ml tris (warmed to 37°C). Incubate at 37°C for 10 minutes.
9. Place tubes under sterile hood, add 10 ml Hank's, and place test tube in centrifuge again as in step 6.
10. Pour off solution. If pellet has red cells, repeat steps 7 and 8. Add tris only twice.
11. Wash cells with Hank's after the last treatment of tris by adding 4 mls of Hanks to each tube and centrifuge. Pour off liquid, repeat.
12. Add 1 ml of media to each test tube.
13. In a tube add .8 ml Hank's, .1 ml methylene blue and .1 ml of the solution in step 11. Mix gently but thoroughly.
14. Using a capillary tube, place this solution on a hemocytometer and count number of cells using a microscope.
15. After counting the cells, multiple by $10^8$ to obtain number of cells per ml.
16. Add appropriate amounts of media to make cell suspension of 2 X $10^6$ cells per ml.
17. Add 100 $\mu$l of each mitogen dilution to three wells in the microtiter. Do this for all six mixtures. Add 100 $\mu$l of media to three control wells.
18. Add 100 $\mu$l of cell suspension to each well in a microtiter plate.
19. Place plate in a 5% $CO_2$, humidified incubator at 37°C for 72 h.
20. Pulse each well by adding 1 $\mu$Ci of $^3$H-thymidine in a RPMI media.
21. After 18 h in the incubator, freeze the plates.
22. Harvest the cells on glass filter paper using a mini-Mash II harvestor (Whittaker: M. A. Bioproducts), placed in 3 ml liquid scintillation cocktail and counted in a Beckman LS 1800 scintillation counter.
23. Data was counted in terms of counts per minute. The average number of counts of each mitogen is divided by the average number of counts of the control and reported as an index number.

APPENDIX TABLE 11. VITAMIN E CONCENTRATION CONTAINED IN THE DIETS

| Item[a] | Supplemental Vitamin E | | | | SE | P |
|---|---|---|---|---|---|---|
| | X[b] | 10X | 20X | 50X | | |
| Vitamin E Concentration ($IU \cdot kg^{-1}$ feed) | 12.0 | 101.1 | 235.1 | 511.9 | 12.82 | .01 |

[a]Mean of five trials.
[b]Recommended NRC levels of Vitamin E supplemented to the diet ($IU \cdot kg^{-1}$).

APPENDIX TABLE 12. LEAST SQUARES MEANS FOR LIVER SELENIUM CONCENTRATION FOR TRIALS 5 AND 6

| Item[a] | Trial | | SE | P |
|---|---|---|---|---|
| | 5 | 6 | | |
| Selenium[b] | 219.3 | 247.5 | 17.1 | .29 |

[a]Number of observations; eight samples per trial; each sample was a composite of two pigs.
[b]Parts per billion.

APPENDIX TABLE 13. PEN LEAST SQUARES MEANS OF THE EFFECT OF TEMPERATURE ON SELENIUM CONCENTRATION IN THE LIVER FOR TRIALS 5 AND 6

| | Temperature (°C) | | | |
|---|---|---|---|---|
| Item[a] | 30 | 19 | SE | P |
| Selenium[b] | 244.0 | 223.0 | 17.0 | .41 |

[a]Number of observations; eight samples per temperature treatment; each sample was a composite of two pigs.
[b]Parts per billion.

APPENDIX TABLE 14. SELENIUM CONCENTRATION IN COMPOSITE FEED SAMPLES FOR TRIALS 1, 3, 4, 5 AND 6

| | Trial | | | | |
|---|---|---|---|---|---|
| Item[a] | 1 | 3 | 4 | 5 | 6 |
| Selenium[b] | 132 | 259 | 128 | 150 | 187 |

[a]Mean of all individual diets for that diet.
[b]Parts per billion.

APPENDIX TABLE 15. THE EFFECT OF SUPPLEMENTAL VITAMIN E ON SELENIUM CONCENTRATION IN THE LIVER FOR TRIALS 5 AND 6

| | Supplemental Vitamin E | | | | | |
|---|---|---|---|---|---|---|
| Item[a] | X[b] | 10X | 20X | 50X | SE | P |
| Selenium[c] | 192.3 | 238.5 | 245.7 | 257.6 | 24.1 | .32 |

[a]Mean of four samples per diet; each sample contains a composite of two pig livers.
[b]Recommended NRC levels of Vitamin E supplemented to diet ($IU \cdot kg^{-1}$).
[c]Parts per billion.

APPENDIX TABLE 16. EFFECT OF WEANING AGE X TRIAL INTERACTIONS ON LEAST SQUARES MEANS OF SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL AND ANTIBODY TITERS FOR TRIALS 1, 2 AND 3

| Item[a] | Weaning age (d) | Trial 1 | Trial 2 | Trial 3 | SE | P |
|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot dl^{-1}$)[b] | 21 | | .18 | .16 | | |
| | 28 | | .16 | .16 | | |
| | 35 | | .16 | .17 | .01 | .16 |
| Cortisol ($ng \cdot dl^{-1}$) | 21 | 22.5 | 7.5 | 6.4 | | |
| | 28 | 22.3 | 10.5 | 7.9 | | |
| | 35 | 24.5 | 17.6 | 9.0 | 1.09 | .01 |
| Antibody Titer[c] | 21 | 1.84 | 2.70 | 2.38 | | |
| | 28 | 2.18 | 2.61 | 1.94 | | |
| | 35 | 1.91 | 1.94 | 2.65 | .17 | .01 |

[a]Number of observations; fifty-two pigs in each weaning treatment; thirty pens; three trials and analyzed by the number of weeks on test.
[b]For trials 2 and 3 only.
[c]Injected with sheep red blood cells (.1 ml per pig) on d 0 and d 17 and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

APPENDIX TABLE 17. LEAST SQUARES MEANS OF SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL AND ANTIBODY TITERS EXPRESSED BY WEEK FOR TRIALS 1, 2 AND 3

| Item[a] | Week 1 | 2 | 3 | 4 | 5 | 6 | SE | P |
|---|---|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot ml^{-1}$)[b] | .21 | .12 | .14 | .15 | .18 | .20 | .01 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | 17.8 | 8.8 | 10.8 | 13.7 | 15.8 | 18.3 | .81 | .01 |
| Antibody Titer[c] | .13 | 1.68 | 1.69 | 2.95 | 4.21 | 3.92 | .13 | .01 |

[a]Number of observations; 156 pigs on test, three trials, sixty pens, and analyzed by the number of weeks on test.
[b]For trials 2 and 3 only.
[c]Injected with sheep red blood cells (.1 ml per pig) on d 0 and d 17 and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

APPENDIX TABLE 18. EFFECT OF DIETS X TRIAL INTERACTIONS ON LEAST SQUARES MEANS OF SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL AND ANTIBODY TITERS FOR TRIALS 1, 2 AND 3

| Item[a] | Supplemental Vitamin E | Trial 1 | Trial 2 | Trial 3 | SE | P |
|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot dl^{-1}$)[b] | X[c] | | .10 | .08 | | |
| | 20X | | .22 | .25 | .01 | .02 |
| Cortisol ($ng \cdot dl^{-1}$) | X | 25.7 | 11.6 | 7.4 | | |
| | 20X | 20.5 | 12.7 | 8.1 | .91 | .01 |
| Antibody Titer[d] | X | 1.79 | 3.30 | 2.32 | | |
| | 20X | 2.16 | 2.68 | 2.33 | .13 | .20 |

[a]Number of observations; 78 pigs in each diet treatment; 30 pens; three trials and analyzed by the number of weeks on test.
[b]For trials 2 and 3 only.
[c]Recommended NRC levels of Vitamin E (11 $IU \cdot kg^{-1}$).
[d]Injected with sheep red blood cells (.1 ml per pig) on d 0 and d 17 and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

APPENDIX TABLE 19. EFFECT OF WEANING AGE X DIET INTERACTIONS ON LEAST SQUARES MEANS OF SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL AND ANTIBODY TITERS FOR TRIALS 1, 2 AND 3

| Item[a] | Weaning age (d) | Supplemental Vitamin E | | SE | P |
|---|---|---|---|---|---|
| | | X[b] | 20X | | |
| Serum Vitamin E ($mg \cdot dl^{-1}$)[c] | 21 | .10 | .24 | | |
| | 28 | .09 | .23 | | |
| | 35 | .09 | .23 | .01 | .94 |
| Cortisol ($ng \cdot dl^{-1}$) | 21 | 13.1 | 11.1 | | |
| | 28 | 14.3 | 12.8 | | |
| | 35 | 16.6 | 17.4 | .95 | .47 |
| Antibody Titer[d] | 21 | 2.38 | 2.24 | | |
| | 28 | 2.20 | 2.29 | | |
| | 35 | 2.84 | 2.64 | .15 | .72 |

[a]Number of observations; fifty-two pigs in each weaning treatment; thirty pens; three trials and analyzed by the number of weeks on test.
[b]Recommended NRC levels of Vitamin E (11 $IU \cdot kg^{-1}$).
[c]For trials 2 and 3 only.
[d]Injected with sheep red blood cells (.1 ml per pig) on d 0 and d 17 and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

APPENDIX TABLE 20. EFFECT OF VITAMIN E SUPPLEMENTATION ON PEN PERFORMANCE MEANS OF WEANLING PIGS IN TRIALS 1,2 AND 3

| Item[a] | Supplemental Vitamin E | | SE | P |
|---|---|---|---|---|
| | X[b] | 20X | | |
| Avg weight (kg) | | | | |
| Initial | 8.0 | 7.8 | .38 | .76 |
| Week 1 | 9.0 | 8.8 | .40 | .60 |
| 2 | 11.0 | 10.8 | .47 | .60 |
| 3 | 14.2 | 13.6 | .60 | .23 |
| 4 | 18.0 | 17.6 | .73 | .70 |
| 5 | 21.8 | 21.5 | .76 | .89 |
| Avg daily feed intake (kg) | | | | |
| Week 1 | .27 | .26 | .03 | .84 |
| 2 | .51 | .49 | .04 | .55 |
| 3 | .80 | .81 | .05 | .57 |
| 4 | .97 | .97 | .05 | .63 |
| 5 | 1.13 | 1.13 | .05 | .80 |
| 1-2 | .39 | .38 | .03 | .57 |
| 1-3 | .53 | .52 | .03 | .98 |
| 1-4 | .64 | .63 | .03 | .84 |
| 1-5 | .74 | .73 | .03 | .80 |
| Avg daily gain (kg) | | | | |
| Week 1 | .14 | .14 | .01 | .70 |
| 2 | .29 | .29 | .02 | .76 |
| 3 | .44 | .41 | .03 | .05 |
| 4 | .54 | .57 | .02 | .14 |
| 5 | .55 | .56 | .03 | .71 |
| 1-2 | .22 | .21 | .01 | .64 |
| 1-3 | .29 | .28 | .01 | .16 |
| 1-4 | .36 | .35 | .01 | .74 |
| 1-5 | .40 | .39 | .03 | .98 |
| Feed/gain | | | | |
| Week 1 | 2.81 | 3.09 | .95 | .88 |
| 2 | 1.87 | 1.83 | .15 | .67 |
| 3 | 1.86 | 2.08 | .12 | .09 |
| 4 | 1.84 | 1.75 | .07 | .26 |
| 5 | 2.28 | 2.08 | .18 | .33 |
| 1-2 | 1.83 | 1.83 | .08 | .89 |
| 1-3 | 1.81 | 1.91 | .06 | .24 |
| 1-4 | 1.80 | 1.82 | .06 | .67 |
| 1-5 | 1.88 | 1.88 | .06 | .99 |

[a]Number of observations; thirty pens, eighty-one pigs per diet.
[b]Recommended NRC levels of Vitamin E (11 $IU \cdot kg^{-1}$).

APPENDIX TABLE 21. EFFECT OF DIET BY TRIAL INTERACTIONS ON PEN PERFORMANCE MEANS OF WEANLING PIGS IN TRIALS 1, 2 AND 3

| | Supplemental Vitamin E | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Trial 1 | | Trial 2 | | Trial 3 | | | |
| Item[a] | X[b] | 20X | X | 20X | X | 20X | SE | P |
| Avg weight (kg) | | | | | | | | |
| Initial | 8.2 | 7.8 | 7.4 | 7.9 | 8.1 | 7.8 | .55 | .39 |
| Week 1 | 9.2 | 8.9 | 8.3 | 8.4 | 9.2 | 8.9 | .56 | .73 |
| 2 | 11.4 | 10.9 | 10.4 | 10.7 | 11.1 | 10.7 | .68 | .62 |
| 3 | 14.4 | 13.7 | 13.7 | 13.4 | 14.1 | 13.7 | .91 | .85 |
| 4 | 18.3 | 17.2 | 17.1 | 17.6 | 18.1 | 18.1 | .90 | .39 |
| 5 | 22.2 | 20.8 | 20.8 | 21.4 | 22.0 | 22.3 | 1.21 | .31 |
| Avg daily feed intake (kg) | | | | | | | | |
| Week 1 | .34 | .28 | .16 | .19 | .26 | .28 | .03 | .26 |
| 2 | .49 | .45 | .45 | .49 | .57 | .54 | .06 | .97 |
| 3 | .83 | .78 | .71 | .76 | .81 | .85 | .09 | .61 |
| 4 | .97 | .89 | 1.00 | 1.04 | .96 | 1.02 | .08 | .30 |
| 5 | 1.09 | 1.08 | 1.12 | 1.28 | 1.17 | 1.10 | .08 | .42 |
| 1-2 | .41 | .36 | .30 | .34 | .41 | .41 | .04 | .57 |
| 1-3 | .55 | .50 | .44 | .48 | .54 | .56 | .05 | .98 |
| 1-4 | .66 | .60 | .58 | .62 | .65 | .68 | .05 | .84 |
| 1-5 | .74 | .70 | .69 | .75 | .75 | .76 | .05 | .80 |
| Avg daily gain (kg) | | | | | | | | |
| Week 1 | .14 | .15 | .12 | .07 | .15 | .16 | .02 | .67 |
| 2 | .32 | .29 | .30 | .33 | .27 | .26 | .08 | .66 |
| 3 | .44 | .40 | .48 | .39 | .44 | .42 | .04 | .45 |
| 4 | .54 | .50 | .49 | .59 | .57 | .63 | .04 | .07 |
| 5 | .56 | .52 | .53 | .55 | .55 | .60 | .04 | .58 |
| 1-2 | .23 | .22 | .21 | .20 | .21 | .21 | .02 | .99 |
| 1-3 | .30 | .28 | .30 | .26 | .29 | .28 | .02 | .76 |
| 1-4 | .36 | .33 | .35 | .34 | .36 | .37 | .02 | .40 |
| 1-5 | .40 | .37 | .38 | .39 | .40 | .41 | .02 | .30 |
| Feed/gain | | | | | | | | |
| Week 1 | 3.51 | 4.72 | 1.62 | 2.00 | 2.71 | 2.01 | 1.56 | .84 |
| 2 | 1.58 | 1.58 | 1.53 | 1.54 | 2.31 | 2.23 | .20 | .94 |
| 3 | 1.85 | 1.94 | 1.58 | 2.03 | 2.00 | 2.25 | .21 | .57 |
| 4 | 1.78 | 1.82 | 2.11 | 1.81 | 1.76 | 1.65 | .14 | .46 |
| 5 | 1.98 | 2.11 | 2.17 | 2.33 | 2.64 | 1.91 | .24 | .29 |
| 1-2 | 1.83 | 1.70 | 1.46 | 1.74 | 2.02 | 2.01 | .12 | .89 |
| 1-3 | 1.82 | 1.79 | 1.48 | 1.83 | 1.96 | 2.07 | .13 | .24 |
| 1-4 | 1.81 | 1.80 | 1.68 | 1.80 | 1.86 | 1.88 | .10 | .67 |
| 1-5 | 1.86 | 1.88 | 1.79 | 1.93 | 1.96 | 1.86 | .11 | .99 |

[a]Number of observations; Trial 1, twenty-four pens, thirty pigs; Trial 2, twelve pens, twenty-one pigs; Trial 3, twenty-four pens, thirty pigs.
[b]Recommended NRC levels of Vitamin E (11 IU per kg)

APPENDIX TABLE 22. EFFECT OF WEANING AGE ON PEN PERFORMANCE MEANS OF WEANLING PIGS IN TRIALS 1, 2 AND 3

| Item[a] | Weaning Age (d) 21 | 28 | 35 | SE | P |
|---|---|---|---|---|---|
| Avg weight (kg) | | | | | |
| Initial | 6.0 | 7.6 | 10.1 | .26 | .01 |
| Week 1 | 6.9 | 8.6 | 11.2 | .27 | .02 |
| 2 | 8.7 | 10.3 | 13.8 | .31 | .01 |
| 3 | 10.8 | 13.4 | 17.6 | .37 | .01 |
| 4 | 13.8 | 17.6 | 22.0 | .45 | .01 |
| 5 | 17.8 | 21.4 | 25.7 | .55 | .01 |
| Avg daily feed intake (kg) | | | | | |
| Week 1 | .22 | .30 | .28 | .03 | .09 |
| 2 | .47 | .42 | .62 | .03 | .02 |
| 3 | .61 | .79 | 1.01 | .02 | .01 |
| 4 | .78 | 1.07 | 1.06 | .05 | .04 |
| 5 | 1.14 | 1.09 | 1.16 | .05 | .16 |
| 1-2 | .35 | .36 | .45 | .02 | .03 |
| 1-3 | .43 | .50 | .63 | .03 | .01 |
| 1-4 | .52 | .65 | .74 | .03 | .03 |
| 1-5 | .64 | .73 | .82 | .03 | .09 |
| Avg daily gain (kg) | | | | | |
| Week 1 | .13 | .14 | .16 | .02 | .34 |
| 2 | .26 | .25 | .37 | .02 | .01 |
| 3 | .29 | .44 | .54 | .02 | .01 |
| 4 | .44 | .60 | .62 | .02 | .01 |
| 5 | .57 | .55 | .54 | .03 | .77 |
| 1-2 | .19 | .19 | .26 | .01 | .03 |
| 1-3 | .22 | .27 | .36 | .01 | .01 |
| 1-4 | .28 | .35 | .42 | .01 | .01 |
| 1-5 | .34 | .39 | .45 | .01 | .01 |
| Feed/gain | | | | | |
| Week 1 | 2.35 | 4.20 | 2.31 | 1.06 | .87 |
| 2 | 1.87 | 1.93 | 1.75 | .17 | .88 |
| 3 | 2.15 | 1.88 | 1.88 | .14 | .56 |
| 4 | 1.83 | 1.82 | 1.74 | .09 | .86 |
| 5 | 2.32 | 2.01 | 2.21 | .20 | .77 |
| 1-2 | 1.80 | 1.97 | 1.73 | .09 | .07 |
| 1-3 | 1.91 | 1.89 | 1.79 | .09 | .55 |
| 1-4 | 1.87 | 1.83 | 1.75 | .07 | .49 |
| 1-5 | 1.93 | 1.87 | 1.85 | .06 | .62 |

[a]Number of observations; twenty pens, fifty-four pigs per weaning treatment.

APPENDIX TABLE 23. EFFECT OF WEANING AGE X TRIAL INTERACTIONS ON PEN PERFORMANCE MEANS FOR TRIALS 1, 2 AND 3

| Weaning age -> | 21 d | | | 28 d | | | 35 d | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item[a] Trial -> | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | SE | P |
| Avg weight (kg) | | | | | | | | | | | |
| Initial | 6.1 | 6.0 | 5.0 | 7.7 | 7.5 | 7.7 | 10.3 | 9.5 | 10.2 | .38 | .41 |
| Week 1 | 7.1 | 6.9 | 6.7 | 8.6 | 7.7 | 9.1 | 11.4 | 10.5 | 11.4 | .38 | .05 |
| 2 | 9.0 | 8.3 | 8.6 | 10.6 | 9.8 | 10.3 | 13.9 | 13.5 | 13.8 | .95 | .69 |
| 3 | 11.2 | 10.4 | 10.4 | 13.5 | 13.4 | 13.2 | 17.3 | 16.9 | 18.1 | .51 | .47 |
| 4 | 14.4 | 13.9 | 13.3 | 17.3 | 17.5 | 17.8 | 21.4 | 20.6 | 23.3 | .65 | .06 |
| 5 | 18.1 | 18.3 | 17.3 | 21.4 | 21.2 | 21.5 | 24.9 | 24.0 | 27.4 | .76 | .13 |
| Avg daily feed intake (kg) | | | | | | | | | | | |
| Week 1 | .23 | .12 | .26 | .33 | .22 | .31 | .36 | .19 | .24 | .03 | .06 |
| 2 | .54 | .39 | .45 | .38 | .38 | .49 | .50 | .64 | .72 | .05 | .09 |
| 3 | .61 | .51 | .65 | .68 | .86 | .86 | 1.13 | .82 | .98 | .07 | .01 |
| 4 | .75 | .87 | .78 | 1.10 | 1.07 | 1.05 | .95 | 1.13 | 1.13 | .07 | .01 |
| 5 | 1.19 | 1.13 | 1.08 | .98 | 1.29 | 1.09 | 1.08 | 1.18 | 1.22 | .09 | .73 |
| 1-2 | .38 | .26 | .35 | .35 | .30 | .40 | .43 | .42 | .48 | .03 | .73 |
| 1-3 | .46 | .34 | .45 | .46 | .48 | .55 | .66 | .55 | .65 | .14 | .37 |
| 1-4 | .53 | .48 | .53 | .62 | .63 | .68 | .74 | .69 | .74 | .05 | .02 |
| 1-5 | .66 | .61 | .64 | .69 | .76 | .76 | .80 | .79 | .86 | .05 | .10 |
| Avg daily gain (kg) | | | | | | | | | | | |
| Week 1 | .14 | .13 | .11 | .13 | .03 | .19 | .16 | .14 | .17 | .02 | .10 |
| 2 | .27 | .21 | .27 | .28 | .31 | .18 | .36 | .43 | .34 | .02 | .11 |
| 3 | .32 | .29 | .27 | .42 | .51 | .41 | .51 | .48 | .61 | .03 | .13 |
| 4 | .45 | .50 | .40 | .55 | .59 | .65 | .56 | .53 | .74 | .04 | .26 |
| 5 | .54 | .63 | .58 | .57 | .52 | .55 | .51 | .48 | .59 | .03 | .37 |
| 1-2 | .21 | .17 | .19 | .21 | .17 | .19 | .26 | .28 | .26 | .02 | .78 |
| 1-3 | .25 | .21 | .21 | .28 | .28 | .26 | .34 | .35 | .38 | .02 | .59 |
| 1-4 | .30 | .28 | .26 | .35 | .36 | .36 | .40 | .40 | .47 | .02 | .88 |
| 1-5 | .34 | .35 | .32 | .39 | .39 | .40 | .42 | .41 | .49 | .02 | .76 |
| Feed/gain | | | | | | | | | | | |
| Week 1 | 1.54 | 1.03 | 3.98 | 7.19 | 2.97 | 1.82 | 3.63 | 1.41 | 1.43 | 1.55 | .57 |
| 2 | 1.97 | 1.92 | 1.74 | 1.33 | 1.18 | 2.89 | 1.42 | 1.51 | 2.19 | .18 | .01 |
| 3 | 1.82 | 1.92 | 2.60 | 1.62 | 1.80 | 2.17 | 2.25 | 1.70 | 1.60 | .22 | .03 |
| 4 | 1.68 | 1.81 | 1.98 | 1.99 | 1.83 | 1.63 | 1.72 | 2.23 | 1.51 | .16 | .04 |
| 5 | 2.25 | 1.82 | 2.63 | 1.74 | 2.43 | 2.08 | 2.15 | 2.50 | 2.12 | .26 | .92 |
| 1-2 | 1.81 | 1.54 | 1.91 | 1.79 | 1.77 | 2.24 | 1.71 | 1.47 | 1.89 | .13 | .60 |
| 1-3 | 1.80 | 1.69 | 2.13 | 1.66 | 1.71 | 2.18 | 1.96 | 1.57 | 1.73 | .13 | .10 |
| 1-4 | 1.76 | 1.70 | 2.05 | 1.79 | 1.75 | 1.91 | 1.85 | 1.77 | 1.64 | .10 | .11 |
| 1-5 | 1.90 | 1.73 | 2.04 | 1.77 | 1.93 | 1.93 | 1.92 | 1.92 | 1.75 | .09 | .21 |

[a]Number of observations; Trial 1, twenty-four pens, thirty pigs; Trial 2, twelve pens,twenty-one pigs; Trial 3, twenty-four pens, thirty pigs.
[b]Recommended NRC levels of Vitamin E (11 IU per kg).

APPENDIX TABLE 24. EFFECT OF TRIALS ON PEN PERFORMANCE MEANS FOR TRIALS 1, 2 AND 3

| Item[a] | Trial 1 | Trial 2 | Trial 3 | SE | P |
|---|---|---|---|---|---|
| Avg weight (kg) | | | | | |
| Initial | 8.0 | 7.7 | 8.0 | .45 | .02 |
| Week 1 | 9.0 | 8.4 | 9.0 | .47 | .01 |
| 2 | 11.1 | 10.6 | 10.9 | .58 | .03 |
| 3 | 14.0 | 13.6 | 13.9 | .73 | .13 |
| 4 | 17.7 | 17.3 | 18.1 | .85 | .15 |
| 5 | 21.5 | 21.1 | 22.1 | .84 | .12 |
| Avg daily feed (kg) | | | | | |
| Week 1 | .31 | .18 | .27 | .02 | .01 |
| 2 | .47 | .47 | .55 | .04 | .20 |
| 3 | .81 | .73 | .83 | .06 | .90 |
| 4 | .93 | 1.02 | .99 | .05 | .49 |
| 5 | 1.09 | 1.20 | 1.13 | .05 | .08 |
| 1-2 | .39 | .32 | .41 | .03 | .07 |
| 1-3 | .53 | .46 | .55 | .03 | .32 |
| 1-4 | .63 | .60 | .66 | .02 | .37 |
| 1-5 | .72 | .72 | .75 | .04 | .57 |
| Avg daily gain (kg) | | | | | |
| Week 1 | .14 | .10 | .16 | .02 | .08 |
| 2 | .30 | .31 | .26 | .02 | .10 |
| 3 | .42 | .43 | .43 | .03 | .82 |
| 4 | .52 | .54 | .60 | .03 | .03 |
| 5 | .54 | .54 | .58 | .03 | .52 |
| 1-2 | .22 | .20 | .21 | .01 | .44 |
| 1-3 | .29 | .28 | .28 | .02 | .80 |
| 1-4 | .35 | .35 | .36 | .02 | .37 |
| 1-5 | .39 | .38 | .41 | .01 | .26 |
| Feed/gain | | | | | |
| 1 | 4.12 | 1.80 | 2.36 | .95 | .47 |
| 2 | 1.58 | 1.54 | 2.27 | .14 | .01 |
| 3 | 1.90 | 1.81 | 2.13 | .15 | .44 |
| 4 | 1.79 | 1.95 | 1.71 | .10 | .27 |
| 5 | 2.05 | 2.25 | 2.28 | .18 | .38 |
| 1-2 | 1.77 | 1.60 | 2.01 | .08 | .02 |
| 1-3 | 1.81 | 1.66 | 2.01 | .09 | .13 |
| 1-4 | 1.80 | 1.74 | 1.87 | .07 | .60 |
| 1-5 | 1.87 | 1.86 | 1.91 | .07 | .60 |

[a]Number of observations; Trial 1, twenty-four pens, sixty pigs; Trial 2, twelve pens, forty-two pigs; Trial 3, twenty-four pens, sixty pigs.

APPENDIX TABLE 25. LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES EXPRESSED BY WEEK FOR TRIAL 4

| Item[b] | Weeks[a] 0 | 1 | 2 | 3 | 4 | 5 | SE | P |
|---|---|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot dl^{-1}$) | .22 | .21 | .17 | .18 | .23 | .28 | .02 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | 13.8 | 6.9 | 8.1 | 10.3 | 15.1 | 14.9 | 1.02 | .01 |
| Antibody Titer[c] | | | | | | | | |
| SRBC | .8 | 2.7 | 2.3 | 2.6 | 2.2 | 2.4 | .38 | .01 |
| Lysozyme | 4.1 | 3.2 | 3.4 | 3.2 | 3.8 | 3.9 | .58 | .24 |
| Stimulation Indices[d] | | | | | | | | |
| Lysozyme | | | | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 1.4 | 1.6 | 1.5 | 1.6 | 3.5 | 3.1 | .61 | .02 |
| .1 $mg \cdot ml^{-1}$ | 1.1 | 1.4 | 1.3 | 1.5 | 2.9 | 3.1 | .42 | .01 |
| Concanavilin A | | | | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 41.2 | 123.5 | 89.0 | 27.8 | 121.1 | 152.1 | 24.21 | .01 |
| 10 $\mu g \cdot ml^{-1}$ | 3.9 | 4.9 | 4.5 | 2.5 | 9.2 | 11.8 | 1.45 | .01 |
| Phytohemagglutinin | | | | | | | | |
| 1:10 | 72.1 | 140.5 | 72.6 | 53.5 | 38.6 | 66.8 | 9.20 | .01 |
| 1:500 | 10.1 | 7.2 | 7.3 | 2.3 | 1.5 | 12.3 | 6.21 | .02 |

[a]Number of weeks on study (after weaning).
[b]Number of observations; eight pigs, four pens (two pigs per pen) on study.
[c]Injected with sheep red blood cells (.1 ml) and lysozyme (10 μg) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.

APPENDIX TABLE 26. LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES EXPRESSED BY WEEK FOR TRIAL 5

| Item[b] | Weeks[a] 0 | 1 | 2 | 3 | 4 | 5 | SE | P |
|---|---|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot dl^{-1}$) | .19 | .15 | .15 | .21 | .27 | .31 | .01 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | 13.6 | 3.9 | 5.5 | 6.6 | 7.9 | 11.8 | 1.15 | .01 |
| Antibody Titer[c] | | | | | | | | |
| SRBC | .1 | 2.0 | 1.7 | 1.9 | 3.9 | 3.8 | .19 | .01 |
| Lysozyme | 6.7 | 6.5 | 6.7 | 6.4 | 6.6 | 6.5 | .27 | .96 |
| Stimulation Indices[d] | | | | | | | | |
| Lysozyme | | | | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 1.2 | 3.1 | 2.5 | 1.7 | | 4.7 | 1.03 | .08 |
| .1 $mg \cdot ml^{-1}$ | 1.2 | 1.2 | 2.8 | 2.0 | | 3.1 | .67 | .13 |
| Concanavilin A | | | | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 119.1 | 176.1 | 193.7 | 133.9 | | 234.4 | .93 | .01 |
| 10 $\mu g \cdot ml^{-1}$ | 52.8 | 45.4 | 18.8 | 16.9 | | 26.5 | 6.33 | .02 |
| Phytohemagglutinin | | | | | | | | |
| 1:10 | 384.9 | 715.1 | 317.9 | 270.9 | | 489.3 | 55.50 | .01 |
| 1:500 | 22.9 | 12.2 | 2.1 | 1.8 | | 39.8 | 3.81 | .01 |

[a]Number of weeks on study (after weaning).
[b]Number of observations; thirty-two pigs, sixteen pens (two pigs per pen) on study.
[c]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.

APPENDIX TABLE 27. LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES EXPRESSED BY WEEK FOR TRIAL 6

| Item[b] | Weeks[a] 0 | 1 | 2 | 3 | 4 | 5 | SE | P |
|---|---|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot dl^{-1}$) | .12 | .11 | .14 | .21 | .27 | .28 | .01 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | 9.2 | 7.8 | 11.7 | 12.1 | 11.2 | 11.7 | .99 | .01 |
| Antibody Titer[c] | | | | | | | | |
| SRBC | .2 | .4 | .3 | 1.0 | 1.9 | 1.6 | .17 | .01 |
| Lysozyme | 2.2 | 2.5 | 3.3 | 3.8 | 6.2 | 5.3 | .28 | .01 |
| Stimulation Indices[d] | | | | | | | | |
| Lysozyme | | | | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 2.7 | 2.5 | 3.8 | 8.2 | 2.0 | .66 | .01 | .01 |
| .1 $mg \cdot ml^{-1}$ | 2.2 | 2.5 | 2.6 | 5.8 | 2.2 | .51 | .01 | .04 |
| Concanavilin A | | | | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 136.0 | 132.1 | 184.6 | 189.4 | 86.8 | 22.72 | .01 | .33 |
| 10 $\mu g \cdot ml^{-1}$ | 12.4 | 9.7 | 15.7 | 21.1 | 5.6 | 3.42 | .02 | .19 |
| Phytohemagglutinin | | | | | | | | |
| 1:10 | 106.3 | 293.4 | 166.2 | 218.3 | 138.6 | 57.5 | .18 | .01 |
| 1:500 | 2.3 | 6.0 | 3.2 | 2.6 | 1.8 | 1.4 | .20 | .01 |

[a]Number of weeks on study (after weaning).
[b]Number of observations; thirty-two pigs, sixteen pens (two pigs per pen) on study.
[c]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbant assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.

APPENDIX TABLE 28. LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES EXPRESSED BY WEEK FOR TRIALS 4, 5 AND 6

| Item[b] | Weeks[a] 0 | 1 | 2 | 3 | 4 | 5 | SE | P |
|---|---|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot dl^{-1}$) | .20 | .15 | .16 | .20 | .27 | .30 | .02 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | 14.5 | 6.2 | 8.6 | 9.7 | 10.7 | 12.2 | .70 | .01 |
| Antibody Titer[c] | | | | | | | | |
| SRBC | .0 | 1.6 | 1.4 | 1.8 | 2.9 | 2.9 | .19 | .01 |
| Lysozyme | 4.9 | 3.8 | 4.3 | 4.3 | 5.5 | 5.1 | .23 | .01 |
| Stimulation Indices[d] | | | | | | | | |
| Lysozyme | | | | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | .7 | 2.4 | 2.2 | 2.3 | 4.0 | 3.2 | .51 | .01 |
| .1 $mg \cdot ml^{-1}$ | 1.2 | 1.5 | 2.2 | 2.1 | 4.6 | 2.6 | .40 | .01 |
| Concanavilin A | | | | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 73.8 | 142.3 | 141.5 | 127.6 | 182.1 | 152.5 | 16.70 | .03 |
| 10 $\mu g \cdot ml^{-1}$ | 36.7 | 21.5 | 10.9 | 12.6 | 22.5 | 14.2 | 3.84 | .01 |
| Phytohemagglutinin | | | | | | | | |
| 1:10 | 169.5 | 303.3 | 229.3 | 163.6 | 257.5 | 227.8 | 41.40 | .17 |
| 1:500 | 17.3 | 7.1 | 5.0 | 2.4 | 9.0 | 21.7 | 3.22 | .01 |

[a]Number of weeks on study (after weaning).
[b]Number of observations; eighty pigs, forty pens (two pigs per pen) on study.
[c]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.

APPENDIX TABLE 29. EFFECT OF INDIVIDUAL DIETS ON SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES EXPRESSED BY WEEK.

| | Diet 1 | | | | | | Diet 2 | | | | | | Diet 3 | | | | | | Diet 4 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item[a] | 0 | 1 | 2 | 3 | 4 | 5 | 0 | 1 | 2 | 3 | 4 | 5 | 0 | 1 | 2 | 3 | 4 | 5 | 0 | 1 | 2 | 3 | 4 | 5 | SE | P |
| Serum Vitamin E ($mg \cdot dl^{-1}$) | .24 | .07 | .05 | .06 | .07 | .07 | .22 | .15 | .13 | .22 | .24 | .29 | .17 | .17 | .19 | .22 | .33 | .34 | .16 | .20 | .25 | .30 | .42 | .48 | .02 | .01 |
| Cortisol ($mg \cdot dl^{-1}$) | 16.4 | 5.7 | 8.1 | 9.6 | 10.4 | 12.7 | 10.6 | 6.2 | 10.0 | 10.1 | 12.2 | 12.7 | 14.4 | 5.3 | 7.0 | 10.0 | 10.5 | 11.8 | 16.7 | 7.1 | 9.3 | 9.2 | 9.4 | 11.5 | 1.5 | .35 |
| Antibody Titers[b] | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SRBC | .06 | 2.14 | 1.77 | 2.32 | 2.80 | 3.09 | 0.0 | 1.01 | 1.38 | 1.64 | 2.80 | 3.19 | 0.0 | 1.61 | 1.08 | 1.37 | 2.16 | 1.87 | 0.0 | 1.68 | 1.21 | 1.78 | 3.62 | 3.28 | .33 | .16 |
| ELISA | 6.02 | 3.42 | 4.45 | 4.60 | 5.34 | 4.55 | 4.09 | 3.89 | 4.42 | 4.42 | 5.39 | 5.18 | 5.54 | 4.08 | 4.66 | 4.23 | 4.97 | 5.10 | 3.93 | 3.95 | 3.61 | 3.86 | 6.14 | 5.33 | .43 | .09 |
| Stimulation Indexes[c] | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lysozyme | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 1.09 | 1.69 | 2.0 | 2.29 | 3.30 | 2.11 | 1.21 | 2.04 | 2.07 | 2.66 | 5.21 | 3.91 | 34.4 | 149.0 | 169.6 | 138.9 | 221.8 | 158.8 | 112.8 | 150.4 | 127.4 | 155.1 | 92.1 | 177.4 | 33.2 | .63 |
| .1 $mg \cdot ml^{-1}$ | .85 | 1.32 | 4.14 | 1.69 | 3.54 | 2.00 | 1.86 | 1.84 | 2.32 | 2.73 | 4.97 | 3.49 | 30.4 | 26.5 | 16.5 | 13.6 | 28.0 | 15.1 | 49.5 | 13.8 | 9.1 | 14.0 | 25.9 | 17.5 | 7.7 | .83 |
| Concanavilin A | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 56.3 | 123.4 | 181.9 | 92.4 | 194.5 | 144.1 | 97.5 | 146.3 | 87.2 | 124.0 | 119.9 | 129.7 | 0.0 | 4.15 | 2.83 | 2.07 | 4.77 | 4.37 | 1.21 | 1.68 | 1.85 | 2.01 | 2.67 | 2.28 | 1.01 | .57 |
| 10 $\mu g \cdot ml^{-1}$ | 21.3 | 26.3 | 10.5 | 15.5 | 20.9 | 11.9 | 45.5 | 19.2 | 7.5 | 7.1 | 15.4 | 12.4 | 1.07 | 1.58 | 1.33 | 2.10 | 6.47 | 2.35 | .98 | 1.38 | 1.16 | 1.79 | 3.47 | 2.63 | .80 | .19 |
| Phytohemagglutinin | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1:10 | 173.4 | 281.0 | 219.4 | 111.0 | 224.9 | 223.1 | 85.7 | 367.4 | 154.8 | 223.6 | 352.9 | 197.3 | 223.4 | 286.8 | 239.5 | 181.5 | 266.9 | 219.8 | 195.4 | 277.9 | 303.4 | 138.4 | 185.5 | 270.9 | 76.4 | .96 |
| 1:500 | 9.5 | 10.7 | 7.6 | 1.3 | 9.4 | 17.2 | 12.3 | 10.0 | 2.9 | 3.0 | 12.3 | 34.1 | 21.8 | 4.5 | 3.2 | 2.5 | 7.3 | 18.2 | 25.7 | 3.1 | 6.1 | 2.8 | 7.0 | 17.2 | 6.4 | .88 |

[a]Number of observations; eighty pigs (two pigs per pen) on study.
[b]Injected with sheep red blood cells (.1 ml per pig) and lysozyme (10 $\mu g$ per pig) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA). Initially, each sample was diluted 1:50 for ELISA. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[c]Nitogen concentration per ml of media.

APPENDIX TABLE 30. EFFECT OF TEMPERATURE ON SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS, AND STIMULATION INDEXES EXPRESSED OVERTIME

| | Temperature (°C) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 | | | | | | 19 | | | | | | | |
| Item[a] | 0 | 1 | 2 | 3 | 4 | 5 | 0 | 1 | 2 | 3 | 4 | 5 | SE | P |
| Serum Vitamin E ($mg \cdot dl^{-1}$) | .22 | .14 | .17 | .21 | .27 | .31 | .17 | .15 | .14 | .19 | .26 | .28 | .01 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | 16.4 | 5.8 | 8.3 | 9.6 | 9.8 | 11.8 | 12.6 | 6.5 | 8.9 | 9.9 | 11.5 | 12.5 | 1.1 | .22 |
| Antibody Titers[b] | | | | | | | | | | | | | | |
| SRBC | 0.00 | 1.56 | 1.32 | 1.64 | 2.72 | 2.73 | 0.00 | 1.67 | 1.40 | 1.92 | 2.97 | 2.99 | .21 | .99 |
| Lysozyme | 4.76 | 3.93 | 4.24 | 4.22 | 5.78 | 5.00 | 5.03 | 3.74 | 4.33 | 4.34 | 5.14 | 5.09 | .32 | .65 |
| Stimulation Indexes[c] | | | | | | | | | | | | | | |
| Lysozyme | | | | | | | | | | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 1.01 | 2.94 | 2.90 | 2.43 | 4.41 | 2.70 | .33 | 1.84 | 2.27 | 2.08 | 3.57 | 3.64 | .72 | .72 |
| .1 $mg \cdot ml^{-1}$ | 1.64 | 1.58 | 2.06 | 2.14 | 5.51 | 2.50 | .75 | 1.48 | 2.43 | 2.00 | 3.71 | 2.74 | .57 | .67 |
| Concanavilin A | | | | | | | | | | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 76.2 | 130.6 | 122.6 | 126.7 | 172.9 | 156.4 | 71.3 | 154.0 | 155.4 | 128.6 | 186.3 | 148.6 | 23.5 | .96 |
| 10 $\mu g \cdot ml^{-1}$ | 30.5 | 22.4 | 8.2 | 11.9 | 25.9 | 16.8 | 42.8 | 20.5 | 13.6 | 13.2 | 19.2 | 11.7 | 5.4 | .59 |
| Phytohemagglutinin | | | | | | | | | | | | | | |
| 1:10 | 93.1 | 314.6 | 173.8 | 158.3 | 302.0 | 213.6 | 245.9 | 291.9 | 284.6 | 168.9 | 208.1 | 242.0 | 58.5 | .52 |
| 1:500 | 10.9 | 8.0 | 5.3 | 2.3 | 9.0 | 22.3 | 23.7 | 6.2 | 4.6 | 2.5 | 9.1 | 21.0 | 4.5 | .74 |

[a]Number of observations; eighty pigs (two pigs per pen) on study.
[b]Injected with sheep red blood cells (.1 ml per pig) and lysozyme (10μg per pig) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA). Initially, each sample was diluted 1:50 for ELISA. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[c]Mitogen concentration per ml of media.

APPENDIX TABLE 31. EFFECT OF DIET ON LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES FOR TRIAL 4

| Item[a] | Supplemental Vitamin E | | | | SE |
|---|---|---|---|---|---|
| | X[b] | 10X | 20X | 50X | |
| Vitamin E | | | | | |
| Serum ($mg \cdot dl^{-1}$) | .10 | .24 | .22 | .32 | .02 |
| Liver ($mg \cdot 100g^{-1}$) | .27 | 2.03 | 3.72 | 7.54 | |
| Cortisol ($ng \cdot dl^{-1}$) | 8.9 | 12.1 | 8.7 | 12.5 | 1.26 |
| Antibody Titer[c] | | | | | |
| SRBC | 3.3 | 2.3 | 1.2 | 2.3 | .31 |
| Lysozyme | 3.8 | 3.0 | 3.4 | 2.5 | .42 |
| Stimulation Indices[d] | | | | | |
| Lysozyme | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 2.0 | 2.6 | 1.5 | 1.3 | .87 |
| .1 $mg \cdot ml^{-1}$ | 1.8 | 2.3 | 1.6 | 1.3 | .69 |
| Concanavilin A | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 96.6 | 91.4 | 80.2 | 104.3 | 28.60 |
| 10 $\mu g \cdot ml^{-1}$ | 4.4 | 10.0 | 10.5 | 11.3 | 6.58 |
| Phytohemagglutinin | | | | | |
| 1:10 | 62.1 | 21.4 | 72.0 | 45.9 | 71.00 |
| 1:500 | 8.7 | 17.5 | 9.3 | 10.0 | 5.50 |

[a]Number of observations; 4 pigs per diet.
[b]Recommended NRC levels of Vitamin E (11 $IU \cdot kg^{-1}$).
[c]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.

APPENDIX TABLE 32. EFFECT OF DIET ON LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS, AND STIMULATION INDICES FOR TRIAL 5

| Item[a] | Supplemental Vitamin E | | | | SE |
|---|---|---|---|---|---|
| | X[b] | 10X | 20X | 50X | |
| Vitamin E | | | | | |
| Serum ($mg \cdot dl^{-1}$) | .10 | .21 | .24 | .33 | .01 |
| Liver ($mg \cdot 100g^{-1}$) | .25 | 1.89 | 1.54 | 4.31 | |
| Cortisol ($ng \cdot dl^{-1}$) | 9.6 | 8.4 | 9.5 | 6.5 | .90 |
| Antibody Titer[c] | | | | | |
| SRBC | 2.3 | 1.7 | 1.8 | 2.8 | .22 |
| Lysozyme | 6.1 | 6.6 | 6.6 | 6.6 | .26 |
| Stimulation Indices[d] | | | | | |
| Lysozyme | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 2.0 | 3.1 | 5.3 | 1.9 | .66 |
| .1 $mg \cdot ml^{-1}$ | 2.7 | 2.7 | 2.8 | 2.0 | .53 |
| Concanavilin A | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 186.5 | 148.3 | 222.6 | 172.4 | 21.70 |
| 10 $\mu g \cdot ml^{-1}$ | 31.4 | 31.0 | 37.6 | 34.1 | 5.00 |
| Phytohemagglutinin | | | | | |
| 1:10 | 407.5 | 476.4 | 486.8 | 411.7 | 54.20 |
| 1:500 | 15.6 | 18.1 | 14.6 | 13.0 | 4.17 |

[a]Number of observations; eight pigs per diet.
[b]Recommended NRC levels of Vitamin E ($11\ IU \cdot kg^{-1}$).
[c]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.

APPENDIX TABLE 33. EFFECT OF DIET ON LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS, AND STIMULATION INDICES FOR TRIAL 6

| Item[a] | Supplemental Vitamin E | | | | | |
|---|---|---|---|---|---|---|
| | X[b] | 10X | 20X | 50X | SE | P |
| Vitamin E | | | | | | |
| Serum ($mg \cdot dl^{-1}$) | .09 | .19 | .25 | .26 | .01 | .11 |
| Liver ($mg \cdot 100g^{-1}$) | .19 | 1.28 | 3.31 | 4.11 | | |
| Cortisol ($ng \cdot dl^{-1}$) | 13.0 | 10.7 | 11.2 | 12.6 | 1.02 | .27 |
| Antibody Titer[c] | | | | | | |
| SRBC | .5 | .9 | .9 | .5 | .30 | .10 |
| Lysozyme | 4.3 | 4.1 | 4.3 | 4.3 | .32 | .64 |
| Stimulation Indices[d] | | | | | | |
| Lysozyme | | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 2.3 | 2.9 | 1.9 | 2.7 | .05 | .07 |
| .1 $mg \cdot ml^{-1}$ | 2.2 | 3.6 | 3.2 | 2.4 | .52 | .89 |
| Concanavilin A | | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 110.2 | 112.5 | 133.5 | 180.9 | 21.6 | .28 |
| 10 $\mu g \cdot ml^{-1}$ | 17.5 | 12.6 | 16.9 | 16.9 | 4.99 | .99 |
| Phytohemagglutinin | | | | | | |
| 1:10 | 146.8 | 193.1 | 150.2 | 228.1 | 53.3 | .73 |
| 1:500 | 3.5 | 1.7 | 4.8 | 8.0 | 4.1 | .47 |

[a]Number of observations; eight pigs per diet.
[b]Recommended NRC levels of Vitamin E (11 $IU \cdot kg^{-1}$).
[c]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[d]Mitogen concentration per ml of media.

APPENDIX TABLE 34. EFFECT OF TEMPERATURE ON LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES FOR TRIAL 4

| Item[a] | Temperature (°C) 30 | 19 | SE |
|---|---|---|---|
| Vitamin E | | | |
| Serum ($mg \cdot dl^{-1}$) | .21 | .23 | .90 |
| Liver ($mg \cdot 100g^{-1}$) | 2.64 | 4.17 | |
| Cortisol ($ng \cdot dl^{-1}$) | 10.3 | 10.8 | .44 |
| Antibody Titer[b] | | | |
| SRBC | 2.1 | 2.5 | .22 |
| Lysozyme | 3.1 | 3.2 | .30 |
| Stimulation Indices[c] | | | |
| Lysozyme | | | |
| 1.0 $mg \cdot ml^{-1}$ | 2.3 | 1.3 | .62 |
| .1 $mg \cdot ml^{-1}$ | 2.1 | 1.4 | .49 |
| Concanavilin A | | | |
| 100 $\mu g \cdot ml^{-1}$ | 98.7 | 87.5 | 20.30 |
| 10 $\mu g \cdot ml^{-1}$ | 10.8 | 7.3 | 4.67 |
| Phytohemagglutinin | | | |
| 1:10 | 49.1 | 51.6 | 50.40 |
| 1:500 | 12.3 | 10.5 | 3.89 |

[a]Number of observations; eight pigs, four pens per temperature treatment.
[b]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[c]Mitogen concentration per ml of media.

APPENDIX TABLE 35. EFFECT OF TEMPERATURE ON LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES FOR TRIAL 5

| Item[a] | Temperature (°C) 30 | 19 | SE |
|---|---|---|---|
| Vitamin E | | | |
| Serum ($mg \cdot dl^{-1}$) | .24 | .19 | .01 |
| Liver ($mg \cdot 100g^{-1}$) | 1.47 | 2.21 | |
| Cortisol ($ng \cdot dl^{-1}$) | 8.4 | 8.6 | .63 |
| Antibody Titer[b] | | | |
| SRBC | 2.2 | 2.2 | .15 |
| Lysozyme | 6.4 | 6.5 | .19 |
| Stimulation Indices[c] | | | |
| Lysozyme | | | |
| 1.0 $mg \cdot ml^{-1}$ | 2.5 | 3.6 | .47 |
| .1 $mg \cdot ml^{-1}$ | 2.3 | 2.9 | .37 |
| Concanavilin A | | | |
| 100 $\mu g \cdot ml^{-1}$ | 169.2 | 195.7 | 15.40 |
| 10 $\mu g \cdot ml^{-1}$ | 32.7 | 34.4 | 3.54 |
| Phytohemagglutinin | | | |
| 1:10 | 418.6 | 472.6 | 38.40 |
| 1:500 | 13.1 | 17.5 | 2.96 |

[a]Number of observations; sixteen pigs, eight pens per temperature treatment.
[b]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[c]Mitogen concentration per ml of media.

APPENDIX TABLE 36. EFFECT OF TEMPERATURE ON LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES FOR TRIAL 6

| Item[a] | Temperature (°C) 30 | 19 | SE | P |
|---|---|---|---|---|
| Vitamin E | | | | |
| Serum ($mg \cdot dl^{-1}$) | .20 | .19 | .01 | .03 |
| Liver ($mg \cdot 100g^{-1}$) | 2.53 | 1.91 | | |
| Cortisol ($ng \cdot dl^{-1}$) | 12.2 | 11.6 | .72 | .88 |
| Antibody Titer[b] | | | | |
| SRBC | .7 | .7 | .21 | .60 |
| Lysozyme | 4.5 | 4.1 | .23 | .54 |
| Stimulation Indices[c] | | | | |
| Lysozyme | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 2.9 | 2.1 | .46 | .08 |
| .1 $mg \cdot ml^{-1}$ | 3.4 | 2.3 | .37 | .11 |
| Concanavilin A | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 129.8 | 138.9 | 15.40 | .66 |
| 10 $\mu g \cdot ml^{-1}$ | 14.5 | 18.8 | 3.55 | .68 |
| Phytohemagglutinin | | | | |
| 1:10 | 162.5 | 196.6 | 32.80 | .73 |
| 1:500 | 3.4 | 5.6 | 2.90 | .57 |

[a]Number of observations; sixteen pigs, eight pens per temperature treatment.
[b]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[c]Mitogen concentration per ml of media.

APPENDIX TABLE 37. LEAST SQUARES MEANS OF SERUM AND LIVER VITAMIN E CONCENTRATION, CORTISOL LEVEL, ANTIBODY TITERS AND STIMULATION INDICES FOR TRIALS 4, 5 AND 6

| Item[a] | Trial 4 | Trial 5 | Trial 6 | SE | P |
|---|---|---|---|---|---|
| Vitamin E | | | | | |
| Serum ($mg \cdot dl^{-1}$) | .22 | .22 | .20 | .01 | .42 |
| Liver ($mg \cdot 100g^{-1}$) | 3.4 | 1.9 | 2.2 | .62 | .11 |
| Cortisol ($ng \cdot dl^{-1}$) | 10.6 | 8.5 | 11.9 | .53 | .17 |
| Antibody Titer[b] | | | | | |
| SRBC | 2.3 | 2.2 | .7 | .14 | .06 |
| Lysozyme | 3.2 | 6.5 | 4.3 | .16 | .01 |
| Stimulation Indices[c] | | | | | |
| Lysozyme | | | | | |
| 1.0 $mg \cdot ml^{-1}$ | 1.8 | 3.1 | 2.5 | .36 | .36 |
| .1 $mg \cdot ml^{-1}$ | 1.7 | 2.6 | 2.8 | .29 | .14 |
| Concanavilin A | | | | | |
| 100 $\mu g \cdot ml^{-1}$ | 93.1 | 182.4 | 134.3 | 11.98 | .09 |
| 10 $\mu g \cdot ml^{-1}$ | 9.0 | 33.5 | 16.6 | 2.77 | .03 |
| Phytohemagglutinin | | | | | |
| 1:10 | 50.4 | 445.6 | 179.5 | 29.8 | .01 |
| 1:500 | 11.4 | 15.3 | 4.5 | 2.3 | .42 |

[a]Number of observations; Trial 4, sixteen pigs; Trial 5, thirty-two pigs; Trial 6, thirty-two pigs (two pigs per pen).
[b]Injected with sheep red blood cells (.1 ml) and lysozyme (10 $\mu g$) on d 0 and d 17 and measured using a hemagglutination assay and an enzyme-linked immunosorbent assay (ELISA), respectively. Initial samples were diluted for ELISA 1:50. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.
[c]Mitogen concentration per ml of media.

APPENDIX TABLE 38. EFFECT OF VITAMIN E SUPPLEMENTATION ON PEN PERFORMANCE LEAST SQUARES MEANS OF WEANLING PIGS IN TRIAL 4

| Item[a] | Vitamin E Supplementation | | | |
|---|---|---|---|---|
| | X[b] | 10X | 20X | 50X |
| Avg weight (kg) | | | | |
| Initial | 5.6 | 6.5 | 6.2 | 6.8 |
| Week 1 | 7.1 | 8.7 | 7.7 | 9.0 |
| 2 | 9.7 | 12.1 | 10.8 | 12.4 |
| 3 | 12.6 | 15.9 | 13.5 | 15.5 |
| 4 | 16.8 | 19.7 | 17.6 | 18.5 |
| 5 | 22.2 | 25.3 | 22.1 | 23.9 |
| Avg daily feed intake (kg) | | | | |
| Week 1 | .35 | .37 | .25 | .50 |
| 2 | .72 | .77 | .66 | .86 |
| 3 | .93 | .79 | .90 | .90 |
| 4 | .87 | .85 | .81 | .84 |
| 5 | 1.62 | 1.64 | 1.52 | 1.60 |
| 1-2 | .54 | .57 | .46 | .68 |
| 1-3 | .67 | .65 | .60 | .75 |
| 1-4 | .72 | .70 | .66 | .78 |
| 1-5 | .90 | .88 | .83 | .94 |
| Avg daily gain (kg) | | | | |
| Week 1 | .22 | .32 | .21 | .31 |
| 2 | .37 | .48 | .45 | .49 |
| 3 | .44 | .55 | .39 | .44 |
| 4 | .57 | .54 | .58 | .43 |
| 5 | .77 | .80 | .65 | .76 |
| 1-2 | .30 | .40 | .33 | .40 |
| 1-3 | .34 | .45 | .35 | .41 |
| 1-4 | .40 | .47 | .41 | .42 |
| 1-5 | .47 | .54 | .45 | .49 |
| Feed/gain | | | | |
| Week 1 | 1.79 | 1.15 | 1.28 | 1.36 |
| 2 | 1.92 | 1.31 | 1.34 | 1.70 |
| 3 | 2.17 | 1.55 | 2.48 | 2.04 |
| 4 | 1.46 | 1.58 | 1.40 | 1.89 |
| 5 | 2.10 | 2.31 | 2.51 | 2.23 |
| 1-2 | 1.78 | 1.34 | 1.29 | 1.57 |
| 1-3 | 2.01 | 1.39 | 1.74 | 1.81 |
| 1-4 | 1.77 | 1.42 | 1.59 | 1.83 |
| 1-5 | 1.90 | 1.65 | 1.84 | 1.94 |

[a]Number of observations; eight pens (two pigs per pen).
[b]Recommended NRC levels of Vitamin E (11 IU·kg$^{-1}$).

APPENDIX TABLE 39. EFFECT OF VITAMIN E SUPPLEMENTATION ON PEN PERFORMANCE LEAST SQUARES MEANS OF WEANLING PIGS IN TRIAL 5

| Item[a] | Vitamin E Supplementation | | | |
|---|---|---|---|---|
| | X[b] | 10X | 20X | 50X |
| Avg weight (kg) | | | | |
| Initial | 7.0 | 7.1 | 7.1 | 7.1 |
| Week 1 | 7.9 | 8.1 | 7.9 | 7.9 |
| 2 | 9.7 | 10.1 | 10.5 | 9.9 |
| 3 | 12.1 | 12.1 | 12.5 | 12.6 |
| 4 | 15.3 | 15.7 | 16.5 | 15.6 |
| 5 | 19.2 | 18.4 | 20.0 | 18.7 |
| Avg daily feed intake (kg) | | | | |
| Week 1 | .27 | .24 | .29 | .25 |
| 2 | .52 | .55 | .52 | .50 |
| 3 | .85 | .84 | .86 | .83 |
| 4 | .86 | .91 | .91 | .86 |
| 5 | .54 | .67 | .88 | .74 |
| 1-2 | .39 | .39 | .41 | .38 |
| 1-3 | .54 | .54 | .56 | .53 |
| 1-4 | .62 | .63 | .65 | .61 |
| 1-5 | .61 | .64 | .69 | .64 |
| Avg daily gain (kg) | | | | |
| Week 1 | .12 | .15 | .12 | .11 |
| 2 | .26 | .28 | .38 | .29 |
| 3 | .39 | .29 | .28 | .39 |
| 4 | .40 | .52 | .58 | .44 |
| 5 | .57 | .39 | .49 | .44 |
| 1-2 | .19 | .21 | .25 | .20 |
| 1-3 | .26 | .24 | .26 | .26 |
| 1-4 | .29 | .31 | .34 | .30 |
| 1-5 | .35 | .32 | .37 | .33 |
| Feed/gain | | | | |
| Week 1 | 3.08 | 1.77 | 2.57 | 2.42 |
| 2 | 2.00 | 2.43 | 1.41 | 1.90 |
| 3 | 2.16 | 2.92 | 3.21 | 2.21 |
| 4 | 2.22 | 1.80 | 1.93 | 1.99 |
| 5 | .96 | 2.05 | 1.83 | 1.82 |
| 1-2 | 2.10 | 1.92 | 1.67 | 2.04 |
| 1-3 | 2.09 | 2.29 | 2.16 | 2.01 |
| 1-4 | 2.13 | 2.09 | 1.99 | 2.02 |
| 1-5 | 1.74 | 1.98 | 1.91 | 1.92 |

[a]Number of observations; sixteen pens (two pigs per pen).
[b]Recommended NRC levels of Vitamin E (11 IU·kg$^{-1}$).

APPENDIX TABLE 40. EFFECT OF VITAMIN E SUPPLEMENTATION ON PEN PERFORMANCE LEAST SQUARES MEANS OF WEANLING PIGS IN TRIAL 6

| Item[a] | Vitamin E Supplementation | | | | SE | P |
|---|---|---|---|---|---|---|
| | X[b] | 10X | 20X | 50X | | |
| Avg weight (kg) | | | | | | |
| Initial | 7.3 | 7.3 | 7.6 | 7.5 | .48 | .98 |
| Week 1 | 9.0 | 9.2 | 9.0 | 9.0 | .63 | .85 |
| 2 | 12.1 | 11.8 | 11.7 | 11.7 | .78 | .61 |
| 3 | 14.6 | 14.7 | 14.6 | 14.4 | 1.03 | .58 |
| 4 | 19.3 | 18.8 | 19.3 | 18.3 | 1.40 | .92 |
| 5 | 24.2 | 23.9 | 24.0 | 23.4 | 1.34 | .77 |
| Avg daily feed intake (kg) | | | | | | |
| Week 1 | .35 | .35 | .37 | .36 | .06 | .44 |
| 2 | .50 | .56 | .63 | .50 | .06 | .44 |
| 3 | .69 | .67 | .73 | .67 | .11 | .99 |
| 4 | .97 | 1.15 | 1.18 | .96 | .10 | .82 |
| 5 | 1.09 | 1.20 | 1.29 | 1.11 | .12 | .69 |
| 1-2 | .42 | .46 | .50 | .43 | .05 | .25 |
| 1-3 | .51 | .53 | .57 | .51 | .06 | .79 |
| 1-4 | .63 | .69 | .73 | .62 | .05 | .61 |
| 1-5 | .72 | .79 | .84 | .72 | .05 | .51 |
| Avg daily gain (kg) | | | | | | |
| Week 1 | .25 | .27 | .20 | .20 | .03 | .49 |
| 2 | .43 | .38 | .38 | .39 | .04 | .24 |
| 3 | .37 | .41 | .42 | .38 | .04 | .07 |
| 4 | .67 | .59 | .67 | .55 | .08 | .85 |
| 5 | .70 | .73 | .67 | .73 | .07 | .48 |
| 1-2 | .34 | .32 | .29 | .30 | .02 | .09 |
| 1-3 | .34 | .35 | .33 | .33 | .02 | .18 |
| 1-4 | .43 | .41 | .42 | .38 | .04 | .78 |
| 1-5 | .48 | .48 | .47 | .45 | .03 | .56 |
| Feed/gain | | | | | | |
| Week 1 | 1.37 | 1.34 | 1.93 | 1.90 | .44 | .62 |
| 2 | 1.15 | 1.51 | 1.85 | 1.35 | .41 | .40 |
| 3 | 1.89 | 1.68 | 1.74 | 1.79 | .32 | .20 |
| 4 | 1.45 | 1.95 | 1.73 | 1.75 | .31 | .62 |
| 5 | 1.58 | 1.67 | 2.03 | 1.54 | .37 | .73 |
| 1-2 | 1.23 | 1.42 | 1.85 | 1.54 | .23 | .21 |
| 1-3 | 1.46 | 1.50 | 1.75 | 1.63 | .17 | .38 |
| 1-4 | 1.46 | 1.66 | 1.75 | 1.64 | .11 | .15 |
| 1-5 | 1.49 | 1.65 | 1.82 | 1.59 | .11 | .32 |

[a]Number of observations; sixteen pens (two pigs per pen).
[b]Recommended NRC levels of Vitamin E (11 IU·kg$^{-1}$).

APPENDIX TABLE 41. EFFECT OF VITAMIN E SUPPLEMENTATION ON PEN PERFORMANCE LEAST SQUARES MEANS OF WEANLING PIGS IN TRIALS 4, 5 AND 6

| Item[a] | Supplemental Vitamin E | | | | | |
|---|---|---|---|---|---|---|
| | X[b] | 10X | 20X | 50X | SE | P |
| Avg weight (kg) | | | | | | |
| Initial | 6.6 | 6.9 | 7.0 | 7.2 | .34 | .74 |
| Week 1 | 8.0 | 8.7 | 8.2 | 8.6 | .40 | .56 |
| 2 | 10.5 | 11.3 | 11.0 | 11.3 | .46 | .56 |
| 3 | 13.3 | 14.2 | 13.5 | 14.2 | .52 | .38 |
| 4 | 17.1 | 18.1 | 17.8 | 17.5 | .83 | .86 |
| 5 | 21.9 | 22.5 | 22.0 | 22.0 | .79 | .93 |
| Avg daily feed (kg) | | | | | | |
| Week 1 | .32 | .31 | .30 | .37 | .03 | .49 |
| 2 | .58 | .63 | .60 | .62 | .04 | .81 |
| 3 | .82 | .77 | .83 | .80 | .06 | .89 |
| 4 | .90 | .97 | .97 | .88 | .06 | .63 |
| 5 | 1.08 | 1.17 | 1.23 | 1.15 | .07 | .51 |
| 1-2 | .45 | .48 | .45 | .49 | .03 | .62 |
| 1-3 | .57 | .57 | .57 | .60 | .04 | .95 |
| 1-4 | .66 | .67 | .68 | .67 | .03 | .97 |
| 1-5 | .74 | .77 | .78 | .77 | .03 | .74 |
| Avg daily gain (kg) | | | | | | |
| Week 1 | .20 | .24 | .17 | .20 | .02 | .09 |
| 2 | .35 | .38 | .40 | .39 | .02 | .60 |
| 3 | .40 | .42 | .36 | .40 | .02 | .42 |
| 4 | .54 | .55 | .61 | .47 | .05 | .34 |
| 5 | .68 | .64 | .61 | .64 | .04 | .75 |
| 1-2 | .28 | .31 | .29 | .30 | .01 | .42 |
| 1-3 | .32 | .35 | .31 | .33 | .01 | .28 |
| 1-4 | .37 | .40 | .39 | .37 | .02 | .75 |
| 1-5 | .44 | .44 | .43 | .42 | .02 | .81 |
| Feed/gain | | | | | | |
| Week 1 | 2.07 | 1.41 | 1.93 | 1.89 | .26 | .34 |
| 2 | 1.68 | 1.75 | 1.53 | 1.65 | .24 | .93 |
| 3 | 2.07 | 2.05 | 2.47 | 2.01 | .19 | .31 |
| 4 | 1.71 | 1.77 | 1.68 | 1.88 | .17 | .86 |
| 5 | 1.55 | 2.01 | 2.12 | 1.86 | .22 | .31 |
| 1-2 | 1.70 | 1.56 | 1.60 | 1.71 | .13 | .81 |
| 1-3 | 1.84 | 1.73 | 1.88 | 1.81 | .10 | .73 |
| 1-4 | 1.78 | 1.72 | 1.77 | 1.83 | .07 | .72 |
| 1-5 | 1.71 | 1.76 | 1.85 | 1.81 | .06 | .40 |

[a]Number of observations; ten pens per diet; two pigs per pen.
[b]Recommended NRC levels of Vitamin E (11 $IU \cdot kg^{-1}$).

APPENDIX TABLE 42. EFFECT OF TRIALS ON PEN PERFORMANCE LEAST SQUARES MEANS IN TRIALS 4, 5 AND 6

| Item[a] | Trial 4 | Trial 5 | Trial 6 | SE | P |
|---|---|---|---|---|---|
| Avg weight (kg) | | | | | |
| Initial | 6.3 | 7.1 | 7.4 | .29 | .06 |
| Week 1 | 8.1 | 7.9 | 9.0 | .31 | .02 |
| 2 | 11.2 | 10.1 | 11.8 | .39 | .01 |
| 3 | 14.4 | 12.3 | 14.6 | .44 | .01 |
| 4 | 18.1 | 15.8 | 18.9 | .70 | .01 |
| 5 | 23.4 | 19.1 | 23.9 | .66 | .01 |
| Avg daily feed (kg) | | | | | |
| Week 1 | .36 | .27 | .36 | .03 | .01 |
| 2 | .75 | .52 | .55 | .03 | .01 |
| 3 | .88 | .84 | .69 | .06 | .03 |
| 4 | .84 | .88 | 1.06 | .06 | .01 |
| 5 | 1.59 | .71 | 1.18 | .06 | .01 |
| 1-2 | .56 | .39 | .45 | .02 | .01 |
| 1-3 | .67 | .54 | .53 | .03 | .02 |
| 1-4 | .71 | .63 | .66 | .03 | .01 |
| 1-5 | .89 | .64 | .77 | .03 | .01 |
| Avg daily gain (kg) | | | | | |
| Week 1 | .27 | .12 | .23 | .01 | .01 |
| 2 | .45 | .30 | .40 | .02 | .01 |
| 3 | .45 | .33 | .40 | .02 | .01 |
| 4 | .53 | .48 | .62 | .04 | .05 |
| 5 | .74 | .47 | .71 | .03 | .01 |
| 1-2 | .36 | .21 | .31 | .01 | .01 |
| 1-3 | .39 | .25 | .34 | .01 | .01 |
| 1-4 | .42 | .31 | .41 | .02 | .01 |
| 1-5 | .49 | .34 | .47 | .01 | .01 |
| Feed/gain | | | | | |
| 1 | 1.39 | 2.46 | 1.65 | .21 | .01 |
| 2 | 1.56 | 1.94 | 1.46 | .20 | .17 |
| 3 | 2.06 | 2.62 | 1.77 | .16 | .01 |
| 4 | 1.68 | 1.99 | 1.72 | .16 | .15 |
| 5 | 2.38 | 1.66 | 1.71 | .20 | .11 |
| 1-2 | 1.49 | 1.93 | 1.51 | .12 | .01 |
| 1-3 | 1.72 | 2.14 | 1.58 | .10 | .01 |
| 1-4 | 1.65 | 2.06 | 1.62 | .06 | .01 |
| 1-5 | 1.83 | 1.89 | 1.64 | .05 | .01 |

[a]Number of observations; Trial 4, eight pens; Trial 5, sixteen pens; Trial 6; sixteen pens (two pigs per pen).

APPENDIX TABLE 43. EFFECT OF TEMPERATURE X TRIAL INTERACTIONS ON PEN PERFORMANCE LEAST SQUARES MEANS OF WEANLING PIGS IN TRIALS 4, 5 AND 6

| | Temperature (°C) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Trial 4 | | Trial 5 | | Trial 6 | | | |
| Item[a] | 30 | 19 | 30 | 19 | 30 | 19 | SE | P |
| Avg weight (kg) | | | | | | | | |
| Initial | 6.3 | 6.3 | 7.1 | 7.1 | 7.4 | 7.4 | .41 | .99 |
| Week 1 | 7.9 | 8.4 | 8.1 | 7.8 | 9.0 | 9.0 | .49 | .78 |
| 2 | 10.9 | 11.6 | 10.1 | 10.0 | 11.6 | 12.1 | .55 | .66 |
| 3 | 13.5 | 15.3 | 12.2 | 12.4 | 14.1 | 15.1 | .45 | .50 |
| 4 | 16.8 | 19.5 | 16.1 | 15.4 | 18.1 | 19.8 | .65 | .23 |
| 5 | 20.8 | 25.9 | 19.2 | 19.0 | 22.6 | 25.2 | 1.03 | .05 |
| Avg daily feed intake (kg) | | | | | | | | |
| Week 1 | .32 | .42 | .25 | .28 | .35 | .36 | .04 | .54 |
| 2 | .71 | .80 | .47 | .57 | .44 | .65 | .05 | .28 |
| 3 | .70 | 1.06 | .86 | .82 | .63 | .75 | .07 | .07 |
| 4 | .77 | .91 | .91 | .86 | .99 | 1.14 | .07 | .21 |
| 5 | 1.39 | 1.80 | .63 | .78 | 1.23 | 1.12 | .03 | .05 |
| 1-2 | .51 | .61 | .36 | .43 | .40 | .51 | .04 | .70 |
| 1-3 | .58 | .76 | .53 | .56 | .47 | .59 | .04 | .22 |
| 1-4 | .62 | .80 | .62 | .63 | .60 | .73 | .04 | .09 |
| 1-5 | .78 | 1.00 | .62 | .66 | .73 | .80 | .04 | .08 |
| Avg daily gain (kg) | | | | | | | | |
| Week 1 | .23 | .30 | .13 | .11 | .23 | .23 | .02 | .21 |
| 2 | .43 | .47 | .30 | .31 | .36 | .43 | .02 | .38 |
| 3 | .39 | .52 | .32 | .35 | .36 | .43 | .03 | .26 |
| 4 | .47 | .60 | .53 | .43 | .57 | .67 | .05 | .11 |
| 5 | .56 | .92 | .44 | .51 | .65 | .77 | .02 | .02 |
| 1-2 | .33 | .38 | .22 | .21 | .30 | .33 | .01 | .22 |
| 1-3 | .35 | .43 | .25 | .25 | .32 | .36 | .02 | .09 |
| 1-4 | .38 | .47 | .32 | .30 | .38 | .44 | .02 | .06 |
| 1-5 | .42 | .56 | .34 | .34 | .43 | .51 | .02 | .01 |
| Feed/gain | | | | | | | | |
| Week 1 | 1.31 | 1.48 | 1.92 | 3.00 | 1.62 | 1.65 | .31 | .14 |
| 2 | 1.63 | 1.50 | 1.63 | 2.24 | 1.38 | 1.55 | .29 | .48 |
| 3 | 1.84 | 2.29 | 2.77 | 2.47 | 1.77 | 1.78 | .23 | .34 |
| 4 | 1.73 | 1.44 | 1.89 | 2.08 | 1.76 | 1.69 | .22 | .52 |
| 5 | 2.59 | 1.99 | 1.76 | 1.57 | 1.94 | 1.47 | .16 | .72 |
| 1-2 | 1.53 | 1.46 | 1.69 | 2.17 | 1.44 | 1.58 | .16 | .26 |
| 1-3 | 1.64 | 1.81 | 2.10 | 2.18 | 1.52 | 1.65 | .12 | .93 |
| 1-4 | 1.64 | 1.66 | 1.99 | 2.13 | 1.60 | 1.66 | .04 | .74 |
| 1-5 | 1.89 | 1.78 | 1.84 | 1.94 | 1.69 | 1.59 | .08 | .22 |

[a]Number of observations; Trial 4, eight pens; Trial 5, sixteen pens; Trial 6, sixteen pens.

APPENDIX TABLE 44. MEAN SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL, AND ANTIBODY TITERS EXPRESSED BY WEEK FOR TRIALS 1, 2 AND 3, AND ANALYZED BY AGE OF PIG ON TEST

| Item[b] | Week[a] 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | SE | P |
|---|---|---|---|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot dl^{-1}$)[c] | .18 | .17 | .16 | .12 | .14 | .17 | .17 | .16 | .04 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | 14.4 | 11.0 | 15.1 | 12.6 | 15.4 | 13.6 | 15.6 | 20.1 | 1.08 | .01 |
| Antibody Titer[f] | .14 | .42 | .94 | 1.92 | 2.72 | 3.32 | 3.90 | 3.88 | .14 | .01 |

[a]Age of pig on test in wk.
[b]Number of total observations; one hundred fifty-six pigs, sixty pens on test.
[c]For Trials 2 and 3 only.
[f]Injected with sheep red blood cells on d 0 and d 17 (.1 ml per pig) and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

APPENDIX TABLE 45. EFFECT OF DIETS ON MEANS OF SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL, AND ANTIBODY TITERS EXPRESSED BY WEEK FOR TRIALS 1, 2 AND 3, AND ANALYZED BY AGE OF PIG ON TEST

| Item[b] | Supp. Vit E. | Week[a] 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | SE | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum Vitamin E ($mg \cdot dl^{-1}$)[c] | | | | | | | | | | | |
| | X | .20 | .14 | .10 | .05 | .05 | .09 | .08 | .07 | | |
| | 20X | .17 | .21 | .23 | .20 | .23 | .27 | .07 | .29 | .01 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | | | | | | | | | | | |
| | X | 13.1 | 11.0 | 15.8 | 13.6 | 16.4 | 13.6 | 15.6 | 20.0 | | |
| | 20X | 15.6 | 10.9 | 14.4 | 11.5 | 14.6 | 13.5 | 15.4 | 20.2 | 1.8 | .93 |
| Antibody Titer[d] | | | | | | | | | | | |
| | X | .00 | .41 | .84 | 2.05 | 2.57 | 3.23 | 3.95 | 4.18 | | |
| | 20X | .30 | .43 | 1.05 | 1.79 | 2.85 | 3.40 | 3.83 | 3.54 | .2 | .67 |

[a]Age of pig on test in wk.
[b]Number of observations; seventy-eight pigs in each diet treatment; thirty pens; three trials.
[c]for Trials 2 and 3 only.
[d]Injected with sheep red blood cells (.1 ml per pig) and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

APPENDIX TABLE 46. EFFECT OF WEANING AGE ON THE MEANS OF SERUM VITAMIN E CONCENTRATION, CORTISOL LEVEL AND ANTIBODY TITERS EXPRESSED BY WEEK FOR TRIALS 1, 2 AND 3, AND ANALYZED BY AGE OF PIG ON TEST

| | Week[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[b] | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | SE | P |
| Serum Vitamin E ($mg \cdot dl^{-1}$)[c] | | | | | | | | | | |
| 21 | .18 | .12 | .15 | .15 | .15 | .21 | | | | |
| 28 | | .23 | .12 | .11 | .13 | .15 | .17 | | | |
| 35 | | | .22 | .11 | .12 | .17 | .16 | .16 | .02 | .01 |
| Cortisol ($ng \cdot dl^{-1}$) | | | | | | | | | | |
| 21 | 14.4 | 7.0 | 11.3 | 15.7 | 17.3 | 13.2 | | | | |
| 28 | | 14.8 | 9.0 | 8.9 | 15.6 | 14.0 | 16.3 | | | |
| 35 | | | 25.5 | 13.1 | 13.5 | 13.5 | 14.9 | 20.1 | 1.68 | .01 |
| Antibody Titer[d] | | | | | | | | | | |
| 21 | .14 | .86 | 1.50 | 2.84 | 3.95 | 3.68 | | | | |
| 28 | | .00 | 1.29 | .86 | 2.37 | 3.66 | 3.43 | | | |
| 35 | | | .04 | 1.87 | 1.75 | 2.64 | 4.32 | 3.89 | .22 | .01 |

[a]Age of pig on test in wk.
[b]Number of total observations; fifty-two pigs, thirty pens in each weaning treatment.
[c]For Trials 2 and 3 only.
[d]Injected with sheep red blood cells on d 0 and d 17 (.1 ml per pig) and measured using a hemagglutination assay. Each positive serial dilution was assigned a value of 1 and the sum for each pig was used for the analysis.

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