Vitamin E: pharmacokinetics of parenteral products and its effect on swine reproduction

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Vitamin E:
Pharmacokinetics of parenteral products and
its effect on swine reproduction

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Animal Nutrition

Approved:
Signature was redacted for privacy.

In Charge of Major Work
Signature was redacted for privacy.

For the Major Department
Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1995
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CHAPTER 1. INTRODUCTION

One of the most important factors to consider when assessing the overall productivity of the swine enterprise is the number of pigs marketed per sow per year. Of course, many factors are involved in such a number, but reproductive performance and mortality during the growth phase have the largest impact. Thus, the objective of a swine enterprise is to maximize reproductive performance and minimize mortality during the growth phase of the swine life cycle.

Many different approaches to the problem have been investigated by university and industry researchers. Regarding mortality during the growing phase, factors such as infections, infestations, management problems, nutritional deficiencies, and their combinations are among its most relevant causes. The economical impact of vitamin E-Selenium deficiency has been identified by the swine industry and, therefore, parenteral supplementation of vitamin E is now a common practice. Moreover, parenteral administration provides a quick and powerful means to improve vitamin E status in the growing pig and therefore, to eliminate the possibility of vitamin E-Selenium deficiency.

Although enteral tocopherol absorption and distribution has been studied considerably, little information is available regarding the kinetics of tocopherol compounds, dosage regimens and product formulation of parenteral vitamin E preparations for monogastric animals. For these reasons, the experiment reported in Chapter 3 was undertaken to investigate the plasma pharmacokinetics of two sources of d-\( \alpha \)-tocopherol (free and acetylated ester) and two routes of administration (i.m. and i.v.) in gilts.

At weaning, pigs have a good vitamin E status which is a reflection of the tocopherol concentration of colostrum and milk. For two weeks after weaning, serum vitamin E declines
because of reduced feed intake and poor development of digestive enzymes. For those reasons, parenteral supplementation is likely to be better utilized than dietary vitamin E supplementation. The experiment reported in Chapter 4 was conducted to investigate the tissue and serum pharmacokinetics of four different injectable sources of vitamin E, two alcohols and two acetates, in weanling pigs.

There are two critical periods in the reproductive cycle of a sow: those 12 to 18 d after breeding when most of the embryonic mortality occurs, and the first few days after farrowing because most of the pig mortality occurs at this time. In recent years, an increased interest in supplementation of specific vitamins at levels higher than those previously thought to be adequate has emerged. Tocopherol is one of those vitamins, and there has been an interest in the effect of supranutritional supplementation of vitamin E on reproductive performance of sows. Besides this effort, there still is not a definitive answer to whether or not those supranutritional levels improve reproductive performance. The experiment in Chapter 5 was conducted to investigate the effect of dietary and/or injectable d-α-tocopherol supplementation on sow reproductive performance and tocopherol status of the dam and her litter over three parities.

**Dissertation Organization**

The thesis is divided into a Literature Review, three papers, and a General Summary. The papers were prepared in the style appropriate for submission to the *Journal of Animal Science*. Each paper includes abstract, introduction, materials and methods, results, discussion, implications, and literature cited sections. The general summary is intended to present the major findings and conclusions of the research performed to partially fulfill the requirements for the Ph.D. degree.
CHAPTER 2. LITERATURE REVIEW

Vitamin E and its Pharmacokinetics

History of vitamin E

In the early 1920s, several groups of researchers found that purified diets containing adequate protein, carbohydrate, and known required minerals supplemented with the newly discovered vitamins A, C, D and thiamine markedly improved the performance of animals (Combs, 1992). Evans and Bishop (1922) observed that in rats fed such supplemented semipurified diets, fetal resorption occurred during late gestation in females and testicular degeneration occurred in males. They reported that the addition of yeast or fresh lettuce to the diet restored fertility in the female and prevented infertility in the male. Evans and Bishop concluded that the diet was missing an unknown fertility factor termed "factor X".

Evans and Bishop (1922) tested numerous feedstuffs, using the prevention of fetal resorption as the bioassay, and found "factor X" activity in dried alfalfa, oats, wheat germ, milk fat, and meats. The active factor was extracted by organic solvents. They showed that daily supplementation with single droplets of wheat germ oil completely prevented fetal resorption, whereas diets that contained cod liver oil, a source high in vitamin A and D, did not. Sure (1924) concluded that the fat-soluble factor was a new vitamin and he proposed the name "vitamin E", the next serial alphabetical designation. This is the currently accepted designation for tocopherols active in preventing certain types of infertility and myopathies in animals.

Olcott and Mattill (1931), at the University of Iowa, found that those preparations which prevented fetal resorption had antioxidant properties. Evans et al. (1936) isolated an alcohol from...
unsaponifiable wheat-germ lipids that had considerable vitamin E activity. They proposed the name "alpha-tocopherol". The term was derived from the Greek words *tokos* (offspring) and *pherein* (to bear), and they used the -ol suffix to indicate that the factor was an alcohol. Emerson et al. (1937) isolated β and γ-tocopherol from vegetable oils. The chemical structure of this new vitamin was proposed one year later (Fernholz, 1938). The same year, the synthesis of α-tocopherol was achieved (Karrer et al., 1938). A decade later, δ-tocopherol was isolated (Stern et al., 1947). In the 1960s, the tocotrienols were isolated from different vegetable oils.

**Structure of vitamin E**

The multiplicity of vitamin E structures has been historically defined over the years. Eight naturally occurring compounds, four tocopherols (α, β, γ, and δ) and four tocotrienols (α, β, γ, and δ), have been isolated. Tocopherols are derived from tocol, hydroxychroman, substituted with a 16-carbon isoprenoid side chain at the 2-position (Figure 1). The full chemical name of α-tocopherol is 2, 5, 7, 8-tetramethyl-2-(4', 8', 12'-trimethyl-tridecyl) chroman-6-ol (IUNS Committee on Nomenclature, 1978). Differences between α, β, γ, and δ are in the number and placement of the methyl groups on the ring. Thus, d-α-tocopherol is 2R-(4'R,8'R)-5,7,8-trimethyl-tocol, β-tocopherol is 2R-(4'R,8'R)-5,8-dimethyl-tocol, γ-tocopherol is 2R-(4'R,8'R)-7,8-dimethyl-tocol and δ-tocopherol is 2R-(4'R,8'R)-8-methyl-tocol (Lynch, 1991).

Tocotrienols (Figure 1) have the same structure as tocopherols with the exception of three double bonds occurring at positions 3',7' and 11' (unsaturated). The full chemical name for α-tocotrienol is 2, 5, 7, 8-tetramethyl-2-(4', 8', 12'-trimethyl-trideca-3', 7', 11'-triene) chroman-6-ol (IUNS Committee on Nomenclature, 1978). The α, β, γ, and δ tocotrienols have methyl groups located on the same position as the tocopherol counterparts.
All-rac- or dl-α-tocopherol is a synthetic compound that is a condensation product of racemic isophytol and trimethylhydroquinone. The result is a totally synthetic mixture of the four possible enantiomeric pairs of the eight diasterisomers (Ullrey, 1981). The d-α-tocopheryl acetate results from the extraction of natural tocopherols from vegetable oils. Those natural tocopherols are then acetylated to produce the ester (Lynch, 1991).

Figure 1. α-tocopherol and α-tocotrienol structure.
In the vitamin E molecule, the chroman ring is responsible for the antioxidant activity, whereas the phytol group determines its transport, and retention within membranes.

**Function of vitamin E**

Vitamin E is essential for the integrity and optimal function of the reproductive, muscular, circulatory, nervous, and immune systems. The most important function of vitamin E is as a biological antioxidant when the phenolic hydroxyl of its chromanol ring is free (unesterified). The free hydroxyl at the sixth position of the chroman ring can function as a scavenger of free radicals, usually being oxidized to the semiquinone or quinone. The active group can be protected from oxidation by esterification with the carboxyl group of organic acids forming esters such as the acetate or succinate derivatives. The esters do not have antioxidant activity (Ullrey, 1981; McDowell, 1989).

**Stability of vitamin E**

Alpha-tocopherol is a yellow oil that is soluble in organic solvents. Tocopherols are readily oxidized. Natural vitamin E is subject to destruction by oxidation, which is accelerated by factors such as heat, moisture, rancid fat, storage time, presence of mold, presence of trace minerals, and storage of feedstuffs. Vitamin E is a natural antioxidant and acting as such is destroyed (Roche, 1991). Placing acetate or succinate in the sixth position of the chroman ring, where the hydroxyl with antioxidant activity is located, leaves the molecule without antioxidant properties, but it is protected against stressors that destroy the vitamin during storage. Therefore, the ester form of vitamin E is the source of choice when formulating dietary vitamin E supplements.
In the diet, the alcohol form of vitamin E is less stable and declines in activity over time (Dove and Ewan, 1987; Chung et al., 1992). Dove and Ewan (1991a) concluded that high levels of Cu, Fe, Zn, or Mn in the diet increased the rate of oxidation of natural tocopherols. Anderson et al. (1995) showed a dramatic decrease in vitamin E content of swine diets supplemented with d- or dl-α-tocopherol after five days of storage, whereas the diets supplemented with acetate forms were stable over the 28-day study.

Data on swine liver and serum showed that α-tocopherol remains stable for at least 12 hours after sample collection when maintained at to 23°C. (Fredrickson, 1990). He concluded that to minimize vitamin E loss between sample collection and further analysis, tissue should be stored at either -20° or -70°C.

Vitamin E acts as an antioxidant in feedstuffs and biological samples. Supranutritional levels of vitamin E in the diet increase vitamin E in muscle and improve the oxidative stability of lipids and color, and reduce drip loss of raw and cooked pork (Monahan et al., 1990; Asghar et al., 1991).

Vitamin E deficiency

Vitamin E deficiency may result from impaired absorption or insufficient dietary intake of the vitamin. Selenium and polyunsaturated fatty acids are the most important factors that can affect the need for vitamin E. This vitamin has a large range of deficiency signs that differ among species and even among animals within the same species. In swine, most vitamin E deficiency signs have been associated with selenium (Se) deficiency. Vitamin E-Se deficiency is an important cause of sporadic mortality in growing swine.

Vitamin E-Se deficiency is expressed as one or several complex lesions in growing swine,
including hepatic necrosis (hepatosis dietetica), myocardial necrosis and hemorrhage (mulberry heart disease or dietetic microangiopathy), skeletal muscle degeneration (white muscle disease), esophagogastric ulceration, and steatitis (yellow fat disease) (Ewan et al., 1969). Other symptoms of vitamin E-Se deficiency that have been reported in swine herds are mastitis-metritis-agalactia complex, spraddle rear legs in newborn, infertility, susceptibility to swine dysentery, and poor skin condition (Ullrey, 1981; McDowell, 1989; Roche, 1991; Combs, 1992). A rise in activity of specific enzymes may be measured before any clinical signs are observed. Lactic acid dehydrogenase can be used to provide the pre-clinical diagnosis of vitamin E/Se deficiency (Hyldgaard-Jensen, 1973).

Vitamin E hypervitaminosis

The manifestation of hypervitaminosis E in rats, chicks and humans indicates a maximum tolerable level of 1000 to 2000 IU of vitamin E per kilogram of diet (NRC, 1987). Dietary supplementation with an excess of vitamin E can interfere with the utilization of other fat-soluble vitamins and produce impaired bone mineralization, reduction in hepatic storage of vitamin A, and coagulation disorders (McDowell, 1989; Combs, 1992).

Assay methods for vitamin E

There are two major assay methods for vitamin E. The first is the chemical assay which is used for qualitative and quantitative analysis. Extraction, purification, and detection are the three basic steps to perform when conducting the analysis. High performance liquid chromatography coupled with fluorescence detection is simple, fast, accurate, and very sensitive technique. There are two common methods of detection: spectrophotometric, and
spectrofluorometric. Fluorometric methods use an activation wavelength of 295 nm and an emission wavelength of 323 nm (Machlin, 1984). The second method of analysis of vitamin E is the biological assay, and monitors the biological activity of vitamin E to reverse or alter symptoms of vitamin E deficiency. Prevention of fetal resorption in rats, encephalomalacia in chicks, nutritional muscular dystrophy, red blood cell hemolysis, plasma concentration, and liver storage tests are the most common forms of bioassay (Coelho, 1991).

**Pharmacokinetics: general concepts**

Pharmacokinetics describes what the body does to a drug and is the mathematical relationship that exists between the dose of a drug and the concentration of the drug in a readily accessible site in the body (i.e., plasma or blood). Several factors determine how rapidly, at what concentration, and for how long the drug will appear at the target organ. Bioavailability, distribution, and clearance represent three major pharmacokinetic variables (Niazi, 1979).

Enteral or parenterally administered vitamin E must reach the general circulation to be distributed throughout the body, and the active form needs to be present at the site of action to exert its effect. The change of vitamin E concentration with time in the blood is a function of the absorption, distribution, and elimination.

Absorption of vitamin E can be defined as the fraction of the dose reaching the systemic circulation as unchanged vitamin E following administration by any route. The specific characteristics of vitamin E absorption will be presented later in this dissertation in the section dealing with route of administration. In the blood, vitamin E distributes rapidly between the plasma and erythrocytes. The transfer of vitamin E between blood, extravascular fluids, and tissues is called distribution. Vitamin E is found bound to lipoproteins in lymph and blood
(Figure 2) and does not seem to have a specific carrier protein (Combs, 1992). About 20% of the tocopherol in blood is located within the erythrocyte membrane (Chow, 1975), but it is probably not a transport form.

Most of the vitamin E in chylomicron and very low density lipoprotein (VLDL) is in the core and is located in the \( \beta \)-lipoprotein fraction (Machlin, 1984). Plasma vitamin E is mainly cleared by the liver in association with chylomicron remnants (Bjorneboe et al., 1990). The majority of hepatic tocopherol in liver is in the parenchymal cells. Liver secretes VLDL, which contains tocopherol, into the circulatory system (Bjorneboe et al., 1990).

The number and position of methyl groups on the chromanol ring influence the biological activity (\( \alpha > \beta > \gamma > \delta \)) of tocopherols and tocotrienols. Tocopherols have greater biological activity than tocotrienol counterparts (Machlin, 1984).

The vitamin E International Unit (IU) was originally defined as the average amount of orally administered vitamin required to prevent fetal resorption in vitamin E-deprived rats, and its currently accepted definition is one mg of dl-\( \alpha \)-tocopheryl acetate. The US Pharmacopeia and National Formulary (1980) uses USP units to define the weight/unit relationships for the isomers of vitamin E (Table 1).

Sato et al. (1991) isolated and characterized a protein from rat liver cytosol that specifically binds \( \alpha \)-tocopherol. This tocopherol binding protein has only been reported in liver cells. Yoshida et al. (1992) and Sato et al. (1993) suggested that in rats the liver takes up \( \alpha \)-tocopherol and preferentially transfers this isomer from the chylomicron to VLDL, discriminating against the \( \gamma \)-tocopherol. Then, \( \gamma \)-tocopherol is excreted in bile. This type of discrimination has also been reported in swine (Hoppe, 1991) and humans (Traber and Kayden, 1989).

Discrimination between RRR-\( \alpha \)-tocopherol and other steroisomers has been reported in humans
Figure 2. Scheme for the intestinal absorption, transfer in plasma lipoprotein, and uptake of vitamin E (VITE) by tissues. Where chylomicron (CHY), chylomicron remnant (CHY-R), very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), tocopherol binding protein (TBP), and gastro-intestinal tract (GIT) are depicted.
Table 1. USP units per mg of vitamin E source

<table>
<thead>
<tr>
<th>Source</th>
<th>USP units/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>dl-α-tocopheryl acetate (all-rac)</td>
<td>1.00</td>
</tr>
<tr>
<td>dl-α-tocopheryl acid succinate (all-rac)</td>
<td>0.89</td>
</tr>
<tr>
<td>dl-α-tocopherol (all-rac)</td>
<td>1.10</td>
</tr>
<tr>
<td>d-α-tocopheryl acid succinate (RRR)</td>
<td>1.21</td>
</tr>
<tr>
<td>d-α-tocopheryl acetate (RRR)</td>
<td>1.36</td>
</tr>
<tr>
<td>d-α-tocopherol (RRR)</td>
<td>1.49</td>
</tr>
</tbody>
</table>


(Acuff et al., 1994), cattle (Hidiroglou et al., 1988), rabbits (Fitch and Diehl, 1965), and rats (Ingold et al., 1987).

Discrimination among stereoisomers may affect the interconversion between mg and IU of the different vitamin E compounds. Machlin (1993) concluded that the values assigned to the RRR- and all-rac-forms of vitamin E are valid for farm animals and that there is not advantage of supplementing animals with the natural form of vitamin E as long as the supplementation is measured in IU. Stuart (1993) questioned the interpretation of data regarding biopotency of the natural and synthetic vitamin E because they are normally expressed on a weight basis and not in IU. He concluded that the actual equivalence of the natural vitamin E is greater than what is currently accepted and that all species cannot utilize the different forms of vitamin E in the same
fashion. Anderson et al. (1995) concluded that the actual bioavailability of d-α-tocopheryl acetate
in swine is greater than the suggested values based on rat bioassays.

The intestinal absorption of vitamin E, its transfer to and transport in blood lipoproteins, and its uptake by tissues are summarized on Figure 2. Machlin and Gabriel (1982) reported that it is difficult to saturate tissue with vitamin E and that not only the concentration, but also the duration of supplementation may affect the concentration of vitamin E in the tissues. Two mechanisms of cellular uptake of vitamin E from plasma seem to be involved. The first is receptor-mediated uptake of low density lipoprotein (Traber and Kayden, 1984), and the second is the lipoprotein lipase-mediated release of lipid from chylomicron and VLDL (Traber et al., 1985).

Vitamin E is stored in many tissues in the body and is concentrated in the cell membrane fractions such as those of the mitochondria and microsome (Diplock, 1985). Adipose tissue, liver and muscle are the major storage sites for this fat soluble vitamin (Bjorneboe et al., 1990).

Pascoe and Reed (1987), using isolated hepatocytes in vitro, suggested that tocopherol esters may be more effective antioxidants than free tocopherol, because they can pass through the cell membrane and reach intracellular sites where they can be later hydrolyzed to the alcohol form. On the other hand, the highly lipophilic free tocopherol may be immobilized in the plasma membrane and may never reach the intracellular sites where it is needed.

Most tissues serve as storage sites for vitamin E. However, not all stored tocopherol is accessible for use. In most non-adipose tissues, the location of vitamin E is almost exclusively in their membranes. Bieri (1972) reported that tissues have two pools of vitamin E: a labile pool that turns over rapidly, and a fixed pool that turns over slowly. Apparently, the labile pool is predominantly in non-adipose tissues like plasma and liver. On the other hand, the fixed pool is predominantly in the adipose tissue (Machlin et al., 1979). Burton et al. (1990) divided the
tissues of an animal in a steady-state into two kinetic groups. One tissue group included brain, testes, muscle and heart; this group had a slow, first order loss of old and gain of new tocopherol. The other tissue group included plasma, liver, lung, adrenal gland, and kidney; and this group had an initial, rapid change in vitamin E concentration compared with later stages. Therefore, the behavior of the fast tissues can be expressed as the sum of two (or more) first order processes.

The body can be seen as a collection of separate compartments, each containing a fraction of the administered vitamin E (Figure 3). The transfer of vitamin E from one compartment to another is associated with a rate constant (K). The magnitude of the rate constant determines how rapidly the transfer occurs. Thus, vitamin E in plasma exists in equilibrium with vitamin E in erythrocytes, other body fluids, and tissues. Because of this equilibrium, changes in the concentration of vitamin E in plasma are indicative of changes in the concentration of this vitamin in other tissues.

The first stage of vitamin E biotransformation is the oxidative conversion of tocopherol to tocopheroxy-radical. This is a reversible reaction, and further oxidation of the radical intermediate is unidirectional and results in tocopheryl-quinone. This tocopheryl-quinone can be reduced to tocopherol-hydroquinone in a reversible reaction. All the quinone metabolites have little vitamin E activity. The liver is able to conjugate tocopheryl-quinone for excretion in bile (Figure 2). Bjorneboe et al. (1987) observed that 24 h after i.v. administration of dl-α-[3H] tocopherol in rats, 14% of the radioactivity was found in bile, and that only a fraction was unchanged α-tocopherol. In sheep, only 3% of the total radioactivity found in bile was identified as unchanged tocopherol (Hidiroglou and Ivan, 1992). Lee-Kim et al. (1988) observed similar results, and also reported that in rats, the magnitude of enterohepatic circulation of vitamin E is very small.
Figure 3. a) Two- and b) three-compartment models. Compartments are shown as circles with their numbers inside. The $K_{ij}$ represents the transfer rates from compartment j to compartment i. The elimination rates are depicted as $K_{0j}$. 
The kidney degrades tocopheryl-quinone to tocopheronic acid and its derivative \( \gamma \)-lactone (Simon et al., 1956). Both acid and lactone metabolites can be excreted in urine or conjugated with glucuronic acid, resulting in a water soluble product of tocopherol that can follow the same route of excretion as the acid and lactone metabolites. Tocopheryl-quinone can also be reduced to a dimer and the dimer can be converted into a trimer (Gallo-Torres, 1980).

The major route of excretion of vitamin E is through the feces, with urinary excretion representing a minor pathway. Dermal elimination has also been suggested to be significant because a large fraction of \( \alpha \)-tocopherol can be found in the skin after injection (Combs, 1992).

The shape of the concentration over time curve depends on the relative rates of absorption and elimination (Figure 4). For instance, an i.v. injection yields an earlier peak than does an i.m. injection due to fast or almost instantaneous absorption, whereas enteral, s.c., and other routes of administration may show delayed peaks due to slower rates of absorption. Generally, the concentration dependant elimination rate is considered to be constant for a given drug. Therefore, the peak in the concentration vs time curve is determined only by the rate of absorption.

A higher or earlier peak in the curve does not necessarily mean larger absorption than a lower or delayed peak. The total absorption depends not only on the peak concentration achieved, but also on the persistence of the drug in the blood. The area under the curve better characterizes the total absorption of a drug. This area under the curve can not be used to determine the extent of absorption unless it is compared with a known standard or by some other method of administration such as i.v., which is assumed to be 100% absorbed (Niazi, 1979).
Figure 4. Phases of plasma concentration vs time profile.

**Approaches to pharmacokinetics studies**

Most of the studies done to date with the objective of better understanding the absorption, transport, and uptake by tissues of vitamin E in vivo have been conducted using one of the following three methods:

*Trace administration of vitamin E marked with radioactive isotopes.* Most of the studies have used $^3$H- (Gallo-Torres and Miller, 1971; Hidiroglou and Karpinski, 1987) or $^{14}$C- (Krishramurthy and Bieri, 1963; Knight and Roberts, 1986) marked vitamin E given through a
enteral or parenteral route. Samples (blood, tissues) were collected at specific time intervals after vitamin E administration, and radioactivity was measured using a liquid scintillation counter.

This method has the advantage that it distinguishes between endogenous and exogenous (radiolabelled) vitamin E in the system. On the other hand, radiolabeled vitamin E is not suitable for studies with humans, and it needs to be purified just before use due to its tendency to decompose.

*Trace administration of vitamin E marked with stable isotopes.* The use of radioactive materials and radiation-producing devices has increased, and so has the understanding of the potential hazards associated with the use of those procedures. The use of stable isotopes eliminates the risk associated with the use of radioactivity.

The use of stable isotopes allows the monitoring of unlabeled endogenous and labeled exogenous vitamin E by selected ion monitoring. Burton and Traber (1990) described the substitution of different amounts of deuterium on the chroman ring of vitamin E. This approach allows the administration of different steroisomers simultaneously. Furthermore, this technique allows the use of the same animal as its own control, and eliminates the need for crossover protocols.

The procedure described by Burton and Traber (1990) involves the determination of the relative amounts of deuterated and nondeuterated α-tocopherol in the sample by silylating the tocopherol fraction purified by HPLC, and injecting the silyl derivative into a gas chromatograph mass spectrometer.

*Large dose of unlabeled vitamin E.* This approach has the disadvantages that it does not distinguish between endogenous and exogenous vitamin E, it may not be physiologically relevant, because large doses are needed to distinguish any change relative to background levels, and it is
necessary to use crossover protocol or a relatively large number of experimental units to minimize the effects of individual variation. Levels used with this methodology are associated with the therapeutic or prophylactic treatment of deficiencies. Therefore, it has clinical significance. High performance liquid chromatography is generally used to determine vitamin E concentration (Cort et al., 1983).

**Pharmacokinetic parameters**

The equations and models used are always oversimplifications of the real system, and their appropriateness in a given experiment can be judged only by their ability to describe the observed data and the accuracy with which they make predictions of future results.

For example, a single exponential curve generally does not best describe the shape of the curve following a bolus injection (Figure 4). This curve better fits by a multiexponential.

Wolfe (1992) described the calculation of substrate kinetics for single- and multiple-pool models. The first step in calculating parameters in a multiple-pool model by noncompartmental modeling of bolus injection data is to fit a multiexponential curve of the form:

\[ Y(t) = \sum K_i e^{-P_i t} \]

Where \( t, K_i \) and \( P_i \) are time, intercept and slope, respectively, of the different components of the multiexponential curve. The values for \( K_i \) and \( P_i \) are determined by the stripping technique or by nonlinear least squares estimation, using a curve-fitting program such as SAAM/CONSAM (Boston et al., 1981). The program determines the value for those parameters that minimizes the sum of squares of the residuals (SSR), defined as the deviation of the model-derived value from the measured value at an specific time:

\[ SSR = \sum W[A - B]^2 \]
Where $A$ is the observed value at time $t$, $B$ is the expected value at time $t$ derived from the function to fit the data, and $W$ is the term to weight the data for experimental error. In general, each datum is weighted by a value inversely proportional to its variance. The evaluation of the best fit is done through the standard deviation of the estimated parameters, and by the goodness of fit ($SSR$, $SD$, $CV$, or $R^2$).

The area under the curve may be calculated by integration of the function or, alternatively, by the trapezoidal rule (Gibaldi, 1984). Other parameters such as pool size, mean residence time, and rates may be calculated.

In compartmental modeling (Figure 3), compartments are used to model various components of the metabolic system. The development of the model is based on the exponential functions of the curve and an understanding of the metabolic system to be modeled. Generally, the number of compartments in the model is coincident with the number of exponential terms. When using the slopes and intercepts of the exponential equation to calculate certain kinetic parameters, the compartmental analysis is called empirical modeling. If plasma data plus any other data (tissue, feces, urine, ...) from the same or another experiment are fitted to a hypothesized compartmental model and later adjusted to the model parameter, the compartmental analysis is called model-based compartmental analysis (Green and Green, 1990).

**Route of administration**

*Enteral.* Vitamin E absorption is dependent upon normal lipid digestion and absorption (Ullrey, 1981). Thus, normal pancreatic function, bile secretion, micelle formation, and penetration across intestinal membranes are needed. Humans and animals with lipid malabsorption syndromes (e.g., cystic fibrosis, premature infants, biliary atresia) have low
vitamin E status (Combs, 1992).

Absorption is primarily in the medial small intestine following hydrolysis of the esterified forms of vitamin E to the alcohol form. No absorption occurs in the large intestine (Gallo-Torres, 1980). The vitamin E alcohol is the main form which appears in mixed micelles, and is the predominant form of absorbed vitamin E (Combs, 1991). Absorbed tocopherol is released into the lymphatic system in mammals, or into the portal system in fish, birds, and reptiles (Combs, 1992).

Gallo-Torres (1980) reported that 20 to 40% of orally administered vitamin E is absorbed. D-α-tocopherol, when added to the diet of weanling pigs, may be better absorbed and retained than dl-α-tocopheryl acetate (Chung et al., 1992). Ingold et al. (1987) found that in rats, the discrimination between RRR- and SRR-α-tocopheryl acetate begins in the gut, due to the incomplete hydrolysis of the SRR- when compared to the RRR- steroisomer. Some minerals and lipids in the diet may cause vitamin E oxidation in the gastrointestinal tract, thus decreasing absorption (Burton et al., 1988).

Factors affecting the bioavailability of oral drugs were reviewed by Wagner (1961) and Koch-Weser (1974). They listed and discussed the most important factors such as: 1) Characteristics of the drug, which included inactivation before gastrointestinal absorption, incomplete absorption, and biotransformation in the intestinal wall or liver, 2) Formulation of the drug product, which included state of the drug, and excipient, 3) Interaction with other substances, such as food and drugs, in the gastrointestinal tract, and 4) Characteristics of the patient which included genetics; hepatic function; and gastrointestinal pH, motility, perfusion flora, and structure.

The delivery systems for oral dosing of vitamin E have been reviewed by Bauernfeind et
al. (1974) and include a sealed gelatin capsule, oil solution, water-dispersible liquid form, and water-emulsifiable form added to dry food or liquid. Bateman and Uccellini (1985) reported that vitamin E was better absorbed from a formulation containing surfactant than from an oily preparation following oral administration.

After administration of a single capsule containing 500 IU of d-α-tocopherol in a water soluble base in humans fasted for at least 12 h, Bateman and Uccellini (1985) reported an absorption constant (Ka) of .87 h⁻¹, and an elimination constant (Ke) of .0032 h⁻¹. They suggested that this high absorption constant was due to a rapid mechanism of absorption that did not require incorporation of vitamin E into micelles.

Ferslew et al. (1993) reported that after oral administration of two 400 mg soft-gelatin capsules of either RRR- or all-rac-α-tocopherol to overnight fasted humans, natural and synthetic vitamin E had a similar lag time (3.2 h), half-life of absorption (t_1/2a = 2.9 h), Ka (.454 h⁻¹), maximum concentration (C_max = 19.5 μg/mL), time to reach C_max (T_max = 13.1 h), half-life of elimination (t_1/2e = 76.9 h), and Ke = .017 h⁻¹. The AUC from 0 to 96 h after administration of the dose (AUC_0-96 h) ratio RRR/all-rac was 1.53.

In chicks (Marusich et al., 1967), an oral dose of d- or dl-α-tocopheryl acetate in a water-based formula; in human (Overman et al., 1954), an oral dose of an aqueous emulsion (capsule) of d-α-tocopheryl acetate; and in pigs (Hoppe, 1991), d-α- and d-γ-tocopherol added to the feed reached Tmax 6 h after administration. The latest experiment, Hoppe (1991) reported that d-α-tocopherol was 2.5 times more bioavailable, based on AUC_0-48 h, than d-γ-tocopherol.

Hidiroglou and Karpinski (1988) reported Tmax at 40, 36, 26, and 32 h; Ke of .034, .016, .019, and .021 h⁻¹, and AUC_0-320 h of 408, 420, 656, and 426 μg/mL h after oral administration in gelatin capsule of 100 IU/kg BW of dl-α-tocopherol, dl-α-tocopheryl acetate, d-
α-tocopherol, and d-α-tocopheryl acetate, respectively.

Doncon and Steele (1988) reported Tmax between 8 and 24 h after oral administration of 1000 or 2000 IU of a commercial α-tocopherol product (Rovimix E-20, Roche) in a study designed to investigate the effect of vitamin E supplementation on the mycotoxicosis lupinosis in sheep.

In sheep, Hidiroglou and Ivan (1992) fitted the change in plasma concentration over time after oral administration of d-α-[3H] tocopherol in ethanol to a two exponential (Ct = K₁ e⁻P₁ t - K₂ e⁻P₂ t). Where the magnitude of the intercepts (dpm/g) were K₁ = 419, and K₂ = 438 and of the slopes (h⁻¹) were P₁ = .0027, and P₂ = .31. The Tmax was reported between 32 and 48 h.

In sheep, Hidiroglou and Karpinski (1987) expressed the change in plasma concentration with time after oral administration of d-α-[3H] tocopherol in capsule as two exponentials (Ct = K₁ e⁻P₁ t - K₂ e⁻P₂ t). The intercepts (dpm/μg) were 3,000 for K₁, and 2,500 for K₂; and for the slopes (h⁻¹) were .008 for P₁, and .25 for P₂. The Tmax was reported at 16 h, and the bioavailability related to the i.v. administration was 28%. They also fitted the change in plasma concentration over time after intraruminal administration of d-α-[3H] tocopherol to a three exponential (Ct = K₁ e⁻P₁ t + K₂ e⁻P₂ t - K₃ e⁻P₃ t). The intercepts (dpm/μg) were 2,000 for K₁ and K₃, and 1,500 for K₂; and of the slopes (h⁻¹) were .01 for P₁, .002 for P₃, and .2 for P₃. The Tmax was 16 h after administration and a relative bioavailability of 21% compared with the i.v. administration. The same authors (Hidiroglou and Karpinski, 1988) reported Tmax at 28 h after intraruminal administration of dl-α-tocopheryl acetate, and a relative bioavailability (based on the AUC₀−180h) of 82% compared with the i.p. route.

Hidiroglou et al. (1994) estimated that the mean retention time of tocopherol in the rumen was between 15 to 19 h, and that a small fraction of the free tocopherol was absorbed by the
ruminal wall. Bioavailability of d-α-tocopherol was four times larger than that of its succinate ester because of the incomplete hydrolysis of the ester.

After a single intraruminal administration of 100 mg of dl-α-tocopherol or dl-α-tocopheryl acetate per kg BW in sheep, Hidiroglou et al. (1989) reported similar Cmax and final concentration (Cf) for the alcohol and ester sources of vitamin E. Tmax was faster for the free (27 h) than for the ester form (40 h) of synthetic vitamin E. The AUC₀-288ₜ ratio of free/ester was 1.12. They also reported similar Cmax, Cf, and Tmax (35-49 h) after a single oral administration of 50 mg of dl-α-tocopherol or dl-α-tocopheryl acetate per kg BW in cattle. The AUC₀-480ₜ ratio of free/acetate was 1.23.

Parenteral. Parenteral administration of a drug is intended to go through or under one or more layers of skin. Subcutaneous, intramuscular, intravenous, intradermal, hypodermal, intra-arterial, intrapleural, intraperitoneal, intra-articular, intracardial, intraspinal, and intracerebral are all parenteral administration routes that differ in the site of injection.

Complications following the parenteral administration of drugs are not uncommon, and may include: pain or discomfort, skin pigmentation, hemorrhage, septic or sterile abscess, fibrosis, necrosis and gangrene in the site of injection (Ballard, 1968).

Complete entry into the general circulation is assured only when the product is administered intravenously, intra-arterially, intraspinaly, and intracerebrally. The other parenteral routes of administration only allow incomplete and variable bioavailability of the drug. Absorption rates after using any of the other routes of administration can be very different from the enteral administration (Wagner, 1961). Most of the factors influencing bioavailability of oral drugs also apply for parenteral administration.

Koch-Weser (1974) remarked on the importance of the injection site on absorption rate,
particularly regarding perfusion at the injection site, circulatory disturbances, muscle activity, etcetera. Total surface area available for diffusion also influences the rate of absorption. High-pressure injection devices and massage of the injection site after administration are effective means of spreading the solution (Greenblatt and Koch-Weser, 1976).

Muscle has a rich supply of capillary vessels, but has a poor supply of lymph vessels. Lymph vessels exist where fascial planes enter the muscle. The subcutaneous region is well supplied by capillary and lymphatic vessels (Ballard, 1968).

Lipid-soluble molecules diffuse through capillary walls (Ballard, 1968), but large lipid-insoluble drug molecules depend on the lymphatic system to be removed from the injection site. The process is slow because the rate of lymph flow is less than .1% of the plasma flow (Greenblatt and Koch-Weser, 1976).

The delivery systems for intramuscular administration of vitamin E have been reviewed by Bauernfeind et al. (1974) and include: water-dispersible, oil solution, and liquid emulsions. Drugs with low water solubility at physiologic pH may precipitate at the injection site and may be unable to diffuse into capillaries. In some parenteral preparations, the drug is in solution until it is placed under the skin, and then precipitation of the drug occurs. This can explain some cases of partial bioavailability after parenteral administration. Precipitated compounds can gradually redissolve and be absorbed, or they can be removed by phagocytosis (Greenblatt and Koch-Weser, 1976).

Overman et al. (1954) reported that i.m. administration of 500 mg of dl-α-tocopherol in sesame oil did not increase the plasma tocopherol concentration in humans. Behrens et al. (1975) reported that the slow rate of release after i.m. administration of vitamin E in oil carrier (peanut or soybean oils) in sheep may be due to myositis and lymphadenitis. Similar clinical observations
were made by Dickson et al. (1986) also in sheep. The oil carrier itself was not responsible for such reaction. They also reported that lymph glands, where lymph drained from the site of injection, stored important amounts of vitamin E for at least 4 months after i.m. administration. Chung (1993) also reported a continued and slow release of tocopherol, reflected as serum and tissue concentration, after i.m. administration of d-α-tocopheryl acetate with olive oil as a carrier in swine. In sheep; similar observation were made by Hidiroglou and McDowell (1987) after 40 mg of d-α-tocopherol in sesame oil were i.m. injected per kg of BW, and by Judson et al. (1991) after s.c. administration of 15, 30, 60, or 120 mg of dl-α-tocopheryl acetate in an oily solution per kg of BW.

Slow absorption can be obtained by the use of injection vehicles of various viscous organic solvents (glycerine, cotton-seed oil, sesame oil, or polyethylene glycol), or by the use of fatty acid ester derivatives. These complexes are gradually hydrolyzed, and slow release of the active drug to the circulatory system takes place. The rate of absorption, however, may be erratic and unpredictable (Greenblatt and Koch-Weser, 1976).

The simplest model of drug absorption after i.m. administration is the first-order absorption, which assumes passive diffusion of the compound from the injection site into the capillary system. The concentration of the compound in the capillaries into which it diffuses is assumed to be negligible as compared to that at the place of injection. Therefore, this model assumes that the rate of absorption is proportional to the concentration (C) of the specific compound at the injection site:

$$\frac{dC}{dt} = -\Pi C$$

Where t is the time after injection, and Pi is the apparent first-order absorption rate constant. After integration of the differential equation, the following relationship is obtained:
\[ C = C_0 e^{-Pt} \]

Indicating that the concentration at the site of injection, having an initial value \( C_0 \) at time zero, declines exponentially. The half-life of the absorption process is:

\[ t_{1/2} = \ln 2/P_i = 0.693/P_i \]

When the rate of disappearance from the site of injection to concentration remaining at the injection site is not constant at all times after injection, the absorption is called nonlinear. This is a common situation, because local and systemic factors that influence the rate of absorption rarely remain constant while absorption takes place (Greenblatt and Koch-Weser, 1976).

Hoppe (1991) reported in swine that after i.m. administration of equimolar doses of d-\( \alpha \)- or d-\( \gamma \)-tocopherol in water based emulsion, \( T_{\text{max}} \) was 4 and 8 h for \( \alpha \)- and \( \gamma \)-tocopherol, respectively; and that based on the AUC_{0-48h} \( \alpha \)-tocopherol was 2.1 times more bioavailable than \( \gamma \)-tocopherol. He also stated that tocopherol was 4-5 times more bioavailable after i.m. administration than after oral administration.

Hidiroglou and Karpinski (1991) noted that absorption (\( .174 \text{ h}^{-1} \)) and elimination (\( .02 \text{ h}^{-1} \)) rates of dl-\( \alpha \)-tocopheryl acetate in liquid emulsion, after i.m. administration to sheep, are independent of dose, but that the extent of the absorption is parallel to dose. They also reported a lag phase following i.m. administration. Njeru et al. (1992) reported in sheep that a single i.m. injection of dl-\( \alpha \)-tocopherol in an emulsifiable solution had an elimination rate independent of dose, whereas the absorption rate increased with increasing dose. They used a three-phase linear model to describe this results.

In man (Rindi and Perri, 1958) and in dog (Bauernfeind et al., 1974; Fujii, 1980) after i.m. administration of dl-\( \alpha \)-tocopheryl acetate, the peak of tocopherol in serum was always later than the peak of the acetate form (8 h). Therefore, hydrolysis of the tocopheryl ester was the
limiting step in bioavailability.

Gallo-Torres and Miller (1971) reported hydrolysis of dl-\(\alpha\)-tocopheryl acetate by the liver in rats. These authors found that, 30 min after i.v. administration, 79% of the radioactivity in the liver was due to quinone derivatives, 15% to the ester form, and only small quantities were due to the alcohol form. They also reported the same biotransformations in several other tissues and also indicated that blood contains enzymes capable of hydrolyzing esters of tocopherol. In contrast, Bauernfeind et al. (1974) reported that the blood does not have enzymes capable of hydrolyzing dl-\(\alpha\)-tocopheryl acetate after an in vitro incubation of dog fresh blood at 37°C for up to 2 h. Newmark et al. (1975) reported similar results after incubation of human and dog blood at 37°C for up to 3 h. The same authors reported that 24 h after i.v. administration of dl-\(\alpha\)-tocopheryl acetate in dogs, 25% of the dose lost the acetate, whereas Ogihara et al. (1985) reported 36% in the case of rats. Therefore, differences seem to exist in the hydrolytic capability among species.

Ogihara et al. (1985) also reported that 24 h after i.v. administration, the ester reached undetectable concentration in plasma, whereas 48 h were needed to reach those undetectable concentrations in red blood cells. Knight and Roberts (1986) observed that not all the \(\alpha\)-tocopheryl acetate was converted to tocopherol. This was probably due to the short duration of the experiment that did not allow total biotransformation of the ester. They also observed \(\alpha\)-tocopheryl acetate uptake by tissues. In premature infants receiving constant i.v. nutrition (including a fat emulsion supplemented with 4.5 mg of all-rac-\(\alpha\)-tocopheryl acetate per kg of BW and day during 5 days), the ester form was readily hydrolyzed and the free form was released, resulting in good bioavailability (Schwalbe et al., 1992). These results indicated that premature infants had the enzymatic capability to hydrolyze the ester.
Hidiroglou and Atwal (1989) reported that the i.p. route was successfully used in cattle for administration of the free and acetylated forms of dl-α-tocopherol in emulsion, using polyoxyethylene sorbitan monooleate (Tween 80), and that the ester form resulted in higher milk tocopherol and lower plasma tocopherol content than the free form. Hidiroglou and Charmley (1990) reported that in sheep, after i.p. administration of 100 mg of dl-α-tocopheryl acetate per kg BW in Tween 80, Tmax was observed after 32 h. They also showed that, based on the AUC<sub>0-192 h</sub>, the i.p. route of administration was 1.2 times more bioavailable than the intraruminal route. Neither reference, however, reported the plasma tocopheryl acetate profile. Therefore, there was no information about whether the disacetylation process took place in the abdominal cavity or once absorbed in the blood stream.

Hidiroglou and Karpinski (1987) studied the kinetics of 200 μCi of d-α-[<sup>3</sup>H] tocopherol in ethanol of a physiological i.v. dose to sheep. The change in plasma tocopherol concentration with time was expressed as a sum of three exponentials \( C_t = K_1 e^{P_1 t} + K_2 e^{P_2 t} + K_3 e^{P_3 t} \), where the intercepts were: \( K_1 = 150,000 \), \( K_2 = 35,000 \), and \( K_3 = 13,000 \) dpm/mL; and the slopes were: \( P_1 = .30 \), \( P_2 = .48 \), and \( P_3 = .014 \) h<sup>-1</sup>. Based on these estimates, a three compartmental model was proposed (Figure 3). In the case of i.m. administration, changes in plasma concentration with time (Figure 4) were characterized by a rapid absorption phase \( C_t = K_1 (1 - e^{-P_1 t}) \) and a slow absorption + elimination phase \( C_t = K_2 e^{-P_2 t} - K_3 e^{-P_3 t} \). The intercepts were \( K_1 = 1,700 \), \( K_2 = 4,700 \), and \( K_3 = 3,700 \) dpm/mL, and the slopes were \( P_1 = 1.73 \), \( P_2 = .0042 \), and \( P_3 = .13 \) h<sup>-1</sup>. The availability of tocopherol following i.m. administration, relative to the i.v. administration, was 51%. In this experiment, Tmax was detected 32 h after administration.

Based on the AUC<sub>0-319 h</sub> [(μg/mL) h], Hidiroglou and Karpinski (1988) reported that i.v.
administration of d-α-tocopheryl acetate (1120) was much better utilized by sheep than dl-α-tocopherol (676) or dl-α-tocopheryl acetate (772). In this experiment, even though they injected the ester form, only the kinetics of the free tocopherol was reported. Therefore, after administration of the ester 62.4 and 28.4 h were needed to reach the maximum concentration of the free form, with a Ke of .028 and .045 h⁻¹ for the dl-α-tocopheryl acetate and the d-α-tocopherol, respectively. When they i.v. injected dl-α-tocopherol, the profile was represented as a sum of three exponentials (Ct = Base + K₁ e⁻P₁ t + K₂ e⁻P₂ t + K₃ e⁻P₃ t), where the intercepts were: K₁ = 46, K₂ = 16, and K₃ = 3 μg/mL h; and the slopes were: P₁ = 50, P₂ = .6, and P₃ = .006 h⁻¹.

Hidiroglou et al. (1990) studied the kinetics of an i.v. dose of 2 μCi of d-α-[³H]tocopherol in emulsion per kg BW in sheep. The change in plasma concentration up to 56 h after administration was expressed as a sum of two exponentials (Ct = K₁ e⁻P₁ t + K₂ e⁻P₂ t), where the intercepts were: K₁ = 11,000, and K₂ = 5,700 dpm/μg/mL; and the slopes were: P₁ = .7, and P₂ = .02 h⁻¹. Based on these estimates, they also were able to fit the data to a two compartmental model (Figure 3).

Karpinski and Hidiroglou (1990) studied the kinetics of i.v. administration of 4 μCi of d-α-[³H]tocopherol in emulsion per kg of BW in sheep. The change in plasma concentration up to 432 h after administration was expressed as a sum of three exponentials (Ct = K₁ e⁻P₁ t + K₂ e⁻P₂ t + K₃ e⁻P₃ t), where the intercepts were: K₁ = 105,000, K₂ = 28,000, and K₃ = 4,000 dpm/μg; and the slopes were: P₁ = 1.56, P₂ = .062, and P₃ = .006 h⁻¹.

Others. The percutaneous route of administration has been the focus of recent attention regarding topical therapy of the skin with vitamin E. Two routes of absorption of tocopheryl acetate by the skin have been proposed by Kamimura and Matsuzawa (1968). The first route is
from the stratum corneum into the epidermis and into the dermis, and the second route is through the hair follicles, by the way of the pilosebaceous canal into the dermal tissue and the connective tissue sheaths.

Tocopheryl esters are hydrolyzed by enzymes in the gastrointestinal tract prior to absorption at the intestinal wall. In contrast, if absorbed through the skin, tocopheryl acetate is not exposed to hydrolytic enzymes. Tocopheryl acetate can diffuse across skin cell membranes, enter the cell, and be associated with mitochondria, endoplasmic reticulum and nuclear membranes. At this level, cellular esterases may release the free alcohol form of this vitamin (Trevithick et al., 1992; Trevithick and Mitton, 1993). These authors also reported that topical application of free tocopherol irritated the skin of mice.

**Sow Reproduction and Nutrition**

Reproductive performance is generally determined by the number of pigs weaned per sow per year and is affected by the number of pigs born and weaned per litter and the number of litters per sow per year. For a meaningful between-herd comparison of number of pigs weaned per sow per year, it is necessary that gilts be considered to be a part of the breeding herd from the time they are selected for breeding and are withheld from market. Thus, to maximize reproductive efficiency, the objective is to mate gilts early and keep them producing healthy and large litters as frequently and for as long as possible.

The effect of nutrition on the reproductive performance of the sow is very difficult to measure because a variety of factors, including environmental and management systems, can mask the sow's responses, and her capability to cope with minor nutritional deficiencies. Nutrient
requirements are different for the various stages of the reproductive life of the sow, and this needs
to be recognized to achieve maximal reproduction.

Around 35% of the sows in a herd are culled each year and replaced with gilts. Gilts
produce smaller litters than sows, and as a result, a high culling rate has a significant impact on
the number of pigs weaned per breeding female. Therefore, the full expression of the
reproductive performance of the gilt is important. Factors that may affect this include age,
weight and body condition at mating, and the ability to be rebred after weaning.

**Age at puberty**

The mean age at puberty of gilts is about 200 days in breeds commonly available in the
U.S. However, relocating gilts to another pen, mixing them with gilts from another pen, and/or
exposing them to a mature boar can induce puberty about 30 days earlier than in non-induced gilts

Conditions of management and nutrition that support acceptable growth rates during
rearing are thought to be adequate for the proper development of the gilt and her subsequent
reproductive performance (Close and Cole, 1986). Newton and Mahan (1993) reported that
breeding weight from 120 to 150 kg at 8 mo of age does not affect the onset of puberty.
Moderate protein restriction does not affect age at puberty. In contrast, severe protein restriction
or amino acid imbalance does delay the age at puberty (Jones and Maxwell, 1974). Increasing
energy intake of gilts for one estrous cycle prior to breeding (flushing) has been reported (Schultz
et al., 1966) to result in an increase of 1.9 corpora lutea. Other factors, such as environment and
management, have a greater impact on the onset of puberty than nutrition.
**Body condition**

Minimum fat cover, at least 10 mm backfat, is required for successful reproduction. Gilts that are bred at younger ages and lighter weights with lower backfat depths have higher subsequent culling rates. In spite of this, the same lean gilts can maintain their original backfat depth and productivity as long as can gilts bred at heavier weights with greater backfat depths if lean gilts are provided good nutrition and management (Aherne, 1991).

**Ovulation rate**

In swine, the main constraint on litter size is ovulation rate. Archibong et al. (1987) suggested that, when puberty occurs at a young age and low body weight, ovulation rate increases with successive estrous periods; thus, delaying mating until the second or third estrus increases the ovulation rate and embryo survival. However, beyond a minimum age and weight, neither age, weight, nor number of estrus periods exhibited influence ovulation rate or litter size (Kirkwood and Thacker, 1988). Flushing gilts for 11 to 14 d before mating increases the ovulation rate, but only to the levels achieved by ad libitum-fed gilts (Den Hartog and van Kempen, 1980). Flushing increases insulin concentration in blood. This elevation in insulin level has been reported to increase ovulation rate, but it is not always accompanied by increases in plasma gonadotrophin level (Robinson, 1990). Thus, ad libitum feeding during the growing-finishing phase and up to mating maximizes ovulation rate.

**Pregnancy and conception rate**

Pregnancy rate may be defined as the proportion of sows selected for breeding that become pregnant, and conception rate may be defined as the proportion of selected sows mated
that become pregnant. In sows fed for 35 days before mating at a level of 1.5 kg, conception rate was not affected, but pregnancy rate was lower than that of gilts fed 2.25 to 3.0 kg/day (Dyck, 1988). Den Hartog and van Kempen (1980) reported that high or low levels of feed intake up to puberty or during the estrous cycle did not significantly affect conception rate.

**Nutrition of the embryo**

Swine have epitheliochorial placentae in which the maternal blood supply is well separated from the absorptive surface of the chorion. Consequently, nutrients need to be transferred from the endometrial surface and glandular epithelium to the chorioallantois (Ashworth, 1991). The uterine milk (histotroph) secreted by the uterine glands furnishes, during early pregnancy, a good part of the nutrients that diffuse through the placental membranes and reach the fetuses. However, in advanced pregnancy, the nutrients come from the maternal circulatory system and are transported across the placenta into the fetal circulation. Different types of placentae have different degree of permeability to minerals, fats, carbohydrates, and proteins. The placental and fetal cells have a greater metabolic rate than the cells of the maternal tissues, and red blood cells from the fetus have a higher oxygen-carrying capacity than maternal ones (Nalbandov, 1976).

**Conceptus secretions**

Blastocysts secrete a variety of compounds prostaglandins, binding proteins for insulin, proteins with antiviral activity, and plasminogen activator. The roles of most of these secretions are not clear, but may increase endometrial prostaglandin synthesis and interact with estrogen to alter prostaglandin (luteolytic agent) movement into the uterine lumen. At 11 to 12 d of pregnancy, estrogen secretion by the pig conceptuses increases. It is at this stage of gestation that
the corpus luteum must be protected from luteolysis to establish pregnancy and maintain progesterone levels to term. Conceptus estrogens increase uterine arterial blood flow, uterine vascular permeability, and increase the concentrations of protein, calcium, prostaglandin, and plasmin inhibitor in uterine secretions (Ashworth, 1991).

Embryo mortality

In pigs, the fertilization rate is close to 100%, but only 60% of the ova shed culminate in the birth of living piglets (Ashworth, 1991). Many factors affect embryonic mortality in the pig, such as feeding, age of gilts at mating, weaning to remating interval, environment, mating sire, breed, level of inbreeding, immunological factors, age and parity of the sow, disease, hormone treatment, length of lactation, time of entry of embryos into the uterus, intra-uterine position and crowding of embryos, chromosomal abnormalities, and anesthesia [see reviews by Stone (1987) and Clark et al. (1988)].

At the time of implantation, when delicate hormonal changes occur, most of the embryo mortality takes place. Any disturbance to the dam at this stage will cause hormonal upset and increase the number of eggs which die (Varley, 1991). Early weaned sows have very high embryonic loss and also show disturbed hormonal profile. Sows injected with pregnant mare's serum gonadotropin, which can stimulate ovarian steroid secretion, on day 15 of pregnancy produced a high number of viable embryos (Stone, 1987). Sows fed progesterone orally for 4 days after breeding had greater embryo survival than sows fed control diets (Schultz et al., 1966).

Great variation exists in the developmental stage among littermate embryos, and a relatively advanced embryo has a greater chance of survival. The relatively advanced embryos secrete increasing quantities of estradiol from d 11 to 12, which alters uterine function in
accordance with their increasing requirements. The lesser developed embryos are consequently exposed to an uterine environment too advanced, from a biochemical point of view, for their needs, and this environment becomes embryocidal (Ashworth, 1991).

Therefore, the increased survival at this early stage of the embryo life could be either the result of a better recognition of live embryos by the hormonal and autocrine mechanisms or the result of an increased viability of the embryos themselves (Buttle, 1991). Youngs et al. (1994), in a reciprocal embryo transfer study involving Meishan and Yorkshire pigs, suggested that the higher prolificacy of Meishan pigs compared with that of Yorkshire pigs may be due not only to the slower growth rate of the embryos and an associated more gradual change in estradiol secretion, but also to uterine factor(s) with a suppressive effect on embryonic growth and estradiol secretion.

The incidence of mummified fetuses increases in sows carrying 10 or more fetuses after 50 d of gestation. This has been associated with a lack of uterine space resulting in intra-uterine crowding. Other factors implicated in such fetal mortality include maternal nutrition, thermal effects, and limitations of blood flow (Ashworth, 1991).

During the first half of pregnancy, a low plane of nutrition does not affect fetal weight adversely if dietary intake is adequate during the second half. An inadequate plane of nutrition during the second half of pregnancy, or competition for available nutrients in polytocous species like swine, reduces the size of the pigs at birth. On the other hand, excessive food intake by the dam does not cause fetal growth beyond the normal size. Thus, fetal size and weight at birth, up to the normal size and weight, depends on the plane of nutrition of the female during the second half of pregnancy (Kirkwood and Thacker, 1988).
Nutrition for gestation

The major energy source in the newborn pig is glycogen. Nevertheless, an increase in glycogen quantity through the diet does not necessarily improve piglet survival. Muscle fiber hyperplasia ceases between 85 and 95 d of gestation, and a reduction in the total number of fibers in muscle of the smallest fetus as compared with the largest one in a litter has been observed. As a consequence, undernutrition of the sow at that time can have a detrimental effect on subsequent performance of the litter (Robinson, 1990).

Feed intake by gestating sows should be limited to control weight gain during pregnancy. Sows should gain a total of 45 kg during the whole gestation period for at least the first three or four parities, 20 kg for the increase in weight of the placenta and other products of conception, and 25 kg for body weight gain. To achieve this goal, gestation feed intake should be maintained between 1.7 and 2.3 kg per day (6.1 Mcal of metabolizable energy per day) (NRC, 1988).

Fasting gilts for 10 days during late gestation after feeding a high-fat diet results in an increase in plasma fatty acid concentration of the dam, but the use of fatty acids by the fetus is limited (Ruwe et al., 1991) by the poor utero-placental fatty acid transfer (Thulin et al., 1989; Ramsay et al., 1991). Overfeeding during pregnancy reduces voluntary feed intake during lactation and seems to impede mammary development (Pettigrew and Tokach, 1991). The nutritional objective during gestation is to conserve body condition, and, as a consequence, sows should gain 1 to 2 mm of backfat in this period and not should lose more than 1 to 2 mm during lactation (Aherne, 1991).

Diets low in protein (.5% to 2%), protein-free diets, or even periods of complete inanition of sows did not adversely affect embryo or fetal development or litter size, but did reduce birth weight of the pigs (Speer, 1982).
Feeding fat during late gestation increases glycogen and fat content of the liver and skeletal muscle of newborn pig. Adding fat, butanediol, fructose, or starch to the gestation diet has minor effects on sow reproductive performance, pig survival, or litter growth (Aherne, 1991).

**Milk production**

There are three important aspects of the lactation period. First, antibodies are transferred via the colostrum during the first 24 hours post-parturition; second, the sow lets down 20 to 25 mL of milk once every 40 min or so for the duration of lactation; and third, the composition of the milk is rich in fat and protein. From an endocrinological point of view, lactation is characterized by elevated prolactin concentrations, and a suppression of gonadotropin secretions (Buttle, 1991).

In pigs, active mammary growth is restricted to the last third of pregnancy. The complex of hormones that stimulate mammary growth consists of the anterior pituitary gland hormones (prolactin and growth hormone), the steroid hormones (estrogen and progesterone), as well as metabolic hormones (e.g., adrenal steroids and insulin). Pigs do not produce a placental lactogen, but their placentae do produce estrogens in large amount, coincident with mammary growth. In ovariectomized pregnant pigs, the administration of relaxin stimulated mammary growth. Relaxin is a hormone similar in structure to insulin. It is formed in the corpora lutea of pigs during pregnancy and plays an important function in mammogenesis, parturition, and lactogenesis of pigs (Buttle, 1991).

The function of the sow is not only to produce large litters of viable pigs, but also to ensure their survival and rapid growth. The most important factors affecting milk yield of the sow are her genetic potential, nutrient and energy intake, body condition, and the demands made
by the litter. The average milk yield of sows ranges from 5 to 10 kg/d, but gilts produce less milk than sows. Milk yield increases up to the third parity after which it plateaus and then declines at the fifth or sixth parity. Milk yield increases gradually postpartum and reaches a peak at about d 21 of lactation (Aherne, 1991). Estimation of milk yield can be made by assuming that each gram of litter weight gain up to 21 days of age requires about 4 g of milk (Noblet and Etienne, 1986).

The composition of milk from the sow varies with nutrient and energy intake in gestation and lactation, and the stage of lactation. The composition of colostrum changes rapidly during the first 12 hours after farrowing. The gamma globulin fraction of colostral protein declines from 8.6% at farrowing to 2% at 12 hours postpartum. After 48 hours postfarrow, the composition of the sow's milk changes little (Aherne, 1991).

**Nutrition for lactation**

Daily feed intake of 4.4 to 6.1 kg of a corn-soybean meal diet containing 3.34 Mcal of DE/kg, 13% CP and 0.6% lysine allows the production of average milk yield of 5 to 7 kg/day, and enable a minimal loss in sow weight or condition throughout lactation. Sows producing greater milk yields than 5 to 7 kg/day require additional feed. A considerable percentage of sows do not consume or obtain the levels of feed recommended by NRC (1988), causing them to mobilize their body reserves, both protein and fat, to maintain milk production (NRC, 1988). Factors affecting feed intake have been reviewed by Aherne (1991), and include appetite, milk yield, gestation-lactation feed intake interactions, temperature, and system of feeding.

Energy intakes of less than 12 Mcal DE per day for first-litter gilts has been reported to increase weight and condition loss, as well as reduce conception rate, embryo survival, and
subsequent litter size. These effects are less obvious in multiparous sows (Reese et al., 1984). Digestible energy intake of a lactating sow can be increased by supplementation of her diet with fat. This supplementation increases litter weaning weights, but a clear benefit on reproductive function has not been shown. Excessive energy intake during pregnancy interferes with mammary development (Pettigrew and Tokach, 1991). In general it is advisable to add fat or oil to the lactating sow diet during the summer, when hot weather can decrease feed intake.

No significant differences in the productivity of the sows fed isonitrogenous and isocaloric diets with or without cereals were found by Van der Peet-Shwering and der Hartog (1991). Feeding sows diets deficient in amino acids resulted in great mobilization of body protein, and this condition has been associated with prolonged weaning-to-estrus interval because of lower mean plasma concentration of luteinizing hormone (Jones and Stahly, 1995).

Excessive loss of either fat or protein body reserves reduces sow reproductive performance. At this point, the critical factor seems to be the amount of body reserves at farrowing and weaning, rather than the amount of tissue that has been mobilized during lactation. As a consequence, weight loss in a thin sow may be more serious than a large weight loss in a sow in good condition (Esbenshade et al., 1986).

There is no reason to think that lactating sows have larger calcium and phosphorus requirements than the levels suggested by NRC (1988). The milk of the sow is rich in calcium and phosphorus, so the requirement for those minerals is high during lactation. Demineralization of the bones is needed to maintain adequate concentration of calcium and phosphorus in milk, but this demineralization does not cause problems unless it reaches an excessive level (Aherne, 1991).
**Weaning-to-rebreeding interval**

Delay of estrus activity is one of the main reasons for early culling of sows in the herd. Protein or energy intake depression during lactation can delay the return to estrus after weaning. This is more evident in gilts than in multiparous sows, and even more accentuated in those gilts mated for the first time at a young age (Robinson, 1990).

**Vitamin E and Reproduction in Swine**

In recent years, an increased interest focus on supplementation of sow diets with specific vitamins at levels higher than those previously thought to be adequate has emerged. Special attention has been paid to six vitamins.

Choline is found at high level in soybean meal, and for many years this level was thought to be adequate for optimal production. Feeding grain-soybean meal diets supplemented with 438 to 800 mg/kg of choline increased litter size and weight of the pigs at farrowing (NRC, 1988). Biotin supplementation of sow diets (.20 mg/kg) improved litter size at farrowing and weaning, litter weight at weaning, and weaning to estrus interval (NRC, 1988).

Parenteral administration of folic acid increased prolificacy of multiparous sows (Friendship and Wilson, 1991). On the other hand, Matte et al. (1993) showed that reproductive capacity of gilts was not affected by dietary supplementation of folic acid. Lindemann (1993) concluded that the positive effect of folic acid during gestation seems to be a consequence of improved embryo or fetal survival rather than an increase in ovulation rate. Supplementation of folic acid in gestation diets could be beneficial for high reproductive capacity animals.

Weekly injection of β-carotene improved embryo survival and litter size in pigs (Brief and
Chew, 1985). Injection of β-carotene or vitamin A at weaning time improved subsequent litter size (Coffey and Britt, 1991). Chew (1993) concluded that β-carotene can affect reproductive performance in swine by influencing ovarian steroidogenesis or changing the uterine environment. The route of administration for β-carotene needs to be studied, because swine absorb little or none of this provitamin from the diet.

Bazer and Zavy (1988) reported that a daily dose of 110 mg of riboflavin from d 4 to 10 of pregnancy produced a transitory increase in riboflavin concentration in uterine flushing, and this event was accompanied by an improvement in litter size. Wiseman et al. (1991) failed to confirm these observations when gilts were treated with 65 or 150 mg of riboflavin per d for 28 consecutive d beginning 5-10 d before breeding.

Vitamin E is the sixth of this group of vitamins and is the subject of the present dissertation. The benefit of supplemental vitamin E in addition to what is contained naturally in the swine diet is unclear (for review see Pharazyn et al., 1990).

**Vitamin E requirement of swine**

Recommendations for vitamin E supplementation of sow diets vary among countries. The NRC (1988) requirement, used in USA and Canada for bred and lactating sows, is 22 IU/kg of diet. In the UK, the ARC (1981) recommendation is 9 IU/kg of diet. In the case of the Netherlands, dietary supplemental levels of vitamin E vary from 10 to 30 IU/kg (Pharazyn et al., 1990). On the other hand, the level of vitamin E used in Denmark is as high as 37 IU/kg of diet.

Many factors need to be considered when assessing vitamin E requirements for animals. The term vitamin E is a generic name, representing the eight structural isomers previously discussed. In the pig, α-tocopherol has the greatest biological activity (McMurray et al., 1983).
There are other dietary, physiological, genetic (Less, 1990), and environmental factors that can affect the requirement of vitamin E for animals of reproductive age.

**Vitamin E, Se and dietary fat**

Many of the experimental studies reported in the literature have evaluated the combination of dietary Se, vitamin E and fat.

Chavez and Patton (1986) considered levels in a basal diet of .1 ppm Se and 15 IU vitamin E/kg of diet to be adequate. In this single parity study, thirty-one sows were assigned to three groups: the basal diet, basal diet plus injection of 3 ml of Dystocel (total 3 mg Se and 408 IU vitamin E) at d 30, 60, and 100 of gestation, or the second treatment provided with an additional injection of Dystocel at weaning of the previous litter. No significant differences in reproductive performance were found among injected groups, but when compared with non-injected animals, the injected ones had a greater litter size at birth, at weaning, and a lower mortality of the pigs prior to weaning. Van Vleet et al. (1973) reported a reduction in perinatal mortality in pigs from Se-vitamin E injected sows within 2 to 3 wk of farrowing and fed a deficient diet in vitamin E. In contrast, Malm et al. (1976) fed a control or a semi-purified diet containing .3 IU per kilogram of vitamin E and .05 ppm as Se supplementation to gestating gilts, and found no evidence of decreased reproductive performance or signs of clinical deficiency. Piatkowski et al. (1979) used a 2x2 factorial design and initiated dietary treatments at 150 days before breeding and continued over one gestation. The semi-purified basal diet contained .02 ppm Se and 6 mg of α-tocopherol/kg of diet and it was supplemented with 0 or 0.1 ppm Se and/or 0 or 22 mg of vitamin E/kg of diet. At 105 days post-coitus, gilts were slaughtered, and no response in litter size and weight was found to dietary vitamin E supplementation. The
conflicting results in the above studies may be due to the differences in dietary level of Se.

The results from the experiment of Piatkowski et al. (1979) indicated that vitamin E and Se, provided by both the semi-purified basal diet and tissue reserves, were adequate for the first parity, but .1 ppm Se and 22 IU vitamin E/kg were necessary to maintain tissue levels. Glienke and Ewan (1977) demonstrated the presence of vitamin E deficiency symptoms in pigs from sows during the second and subsequent parities that were fed a vitamin E-deficient diet for five parities. No symptoms were observed in the sows. Sows were able to buffer short-term deficiencies of vitamin E in the diet for a limited period of time, but deficiency symptoms appeared in the offspring.

The metabolism of vitamin E and its requirement by mammals is affected by the type of dietary fat. The use of fat, especially polyunsaturated fat, in late gestation and lactation to increase the energy intake of the animal (Pettigrew, 1981) may increase the requirement of the sow for vitamin E. As dietary intake of polyunsaturated fatty acids increases, the requirement for tissue antioxidant and, therefore, for vitamin E, increases. Tissue levels of \( \alpha \)-tocopherol are reduced with high dietary intake of polyunsaturated fats (Bieri et al., 1978). For growing pigs, the ARC (1981) suggested an increase of .25 mg of d-\( \alpha \)-tocopherol per gram of polyunsaturated fat added in the diet. A trend was observed for lower serum \( \alpha \)-tocopherol when corn oil rather than lard (both stripped of vitamin E) was provided in the diets of gilts at the same levels (Malm et al., 1976). Two experiments were conducted by Babinszky et al. (1992a) to assess the effect of fat and tocopherol levels in the lactation diet on the performance and milk production of pigs. In Experiment 1, they used two different levels of animal fat, low (43 g/kg of DM) and moderate (75 g/kg of DM) with tapioca starch, and two levels of vitamin E, low (13 to 16 mg/kg of diet) and high (117 to 135 mg/kg of diet). In Experiment 2, the two different levels of animal fat
were: low (37 g/kg DM) and high (125 g/kg DM) with corn starch, and two levels of vitamin E were: low (21 to 24 mg/kg of diet) and high (140 to 163 mg/kg of diet). They concluded that the different fat levels did not affect liver weight, backfat thickness, or milk production of the sows, and utilization of dry matter, fat, protein and energy of milk for baby pig growth. The high dietary fat with corn starch increased the dry matter, fat, and energy content of the milk and the daily gain of piglets between d 18 and 25 of lactation. The dietary vitamin E levels had no affect on any of the variables under investigation. In a different study (Babinszky et al., 1992b), gilts were fed two different sources of fat (animal fat or sunflower oil) with three levels of vitamin E in the diet during gestation and lactation. No effect of the type of fat on reproductive performance was reported. Also, milk production was not affected by the different levels of vitamin E: Low (12 to 16 mg/kg of feed), medium (45 to 50 mg/kg), or high (117 to 140 mg/kg). The authors concluded that colostral vitamin E concentration may increase with increasing levels of vitamin E combined with animal fat, and milk vitamin E concentration at weaning was higher when the diet contained animal fat.

**Vitamin E and tissue concentration in post-puberal female pigs**

The time necessary to develop deficiency symptoms after feeding a diet deficient in vitamin E and Se is longer in gilts or sows than in younger animals (Mahan et al., 1974; Piatkowski et al., 1979). Sows fed a corn-soybean meal diet without supplemental vitamin E or Se did not show any sign of myopathies until after the second parity (Mahan et al., 1974).

The status of vitamin E and Se in an animal reflects the amount provided by the diet and body reserves. During the growth phase, the vitamin E and Se requirement is high, but during the reproduction process this requirement is increased. The contribution of these two nutrients
from the feedstuff can vary and consequently can affect the body pool size of each nutrient. These stores can be mobilized when reproductive demands exceed the supply from the diet. As a result, the concentration of these nutrients accumulated in the tissue during the growth of the animal, the vitamin E and Se levels in the diet, and the reproductive capacity of the female will dictate whether an animal will show deficiency during her life.

**Vitamin E transfer to the progeny**

*Placental.* The nutrients present in the maternal blood reach the fetal blood after crossing the epitheliochorial placenta in swine. Beside this anatomical barrier, there are many other factors that affect the rate of maternal-fetal transfer such as uterine-placental blood flow, the size and chemical characteristics of the compound to be transported, the metabolic activity of the placenta, and the fetal circulation. The concentration of lipid-soluble vitamins in the fetal bloodstream is lower than that in the maternal blood. Passive diffusion has been suggested to be the transport mechanism of vitamin E by the human placenta (Rosso, 1990).

Pigs are born with a poor vitamin E status, whereas their Se status reflects the level of Se fed to the sow (Malm et al., 1976; Mahan et al., 1977; Young et al., 1977; Loundenslager et al., 1986). Mahan (1991b) reported that placental transfer of vitamin E is poor but reflects the level in the diet of the sow. Placental transfer of vitamin E is lower than for Se. Consequently, increasing the levels of tocopherol and/or Se in the pregnant animal diet increases fetal Se reserves but has little effect on fetal vitamin E status (Mahan et al., 1977; Mahan, 1991b). This effect on Se status of neonates was not found when an injectable Se and vitamin E was administered along the gestation period of sows fed a diet supplemented with 0.1 ppm Se and 15 IU vitamin E/kg (Chavez and Patton, 1986). Therefore, newborn pigs are dependent on
Vitamin E and Se effectively traverse mammary tissue. Schweigert (1988) reported that low-density lipoprotein receptors increase in mammary tissue during the formation of colostrum, and that they may help transfer lipophilic substances into the colostrum. Colostrum has a higher concentration of Se and vitamin E than does milk, and both nutrients are elevated in mature milk secretions as the dietary concentrations are increased (Piatkowski et al., 1979; Loudenslager et al., 1986; Mahan, 1991b). Therefore, the requirement of the dam during lactation is greater for these nutrients, and this is accentuated when the sow has a larger litter size and for a longer lactation period. Older sows had a lower vitamin E and Se concentration in the milk than younger sows, suggesting a depletion of those nutrients with age. There is no depletion of vitamin E in milk associated with parity if vitamin E is supplemented (Mahan, 1991b; Mahan et al., 1974, 1977). Furthermore, over one parity, tissue reserves of vitamin E are adequate to
meet demands for secretion into both colostrum and milk (Hardy and Frape, 1982).

Newborn pigs have low circulating levels of vitamin E. Subsequent changes in plasma vitamin E and Se concentration after birth are influenced by the vitamin E, Se and polyunsaturated fatty acid contents of milk, the daily milk intake, and the efficiency of intestinal fat absorption. Greater tissue reservoirs of those nutrients in pigs at weaning time are a reflection of those factors, and this condition is beneficial in preventing the onset of deficiency later in the growth phase (Cline et al., 1974; Mahan et al., 1977). This greater colostrum and milk tocopherol may be even more beneficial to pigs when they are housed in a dirty environment (Mahan, 1994).

Vitamin E and parturition problems

In dairy cattle, retained placenta has been reported as a sign of Se-vitamin E deficiency; however, this has not been demonstrated in swine. Signs of this deficiency in reproducing sows are an inadequate prostaglandin production resulting in an impaired smooth muscle contraction that is expressed as extended parturition time, poor milk letdown, weak newborn pigs, and pigs with spreadlegs (Mahan, 1991a). Mastitis, metritis, and agalactia (MMA) in sows are a complex syndrome. Sows in poor sanitary conditions are exposed to a greater concentration of pathogens and toxic substances. Consequently, those sows have a high incidence of MMA. Sows in poor sanitary conditions tend to respond more positively to dietary vitamin E supplementation than sows in good sanitary conditions (Mahan, 1994).
**Vitamin E and Fe tolerance in young pig**

Low Fe reserves found in neonatal pigs lead to anemia if iron is not available (ARC, 1981). To prevent the development of anemia, the newborn piglet is injected i.m. with iron-dextran. In vitamin E and Se-deficient pigs, an i.m. injection of Fe-dextran may lead to severe bruising, high serum aspartate amino transferase, and in some cases, death 8 to 12 h post-injection. Synthetic antioxidants are able to prevent the Fe-induced myopathy and bruising observed in vitamin E-deficient pigs. The prevention of this symptom by vitamin E is attributable to its antioxidant function in cell membranes (Tollerz, 1973). Loudenslager et al. (1986) conducted an experiment to assess the biological antioxidant status of neonatal pigs and their subsequent tolerance to parenteral Fe. The biological antioxidant status of the newborn unsuckled piglets, measured by serum α-tocopherol concentration and glutathione peroxidase (GSH Px) activity, was low and unaffected by the maternal diet during gestation. Symptoms of Fe toxicity were not observed, but injection of Fe altered the antioxidant status of the neonates by decreasing plasma tocopherol, increasing plasma Se concentration, and increasing plasma GSH Px activity.

**Vitamin E and immunity**

There is little placental transfer of immunoglobulins to the fetus; consequently newborn pigs are essentially devoid of circulating antibodies. Antibodies in colostrum are derived from the serum of the dam and provide systemic immunity to the newborn pig. Immunoglobulin G is the main component of the colostrum immunoglobulin fraction. Milk provides local immunity to the intestinal tract of the pig during the lactation period. Antibodies present in milk are produced in the mammary tissue, with immunoglobulin A as the main component (Bourne and Curtis, 1973; Bourne, 1976). Therefore, piglets are dependent on the sow for both systemic and intestinal tract
immunity during the first few weeks of life.

Immune system function and disease resistance are impaired in vitamin E deficiency. Moreover, vitamin E supplementation may improve disease resistance and immune system function (Nockels, 1983; Tengerdy, 1990). Vitamin E supplementation has been shown to enhance tumor immunity, reduce and (or) delay the growth of tumors, improve protection against infections, increase vaccination responses, abolish radiation-induced immunosuppression, and improve wound healing (Bendich, 1990).

Cell-mediated immunity was impaired in weanling pigs inoculated with Salmonella typhimurium and fed a vitamin E-Se-deficient diet (Lessard et al., 1991). Hayek et al. (1989) reported that sows fed the NRC (1988) requirement and injected with 5 mg of Se and (or) 1,000 IU of vitamin E as a single injection at d 100 of gestation transferred more antibodies to their offspring. An earlier study by Teige et al. (1978) showed that the combination of vitamin E and Se increased resistance of pigs to swine dysentery. Further studies demonstrated that most of the beneficial effect was due to supplemental Se rather than vitamin E (Teige et al., 1978). In another study with weanling pigs, Peplowski et al., (1981) suggested an additive effect of Se and vitamin E, provided either via injection or in the diet, in increasing the humoral response against sheep red blood cells. Spallholz (1990) reviewed the role of Se in immunity.

Dove and Ewan (1991b), in a 2 x 2 factorial arrangement of two levels of Cu (5 or 250 ppm) and two levels of α-tocopheryl acetate (0 or 22 IU/kg) added to the diet of growing pigs, reported no effect of vitamin E on antibody production against sheep red blood cells.

Diets unsupplemented with Se during gestation and up to d 4 of lactation in sows impaired neutrophil function when compared with diets supplemented with .3 ppm. Moreover, diets unsupplemented with vitamin E during the same time-period depressed the number of lymphocytes
and the phagocytic activity of blood, colostrum and milk when compared with dietary supplementation of 60 IU of vitamin E per kg (Wuryastuti et al., 1993). Nemec et al. (1994) studied the effect of dietary vitamin E supplementation above NRC recommendations and fat source (tallow or fish oil) of gestation and lactation diets on humoral and cellular immunity of gilts and their offspring. They reported that only the higher levels of dl-α-tocopheryl acetate supplementation (110 and 220 IU/kg) improved cellular immunity in newborn pigs.

Babinszky et al. (1991) concluded that pigs from sows fed 136 mg of α-tocopherol/kg of diet increased their immune response after weaning when they were challenged with ovalbumin and tetanus toxoid, but that the fat source (5% sunflower oil or animal fat) had no consistent effect on immunity. All the weanling pigs received a diet containing 20 mg of α-tocopherol per kg of diet.

The cost associated with dietary supplementation of vitamin E for effective disease protection is excessive in many situations. Alternate uses of vitamin E for this purpose are to feed the animal with a high dose of vitamin E for 3 to 4 wk prior to vaccination or the use of vitamin E in adjuvant vaccines for immunomodulation and increased disease protection, especially under stress conditions (Tengerdy, 1990).

**Vitamin E and reproductive performance**

There are many factors that can affect vitamin E status in swine. First, factors that can lower the vitamin E content of cereal grains include processing, storage and disease. Second, factors exist that can lower the vitamin E content of the diet such as processing, addition of unsaturated fats, and (or) addition of excess trace minerals to the diets. Finally, there are some other factors that can increase the vitamin E requirements of the animal such as confinement,
genetics, early weaning, and intensified reproductive programs in the farm (Adams and Zimmerman, 1982; Mahan, 1991a).

Litter size was not reduced in primiparous sows fed cereal grain-based diets unsupplemented with Se or vitamin E (Mahan et al., 1974; Wilkinson et al., 1977; Young et al., 1977; Nielsen et al., 1979; Piatkowski et al., 1979; Chavez and Patton, 1986; Loudenslager et al., 1986), because of the high body reserves in gilts. But after the second parity litter size was smaller (Mahan et al., 1974; Chavez and Patton, 1986). This reduction in litter size has been attributed by Adamstone et al. (1949) to embryonic death rather than to impaired ovulation rate or implantation failure. Adamstone et al. (1949) reported muscular incoordination and necrosis of muscle fibers in pigs born from deficient sows. On the other hand, Piatkowski et al. (1979) did not report fetal atrophy in primiparous sows fed a vitamin E and Se-deficient diet.

Myer (1992) reported no effect of a single injection of vitamin E (500 to 600 IU) and Se (10 to 12 mg) at weaning time, or about 4 d before rebreeding on the farrowing rate, number of pigs born alive, stillbirths, birth weight, or number of pigs at d 21 of lactation. No significant effect of vitamin E supplementation in the diet on reproductive performance was reported by Babinszky et al. (1992b). In both cases, the levels of vitamin E in the control diet were considered as normal.

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CHAPTER 3. KINETICS OF ALCOHOL AND ACETATE FORMS OF VITAMIN E AFT ER i.m. AND i.v. ADMINISTRATION IN GILTS

A paper to be submitted to the Journal of Animal Science

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Abstract

The objective of this study was to investigate the plasma pharmacokinetics of two sources of d-α-tocopherol (alcohol and ester) and two routes of administration (i.v. and i.m.) in gilts. After a 4-d adaptation period, eight crossbred (YxLxHxD) gilts (100 ± 4 kg) were surgically cannulated for chronic blood collection. After a 9-d recovery-period, gilts (105 ± 3 kg) were used in two 4x4 Latin squares. Treatments were 20 IU/kg of BW of d-α-tocopherol or d-α-tocopheryl acetate in an emulsion by i.m. injection (IMOL and IMAC), or i.v. administration through the cannula (IVOL and IVAC). Pigs were fed 2.5 kg of corn-soy bean meal diet fortified with vitamins (except vitamin E) and minerals to meet their requirements. Blood samples were collected at 0, 40, and 80 minutes; then at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, 36, 42, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, and 168 h after administration. Changes in plasma concentration over time profile was fitted to a sum of two exponentials ($R^2 = .996, .975, .980,$ and .879 for IVOL, IMOL, IVAC, and IMAC, respectively). IMAC failed to maintain plasma α-tocopherol concentration for at least 7 d after administration. The absorption from the site of injection was calculated to be 43 (IMOL) and 40% (IMAC). Although the rate of elimination was
similar (.026 vs 0.029 h⁻¹), the rate of absorption was greater for IMOL than IMAC (2.168 vs 1.757 h⁻¹). The AUC_{IVOL}/AUC_{IVAC} and AUC_{IMOL}/AUC_{IMAC} ratios were 1.26 and 1.33, respectively. Under our experimental conditions, i.v. administration of vitamin E resulted in adverse systemic reactions, whereas the i.m. route did not. Therefore, the i.m. route is the therapeutic tool of choice to rapidly increase plasma α-tocopherol concentration of deficient animals.

**Key words:** Pigs, Vitamin E, Tocopherol, i.m, i.v., Kinetics

**Introduction**

Vitamin E is a fat soluble vitamin that is routinely supplemented in swine diets. There is a tendency by the industry to increase vitamin E supplementation above the NRC recommendations (Cousins et al., 1995). The reasons for the increase in supplementation are: partial destruction of tocopherol in dietary ingredients; changes in the production system, going from extensive (outdoor-pasture) to intensive (confinement) to more sophisticated schemes of production (segregated early weaning-medicated early weaning); and improvement in the growth and reproductive potential of the new genetic lines. Supplementation with vitamin E is not restricted to dietary addition. Parenteral supplementation at specific points of the life cycle are recommended by some practitioners under conditions that may decrease feed intake (weaning) and vitamin E content of the diet ingredients (storage, processing), or when poor tocopherol status is present.

There is a variety of parenteral vitamin E products commercially available. Some products only provide tocopherol, whereas others are combination of tocopherol with selenium or
vitamin A. Most products are a liquid emulsion, while others use vegetable oils as a carrier.

Although intestinal absorption, distribution, and elimination of vitamin E have been studied (Bjorneboe et al, 1990); little information regarding kinetics of tocopherol compounds, dosage regimens, and product formulation of parenteral vitamin E preparations is available for monogastric animals.

Therefore, the objective of this experiment was to determine the effect of two sources of d-α-tocopherol, acetate ester and alcohol, and two routes of administration, i.m. and i.v. in gilts on serum tocopherol concentration.

Materials and Methods

Eight crossbred (YxLxHxD) gilts (100 ± 4 kg) were individually penned in a mechanically ventilated room. Room temperature was maintained at 20 ± 4 °C. Animals were individually fed 2.5 kg of a diet based on corn and solvent extracted soybean meal once a day (Table 1). The diet was formulated to be adequate in all nutrients and energy (NRC, 1988), with the exception of vitamin E. Gilts were allowed to consume water ad libitum.

After a 4-d adaptation period, surgical cannulation of the vena cava for chronic blood collection was performed. Catheters (Tygon micro-bore tubing, .03 internal diameter x .09 outer diameter, medical grade) were heparinized and gas-sterilized prior to the surgical procedure. Animals were anesthetized with ketamine: xylazine (2:2 mg/kg of BW) given i.v. via marginal ear vein. The procedure has been described by Ford and Maure (1978). A nine d recovery-period was allowed. The catheters were filled with heparinized sterile saline (100 IU heparin/mL) containing 1.5% benzyl alcohol between sampling periods.
Treatments were an i.m. injection in the neck (.9 mm x 25 mm needle) or i.v. administration through the cannula of 20 IU of d-α-tocopherol or d-α-tocopheryl acetate per kg of BW. Ten minutes per animal were allowed for i.v. infusion of the calculated dose. The canula was flushed with sterile saline (5 mL) after the i.v. administration to insure complete infusion. Thus, the treatments were: i.m. administration of d-α-tocopherol (IMOL), i.v. administration of d-α-tocopherol (IVOL), i.m. administration of d-α-tocopheryl acetate (IMAC), and i.v. administration of d-α-tocopheryl acetate (IVAC).

After the adaptation (4d) and recovery-periods (9d), gilts (105 ± 3 kg) were used in two 4x4 Latin squares. Treatments were assigned to four 7-d periods and to four gilts within square with the constraint that gilts were administered each treatment in only one period. Therefore, each gilt received all four treatments, and each animal within square received a different treatment in the same period.

Both injectable vitamin E sources, d-α-tocopherol and d-α-tocopheryl acetate, were liquid emulsion products prepared by the procedure outlined in Table 2. The preparation contained 300 IU of vitamin E per mL. The same product was used for i.v. and i.m. administration.

Gilts were weighed each period, before administration of treatments. Then, exact amount (D) of vitamin E was calculated for each animal. Feed efficiency was also determined for the 41 d that the animals were involved in the experiment.

Blood samples were collected at 0, 40, and 80 minutes; then at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, 36, 42, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, and 168 h after administration of vitamin E. The exact time after administration of the treatments, when each sample was collected was recorded. Plasma samples were frozen and stored at -20°C. The protocol was approved by the Committee on Animal Care and Use at Iowa State University.
Vitamin E was isolated from plasma (2 mL) by deproteinizing with redistilled absolute ethanol containing 1% ascorbic acid (3 mL) and extracting with hexane (1 mL). Tocopherol isomers were separated by HPLC. The hexane extract was injected directly and separation achieved with silica gel column 25 cm x 4.6 mm CHROMAGESPHERE SI-60 with a particle diameter of 5 μm and a pore diameter of 60 Å (ES Industries; Berlin, NJ 08009) with a mobile phase of 3.5% tetrahydrofuran (vol/vol) in HPLC-grade hexane with a flow rate of 2.0 mL/min. Tocopherol was detected fluorometrically with an excitation wavelength of 294 nm and an emission wavelength of 323 nm. Peak areas were determined by integration and compared with standards for quantification.

Plasma concentrations in mg/L were converted to IU/L to make both sources of vitamin E comparable: mg of d-α-tocopherol/L x 1.49 IU/mg, plus mg of d-α-tocopheryl acetate/L x 1.36 IU/mg.

Data were fitted to a multiexponential curve of the form:

\[ C_t = \sum K_i e^{-P_i t} \]

Where \( C_t \) is the serum concentration at time \( t \) in IU/L, \( K_i \) is the intercept in IU/L, \( P_i \) is the rate in h\(^{-1}\), and \( t \) is the time in h. The initial values for \( K_i \) and \( P_i \) were determined by the stripping technique (Wagner, 1975). These estimates were refined by nonlinear least squares regression using the SAAM/CONSAM curve-fitting program (Boston et al., 1981). The program determined the value for the parameters that minimized the sum of squares of the residuals (SSR), defined as the deviation of the predicted concentration from the observed concentration at a specific time:

\[ \text{SSR} = \sum W[A - B]^2 \]

Where \( A \) is the observed value at time \( t \), \( B \) is the predicted value at time \( t \), and \( W \) is a
term to weight the data for experimental error.

The area under the curve (AUC) was calculated by the trapezoidal rule (Gibaldi, 1984).

In compartmental modeling, compartments are used to represent components of the metabolic system. The development of the model was based on the exponential functions and an understanding of the metabolic system to be modeled. When using the slopes and intercepts of the exponential equation to calculate kinetic parameters, the compartmental analysis is called empirical modeling (Green and Green, 1990).

For the empirical modeling, a two compartment open model after a single bolus i.v. injection (Figure 1), where elimination only takes place from the central compartment was used Wagner (1975):

\[
\begin{align*}
k_{1,2} &= [(K_1 \times P_2) + (K_2 \times P_1)] / (K_1 + K_2) \\
k_e &= P_1 \times P_2 / k_{1,2} \\
k_{2,1} &= P_1 + P_2 - k_{1,2} - k_e
\end{align*}
\]

One compartment open model with first order absorption and elimination was used for a single i.m. injection of \(\alpha\)-tocopherol (Figure 1):

\[
\begin{align*}
ka &= P_1 \\
ke &= P_2
\end{align*}
\]

Where: \(ka\) is the absorption rate, \(h^{-1}\); \(ke\) is the elimination rate, \(h^{-1}\); and \(k_{ij}\) is the rate of transfer from compartment \(i\) to \(j\), \(h^{-1}\). The volume of distribution for the central compartment (\(V\)) was also calculated after i.v. administration of \(\alpha\)-tocopherol:

\[
V = D/(K_1 + K_2)
\]

Observed pharmacokinetic parameters (initial concentration, maximum concentration, final concentration, time to maximum concentration, and AUC) were analyzed as latin square
designs, with gilts as columns and time periods as rows using the GLM procedure of SAS (1988). The evaluation of the best fit was done by the $R^2$ for the regression of the observed vs predicted values.

Results

At the end of the experiment, animals weighed 127 ± 7 kg, and had a G:F ratio of .26 for the total 41 d-period. The initial plasma $\alpha$-tocopherol concentration prior to dosing, during the first experimental period, was 1.55 ± .31 IU/L. Both sources of i.m. vitamin E (ester and alcohol) resulted in local reaction, characterized by transitory redness and induration at the site of injection, that generally disappeared by three days after administration. During i.v. infusion of both vitamin E sources, a systemic reaction was observed and was characterized by fainting, muscular spasms, and skin erythema. Shortly after infusion those symptoms disappeared, and the animals stood up and ate normally. The severity of the systemic reaction was greater after i.v. administration of d-$\alpha$-tocopherol than of d-$\alpha$-tocopheryl acetate. Once the experiment was completed, the vehicle without vitamin E, was infused i.v. in the gilts without any systemic reaction.

Plasma $\alpha$-tocopheryl acetate was under detectable limit (< .01 mg/L) in all animals at the beginning and end of each period. Only gilts injected with d-$\alpha$-tocopheryl acetate had $\alpha$-tocopheryl acetate in plasma, and their concentration remained detectable for 5 h after i.v. and for 24 h after i.m. administration.

There was no effect of square ($P > .05$) for the observed pharmacokinetic values (Table 3). Observed pharmacokinetic values represents outcome from the experiment previous
mathematical approach. IVAC-treated pigs had lower initial observed concentration (Co) than pigs in the other treatment groups. IVOL-treated pigs had greater (P < .01) observed plasma maximum concentration (Cmax) than the rest of the animals. IMAC-injected gilts had lower (P < .01) final observed concentration (Cf) than that of animals in the other treatment groups. IVOL and IVAC-treated pigs reached Cmax earlier (P < .05) than IMAC-treated pigs. The time to maximum observed concentration (Tmax) was similar among IMOL and IMAC-treated gilts. Pigs injected with IVOL or IVAC had larger (P < .01) AUC than pigs that received IMOL or IMAC. IVOL-treated pigs had larger (P < .05) AUC than IVAC-treated pigs.

Changes in plasma α-tocopherol concentration with time were fitted to a sum of two exponentials. Figures 2 to 5 represent observed and predicted concentration of the plasma α-tocopherol with time after administration of IVOL, IMOL, IVAC, and IMAC, respectively. Those figures also show the equation; rates, slopes and R² for the best fit; and predicted half life, Co, Cmax, Cf, and Tmax.

In the case of i.v. administration (IVOL, Figure 2; and IVAC, Figure 4), the exponential components of the equation (K₁ e⁻P₁ t + K₂ e⁻P₂ t) represent distribution and elimination. The second exponential term (K₂ e⁻P₂ t) represents the terminal decay phase, which was evident when K₁ e⁻P₁ t become smaller than K₂ e⁻P₂ t, at about 2 to 3 h after dose.

In the case of i.m. administration (IMOL, Figure 3; and IMAC, Figure 5), the first exponential component of the equation (K₁ e⁻P₁ t) represents the first order absorption. The peak plasma concentration represents the time at which absorption becomes equal to distribution and elimination. As the amount of vitamin E remaining at the injection site decreases, the rate of absorption also decreases, until K₁ e⁻P₁ t approximate zero. Thereafter, the plasma concentration is described only by the distribution and elimination portion of the equation (K₁ e⁻P₂ t).
Results from the empirical compartmental model are presented in Table 4, whereas the scheme of the compartments is depicted in Figure 1. IMOL had a numerically greater absorption rate ($k_0$) and smaller distribution and elimination rate ($k_e$) than IMAC. Whereas IVOL had similar rate of transfer from compartment 2 to 1 ($k_{1,2}$), but greater rates of transfer from compartment 1 to 2 ($k_{2,1}$) and disappearance from the central compartment ($k_e$) than IVAC.

Discussion

Plasma $\alpha$-tocopherol concentration increased following i.v. or i.m. administration of $d$-\-tocopherol or $d$-$\alpha$-tocopheryl acetate in gilts. This is in agreement with reports using i.m. supplementation of liquid emulsion vitamin E products in human (Rindi and Perri, 1958), pig (Hoppe, 1991; Carrion et al., 1995), and sheep (Hidiroglou and Karpinski, 1991; Njeru et al., 1992); and with studies using i.v. injection in human (Schwalbe et al., 1992), dog (Fujii, 1980), rabbit (Knight and Roberts, 1986), and rat (Gallo-Torres and Miller, 1971).

Our results showed that IMAC was the only treatment where $C_f$ was lower than $C_o$. Therefore, IMAC was not able to maintain $\alpha$-tocopherol concentration when supplemented at 20 IU/kg of BW. As a consequence, $C_o$ for IVAC was significantly lower than in the other treatments, since randomization dictated for 6 out of 8 animals to receive IVAC after IMAC.

Hoppe (1991) reported that, in swine after i.m. administration of equimolar doses of $d$-$\alpha$- or $d$-$\gamma$-tocopherol in water based emulsion, the $T_{max}$ was 4 and 8 h for the $\alpha$- and $\gamma$-tocopherol, respectively. They also observed that based on the $AUC_{0-48,8}$, $\alpha$-tocopherol was 2.1 times more bioavailable than $\gamma$-tocopherol; and that i.m. administration was 4-5 times more bioavailable than oral.

Hidiroglou and Karpinski (1991) noted that absorption ($0.174 \text{ h}^{-1}$) and elimination ($0.02 \text{ h}^{-1}$)
rates of dl-α-tocopheryl acetate in liquid emulsion, after i.m. administration in sheep, are independent of dose, but that the extent of that absorption is parallel to dose. In our case, IMAC had a faster rate of absorption, and similar elimination rate than those reported in the former study. Njeru et al. (1992) reported that after a single i.m. injection of dl-α-tocopherol in an emulsifiable solution, sheep had elimination rate independent of dose, whereas the absorption rate increased with dose. They fitted their data to a three-phase linear model.

IVAC had longer half life for the P₁ and P₂ phases of the concentration vs time profile than IVOL, whereas IVOL had greater Cmax than IVAC, resulting in similar Cf among them.

In man (Rindi and Perri, 1958), dog (Bauernfeind et al., 1974; Fujii, 1980) and pig (Carrion et al., 1995) the peak of tocopherol in serum was always later than the peak of the acetate form after i.m. administration of tocopheryl acetate. Therefore, once the ester leaves the place of injection and reaches blood, hydrolysis of tocopheryl ester is the limiting step in bioavailability.

Gallo-Torres and Miller (1971) reported rapid hydrolysis of dl-α-tocopheryl acetate by the rat liver. Those authors found that, 30 min after i.v. administration, 79% of the chromatographed radioactivity in the liver was due to quinone derivatives, 15% to the ester form, and only small quantities due to the alcohol form. They also reported the same biotransformations in several other tissues and that the blood contains enzymes that hydrolyze esters of tocopherol.

Ogihara et al. (1985) also reported that, 24 h after i.v. administration of 10 mg of dl-α-tocopheryl acetate/kg of BW in rats, the ester reached undetectable concentration in plasma, whereas 48 h were needed to reach those undetectable concentrations in red blood cells. Under our conditions after IVAC administration, d-α-tocopheryl acetate was undetectable in plasma after
Based on the $\text{AUC}_{0.319\ h}$ $[\mu g/mL\ h]$, Hidiroglou and Karpinski (1988) reported that i.v. administration of $d$-$\alpha$-tocopheryl acetate (1120) was much better utilized by sheep than $dl$-$\alpha$-tocopherol (676) or $dl$-$\alpha$-tocopheryl acetate (772). In this experiment, even though they injected the ester form, only the kinetics of the free $\alpha$-tocopherol was reported with an elimination rate ($ke$) of .028 and .045 h$^{-1}$ for the $dl$-$\alpha$-tocopheryl acetate and the $d$-$\alpha$-tocopherol, respectively.

When they i.v. injected $dl$-$\alpha$-tocopherol, the profile was represented as a sum of three exponentials ($C_t = \text{Base} + K_1 e^{\cdot P1\ t} + K_2 e^{\cdot P2\ t} + K_3 e^{\cdot P3\ t}$), where the intercepts were: $K_1 = 46$, $K_2 = 16$, and $K_3 = 3 \mu g/mL\ h$; and the slopes were: $P1 = 50$, $P2 = .6$, and $P3 = .006\ h^{-1}$.

The lower calculated $C_{\text{max}}$ after IVAC (35.78 IU/L) compared to IVOL (124.84 IU/L) may be explained by the larger volume of distribution for $d$-$\alpha$-tocopheryl acetate (64.28 L) compared to $d$-$\alpha$-tocopherol (18.76 L). In both cases the volume of distribution was larger than plasma volume for the size of animals used. Therefore, the central compartment ($Q_1$, Figure 1) after i.v. administration includes non-plasma sources of vitamin E with which plasma $\alpha$-tocopherol can exchange rapidly. The larger $V$ observed for $d$-$\alpha$-tocopheryl acetate may also explain the milder systemic reaction observed after IVAC compared to IVOL.

Hidiroglou et al. (1990) studied the kinetics of an i.v. dose of 2 $\mu$Ci (.036 $\mu$g) of $d$-$\alpha$-$[^3H]$ tocopherol in emulsion per kg of BW in sheep. The plasma data were expressed up to 56 h as a sum of two exponentials ($C_t = K_1 e^{\cdot P1\ t} + K_2 e^{\cdot P2\ t}$), where the intercepts were of the magnitude: $K_1 = 11,000$, and $K_2 = 5,700\ \text{dpm/} \mu \text{g/mL}$; and the slopes were: $P1 = .7$, $P2 = .02\ h^{-1}$. Based on these estimates, they were also able to fit the data to a two compartmental model.

Karpinski and Hidiroglou (1990) studied the kinetics of i.v. administration of 4 $\mu$Ci (.072 $\mu$g) of $d$-$\alpha$-$[^3H]$ tocopherol in emulsion per kg of BW in sheep. The plasma data were expressed
up to 432 h as a sum of three exponentials \( Ct = K_1 e^{-P_1 t} + K_2 e^{-P_2 t} + K_3 e^{-P_3 t} \), where the intercepts were: \( K_1 = 105,000 \), \( K_2 = 28,000 \), and \( K_3 = 4,000 \) dpm/\( \mu \)g; and the slopes were of the magnitude: \( P_1 = 1.56 \), \( P_2 = .062 \), and \( P_3 = .006 \) h\(^{-1}\).

Hidiroglou and Karpinski (1987) studied the kinetics of 200 \( \mu \)Ci of d-\( \alpha \)-\[^3\text{H}\]tocopherol (.077 \( \mu \)g/kg of BW) in 2 mL ethanol of a physiological i.v. dose in sheep. They fitted the plasma profile to a sum of three exponentials \( Ct = K_1 e^{-P_1 t} + K_2 e^{-P_2 t} + K_3 e^{-P_3 t} \), where the intercepts were of the magnitude: \( K_1 = 150,000 \), \( K_2 = 35,000 \), and \( K_3 = 13,000 \) dpm/mL; and the slopes were: \( P_1 = .30 \), \( P_2 = .48 \), and \( P_3 = .014 \) h\(^{-1}\). Based on these estimates, they were also able to fit the data to a three compartmental model. In the case of i.m. administration, a more complicated profile had to be divided in a rapid absorption phase \([Ct = K_1 (1 - e^{-P_1 t})]\) and a slow absorption + elimination phase \([Ct = K_2 e^{-P_2 t} - K_3 e^{-P_3 t}]\), where the intercepts were: \( K_1 = 1,700 \), \( K_2 = 4,700 \), and \( K_3 = 3,700 \) dpm/mL; and the slopes were of the magnitude: \( P_1 = 1.73 \), \( P_2 = .0042 \), and \( P_3 = .13 \) h\(^{-1}\).

All these studies using trace administration of vitamin E marked with radioactive isotopes resulted in slopes of the same magnitude as the ones reported herein using large dose of unlabeled vitamin E. Our conditions resulted in a plasma profile that was described by a sum of two exponentials. Consequently, two compartment open model after a single bolus i.v. injection or a one compartment open model with first order absorption and elimination after a single i.m. dose of vitamin E were developed.

Intravenous, intra-arterial, intraspinal, and intracerebral route of administration allows total entry of any compound into the general circulation (Wagner, 1961). This author also reported that the other parenteral routes only allow incomplete and variable degree of incorporation into the blood stream. Absorption rates may be affected by different factors such as
specificities in formulation of the parenteral product and injection site. For those reasons, the rate of appearance into blood may vary to a larger extent than the rate of disappearance from the general circulation, where conditions are more homogeneous, although individual differences in metabolic states always exist.

Based on the $\frac{\text{AUC}_{\text{i.m.}}}{\text{AUC}_{\text{i.v.}}}$ ratio, 43 and 40% of the original dose was absorbed from the place of injection after administration of d-α-tocopherol and d-α-tocopheryl acetate, respectively. Hidiroglou and Karpinski (1987), using trace of radioactive isotopes of d-α-tocopherol, reported 51% availability of tocopherol following i.m. administration relative to the i.v. in sheep.

From our experiment, we can also estimate the relative availability of the free:acetate ester sources of d-α-tocopherol. The $\frac{\text{AUC}_{\text{IVOL}}}{\text{AUC}_{\text{IVAC}}}$ and $\frac{\text{AUC}_{\text{IMOL}}}{\text{AUC}_{\text{IMAC}}}$ ratios were 1.26 and 1.33, respectively. The slight difference observed among the i.v. and the i.m. ratios may be attributed to the difference in absorption rate observed between the acetate ester and the free α-tocopherol. This ratio is slightly higher than what we expected based on molecular weight (472 g d-α-tocopheryl acetate / 430 g d-α-tocopherol = 1.10) or IU (1.49 IU/mg d-α-tocopherol/1.36 IU/mg d-α-tocopheryl acetate = 1.10).

**Implications**

Under our experimental conditions, i.v. administration of vitamin E resulted in adverse systemic reactions, whereas the i.m. route did not. Therefore, the i.m. route is the therapeutic tool of choice to rapidly increase α-tocopherol status of deficient animals. Single i.m. dose of 20 IU of d-α-tocopherol per kg of BW improves vitamin E status of pig for at least 7 d after
administration when no dietary vitamin E supplementation is provided.

**Literature Cited**


Table 1 Composition of the diet (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredients:</strong></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>81.73</td>
</tr>
<tr>
<td>Soybean meal, solvent</td>
<td>15.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.50</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>.94</td>
</tr>
<tr>
<td>Vitamin premix*</td>
<td>.20</td>
</tr>
<tr>
<td>Trace mineral premix*</td>
<td>.05</td>
</tr>
<tr>
<td>Salt</td>
<td>.50</td>
</tr>
<tr>
<td><strong>Calculated analysis:</strong></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>13.57</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>3,302</td>
</tr>
<tr>
<td>Ca</td>
<td>.75</td>
</tr>
<tr>
<td>P</td>
<td>.60</td>
</tr>
<tr>
<td>Lysine</td>
<td>.64</td>
</tr>
</tbody>
</table>

*Contributed the following per kilogram of diet: vit. A, 4,400 IU; vit. D₃, 1,100 IU; vit. B₁₂, 0.022 mg; riboflavin, 6.6 mg; niacin, 33.1 mg; d-pantothenic acid, 18 mg.

*Contributed the following per kilogram of diet: Zn, 75 mg; Fe, 87.5 mg; Mn, 30 mg; Cu, 8.8 mg; and I, 1 mg.
Table 2. Formulation of the parenteral vitamin E products\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Item</th>
<th>Product</th>
<th>d-(\alpha)-tocopherol</th>
<th>d-(\alpha)-tocopheryl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-(\alpha)-tocopherol, mg</td>
<td></td>
<td>40,268</td>
<td>-</td>
</tr>
<tr>
<td>d-(\alpha)-tocopheryl acetate, mg</td>
<td></td>
<td></td>
<td>44,118</td>
</tr>
<tr>
<td>Polyoxyethylated fatty acid derivative\textsuperscript{c}, mg</td>
<td>30,000</td>
<td>30,000</td>
<td></td>
</tr>
<tr>
<td>Benzyl alcohol, mL</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sterile saline, mL</td>
<td>up to volume</td>
<td>up to volume</td>
<td></td>
</tr>
<tr>
<td>Total volume, mL</td>
<td>200</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Vitamin E was added to the polyoxyethylated fatty acid derivative and the mixture was shaken and sonicated for 15 minutes. Benzyl alcohol was added and diluted to volume with sterile saline. The mixture was shaken and sonicated until total emulsified.

\textsuperscript{b}300 IU per mL.

\textsuperscript{c}Tween 80 (Fisher Sci. Co.).
Table 3. Observed plasma vitamin E pharmacokinetic values in gilts after administration of 20 IU of vitamin E/kg BW

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IVOL</th>
<th>IMOL</th>
<th>IVAC</th>
<th>IMAC</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co, IU/L</td>
<td>2.27c</td>
<td>2.60c</td>
<td>1.79d</td>
<td>2.50e</td>
<td>.12</td>
</tr>
<tr>
<td>Cmax, IU/L</td>
<td>64d</td>
<td>9c</td>
<td>25c</td>
<td>7c</td>
<td>7</td>
</tr>
<tr>
<td>Cf, IU/L</td>
<td>3.02eg</td>
<td>2.64f</td>
<td>2.86fg</td>
<td>2.09e</td>
<td>.10</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>.85e</td>
<td>6.87ef</td>
<td>.65e</td>
<td>10.44f</td>
<td>2.62</td>
</tr>
<tr>
<td>AUC, (IU/L) x h</td>
<td>446e</td>
<td>190f</td>
<td>353g</td>
<td>143f</td>
<td>25</td>
</tr>
</tbody>
</table>

*IMOL = i.m. d-α-tocopherol, IVOL = i.v. d-α-tocopherol, IMAC = i.m. d-α-tocopheryl acetate, and IVAC = i.v. d-α-tocopheryl acetate.

bCo = initial concentration, Cmax = maximum concentration, Cf = final concentration, Tmax = time to maximum concentration, and AUC = area under the plasma concentration vs time curve, calculated by the trapezoidal rule and corrected for Co.

cdMeans in a row with different superscripts differ (P < .01).

efgMeans in a row with different superscripts differ (P < .05).
Table 4. Parameters for the empirical compartmental model of a single parenteral injection of 20 IU of vitamin E as d-α-tocopherol or tocopheryl acetate

<table>
<thead>
<tr>
<th>Item</th>
<th>IVOL</th>
<th>IMOL</th>
<th>IVAC</th>
<th>IMAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$</td>
<td>-</td>
<td>2.168</td>
<td>-</td>
<td>1.757</td>
</tr>
<tr>
<td>$k_{1,2}$</td>
<td>.092</td>
<td>-</td>
<td>.091</td>
<td>-</td>
</tr>
<tr>
<td>$k_{2,1}$</td>
<td>1.169</td>
<td>-</td>
<td>.580</td>
<td>-</td>
</tr>
<tr>
<td>$k_e$</td>
<td>.234</td>
<td>.026</td>
<td>.084</td>
<td>.029</td>
</tr>
<tr>
<td>$V$</td>
<td>18.76</td>
<td>-</td>
<td>64.28</td>
<td>-</td>
</tr>
</tbody>
</table>

*aCalculated using slopes and intercepts of the exponential equations. $R^2$ were .996, .975, .980, and .876 for IVOL, IMOL, IVAC, and IMAC, respectively.

*bIVOL = i.v. d-α-tocopherol, IMOL = i.m. d-α-tocopherol, IVAC = i.v. d-α-tocopheryl acetate, and IMAC = i.m. d-α-tocopheryl acetate.

$c$k_a = rate of absorption, h^{-1}; k_{1,2} = rate of transfer from compartment 2 to compartment 1, h^{-1}; k_{2,1} = rate of transfer from compartment 1 to compartment 2, h^{-1}; k_e = rate of elimination, h^{-1}; and $V$ = volume of distribution, L.
Figure 1. a) two compartment open model with single bolus i.v. injection, where elimination occurs from compartment number one, b) one compartment open model with first order absorption and elimination after a single i.m. dose. $k_a =$ absorption rate, $h^{-1}$; $k_e =$ elimination rate, $h^{-1}$; $D =$ dose, IU; $F =$ fraction of dose; $Q =$ size of compartment, IU; $k_{j,i} =$ rate of transfer from compartment $i$ to $j$, $h^{-1}$. 
Figure 2. Concentration of α-tocopherol vs time observed (●) and predicted (-) values after a single i.v. injection of 20 IU of d-α-tocopherol per kg of BW. Tmax = time to maximum concentration, T1/2 = half life, t = time, Co = initial concentration, Cmax = maximum concentration, Cf = concentration at 168 h, Ct = concentration at time t, K1 = intercept, and Pj = rate.
Figure 3. Concentration of α-tocopherol vs time observed (●) and predicted (-) values after a single i.m. injection of 20 IU of d-α-tocopherol per kg of BW. Tmax = time to maximum concentration, T1/2 = half life, t = time, Co = initial concentration, Cmax = maximum concentration, Cf = concentration at 168 h, Ct = concentration at time t, Ki = intercept, and Pi = rate.
Figure 4. Concentration of α-tocopherol vs time observed (●) and predicted (-) values after a single i.v. injection of 20 IU of d-α-tocopheryl acetate per kg of BW. T\text{max} = time to maximum concentration, T_{1/2} = half life, t = time, C_0 = initial concentration, C_{\text{max}} = maximum concentration, C_f = concentration at 168 h, C_t = concentration at time t, K_i = intercept, and P_i = rate.
Figure 5. Concentration of α-tocopherol vs time observed (○) and predicted (−) values after a single i.m. injection of 20 IU of d-α-tocopheryl acetate per kg of BW. T_max = Time to maximum concentration, T_1/2 = half life, t = time, C_0 = initial concentration, C_max = maximum concentration, C_f = concentration at 168 h, C_t = concentration at time t, K_i = intercept, and P_i = rate.
CHAPTER 4. PHARMACOKINETICS OF INTRAMUSCULARLY INJECTABLE SOURCES OF VITAMIN E IN WEANLING PIGS

A paper to be submitted to the *Journal of Animal Science*

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Abstract

An experiment was conducted with weanling pigs to investigate the pharmacokinetics of four different injectable sources of d-α-tocopherol, two alcohols and two acetates, in tissue and serum. Three groups of six littermate crossbred (YxLxHxD) pigs (6.36 ± .40 kg) were used. After a 14-d adaptation period, pigs within littermate groups were randomly assigned to one of six groups. One group was killed and used as base line. The other 5 groups were the treatments: i.m. injection in the right ham of either saline or 138 IU/kg BW of one of two sources of d-α-tocopheryl acetate (A-act and B-act) or d-α-tocopherol (A-ol and B-ol). Both of the d-α-tocopherol and one of the d-α-tocopheryl acetate sources of injectable vitamin E were products prepared as emulsions, whereas, the other d-α-tocopheryl acetate (A-act) was prepared with olive oil as a carrier. Similar serum and tissue tocopherol concentrations were detected among A-ol and B-ol treated pigs. Therefore, the data were pooled for animals injected with d-α-tocopherol (Ol). Pigs injected with A-act did not show a response in the serum tocopherol concentrations. For B-act ($R^2 = .93$ and $.65$ for serum tocopheryl acetate and serum tocopherol respectively) and Ol ($R^2 = .96$ for serum tocopherol), the serum profiles were characterized by exponential
equations. Based on the half life of the curve and the absorption rates of the compartmental approaches, the tocopheryl acetate was absorbed faster from the site of injection than the free form. In animals receiving the acetate sources, serum and tissue vitamin E concentrations were only detected as alcohol 336 h after administration. B-act and Ol had similar tissue tocopherol concentrations, with the exception of spleen. However the injection of the alcohol increased total muscle vitamin E content. These results indicate that: pigs were able to hydrolyze the acetate to alcohol vitamin E; olive oil was not a good carrier for i.m. vitamin E preparations; and when vitamin E was formulated with an adequate carrier, it was readily available.

Key words: Pigs, Vitamin E, Tocopherol, Intramuscular Injection, Kinetics

Introduction

Serum vitamin E declines for approximately two weeks after weaning (Chung, 1993) because of reduced feed intake and poor development of digestive enzymes (Leibbrandt et al., 1975). Therefore, an injectable form of vitamin E may be utilized more efficiently than an equal amount given orally (Caravaggi et al., 1968; Reddy et al., 1985), providing a quick and powerful method of improving vitamin E status in weanling pigs and eliminate the possibility of vitamin E-Se deficiency. Although intestinal tocopherol absorption and distribution has been studied in monogastric animals (Gallo-Torres, 1980), very little information is available for parenteral administration. Most of the studies regarding disposition kinetics of dosage regimens and vitamin E compounds following their administration by parenteral routes have been performed in ruminant animals (Hidiroglou and Karpinski, 1987). Studies of the bioavailability of different sources of vitamin E in swine are essential for prevention and therapy of vitamin E deficiency. This
experiment was undertaken with weanling pigs to investigate the pharmacokinetics of four different injectable sources of vitamin E, two alcohols and two acetates, in tissue and serum.

Materials and Methods

Three groups of six littermate crossbred (YxLxHxD) pigs were weaned at an average age of 25 days and weight of 6.36 ± .40 kg. Pigs were housed in individual pens, and allowed to consume feed and water ad libitum. After weaning, the pigs were fed a basal diet (Table 1) for a two-week adaptation period and a two-week experimental period.

After the adaptation period, pigs within littermate groups were randomly assigned to one of six groups. One randomly selected animal per littermate group was killed on the day of injection, and their tissue tocopherol concentrations used as base line. Treatments consisted of an i.m. injection in the right ham of either saline or one of two sources of d-α-tocopheryl acetate (A-act and B-act) or d-α-tocopherol (A-ol and B-ol). The amount of α-tocopherol that was injected (138 IU/kg of BW) was equivalent to the amount that would have been consumed if a diet supplemented with 50 IU of α-tocopherol/kg of diet were fed during a 4-wk period after weaning.

Both of the d-α-tocopherol and one of the d-α-tocopheryl acetate sources of injectable vitamin E were commercially available liquid emulsion products. The other d-α-tocopheryl acetate (A-act) was prepared with olive oil as a carrier (300 IU/mL) as follows: The d-α-tocopheryl acetate (Sigma Chemical) was weighed (5.51 g) in a 25 mL volumetric flask containing 5 mL of absolute ethanol. Then, the solution was brought up to volume (25 mL) with olive oil. The new solution was transferred to a serum bottle, sealed and stored in a refrigerator until used.
Pigs were weighed at weaning, and at two, three, and four weeks after weaning. Feed intake and efficiency were determined. Blood samples were obtained from the orbital sinus at 0, 2, 4, 8, 16, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 288, and 336 h after injection. At the end of the experimental period, pigs were electrically stunned and killed by exsanguination. Psoas muscle, longissimus muscle, left ham, heart, adrenal gland, kidney, liver, lung, pancreas, and spleen were excised and weighed. Serum and tissues samples were frozen and stored at -20°C until analysis. The protocol was approved by the Committee on Animal Care and Use at Iowa State University.

Tissues were prepared for tocopherol analysis by homogenizing 2 to 3 g of tissue in 10 mL (wt/vol) of phosphate-EDTA-buffer (ph 7.0). Serum and tissue homogenates (2 mL) were deproteinized with redistilled absolute ethanol (3 mL) and tocopherols were extracted with hexane (1 mL). The hexane extract was injected directly and separation achieved with silica gel column 25 cm x 4.6 mm CHROMAGESPHERE SI-60 with a particle diameter of 5 μm and a pore diameter of 60 Å (ES Industries; Berlin, NJ 08009) with a mobile phase of 3.5% tetrahydrofuran (vol/vol) in HPLC-grade hexane with a flow rate of 2.0 mL/min. Tocopherol was detected fluorometrically with an excitation wavelength of 294 nm and an emission wavelength of 323 nm. Peak areas were determined by integration and compared with standards for quantification.

Data were fitted to a multiexponential curve of the form:

\[ C_t = \sum K_i e^{P_i t} \]

Where \( C_t \) is the serum concentration at time "t" in mg/L, \( K_i \) is the intercept in mg/L, \( P_i \) is the rate in h\(^{-1}\), and \( t \) is the time in h. The initial values for \( K_i \) and \( P_i \) were determined by the stripping technique (Wagner, 1975). These estimates were refined by nonlinear least squares regression using the curve-fitting program SAAM/CONSAM (Boston et al., 1981). The program
determines the value for those parameters that minimize the sum of square of the residuals.

The area under the curve (AUC) was calculated by integration of such a function or by the trapezoidal rule (Gibaldi, 1984).

In compartmental modeling, compartments are used to model various components of the metabolic system. The development of the model is based in the exponential functions of the curve and an understanding of the metabolic system to be modeled. When using the slopes and intercepts of the exponential equation to calculate certain kinetic parameters, the compartmental analysis is called empirical modeling. When fitting plasma data plus any other data from the same or other experiment (tissue, feces, urine, ...) to a hypothesized compartmental model and later adjusting the model parameter, the compartmental analysis is called model-based compartmental analysis (Green and Green, 1990).

For the empirical modeling, we used the equations given by Wagner (1975) for one (serum d-α-tocopheryl acetate) and two (serum d-α-tocopherol) compartment open model with first order absorption and elimination after a single dose (Figure 1):

a) Serum d-α-tocopheryl acetate:

\[ k_a = P_1 \]
\[ k_e = P_2 \]

b) Serum d-α-tocopherol:

\[ k_a = P_1 \]
\[ k_{1,2} = \frac{[K_2 \times P_2 \times P_1] + (K_1 \times P_3 \times P_1) + (-K_1 \times P_3 \times P_2)}{[K_2 \times (P_1 - P_3) + K_1 \times (P_1 - P_2)]} \]
\[ K_e = P_3 \times P_2 \div k_{1,2} \]
\[ K_{2,1} = P_3 + P_2 + k_{1,2} - k_e \]

Where \( K_a \) = absorption rate, \( h^{-1} \); \( K_e \) = elimination rate, \( h^{-1} \); and \( K_{j,i} \) = rate of transfer
from compartment i to j, h⁻¹.

Data were analyzed by the GLM procedure of SAS (1988). Growth data were analyzed by analysis of covariance using initial body weight (weight at injection time) as a covariate. For tocopherol concentration in tissues and observed pharmacokinetic values, non-orthogonal contrasts were performed. The evaluation of the best fit was done through the fractional standard deviation (FSD) defined as the standard deviation/mean ratio of the estimated parameters or by the R² for the regression of the observed vs predicted values.

Results

Growth Performance

Growth performance for the two week experimental period was not affected by treatment (P > .1). Average daily gain, ADFI, and gain:feed ratio were .52 ± .07 kg, .81 ± .08 kg, and .65 ± .07, respectively. Pigs weighed 7.6 ± 1.1 kg at 14 d post-weaning (day of injection), and 14.9 ± .8 kg at the end of the experimental period.

Vitamin E in Serum

During the first two weeks after weaning, there were some problems with diarrhoea and low feed intake. One animal in the B-ol treatment had low feed intake during the experimental period and was culled out. Similar serum and tissue tocopherol concentrations were observed among pigs injected with A-ol and B-ol. Therefore, the data were pooled (OI). A local reaction occurred at the injection site of animals injected with the alcohol form of vitamin E.

The initial (Co) and final (Cf) concentrations of d-α-tocopheryl acetate in serum were
below the detectable limit (< .01 mg/L) in all treatments. Animals injected with B-act had
greater serum tocopheryl acetate concentration (Cmax = 301 ± 52 mg/L) at 16 h after injection
and area under the concentration vs time curve [AUC = 10,257 ± 2099 (mg d-α-tocopheryl
acetate/L) x h] than animals in the other treatment groups. Nine per cent of this AUC for
tocopheryl acetate represented the AUC for tocopherol in serum.

Observed pharmacokinetic values (Table 2) represents outcome from the experiment
previous mathematical approach. All the treatment groups had similar Co of serum α-tocopherol.
Pigs injected with d-α-tocopherol had greater (P < .001) serum observed d-α-tocopherol Cmax
and AUC, calculated by the trapezoidal rule, than pigs that received saline or d-α-tocopheryl
acetate. The time to maximum observed d-α-tocopherol concentration in serum (Tmax) occurred
significantly later (P < .001) for the A-act treatment than for the other treatment groups. All
sources of injectable vitamin E (alcohol and acetate) increased (P < .01) the observed Cf of α-
tocopherol at the end of the experimental period when compared with saline. There were no
differences among the Cf of the vitamin E injected pigs. The average concentration of α-
tocopherol detected at this time in serum was 1.44 mg/L for pigs injected with any of the vitamin
E sources, and .56 mg/L for pigs injected with saline.

There was no peak (Cmax) in the serum observed α-tocopherol with time profile for
saline and A-act treatments. Therefore, we only attempted to fit the serum α-tocopherol profile to
a sum of exponentials in those cases where a clear response to injectable vitamin E was observed
(Table 3). Those cases were: a) serum α-tocopheryl acetate concentration after injection of B-act
(Figure 2); b) serum α-tocopherol concentration after injection of B-act (Figure 3); and c) serum
α-tocopherol concentration after injection of Ol (Figure 4).

In the case of serum α-tocopheryl acetate concentration vs time, the profile was best fitted
to a sum of two exponentials, whereas serum α-tocopherol concentration vs time profile was best represented as a sum of three exponentials.

The estimated concentration at time zero for the best fit was always negative and far from the observed value. Therefore, a lag phase between injection time (t = 0) and the time at which first-order absorption began (t = T), was hypothesized. Because the observed Co was subtracted from the concentration at each specific time when modeling, Co was equal to zero. Therefore, T was calculated for the best fit as the time at which Ct was equal to zero, and the final model fit in the form:

For t < T, then

\[ Ct = Co \]

For serum d-α-tocopheryl acetate and t ≥ T, then

\[ Ct = Co - K_1 e^{-P_1(t-T)} + K_1 e^{-P_2(t-T)} \]

If serum d-α-tocopherol and t ≥ T, then

\[ Ct = Co - K_1 e^{-P_1(t-T)} + K_1 e^{-P_2(t-T)} + K_2 e^{-P_3(t-T)} \]

Parameter estimates for B-act and Ol are presented in Table 3. Figures 2 to 4 represent observed and predicted profiles of the serum concentration vs time curve.

The predicted lag phase was shorter in animals injected with Ol than with B-act, reflecting the early appearance of the first-order absorption.

The first exponential component of the equation \( K_1 e^{-P_1(t-T)} \) represents the first order absorption. The peak plasma concentration represents the time at which absorption becomes equal to distribution and elimination. As the amount of vitamin E remaining at the injection site decreases, the rate of absorption also decreases until \( K_1 e^{-P_1(t-T)} \) approximates zero. Thereafter, the plasma concentration is described only by the distribution and elimination portion of the
equation: $K_1 e^{P2 \cdot (t-T)}$ in case of serum $d-\alpha$-tocopheryl acetate, or $K_1 e^{P2 \cdot (t-T)} + K_2 e^{P3 \cdot (t-T)}$ in case of $d-\alpha$-tocopherol. The terminal decay phase was $K_2 e^{P3 \cdot (t-T)}$, which was evident when $K_1 e^{P2 \cdot (t-T)}$ became smaller than $K_2 e^{P3 \cdot (t-T)}$.

Predicted serum pharmacokinetic values calculated from the exponential equation are displayed in Table 4. The absorption rate constant, $P_1$, was always larger than the distribution and elimination rate constants, $P_2$ and $P_3$. The faster vitamin E is absorbed ($P_1 T_{1/2}$), the higher is the serum maximum concentration ($C_{max}$), and the shorter is the time to maximum concentration after administration ($T_{max}$, Table 4).

Based on equimolar equivalence, 91% of the ester corresponds with the free form, and expressing the total observed AUC in (mg of $\alpha$-tocopherol per liter) x hour:

a) Observed values (Table 2).

\[B_{act} = [10,257 \text{ (mg of } d-\alpha\text{-tocopheryl acetate/ L)x h)} \times .91 + 912 \text{ (mg of } d-\alpha\text{-tocopherol/ L)} \times h = 10,246 \text{ (mg } d-\alpha\text{-tocopherol/ L)} \times h.

\[C_{max} = 4,872 \text{ (mg of } d-\alpha\text{-tocopherol/ L)} \times h

b) Predicted values (Table 4).

\[B_{act} = [8,156 \text{ (mg of } d-\alpha\text{-tocopheryl acetate/ L)x h)} \times .91 + 939 \text{ (mg of } d-\alpha\text{-tocopherol/ L)} \times h = 8,361 \text{ (mg } d-\alpha\text{-tocopherol/ L)} \times h.

\[C_{max} = 4,914 \text{ (mg of } d-\alpha\text{-tocopherol/ L)} \times h

In this way, the AUC for the B-act was between 2.1 (observed) and 1.7 (predicted) times greater than $C_{max}$.
Vitamin E in Tissues

At the end of the 14 d experimental period, α-tocopherol concentration of all tissues, except adrenal gland and lung, were similar among Saline and A-act treated pigs (Table 5). Tocopherol concentrations of lung and adrenal gland from pigs injected with A-act were greater (P < .05) than those of pigs injected with saline, but lower (P < .06) than those of pigs injected B-act or Ol. B-act and Ol treated pigs had similar tissue α-tocopherol concentration (P > .05). Pigs receiving B-act or Ol had greater (P < .05) longissimus muscle, heart, adrenal gland, kidney, and pancreas α-tocopherol concentration than pigs receiving no supplemental vitamin E. Ham and psoas muscle α-tocopherol concentration of B-act injected pigs were similar to that of saline and A-act injected pigs, but lower (P < .06) than α-tocopherol concentration of Ol injected pigs. Pigs injected with B-act had extremely high (P < .01) spleen tocopherol concentration (62 mg/kg), compared with the rest of the animals (3 mg/kg).

Total α-tocopherol content of organs (Table 6) followed the same pattern observed for their respective α-tocopherol concentrations (Table 5), with a few exceptions: 1) pigs receiving injectable A-act had similar total α-tocopherol content in adrenal gland and liver as pigs receiving saline and B-act; and 2) pigs receiving injectable A-act had similar total α-tocopherol content in lung than pigs receiving saline, but lower (P < .01) vitamin E concentration than pigs receiving B-act and Ol treatments.

Total α-tocopherol content of the organs were similar (Table 6) at the beginning (basal) and the end of the experimental period when no supplemental vitamin E was administered (saline), but tissue α-tocopherol concentrations were generally lower (Table 5) in the animals injected with saline than the ones used as basal line.

Tocopherol content of injectable origin (Table 7) was similar among B-act and Ol treated
pigs with the exception of that of spleen and muscle. Total and muscle α-tocopherol content of injectable origin were greater (P < .03) for Ol than for B-act treated pigs.

Results from the empirical compartmental model are presented in Table 8, whereas the scheme of the compartments is depicted in Figure 1. Similar absorption rate (kₐ) and rate of transfer from compartment 2 to 1 (k₁₂) were observed for serum α-tocopherol after injection of either B-act or Ol treatment. On the other hand, rate of transfer from compartment 1 to 2 (k₂₁) and elimination rate (kₑ) were smaller for the B-act than for the Ol treated pigs. Faster absorption rate for B-act than Ol was observed (.15 vs .08 h⁻¹).

The rate of disappearance from the central compartment of α-tocopheryl acetate after B-act administration (kₑ = .081 h⁻¹) was similar to that for α-tocopherol after injection of Ol (kₑ + k₂₁ = .06 + .023 = .083 h⁻¹).

Figures 5 and 6 depict the model-based compartments for i.m. d-α-tocopheryl acetate and d-α-tocopherol respectively. Values for the parameters defined in those figures are reported in Tables 9 and 10. As a result, the size of the different pools over time can be predicted (Figures 7 and 8).

Discussion

Serum α-tocopherol concentration of the saline-injected pigs was similar for all periods, and within the normal range for pigs of the same age fed a corn-soybean-whey starter diet meeting the NRC (1988) nutrient recommendations.

In spite of the relatively large amount of d-α-tocopheryl acetate (138 IU/kg of BW) in olive oil (A-act), no detectable changes in the serum vitamin E concentration with time were
observed until the end of the experiment. Similar results were obtained in our laboratory by Chung (1993) when studying the effect of source and method of administration of vitamin E on weanling pigs. He used d-α-tocopheryl acetate in olive oil as one of the injectable sources. In the present experiment, a slight but significant increment of serum α-tocopherol was detected in A-act injected pigs as compared to Saline injected pigs at 336 h, suggesting an initial sequestration phase, followed by a very slow rate of release. Another explanation for this is an interaction between the carrier (olive oil) and the vitamin E that impeded the distribution of the vitamin into the blood at high rate immediately after injection. The use of high viscosity injection vehicle (glycerin, cottonseed oil, sesame oil, or polyethylene glycol) slows the absorption of i.m. administered drugs (Niazi, 1979). The same author also indicated that after i.m. administration, small molecules are reported to be absorbed directly into the capillary system, while large molecules (vitamin E-oleate complex) follow the lymphatic system before reaching the general circulation. At this point, the limiting factor would be the slow flow rate of lymph (.1% of the plasma flow). Hidiroglou and McDowell (1987) also observed a similar delay (Tmax = 254 h) after 40 mg of d-α-tocopherol in sesame oil per kg of BW were i.m. injected in sheep. Njeru et al. (1994) observed no response of serum vitamin E concentration to i.m. injection of dl-α-tocopheryl acetate in sesame oil in wethers. Behrens et al. (1975) reported that in sheep the slow rate of vitamin E release after i.m. administration of vitamin E in oil carrier (peanut or soybean oils) may be due to myositis and lymphadenitis. Similar clinical observations were made by Dickson et al. (1986). The oil carrier by itself was not responsible for the myositis and lymphadenitis. They also reported that lymph glands, where lymph drained from the place of injection, stored important amounts of vitamin E for at least 4 months after i.m. administration. In our experiment, there was no attempt to make histopathological observations, but we were able
to detect local swellings in the place of injection when both sources of vitamin E as alcohol (A- and B-ol) were administered. Topical application of free tocopherol irritated the skin of mice, whereas the acetylated form did not (Trevithick et al., 1992).

In the cases where a clear response to injectable vitamin E administration was observed, serum profiles were characterized by exponential equations. Absorption was represented by a single exponential term, whereas elimination + distribution was represented by a single exponential term in case of serum α-tocopheryl acetate, or two exponential terms in the case of α-tocopherol. The single elimination + distribution exponential term in B-act is explained because after the i.p. injection, serum α-tocopheryl acetate declined quickly to non detectable levels. Hidiroglou and Karpinski (1991) reported similar absorption and elimination rates after fitting plasma α-tocopherol to a two exponential equation following a single i.m. injection of dl-α-tocopheryl acetate in sheep. They also reported a lag phase between the injection time and the beginning of the first-order absorption.

Vitamin E, either as acetate or as alcohol, was released at very rapid rates from the injection site into the blood stream (absorption > distribution + elimination), peaking between 10 and 24 h after injection (absorption = distribution + elimination). Within the next 48 h, there was a rapid removal of the vitamin from blood for either utilization and (or) storage by the tissues, or excretion as metabolites (absorption < distribution + elimination). After 72 h post-injection, the concentration of vitamin E in blood decreased (absorption = 0, distribution + elimination > 0) at slow rate (Figures 2 to 4).

In animals receiving the A-act treatment, the vitamin E was only detected in serum in the alcohol form, indicating hydrolysis of the ester by muscle esterases before its release from the place of injection, and/or by plasma and/or tissue esterases immediately after its slow release rate
from muscle. In contrast, when B-act was i.m. administered, the ester form rapidly increased in serum (Figure 2), and this increment was followed by a slight rise in the alcohol form of vitamin E in serum (Figure 3). The peak of the alcohol form in serum was always later than the peak of the acetate form (Table 4). Similar results have been reported after i.m. administration of dl-α-tocopheryl acetate in man (Rindi and Perri, 1958), and in dogs (Bauernfeind et al., 1974; Fujii, 1980). The differences observed in the AUC data between the free and the ester sources of vitamin E (Tables 2 and 4), were partially explained by the rate of absorption from the injection site (Tables 8 to 10).

Gallo-Torres and Miller (1971) reported rapid hydrolysis of dl-α-tocopheryl acetate by the rat liver. They found that, 30 min after i.v. administration, 79% of the chromatographed radioactivity in the liver was due to quinone-derivatives, 15% to the ester form, and only small quantities were due to the alcohol form. They also reported the same biotransformations in several other tissues, and that the blood contained enzymes able of hydrolyzing esters of tocopherol. In contrast, Bauernfeind et al. (1974) reported that the blood does not have enzymes capable of hydrolyzing dl-α-tocopheryl acetate after an in vitro incubation of dog fresh blood at 37°C for up to 2 h. The same results were reported by Newmark et al. (1975) after incubation of human and dog blood at 37°C for up to 3 h. These authors reported that, 24 h after i.v. administration of dl-α-tocopheryl acetate in dogs, 25% of the dose was hydrolyzed, whereas Ogihara et al. (1985) reported 36% in the case of rats. Therefore, there seem to be differences in the hydrolytic capability among animal species.

Ogihara et al. (1985) also reported that at 24 h after i.v. administration in rats, the ester reached undetectable concentrations in plasma, whereas 48 h were needed to reach those undetectable concentration in red blood cells. In rabbits, Knight and Roberts (1986) observed that
not all the α-tocopheryl acetate was converted to tocopherol and that there was some α-tocopheryl acetate uptake by tissues. Our observations showed a total conversion of α-tocopheryl acetate to α-tocopherol, because by the end of the experiment, the acetate was not observed in serum and tissues. A possible reason for these differences may be the short duration of the experiment carried out by Knight and Roberts (1986), that did not allow total biotransformation of the ester. From our observations, there seems to be an enzymatic machinery in the organism able to convert the acetate to alcohol. Fujii (1980) reported that as much as 10% of the acetate ester of vitamin E is hydrolyzed to the free form after i.m. administration of dl-α-tocopheryl acetate in dogs.

Pancreas and adrenal gland had the greatest α-tocopherol concentrations of all tissues analyzed (Table 5), but the lowest total α-tocopherol content (Table 6). Similar results were observed by Karpinski and Hidiroglou (1990) in sheep.

Immediately after parenteral administration of radiolabeled α-tocopheryl acetate, most of the recovered radioactivity was present in spleen, lung, and liver of rats (Gallo-Torres and Miller, 1971) and rabbits (Knight and Roberts, 1986). Therefore, most of the biotransformations leading to excretion (quinones) or utilization (free alcohol) of the ester may take place in those three tissues belonging to the reticuloendothelial system. Our results showed that spleen, lung, and liver were the organs with the greatest content of α-tocopherol 14 d after i.m. injection of B-act, whereas only liver and lung were the tissues with the greatest content of α-tocopherol in the case of Ol administration (Table 6).

Of those three organs, concentration of α-tocopherol in spleen of B-act injected pigs was 17-fold greater than in the rest of the animals, and about 10-fold greater than that of the other tissues analyzed. Hidiroglou and Karpinski (1991) also reported a 66-fold increase in α-
tocopherol concentration in the spleen of sheep 80 h after i.m. administration of 60 mg of dl-α-tocopheryl acetate per kg BW, compared to control sheep. Spleen is considered as the main filter for foreign substances present in blood, whereas lymph glands are the filters for foreign substances present in lymph. If the acetate form of vitamin E were considered as a foreign substance, it would be retained by the lymph glands if its absorption from the place of injection were via the lymphatic pathway (Dickson et al., 1985), or by the spleen if the absorption were via blood. In our experiment, the lymphatic pathway has been hypothesized as the via of absorption for treatment A-act, but we did not collect lymph or lymph gland samples to confirm the data reported by Dickson et al. (1985). In contrast, for treatments B-act, and Ol, in which a quick response in serum vitamin E concentration was observed (Figure 2 to 4), the absorption from the place of injection was hypothesized to be via blood. The slow lymph flow rate preclude such a rapid response.

For all the tissues analyzed but spleen, tocopherol concentration was similar among B-act and Ol treated pigs. Therefore, swine seem to have the enzymatic capability to effectively hydrolyze d-α-tocopheryl acetate to α-tocopherol and make the biologically active form of this vitamin available for the site of action.

Physicochemical and technologic implementations in the design of the dose forms may result in differences in the bioavailability of drugs (Niazi, 1979). The principal factors controlling the bioavailability of drugs from the site of injection are: 1) the vascularization of the injection site, 2) the lipid solubility and ionization of the drug, and 3) the volume of the injection, and the osmolality of the solution (Greenblatt and Koch-Weser, 1976). Therefore, the release of a parenterally administered drug and the subsequent shape of the serum concentration vs time profile can be controlled by the type of administration (i.m., i.v., i.p.), the type of salts (ether,
ester, or complexes of the active ingredient with low solubility), the type of vehicle used, etc.

Njeru et al. (1992) reported that after a single i.m. injection of dl-α-tocopherol in an emulsifiable solution, sheep had elimination rate independent of dose, whereas the absorption rate increased with dose. They fitted the data to a three-phase linear model. Our results suggest faster absorption of vitamin E from the place of injection when i.m. supplementation was done as the acetylated ester, compared to the free form [\(P_{1}\), Table 4; \(k_a\), Table 8; \(k(8,7)\), Table 9; and \(k(6,1)\), Table 10]. And that once the acetylated ester is hydrolyzed to free the form, serum α-tocopherol is maintained longer than when d-α-tocopherol is i.m. injected as such [\(P_{3}\), Table 4]. This was probably because, when d-α-tocopheryl acetate was injected, larger and more sustained inputs than outputs into compartment \(q_3\) (serum d-α-tocopherol) occurred, compared with i.m. administration of d-α-tocopherol (Tables 9 and 10). Reasons for this, may be: first, that the esterified source (Table 10 and Figure 6) is absorbed from the place of injection [\(k(6,1)\)], distributed by the circulatory system (\(q_2\)), and taken up by different tissues [\(k(4,2)\) and \(k(9,2)\)]; second, that the hydrolysis of d-α-tocopheryl acetate to free tocopherol may have taken place at the injection site [\(k(7,1)\)], circulatory system [\(k(3,2)\)], and tissue level [\(k(5,4)\)]; and third, that once hydrolysis took place, d-α-tocopherol had the same behavior as when it was directly injected into the system [\(k(8,7)\), \(k(5,3)\), \(k(3,5)\), and \(k(3,9)\)].

The anatomical distribution of the pools for the model-based compartmental analysis was based on actual knowledge of vitamin E metabolism, experimental results, and some assumptions. This distribution was as follows: \(q_1\) = d-α-tocopheryl acetate in place of injection, \(q_2\) = d-α-tocopheryl acetate in serum, \(q_3\) = d-α-tocopherol in serum, \(q_4\) = d-α-tocopheryl acetate in extrahepatic tissues, \(q_5\) = d-α-tocopherol in extrahepatic tissues, \(q_6\) = d-α-tocopherol in place of injection, \(q_7\) = vitamin E and its metabolites in liver, \(q_{10}\) = vitamin E and its metabolites in bile,
$d_6$ and $d_8 = \text{delays.}$

Even though tocopherol concentration of tissues (Table 5) and tocopherol content of organs (Table 6) 336 h after application of the treatments were similar among B-act and O1 treated pigs, with the exception of spleen; total and muscle $\alpha$-tocopherol content of injectable origin (Table 7) was greater for O1 than for B-act treated pigs. Muscle had the largest contribution to total analyzed vitamin E content. The total amount of tissues analyzed for tocopherol content of injectable origin accounted for about 56% of BW.

Results from the model-based compartmental analysis showed similar total content of $\alpha$-tocopherol in extrahepatic tissue ($q_0$) in B-act and O1 treated pigs (48.9 vs 48.6 mg respectively) at 336 h after injection. This may suggest preferential uptake of the acetylated form of vitamin E by specific tissues, such as spleen (Table 5). Hidiroglou and Atwal (1989) reported that, after parenteral administration of an ester or free vitamin E to dairy cows, the acetate ester resulted in greater milk tocopherol concentration than the free form. This result may also be due to a preferential uptake of tocopheryl acetate by the mammary gland. Njeru et al. (1994) observed that after i.m. administration of dl-$\alpha$-tocopheryl acetate in sheep, an increase in tocopherol concentration in liver and pancreas but not in diaphragm, kidney, heart, and neck.

Total analyzed liver $\alpha$-tocopherol content of injectable origin (Table 7) was similar in B-act and O1 treated pigs and this results were in agreement with the output from the model-based compartmental analysis ($q_0$).

The existence of a tocopherol binding protein (TBP) in rat liver has been reported (Murphy and Mavis, 1981). This protein is able to discriminate between $\alpha$- and $\gamma$-tocopherol. This could be the reason why, even though $\gamma$-tocopherol is predominant in the diet, $\alpha$-tocopherol is more abundant in blood and tissue. Although TBP has not been reported in swine, Hoppe (1991) found
that weanling pigs fed a single dose of a 1:1 mixture of α-tocopherol and γ-tocopherol lost the γ-isomers more rapidly than the α-isomers. Discrimination between RRR- and other stereoisomers has been reported. Hidiroglou and Karpinski (1988) reported that i.v. administration of d-α-tocopheryl acetate was much better utilized by sheep than i.v. administration of dl-α-tocopherol or dl-α-tocopheryl acetate.

The volume of distribution of the central compartment for B-act injected pigs (Table 10) was similar to the calculated serum volume of a 15 kg-pig (.84 kg). In contrast, O1 injected pigs (Table 9) had greater volume of distribution of the central compartment than the calculated serum volume. This implies that pool q3 must include non-serum sources of vitamin E rapidly exchangeable with serum tocopherol.

When using the empirical compartmental model, we fitted one compartmental model for every exponential curve defined. In cases such as B-act treated pigs, two equations were needed to describe vitamin E vs time profile, one for the acetylated and the other for the free tocopherol, and therefore, two different empirical compartmental models were developed based on those equations (Figure 1 and Table 8). In biological systems, serum tocopheryl acetate and free tocopherol are not unrelated, they are metabolically interconnected. For that reason, even though the empirical model may be a good start-point, model-based compartmental analysis would be the method of choice (Figure 6). Besides, model-based compartmental analysis is more flexible dealing with non-steady state conditions, allows us to include information from other sources (literature,...) in the model, and allows us to generate new hypothesis and develop new experiments (Green and Green, 1990). For instance, based on the information provided by model-based compartmental analysis, it would be possible to define the best protocol (route, source, preparation, dose, and time of vitamin E application) to obtain the α tocopherol
concentration in muscle at slaughter time that would optimize meat quality, or to maintain optimal vitamin E status to prevent the onset of deficiency in the herd.

In the current study, we only used the d-α isomers of vitamin E, either as acetylated ester or alcohol. When d-α-tocopheryl acetate was used in conjugation with an adequate carrier, and given equal amounts based on IU of vitamin E activity, both sources (acetate and alcohol) of the same isomer (d-α-) had similar responses regarding α-tocopherol concentration in tissues, with the exception of spleen; but the alcohol source had greater total muscle vitamin E content of injectable origin.

Implications

Vitamin E dissolved in olive oil was poorly utilized after i.m. administration. However, when an adequate carrier was used in the formulation of i.m. injectable preparations, the acetate and alcohol sources of the same vitamin E isomer (d-α-) resulted in similar serum and tissue α-tocopherol concentrations in pigs 336 h after administration, except in spleen. The alcohol form was superior to the acetate source in increasing the total muscle vitamin E content of injectable origin. The α-tocopheryl acetate has greater stability during storage, greater serum AUC, and maintains serum tocopherol longer than the alcohol. The α-tocopheryl acetate was not an irritant when i.m. administered. Regarding the different approaches to compartmental modeling, model-based compartmental analysis is the method of choice.
Literature Cited


Table 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>55.93</td>
</tr>
<tr>
<td>Soybean meal, dehulled, solvent</td>
<td>29.10</td>
</tr>
<tr>
<td>Whey</td>
<td>10.00</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.51</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>.76</td>
</tr>
<tr>
<td>Vitamin premix^a</td>
<td>.20</td>
</tr>
<tr>
<td>Trace mineral premix^b</td>
<td>.05</td>
</tr>
<tr>
<td>Salt</td>
<td>.25</td>
</tr>
<tr>
<td>Antibiotic^c</td>
<td>1.00</td>
</tr>
<tr>
<td>L-lysine-HCl</td>
<td>.20</td>
</tr>
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</table>

Calculated analysis:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>20.19</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td>3,280</td>
</tr>
<tr>
<td>Ca</td>
<td>.86</td>
</tr>
<tr>
<td>P</td>
<td>.70</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.30</td>
</tr>
</tbody>
</table>

^aContributed the following per kilogram of diet: vit. A, 4,400 IU; vit. D₃, 1,100 IU; vit. E, 22 IU; vit. B₁₂, 0.022 mg; riboflavin, 6.6 mg; niacin, 33 mg; d-pantothenic acid, 17.6 mg.

^bContributed the following per kilogram of diet: Zn, 75 mg; Fe, 87.5 mg; Mn, 30 mg; Cu, 8.75 mg; I, and 1 mg.

^cContributed .9 mg of carbadox per kilogram of diet.
Table 2. Observed serum α-tocopherol pharmacokinetic values in pigs after a single i.m. injection of 138 IU of vitamin E/kg of BW

<table>
<thead>
<tr>
<th>Item</th>
<th>Saline</th>
<th>A-act</th>
<th>B-act</th>
<th>Ol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co, mg/L</td>
<td>.60</td>
<td>.54</td>
<td>.54</td>
<td>.68</td>
<td>.12</td>
</tr>
<tr>
<td>Cmax, mg/L</td>
<td>1c</td>
<td>1c</td>
<td>14c</td>
<td>129d</td>
<td>11</td>
</tr>
<tr>
<td>Cf, mg/L</td>
<td>.56c</td>
<td>1.21d</td>
<td>1.58d</td>
<td>1.43d</td>
<td>.16</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>96c</td>
<td>272d</td>
<td>24c</td>
<td>10c</td>
<td>31</td>
</tr>
<tr>
<td>AUC, (mg/L) (\times) h</td>
<td>5c</td>
<td>114c</td>
<td>912c</td>
<td>4,872d</td>
<td>418</td>
</tr>
</tbody>
</table>


bCo = Initial concentration. Cmax = Maximum concentration. Cf = Final concentration. Tmax = Time to maximum concentration. AUC = Area under the serum concentration vs time curve. Calculated by the trapezoidal rule and corrected for Co.

cdMeans in a row with different superscripts differ \((P < .01)\).
Table 3. Exponential equation parameters estimated for serum vitamin E in pigs after a single i.m. injection of 138 IU of vitamin E/kg of BW<sup>ab</sup>

<table>
<thead>
<tr>
<th>Item</th>
<th>B-act</th>
<th>Ol</th>
<th>B-act</th>
<th>Ol</th>
</tr>
</thead>
<tbody>
<tr>
<td>T, h</td>
<td>2.97</td>
<td>.62</td>
<td>1.24</td>
<td>-</td>
</tr>
<tr>
<td>K&lt;sub&gt;1&lt;/sub&gt;, mg/L</td>
<td>35</td>
<td>1580</td>
<td>1440</td>
<td>-</td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;, mg/L</td>
<td>2.46</td>
<td>4.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P&lt;sub&gt;1&lt;/sub&gt;, h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>.077</td>
<td>.086</td>
<td>.151</td>
<td>-</td>
</tr>
<tr>
<td>P&lt;sub&gt;2&lt;/sub&gt;, h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>.04</td>
<td>.07</td>
<td>.08</td>
<td>-</td>
</tr>
<tr>
<td>P&lt;sub&gt;3&lt;/sub&gt;, h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>.003</td>
<td>.006</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>.65</td>
<td>.96</td>
<td>.93</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Saline = Injection of saline. A-act = Injection of d-α-tocopheryl acetate in olive oil. B-act = Injection of d-α-tocopheryl acetate as emulsion. Ol = Injection of d-α-tocopherol as emulsion. Saline and A-act treatments did not show a response.

<sup>b</sup>If t < T, then: Ct = Co. If serum d-α-tocopheryl acetate and t ≥ T, then: Ct = Co - K<sub>1</sub> e<sup>P<sub>1</sub>(t-T)</sup> + K<sub>1</sub> e<sup>P<sub>2</sub>(t-T)</sup>. If serum d-α-tocopherol and t ≥ T, then: Ct = Co - K<sub>1</sub> e<sup>P<sub>1</sub>(t-T)</sup> + K<sub>1</sub> e<sup>P<sub>2</sub>(t-T)</sup> + K<sub>2</sub> e<sup>P<sub>3</sub>(t-T)</sup>. T = time at which first-order absorption begins; t = time; Ct = concentration at t time; Co = initial concentration; K<sub>i</sub> = intercept; and P<sub>j</sub> = rate.

<sup>c</sup>Serum d-α-tocopheryl acetate was not detected (< .01 mg/L) after injection of d-α-tocopherol (Ol).
Table 4. Predicted serum pharmacokinetic values calculated from exponential equations after a single i.m. administration of 138 IU of vitamin E/kg of BW

<table>
<thead>
<tr>
<th>Item</th>
<th>B-act</th>
<th>Ol</th>
<th>B-act</th>
<th>Ol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co, mg/L</td>
<td>.54</td>
<td>.68</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Cmax, mg/L</td>
<td>10.42</td>
<td>124</td>
<td>325</td>
<td>-</td>
</tr>
<tr>
<td>Cf. mg/L</td>
<td>1.36</td>
<td>1.43</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>20.01</td>
<td>13.45</td>
<td>10.14</td>
<td>-</td>
</tr>
<tr>
<td>P1 T1/2, h</td>
<td>9.06</td>
<td>8.06</td>
<td>4.59</td>
<td>-</td>
</tr>
<tr>
<td>P2 T1/2, h</td>
<td>16.42</td>
<td>9.90</td>
<td>8.56</td>
<td>-</td>
</tr>
<tr>
<td>P3 T1/2, h</td>
<td>210</td>
<td>126</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AUC, (mg/L)xh</td>
<td>939</td>
<td>4914</td>
<td>8156</td>
<td>-</td>
</tr>
</tbody>
</table>

*Saline = Injection of saline. A-act = Injection of d-α-tocopheryl acetate in olive oil. B-act = Injection of d-α-tocopheryl acetate as emulsion. Ol = Injection of d-α-tocopherol as emulsion. Saline and A-act treatments did not show a response. For B-act (R² = .93 and .65 for serum α-tocopheryl acetate and serum tocopherol respectively) and Ol (R² = .96 for serum α-tocopherol).

*Co = initial concentration; Cmax = maximum concentration; Cf = final concentration; Tmax = Time to maximum concentration; P1 T1/2 = half life for the P1 phase; AUC = Area under the serum concentration vs time curve, estimated after integration of the exponential equation from time 0 to 336 h.

*Serum α-tocopheryl acetate was not detected (< .01 mg/L) after injection of d-α-tocopherol (Ol).
Table 5. Concentration of α-tocopherol in tissues before and 336 h after a single i.m. administration of 138 IU of vitamin E/kg of BW (mg/kg)

<table>
<thead>
<tr>
<th>Item</th>
<th>Basal*</th>
<th>SE</th>
<th>Treatmentsb</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Saline</td>
<td>A-act</td>
<td>B-act</td>
<td>Ol</td>
<td>SE</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td>3.1</td>
<td>2.2</td>
<td>1.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>8.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Longissimus</td>
<td>2.4</td>
<td>.6</td>
<td>1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.4</td>
<td></td>
</tr>
<tr>
<td>Psoas</td>
<td>3.5</td>
<td>1.3</td>
<td>1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>6.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>4.2</td>
<td>.6</td>
<td>2.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.4</td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>2.6</td>
<td>.8</td>
<td>2.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.6</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>2.0</td>
<td>.4</td>
<td>1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.3</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.4</td>
<td>1.0</td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.7</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>3.1</td>
<td>.6</td>
<td>1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.4</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>3.4</td>
<td>.6</td>
<td>2.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.5</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>7</td>
<td>14</td>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

*Base line. One animal per littermate group was killed before i.m. administration of vitamin E.

Table 6. Content of α-tocopherol in organs before and 336 h after a single i.m. administration of 138 IU of vitamin E/kg of BW (mg)

<table>
<thead>
<tr>
<th>Item</th>
<th>Basal(^a)</th>
<th>Saline</th>
<th>A-act</th>
<th>B-act</th>
<th>Ol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>.21</td>
<td>.19(^c)</td>
<td>.25(^c)</td>
<td>.58(^d)</td>
<td>.63(^d)</td>
<td>.04</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>.007</td>
<td>.006(^c)</td>
<td>.011(^{cd})</td>
<td>.016(^{de})</td>
<td>.019(^e)</td>
<td>.002</td>
</tr>
<tr>
<td>Kidney</td>
<td>.10</td>
<td>.22(^c)</td>
<td>.26(^c)</td>
<td>.55(^d)</td>
<td>.55(^d)</td>
<td>.05</td>
</tr>
<tr>
<td>Liver</td>
<td>.47</td>
<td>1.04(^c)</td>
<td>1.46(^{cd})</td>
<td>2.32(^{de})</td>
<td>2.27(^e)</td>
<td>.28</td>
</tr>
<tr>
<td>Lung</td>
<td>.32</td>
<td>.34(^c)</td>
<td>.56(^c)</td>
<td>.97(^d)</td>
<td>1.05(^d)</td>
<td>.09</td>
</tr>
<tr>
<td>Pancreas</td>
<td>.07</td>
<td>.12(^c)</td>
<td>.15(^c)</td>
<td>.40(^d)</td>
<td>.42(^d)</td>
<td>.04</td>
</tr>
<tr>
<td>Spleen</td>
<td>.03</td>
<td>.06(^c)</td>
<td>.12(^c)</td>
<td>1.80(^d)</td>
<td>.11(^c)</td>
<td>.27</td>
</tr>
</tbody>
</table>

\(^a\)Basal line. One animal per littermate group was killed before i.m. administration of vitamin E.


\(^{cd}\)Means in a row with different superscripts differ (P < .06).
Table 7. Content of α-tocopherol of injectable origin\(^a\) 336 h after a single i.m. administration of 138 IU of vitamin E/kg of BW (mg)

<table>
<thead>
<tr>
<th>Item</th>
<th>A-act</th>
<th>B-act</th>
<th>Ol</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>.060(^f)</td>
<td>.384(^g)</td>
<td>.437(^g)</td>
<td>.047</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>.005(^f)</td>
<td>.010(^g)</td>
<td>.013(^g)</td>
<td>.002</td>
</tr>
<tr>
<td>Kidney</td>
<td>.047(^f)</td>
<td>.335(^g)</td>
<td>.329(^g)</td>
<td>.057</td>
</tr>
<tr>
<td>Liver</td>
<td>.419</td>
<td>1.273</td>
<td>1.250</td>
<td>.299</td>
</tr>
<tr>
<td>Lung</td>
<td>.222(^f)</td>
<td>.627(^g)</td>
<td>.699(^g)</td>
<td>.095</td>
</tr>
<tr>
<td>Pancreas</td>
<td>.032(^f)</td>
<td>.278(^g)</td>
<td>.298(^g)</td>
<td>.043</td>
</tr>
<tr>
<td>Spleen</td>
<td>.057(^f)</td>
<td>1.741(^g)</td>
<td>.031(^f)</td>
<td>.291</td>
</tr>
<tr>
<td>Muscle(^c)</td>
<td>3.380(^f)</td>
<td>17.433(^g)</td>
<td>34.877(^b)</td>
<td>4.295</td>
</tr>
<tr>
<td>Serum(^d)</td>
<td>.552</td>
<td>.863</td>
<td>.736</td>
<td>.148</td>
</tr>
<tr>
<td>TOTAL(^c)</td>
<td>4.774(^f)</td>
<td>22.944(^g)</td>
<td>38.670(^b)</td>
<td>4.076</td>
</tr>
</tbody>
</table>

\(^a\) Calculated as follows: Tissue α-tocopherol content minus tissue vitamin E content of its littermate in the saline injected group.

\(^b\) A-act = Injection of d-α-tocopheryl acetate in olive oil. B-act = Injection of d-α-tocopheryl acetate as emulsion. Ol = Injection of d-α-tocopherol as emulsion.

\(^c\) Calculated assuming that a 15 kg pig has 6.57 kg muscle, and using the average vitamin E concentration of the three skeletal muscle analyzed for each animal.

\(^d\) Calculated assuming that a 15 kg pig has 0.84 kg serum, and using the α-tocopherol concentration in serum 336 h after i.m. administration.

\(^e\) Calculated adding up total α-tocopherol content of all the tissues analyzed for each animal. The total amount of tissues analyzed for α-tocopherol content of injectable origin accounted for about 56% of BW.

\(^f, g\) Means in a row with different superscripts differ (P < .07).
Table 8. Parameters for the empirical compartmental model of a single i.m. injection of 138 IU of vitamin E as d-α-tocopherol or tocopheryl acetate

<table>
<thead>
<tr>
<th>Item(^{b})</th>
<th>Serum α-tocopherol</th>
<th>Serum α-tocopheryl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B-act</td>
<td>Ol</td>
</tr>
<tr>
<td>(k_a)</td>
<td>.077</td>
<td>.086</td>
</tr>
<tr>
<td>(k_{1,2})</td>
<td>.008</td>
<td>.007</td>
</tr>
<tr>
<td>(k_{2,1})</td>
<td>.015</td>
<td>.023</td>
</tr>
<tr>
<td>(k_e)</td>
<td>.036</td>
<td>.060</td>
</tr>
</tbody>
</table>

\(^{a}\)Calculated using slopes and intercepts of the exponential equations. For B-act (\(R^2 = .93\) and .65 for serum α-tocopheryl acetate and serum α-tocopherol, respectively) and Ol (\(R^2 = .96\) for serum α-tocopherol).

\(^{b}\)\(k_a\) = rate of absorption, \(k_{1,2}\) = rate of transfer from compartment 2 to compartment 1, \(k_{2,1}\) = rate of transfer from compartment 1 to compartment 2, and \(k_e\) = rate of elimination (h\(^{-1}\)).

\(^{c}\)Serum α-tocopheryl acetate was not detected (< .01 mg/L) after injection of d-α-tocopherol (Ol).
Table 9. Parameters for the model-based compartmental analysis of a single i.m. injection of 138 IU of vitamin E as d-α-tocopherol (Ol)

<table>
<thead>
<tr>
<th>Parameter (k_{(j,i)})</th>
<th>Characteristic</th>
<th>Value</th>
<th>FSD (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{(8,7)})</td>
<td>Adjustable</td>
<td>.060</td>
<td>.126</td>
</tr>
<tr>
<td>(k_{(3,5)})</td>
<td>Adjustable</td>
<td>.004</td>
<td>.147</td>
</tr>
<tr>
<td>(k_{(5,3)})</td>
<td>Adjustable</td>
<td>.022</td>
<td>.097</td>
</tr>
<tr>
<td>(k_{(3,9)})</td>
<td>Adjustable</td>
<td>.004</td>
<td>.688</td>
</tr>
<tr>
<td>(k_{(9,3)})</td>
<td>Adjustable</td>
<td>.087</td>
<td>.181</td>
</tr>
<tr>
<td>(k_{(10,9)})</td>
<td>Adjustable</td>
<td>.151</td>
<td>.618</td>
</tr>
<tr>
<td>(V)</td>
<td>Adjustable</td>
<td>1.490</td>
<td>.063</td>
</tr>
<tr>
<td>(d)</td>
<td>Fixed(c)</td>
<td>.620</td>
<td>-</td>
</tr>
</tbody>
</table>

\(a\) \(k_{(j,i)}\) = rate of transfer from compartment \(i\) to compartment \(j\), h\(^{-1}\); \(V\) = volume of distribution, L; and \(d\) = delay or lag time, h.

\(b\) Fractional standard deviation (FSD) is defined as the standard deviation/mean ratio.

\(c\) Parameter from numerical model of Ol treatment.
Table 10. Parameters for the model-based compartmental analysis of a single i.m. injection of 138 IU of vitamin E as d-α-tocopheryl acetate (B-act)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Characteristic</th>
<th>Value</th>
<th>FSDb</th>
</tr>
</thead>
<tbody>
<tr>
<td>k(6,1)</td>
<td>Fixedc</td>
<td>.131</td>
<td></td>
</tr>
<tr>
<td>k(7,1) &amp; k(5,4)</td>
<td>Fixedd</td>
<td>.010</td>
<td></td>
</tr>
<tr>
<td>k(4,2)</td>
<td>Adjustable</td>
<td>.013</td>
<td>.601</td>
</tr>
<tr>
<td>k(3,2)</td>
<td>Fixede</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>k(9,2)</td>
<td>Adjustable</td>
<td>.071</td>
<td>.126</td>
</tr>
<tr>
<td>k(5,3)</td>
<td>Fixedf</td>
<td>.022</td>
<td></td>
</tr>
<tr>
<td>k(3,5)</td>
<td>Fixedf</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>k(9,3)</td>
<td>Adjustable</td>
<td>.157</td>
<td>.639</td>
</tr>
<tr>
<td>k(3,9)</td>
<td>Fixedf</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>k(8,7)</td>
<td>Fixedf</td>
<td>.060</td>
<td></td>
</tr>
<tr>
<td>k(10,9)</td>
<td>Adjustable</td>
<td>.156</td>
<td>.169</td>
</tr>
<tr>
<td>V</td>
<td>Adjustable</td>
<td>.982</td>
<td>.065</td>
</tr>
<tr>
<td>d6</td>
<td>Fixedg</td>
<td>1.700</td>
<td></td>
</tr>
<tr>
<td>d8</td>
<td>Fixedg</td>
<td>2.970</td>
<td></td>
</tr>
</tbody>
</table>

a$k(j,i)$ = rate of transfer from compartment $i$ to compartment $j$, $h^{-1}$; $V$ = volume of distribution, L; and $d_i$ = delay or lag time, h.

bFractional standard deviation (FSD) is defined as the standard deviation/mean ratio.

c$k(6,1) + k(7,1) = 0.143; \text{FSD} = 0.065$.

dAssuming that 1% of tissue tocopherol acetate is hydrolyzed to free tocopherol per hour.

e$k(3,2) + k(4,2) + k(9,2) = 0.087; \text{FSD} = 0.014$.

fParameter from compartmental model of O1 treatment.

gParameter from numerical model of the B-act treatment.
Figure 1. a) One compartment open model with first order absorption and elimination of α-tocopheryl acetate after a single i.m. dose of d-α-tocopheryl acetate (B-act), b) two compartment open model with first order absorption and elimination of α-tocopherol only from compartment 1 after a single i.m. dose of d-α-tocopherol (Ol) or d-α-tocopheryl acetate (B-act). Ka = absorption rate, h⁻¹; Ke = elimination rate, h⁻¹; D = dose, mg; F = fraction of dose; Q = Size of compartment, mg; Kj,i = rate of transfer from compartment i to j, h⁻¹.

\[ Q = V \times C \]

\[ Q_1 = V_1 \times C_1 \]

\[ K_{2,1} \quad K_{1,2} \]

\[ Q_2 \]
Figure 2. Serum α-tocopheryl acetate concentration with time after a single i.m. injection of 138 IU/kg of d-α-tocopheryl acetate (B-act). Observed (●) and predicted values (-). Co = initial concentration, Cmax = maximum concentration, Ct = concentration at time t, t = time, T = time at which first-order absorption begins, Tmax = Time to maximum concentration, T1/2 = half life, K1 = intercept, and P1 = rate.
If \( t < T \), then: 
\[ C_t = C_0 - P_1(t-T) - P_2(t-T) - P_3(t-T) \]

If \( t \geq T \), then: 
\[ C_t = C_0 - K_1 e^{-K_1 t} + K_1 e^{-K_1 T} + K_2 e^{-K_2 t} \]

Figure 3. Serum \( \alpha \)-tocopherol concentration with time after a single i.m. injection of 138 IU/kg of \( \alpha \)-tocopheryl acetate (B-act). Observed (●) and predicted values (-). \( C_0 = \) initial concentration, \( C_{\text{max}} = \) maximum concentration, \( C_t = \) concentration at time \( t \), \( t = \) time, \( T = \) time at which first-order absorption begins, \( T_{\text{max}} = \) Time to maximum concentration, \( T_{1/2} = \) half life, \( K_1 = \) intercept, and \( P_i = \) rate.
Figure 4. Serum α-tocopherol (α-T) concentration with time after a single i.m. injection of 138 IU/kg of d-α-tocopherol (Ol = Pooled A & B-ol). Observed (○) and predicted values (●). Co = initial concentration, Cmax = maximum concentration, Ct = concentration at time t, t = time, T = time at which first-order absorption begins, Tmax = Time to maximum concentration, T1/2 = half life, K1 = intercept, and P1 = rate. 

If t < T, then: 

\[ Ct = Co - P1(t-T) - P2(t-T) - P3(t-T) \]

If t ≥ T, then: 

\[ Ct = Co - K1 e^{-K1 t} - K2 e^{-K2 t} \]

Cmax = 124.46 mg/L
Tmax = 13.45 h
Co = 0.66 mg/L
T = 0.62 h
K1 = 1590 mg/L
K2 = 4.68 mg/L
P1 = 0.088 1/h \( \Rightarrow T_{1/2} = 8.06 \) h
P2 = 0.07 1/h \( \Rightarrow T_{1/2} = 9.90 \) h
P3 = 0.0055 1/h \( \Rightarrow T_{1/2} = 126 \) h
Figure 5. Model-based compartmental analysis of a single i.m. injection of 138 IU of vitamin E as d-α-tocopheryl acetate (B-act).

$q_i$ = size of compartment $i$, mg; $d_6$ and $d_8$ = delay or lag time, h; $S_i$ = sample; and $k(j,i)$ = rate of transfer from compartment $i$ to compartment $j$, h$^{-1}$. 
Figure 6. Model-based compartmental analysis of a single i.m. injection of 138 IU of vitamin E as d-α-tocopherol (Ol = Pooled A & B-ol). $q_i =$ size of compartment $i$, mg; $d_8 =$ delay or lag time, h; $S_j =$ sample; and $k(j,i) =$ rate of transfer from compartment $i$ to compartment $j$, h$^{-1}$. 
Figure 7. Pool sizes vs time profile after model-based compartmental analysis was performed on a single i.m. injection of 138 IU of vitamin E as d-α-tocopheryl acetate B (B-act).
Figure 8. Pool sizes vs time profile after model-based compartmental analysis was performed on a single i.m. injection of 138 IU of vitamin E as d-α-tocopherol (Ol = Pooled A & B-ol).
CHAPTER 5. EFFECT OF DIETARY AND INJECTABLE VITAMIN E ON
REPRODUCTIVE PERFORMANCE AND TOCOPHEROL STATUS OF SOWS AND PIGS
OVER THREE PARITIES

A paper to be submitted to the *Journal of Animal Science*

D. Carrion, R. C. Ewan, and J. Coma

Abstract

The effect of method of administration of vitamin E on reproductive performance and
tocopherol status of the sows and their progeny was evaluated over three parities. Twenty
Yorkshire x Landrace sows were allotted in a complete randomized design. Treatments were a 2
x 2 factorial arrangement of none or 600 IU of d-α-tocopherol injected i.m. at breeding and at
110 d of gestation and a corn-soybean diet or the diet supplemented with 50 IU of d-α-tocopheryl
acetate per kilo. During gestation, sows were individually fed 1.8 kg per d and were full hand
fed at least twice a day during lactation. Treatments had no effect on BW, ADFI, number of pigs
born dead, number of mummies per litter, or percentage of mortality during lactation. The total
number of pigs born and pigs alive at 21 d of lactation per litter decreased (P < .01) in sows fed
the vitamin E-supplemented diets, and increased (P < .01) by parity. The serum activity of
glutathione peroxidase and lactic acid dehydrogenase were not affected by treatment and were
within their respective normal ranges for sows and pigs. Sows fed diets supplemented with d-α-
tocopheryl acetate had greater serum (P < .05) and milk (P < .001) α-tocopherol
concentrations, and their litters had greater (P < .05) serum and tissue α-tocopherol
concentrations than litters from sows fed the basal diet. Sows injected with supplemental d-α-tocopherol had greater (P < .01) serum and milk α-tocopherol concentrations at farrowing, and their litters had greater (P < .05) α-tocopherol concentrations in kidney at 21 d of lactation than those from sows that did not receive the injection. Pigs were born depleted of vitamin E, but the immediate uptake of colostrum repleted them. These results suggest that when sows fed diets containing .3 ppm of selenium for three consecutive parities and when reproductive performance is used as response criterion, 11 IU of vitamin E per kg of diet during gestation and 22 IU of vitamin E per kg during lactation was adequate. Moreover, dietary and injectable tocopherol supplementation of sows can improve the tocopherol status of both sows and their offspring.

Key words: Pigs, Vitamin E, Tocopherol, Reproductive Performance

Introduction

Swine diets are generally supplemented with vitamin E because the vitamin E in ingredients is readily destroyed. Factors contributing to this destruction are: storage and processing of feedstuffs, and dietary factors such as presence of trace minerals or polyunsaturated fatty acids. Other factors affecting the requirement for vitamin E supplementation include the management system and the genetic background of the pigs (Adams and Zimmerman, 1982; Mahan, 1991a).

Sows fed a vitamin E-deficient diet farrow fewer pigs because of embryonic death (Adamstone et al., 1949), occurring primarily between d 10 to 18 after breeding. Another critical time period during the reproductive process is the first few days after farrowing because most of the pig mortality occurs at that time.
Vitamin E supplementation through the diet (Babinszky et al., 1992) or through a single i.m. injection about 4 days before rebreeding (Myer, 1992) did not affect reproductive performance when the concentration of vitamin E in the control diets was normal. Mahan (1991b, 1994) reported an increase in litter size in response to dietary vitamin E supplementation above the NRC (1988) recommendation.

Vitamin E is inefficiently transferred across the placenta, and low concentrations of vitamin E have been reported in pigs prior to first suckling (Malm et al., 1976; Mahan et al., 1977; Young et al., 1977). However, vitamin E is effectively transported by mammary tissue with a greater concentration of vitamin E in colostrum than in milk. The concentration of vitamin E in milk can be increased by additional vitamin E supplementation (Mahan, 1991b; Babinszky et al. 1991; 1992). The following study was conducted to investigate the effect of dietary d-α-tocopheryl acetate supplementation and injectable d-α-tocopherol at breeding and at d 110 of gestation on sow reproductive performance and tocopherol status of the dam and its offspring over three parities.

Materials and Methods

Experimental Design and Management

Twenty Yorkshire x Landrace primiparous sows were reared under common housing, environment, nutrition and management conditions. The sows were allocated in a complete randomized design. Treatments were a 2 x 2 factorial arrangement of none or 600 IU of d-α-tocopherol injected i. m. at breeding and at d 110 of gestation, and of a corn-soybean meal diet or the diet supplemented with 50 IU of d-α-tocopheryl acetate per kilogram fed during the whole
experimental period. The procedures were approved by the Committee on Animal Care and Use at Iowa State University.

Females exhibiting estrus were hand-mated to boars twice at 24 h intervals. During gestation, sows were housed in a naturally ventilated building in individual stalls on partly slatted concrete floor. They were individually fed once daily at 1.8 kg commencing at breeding. The gestation diets were based on corn and solvent extracted soybean meal (Table 1). On d 110 of gestation, each sow was moved to a farrowing room and placed in a farrowing crate until weaning. The farrowing room was a completely closed facility with underslat waste storage. Fans and air inlets provided ventilation, and artificial cooling or heating was used. In the farrowing room, the animals received 1.8 kg/d of gestation diet until farrowing. During lactation, gilts were full hand fed, at least twice daily, a corn-soybean meal lactation diet (Table 1). The basal diets were formulated to meet or exceed the NRC (1988) recommendations. Pigs had no access to creep feed during lactation. Sows and pigs were allowed ad libitum access to water during the experimental period.

Pigs were earnotched, tail docked and injected with iron dextran within 12 h of parturition. Male piglets were castrated at 14 d of lactation.

Sample Collection and Measurements

Diet. The vitamin E premix was prepared by adding 16.68 g of d-α-tocopheryl acetate (Sigma Chemical Co.) in 100 mL distilled ethanol to 25 kg of ground corn. Batches of 455 kg of gestation and lactation diets were mixed at least once per month for immediate consumption. A sample from each batch was taken and stored at -20°C for determination of dry matter, energy, fat, protein, ash, selenium and tocopherol isomers. Feed samples were ground through a 1.0 mm
Dry matter was determined by drying in an oven for 24 hours at 105°C, energy by an adiabatic calorimeter (Parr Instrument Co.), fat by the ether extract procedure (AOAC, 1990, method 920.39C), ash by using a muffle oven at 600°C for 3 h, and N by the Kjeldahl method (AOAC, 1990, method 954.01).

Selenium was determined by the method of Olson et al. (1975). Selenium in the form of selenite reacts with aromatic ortho-diamines to form piazselenols which fluoresce in certain organic solvents. Therefore, the sample was ashed under strong oxidizing conditions. When the Se was in the inorganic form, it was converted to the +4 oxidation state. It is then reacted with 2,3-diaminonaphthalene. The piazselenol was extracted into decahydronaphthalene and the fluorescence was measured. The calculations were done by linear regression based on standards.

Tocopherol isomers and \( \alpha \)-tocopheryl acetate were extracted with acetone in a soxhlet extractor. The extract was evaporated to dryness and dissolved in hexane. Tocopherol isomers were separate by HPLC. The hexane extract was injected directly and separation achieved with silica gel column 25 cm x 4.6 mm CHROMAGESPHERE SI-60 with a particle diameter of 5 \( \mu \)m and a pore diameter of 60 Å (ES Industries; Berlin, NJ 08009) with a mobile phase of 3.5% tetrahydrofuran (vol/vol) in HPLC-grade hexane with a flow rate of 2.0 mL/min. Tocopherol was detected fluorometrically with an excitation wavelength of 294 \( \eta \)m and an emission wavelength of 323 \( \eta \)m. Peak areas were determined by integration and compared with standards for quantification.

**Weighing and reproductive performance.** Data recorded included: weight of the gilts at breeding, at 110 d of gestation, within 12 h after parturition, and at 21 d of lactation, weight of the pigs within 12 h after birth, at 14 d, and at 21 d of lactation, number of stillborn and
mummies, pig mortality, feed consumption, and weaning to remating interval.

Gestation weight gain was calculated as d 110 of gestation weight minus breeding weight, farrowing weight loss was calculated as d 110 of gestation weight minus weight within 12 h after parturition, and lactation weight change was calculated as 21 d of lactation weight minus within 12 h after parturition weight.

**Colostrum and milk.** Milk samples were obtained within 12 h of farrowing (colostrum) and at d 14 and 21 of lactation. An intravenous injection of oxytocin was used to stimulate milk let down. Milk samples were stored at -20°C until analyzed for tocopherol. Milk (2 mL) was deproteinized with redistilled absolute ethanol (3 mL), and tocopherols were extracted with hexane (1 mL). The hexane was injected directly into the HPLC using fluorescence detection as described for the feed analysis.

**Blood.** Blood samples were obtained from the vena cava of the sows at breeding, d 110 of gestation, farrowing, and d 14 and 21 of lactation; and from the orbital sinus of one littermate pig within 12 h after birth, and at d 14 and 21 of lactation (at each lactation period the littermate pig was randomly selected among the pigs alive in the litter). Serum was frozen and stored at -20°C until analysis. The analysis for tocopherol was performed using procedures identical to those described above for milk.

Serum lactate dehydrogenase (LDH) was determined by the method of Amador et al. (1963). The conversion of lactic acid to pyruvic acid is catalyzed by LDH. In this conversion, β-diphosphopyridine nucleotide (β-DPN) is reduced to β-DPNH. This reaction can be followed spectrophotometrically because β-DPNH absorbs at 340 nm wavelength, whereas β-DPN does not. Thus, LDH activity can be measured.

Serum glutathione peroxidase (GSH Px) activity was determined within 12 h of blood
collection by the method of Paglia and Valentine (1967). GSH Px catalyzes the conversion of hydrogen peroxide to water with the oxidation of glutathione. Oxidized glutathione is reduced by glutathione reductase. In this conversion, \( \beta \)-NADPH is oxidized to \( \beta \)-NADP. This reaction can be followed spectrophotometrically because \( \beta \)-NADPH absorbs at 340 nm wavelength and \( \beta \)-NADP does not. Thus, GSH Px activity can be measured.

**Tissues.** The pig randomly selected for bleeding within 12 h after parturition and at d 21 of lactation was electrically stunned and killed by exsanguination. Liver, kidney, heart, longissimus muscle and psoas muscle were excised, weighed, frozen and stored at -20°C. All the dissection work was performed by the same person and following anatomical references for consistency. Longissimus and psoas major muscles were excised from the intervertebral space between the 15th thoracic and the first lumbar vertebrae to the intervertebral space between the 6th and 7th lumbar vertebrae.

Tissue (2 to 3 g) were homogenized in 10 mL (wt/vol) of phosphate-EDTA-buffer (pH 7.0) and deproteinized and extracted as described for milk. Tissues were analyzed for tocopherols by HPLC with fluorescence detection as described for feed. Liver GSH Px activity in the homogenate was determined as described for serum.

**Statistical Analysis**

Data were evaluated by the GLM procedure of SAS (1988). A complete randomized design with an split-split-plot analysis was performed. The whole-plot was treatment with the factorial arrangement of dietary and injectable vitamin E supplementation, the sub-plot was parity, and the sub-sub-plot was gestation and/or lactation stages. Variables measured in the same experimental unit, sow or litter, at different time intervals were analyzed as repeated measures.
Data are reported as least squares means.

**Results**

Three sows were removed from the experiment after completion of the first parity. Two of the sows were in the dietary vitamin E supplemented group and failed to conceive, whereas the other sow was in the vitamin E unsupplemented group and presented legs problems.

**Performance of the Sows**

*Gestation performance of the sows.* Treatments had no effect on the weight of the sows at breeding or 110 d of gestation (Table 2). Those weights increased by parity (P < .001). Weight gain during gestation was also similar among treatments and parities.

*Post-farrowing performance of the sows.* No effect of vitamin E administration on post-farrowing performance of the sows was observed (Table 2). The weight of the sows within 12 h after farrowing, and at 21 d of lactation increased by parity (P < .001). Sow weight change during lactation increased with parity (P < .01). Daily feed intake during lactation also increased (P < .001) by parity. Neither farrowing weight loss nor weaning to remating interval were affected by vitamin E administration or parity.

*Farrowing performance.* The litter weight (live piglets only) and the pig weight at birth increased (P < .05) by parity, but was similar among treatments. The total number of pigs born and born alive per litter decreased (P < .01) in sows fed supplemented diets, and increased (P < .001) with parity (Table 2). The number of born dead and mummies per litter were similar among treatments and parities.
Lactation performance. Neither treatment nor parity had an effect on the weight of the pigs at 21 d of lactation, growth rate to 21 d of lactation, or percentage of mortality during the same time interval (Table 2). Litter weight ($P < .001$) and the number of pigs alive at 21 d of lactation ($P < .01$) increased with parity. At 21 d of lactation, litter size decreased for sows fed unsupplemented diets with injectable d-α-tocopherol, whereas litter size was similar when sows were fed supplemented diets or supplemented diets plus injectable d-α-tocopherol, resulting in a diet x injection interaction ($P < .05$).

Serum Enzymes of the Sows

Treatment did not affect GSH Px or LDH (Table 3) activity ($P > .1$) of the sows. Serum GSH Px activity was greater at breeding than at d 110 of gestation, and serum LDH activity consistently increased at farrowing ($P < .001$). GSH Px activity at d 110 of gestation decreased ($P < .001$) with parity. GSH Px activity at d 14 of lactation increased ($P < .01$) with parity. LDH activity at d 14 of lactation ($P < .05$) and at d 21 of lactation decreased ($P < .01$) with parity.

Tocopherol Status of the Sows

Serum α-tocopherol concentration of sows fed a diet supplemented with d-α-tocopherol increased at breeding ($P < .05$), at d 110 of gestation ($P < .001$), at farrowing ($P < .01$), and at d 14 ($P < .01$) and d 21 ($P < .001$) of lactation when compared with that of sows fed unsupplemented diets (Table 4). Also, serum α-tocopherol concentration of sows injected with d-α-tocopherol was greater ($P < .01$) at farrowing than that of sows that were not injected. Serum α-tocopherol concentration of first parity sows was greater than the concentration of second and
third parity sows at d 110 of gestation (P < .01), at farrowing (P < .05), and at d 14 (P < .001) and d 21 (P < .05) of lactation. Serum α-tocopherol concentration changed with the different stages (P < .01). Serum α-tocopherol concentration was greater at breeding than at d 110 of gestation, but subsequently increased from farrowing to d 21 of lactation.

Concentration of α-tocopherol was 2- to 4-fold greater (P < .001) in colostrum than in d 14 and 21 milk (Table 4). Concentration of α-tocopherol was greater in colostrum of sows supplemented with dietary (P < .001) and/or injectable (P < .01) vitamin E than in colostrum of non-supplemented gilts. The concentration of α-tocopherol in milk from sows fed diets supplemented with vitamin E was increased (P < .001) at d 14 and 21 of lactation as compared with that of sows fed basal diets. At d 14 and 21 of lactation, milk α-tocopherol concentration of the sows fed unsupplemented diets with injectable tocopherol was increased, whereas the combination of dietary and injectable tocopherol supplementation decreased vitamin E concentration, resulting in a diet x injection interaction (P < .05). First parity sows had lower milk α-tocopherol concentration than second and third parity sows at farrowing (P < .001), and d 14 (P < .01) and 21 (P < .001) of lactation.

Enzymes of the Progeny

The serum activities of GSH Px and LDH (Table 5) were not affected (P > .1) by treatment, but increased (P < .001) from birth to d 21 of lactation. Serum GSH Px activity at birth of pigs from second parity sows was greater (P < .01) than that of pigs from first and third parity sows, whereas GSH Px activity at d 14 of lactation of pigs from first parity sows was lower (P < .001) than that of pigs from second and third parity sows, resulting in parity x stage interaction (P < .01). Serum LDH activity at birth of pigs from first parity sows was greater (P
< .05) than that of pigs from second and third parity sows. In contrast, activity of LDH at d 21 of lactation of pigs decreased (P < .01) with parity, resulting in parity x stage interaction (P < .05).

GSH Px activity in liver (Table 5) was not affected by treatment (P > .1), but it increased (P < .001) from birth to d 21 of lactation. At birth, GSH Px activity in liver of pigs from second parity sows was greater (P < .05) than that of pigs from first and third parity sows, whereas at d 21 of lactation, liver GSH Px activity of pigs from first parity sows was lower (P < .001) than that of pigs from second and third parity sows, resulting in parity x stage interaction (P < .001).

Tocopherol Status of the Progeny

**Serum.** Serum α-tocopherol concentration (Table 6) increased over 10-fold from birth to d 14 and 21 of lactation. At birth, serum α-tocopherol concentration was low or not detectable in some samples. Serum α-tocopherol concentration of pigs from sows fed supplemented diets was increased at birth (P < .05) and at d 21 of lactation (P < .01) when compared with that of pigs from sows fed basal diet. On the other hand, serum α-tocopherol concentration of pigs from sows injected with vitamin E was lower (P < .05) than that of pigs from sows not injected with vitamin E only at birth. At d 21 of lactation, the serum α-tocopherol concentration of pigs from sows fed basal diets and injected with d-α-tocopherol was increased, whereas the combination of dietary and injectable supplementation did not increase serum α-tocopherol, resulting in diet x injection interaction (P < .05). At d 21 of lactation, serum α-tocopherol concentration of pigs decreased (P < .05) by parity.

**Tissues.** Concentrations of α-tocopherol in tissues (Table 6) were lower at farrowing than
at d 21 of lactation (P < .001). At birth, dietary vitamin E supplementation of the sows increased α-tocopherol concentration of liver (P < .05), kidney, psoas muscle (P < .01), heart, and longissimus muscle (P < .001) as compared with feeding the control diets. At d 21 of lactation, tissue α-tocopherol concentrations in the offspring increased (P < .001) when sows were fed supplemental vitamin E, but were not affected by injection of vitamin E except for kidney (P < .05).

Treatment and parity did not affect organ weight (Table 7) at birth and at d 21 of lactation, with the following exceptions at birth: psoas and longissimus muscle weights increased (P < .05) by parity, and longissimus muscle weight was similar when sows were fed the basal diets or basal diet plus injectable vitamin E, whereas dietary vitamin E supplementation increased its weight, resulting in a diet x injection interaction (P < .05).

At birth and at 21 d of lactation, total liver, kidney, and heart α-tocopherol content of pigs (Table 8) from sows fed supplemental d-α-tocopheryl acetate was greater (P < .001) than in pigs from sows fed basal diets. At 21 d of lactation, liver α-tocopherol content of pigs decreased (P < .001) by parity. On the other hand, pigs from second parity sows had lower (P < .001) kidney α-tocopherol content than pigs from third parity sows, whereas pigs from second parity sows had lower (P < .001) heart α-tocopherol content than pigs from first and second parity sows.

Discussion

The weight of the sows increased by parity as a result of physiological growth of animals fed diets that met the NRC (1988) requirements which were placed in a sanitary and
environmental controlled facility. Average daily feed intake during lactation was similar among treatment groups, but increased by parity. Therefore, all the animal groups gained weight in a similar manner during lactation, and this weight gain increased by parity.

The pharmacokinetics of the source of injectable vitamin E used in this experiment has been previously described in swine by our group (Carrion et al., 1995). Concentration of α-tocopherol in serum reached a peak around 12 h after i.m. injection. By 72 h, α-tocopherol concentration in serum drastically declined, with a subsequent slow rate of disappearance from serum. Fairly high serum α-tocopherol concentrations were maintained for at least 14 d after i.m. injection.

Ashworth (1991) reported that ovulation in swine takes place 24 to 48 h after the onset of estrus. Therefore, α-tocopherol concentration in serum after i.m. injection at breeding reaches a peak about ovulation time. Around 48 to 56 h after ovulation, the 4-cell embryos enter the uterus, where cell division continues. At d 5 to 6, the blastocyst stage of embryonic development is reached, and hatching of the pig embryo from the zona pellucida occurs 6 to 7 d after breeding. Migration of the embryo within the lumen of the uterus lasts up to 12 d after mating, and then elongation takes place. Most prenatal mortality in swine occurs before the attachment of the embryo in the uterus, when histotroph produced by the uterus is the only source of nutrients (Archibong et al., 1987). It is well established that high uterine blood flow is important for fetal growth during late pregnancy, and that the mammary system develops at the end of gestation. Therefore, i.m. injection of d-α-tocopherol at d 110 of gestation may improve vitamin E status in the neonate, and vitamin E contents of colostrum and milk.

The number of pigs born, and born alive per litter was decreased by dietary vitamin E supplementation of sows, but the number of mummies per litter was similar among treatments.
Therefore, the number of mummies, reflecting mortality of the fetuses after 50 d of gestation, cannot explain the differences found among dietary groups. Thus, these differences must be attributed to reasons such as low ovulation rate, low fertilization rate, or high embryonic death in sows fed the vitamin E supplemented diets, but because we did not estimate any of these traits, we can not conclusively determine which of these factors were responsible for such a response.

Previous reports have suggested greater litter size (Adamstone et al., 1949; Cline et al., 1974; Mahan, 1991b, 1994) and lower mortality (Van Vleet et al., 1973; Mahan, 1991b) in response to supplemental vitamin E. Grandhi et al. (1993) recommended a minimum of 50 IU of vitamin E per kg during pre-breeding and gestation for improved ovulation rate. The same authors found a lack of effect of 50 or 100 IU of dl-α-tocopheryl acetate per kg of diet on progesterone-mediated embryo survival. Vitamin E supplementation in the diet (Babinszky et al., 1992) or through single i. m. injection about 4 days before rebreeding (Myer, 1992) did not affect reproductive performance when the level of vitamin E in the control diets was considered as normal. Feeding sows a cereal grain-based diet without vitamin E or selenium did not result in any sign of myopathies (Mahan et al., 1974) or reduction of litter size (Mahan et al., 1974; Young et al., 1977; Nielsen et al., 1979) until the second reproductive cycle (Mahan et al., 1974).

First parity sows had lighter litters at farrowing and at d 21 of lactation than second and third parity sows due to their smaller litter size. The lower number of pigs alive at d 21 of lactation for sows fed supplemented diets is the result of a reduction in litter size at birth by dietary vitamin E supplementation and no effect of treatment on litter mortality during lactation. Chavez and Patton (1986), Loudenslager et al. (1986), and Mahan (1994) reported no affect of vitamin E supplementation on mortality of pigs during lactation. In our experiment, vitamin E supplementation did not affect pig weight. This lack of response on pig weight is in agreement
with Malm et al. (1976) and Loudenslager et al. (1986).

Plasma GSH Px activity correlates well with Se content of plasma (Meyer et al., 1981). The serum level of Se in the newborn pig reflected the Se content of the sow diet (Mahan et al., 1977; Young et al., 1977; Piatkowski et al., 1979). Supplemental vitamin E had no effect on serum or liver GSH Px activities in our experiment. Serum and liver GSH Px activity of the pigs increased from birth to d 21 of lactation, probably as a consequence of a higher feed (Se) intake of the sows during lactation, and transfer of Se across mammary tissue.

A rise in activity of specific enzymes can be measured before any clinical signs of vitamin E/Se deficiency are observed. Lactate dehydrogenase is one of the enzymes released from damaged cells of skeletal muscle, liver, or heart into the bloodstream. Therefore, LDH can be used to provide the preclinical diagnosis of vitamin E/Se deficiency (Hyldgaard-Jensen, 1973). The activity of LDH in serum of sows and pigs in the current trial suggested that vitamin E/Se deficiency was not present, and this finding was consistent with the studies of Less (1990) and Grandhi et al. (1993). Within those normal values, a consistent increase in serum LDH activity for all the treatments was observed at the time of parturition, coincident with cell damage in the reproductive tract. A similar increase was observed in first parity sows during lactation. On the other hand, serum LDH activity in the sows was lower than that in the pigs. The LDH activity values measured in the newborn pig indicated that the neonates did not have vitamin E/Se deficiency upon birth. These results are in agreement with those of Mahan (1991b).

Serum \( \alpha \)-tocopherol concentration of the pigs at birth was always less than that of the dam. Vitamin E concentrations in tissues and serum of the neonate suggest low quantitative transfer of vitamin E across the pig placenta even though 600 IU of d-\( \alpha \)-tocopherol was injected at d 110 of gestation, coincident with the high placenta blood flow at the end of pregnancy.
Therefore, only a limited and saturated mechanism of vitamin E transport across placenta tissue can explain the lack of response to supplementation of the sow observed in the neonate. Fusion of the chorion and allantois in swine occurs between 30 and 60 d of pregnancy, forming an epitheliochorial placenta in which there is a good separation between maternal blood supply and the absorptive surface of the chorioallantois (Ashworth, 1991). Some studies have reported lower plasma α-tocopherol concentration in the newborn than in the dam (Mino and Nishino, 1973; Young et al., 1977; Loudenslager et al., 1986). In contrast, Malm et al. (1976) reported greater plasma α-tocopherol concentration of the neonate than in the dam. Mahan (1991b) showed lower liver tocopherol concentration in the newborn than in the dam, whereas Kelly et al. (1992) reported greater liver α-tocopherol concentration in the neonatal guinea pig than in the dam. They suggested that vitamin E stored in the liver could be utilized to increase plasma α-tocopherol levels in the neonate. In the present experiment, during dissection of the neonatal pig within 12 h after birth, we recorded whether or not the stomach of the pig was full of colostrum. After comparing this record with the α-tocopherol concentrations in serum and tissues of the pigs, we can state that: first, all the pigs with an empty stomach had low α-tocopherol concentrations in serum and liver; and second, all the pigs with high serum and liver tocopherol concentration had a full stomach, but not all the pigs with a full stomach had high serum and liver α-tocopherol concentrations. This observation suggests that, in a matter of hours after nursing, vitamin E in colostrum would be absorbed, placed in the blood stream (probably in chylomicron), and taken up by the liver. This would imply that, depending on whether or not the neonatal animal nursed and on the time interval between nursing and killing for sample collection, different values would be obtained for α-tocopherol concentration, resulting in a higher variability in α-tocopherol values.

Serum α-tocopherol concentration of sows declined from breeding to parturition and was
associated with high α-tocopherol content of colostrum. This suggests efficient transfer of vitamin E by mammary tissue to colostrum at farrowing. It has been reported that low density lipoprotein receptors increase in mammary gland during formation of colostrum and may help transfer lipophilic substances into the first milk (Schweigert, 1988). Weiss et al. (1992) showed that in cows, this decline in vitamin E concentration in blood at the end of gestation was a result of plasma lipoprotein utilization by the mammary gland, and not a result of a selective removal of vitamin E from blood because the α-tocopherol:cholesterol ratio did not decrease. A similar decline in serum α-tocopherol concentration was reported by Malm et al. (1976), Mahan (1991b), and Grandhi et al. (1993). The concentration of α-tocopherol in colostrum was several fold greater than that in serum of the sows at 110 d of gestation or at parturition. Supplementation of injectable and dietary vitamin E increased colostrum and serum α-tocopherol concentration of the dam at farrowing. Therefore, the newborn pig is dependent on colostrum intake to establish an adequate antioxidant capacity.

Serum α-tocopherol of the dam increased from parturition to d 14 of lactation because feed intake increased and perhaps lower permeability of the mammary tissue to vitamin E compared with parturition time. A decline in α-tocopherol content of milk from 14 to 21 d of lactation was observed, and this observation was consistent with those of Mahan (1994). Vitamin E content of the milk reflected the serum vitamin E concentration of the sow at 14 and 21 d post-parturition. Transfer of vitamin E by mammary tissue to milk is lower in primiparous than in multiparous sows. This may be because the mammary system is immature. As serum and milk α-tocopherol increased, a decline in γ-tocopherol occurred (Loundenslager et al., 1986; Mahan, 1991b). Although the incidence of mastitis, metritis, and agalactia was fairly low, it has been suggested that levels of supplemental vitamin E (44 to 66 IU/kg of diet) higher than the NRC
(1988) recommendations decreased the incidence of this syndrome (Mahan, 1994).

Some of the differences observed between dietary and injectable vitamin E can be explained not only by the frequency of exposure via injectable (acute single dose) or dietary (chronic and continuous dose) treatment, but also by the total amount of vitamin E supplemented, which was 14 times larger for the dietary than the injectable route.

Implications

Vitamin E crosses placental tissue in limited quantities. Therefore, the neonatal pig is dependent on colostrum and milk intake to establish and maintain adequate antioxidant capacity and avoid vitamin E-Se deficiency after weaning. Colostrum and mature milk α-tocopherol content can be increased by dietary or injectable vitamin E supplementation of the sow. Eleven IU of vitamin E per kg of diet for gestating and 22 IU/kg for lactating sows may be adequate for animals in confinement with a good sanitary condition that are fed diets containing .3 ppm of Se for three consecutive parities, when reproductive performance is used as response criterion.

Literature Cited


Table 1. Composition of the basal diets (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>82.20</td>
<td>74.30</td>
</tr>
<tr>
<td>Solvent-extracted SBM</td>
<td>14.30</td>
<td>20.60</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>.90</td>
<td>1.00</td>
</tr>
<tr>
<td>Vitamin premix*</td>
<td>.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Trace mineral premix^b</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Selenium premix^c</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Salt</td>
<td>.50</td>
<td>.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Determined composition of the diet^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>GE, kcal/g</td>
</tr>
<tr>
<td>Dry matter, %</td>
</tr>
<tr>
<td>Protein, %</td>
</tr>
<tr>
<td>Ether extract, %</td>
</tr>
<tr>
<td>Ash, %</td>
</tr>
<tr>
<td>Selenium, ppm</td>
</tr>
<tr>
<td>α-tocopherol, μg/g</td>
</tr>
</tbody>
</table>

*Vitamin composition per kg of diet. Gestation: 2,200 IU of vit. A; 550 IU of vit. D3; 11 IU of vit. E; .011 mg of vit. B12; 3.3 mg of riboflavin; 16.5 mg of niacin; and 8.8 mg of d-pantothenic acid. Lactation: 4,400 IU of vit. A; 1,100 IU of vit. D3; 22 IU of vit. E; .022 mg of vit. B12; 6.6 mg of riboflavin; 33 mg of niacin; and 17.6 mg of d-pantothenic acid.

^bTrace mineral composition per kg of diet: 75 mg of Zn; 87.5 mg of Fe; 30 mg of Mn; 8.75 mg of Cu; and 1 mg of I.

^cProvided .3 mg of Se per kg of diet.

^dValues represent the mean of 18 batches of the diets that were analyzed.
Table 2. Effect of treatment and parity on performance of the sows

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B+I</td>
</tr>
<tr>
<td><strong>Gestation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding wt, kg</td>
<td>150</td>
<td>153</td>
</tr>
<tr>
<td>D 110 gestation wt, kg</td>
<td>197</td>
<td>198</td>
</tr>
<tr>
<td>Gestation wt gain, kg</td>
<td>47</td>
<td>45</td>
</tr>
<tr>
<td><strong>Post-farrowing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-farrowing wt, kg</td>
<td>180</td>
<td>187</td>
</tr>
<tr>
<td>Farrowing wt loss, kg</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>D 21 lactation wt, kg</td>
<td>183</td>
<td>197</td>
</tr>
<tr>
<td>Lactation wt change, kg</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>ADFI lactation, kg</td>
<td>5.93</td>
<td>6.09</td>
</tr>
<tr>
<td>Wean-remating interval, d</td>
<td>4.6</td>
<td>5.4</td>
</tr>
<tr>
<td><strong>Farrowing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total born alive wt, kg</td>
<td>16.0</td>
<td>13.5</td>
</tr>
<tr>
<td>Avg. pig wt, kg</td>
<td>1.25</td>
<td>1.21</td>
</tr>
<tr>
<td>No born/litter</td>
<td>12.9</td>
<td>11.2</td>
</tr>
<tr>
<td>No born alive/litter</td>
<td>12.8</td>
<td>10.8</td>
</tr>
<tr>
<td>No born dead/litter</td>
<td>.12</td>
<td>.40</td>
</tr>
<tr>
<td>No Mummies/litter</td>
<td>.34</td>
<td>.34</td>
</tr>
<tr>
<td><strong>Lactation, at 21 d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total litter wt, kg</td>
<td>53</td>
<td>42</td>
</tr>
<tr>
<td>Pig wt, kg</td>
<td>5.42</td>
<td>5.41</td>
</tr>
<tr>
<td>Growth rate, g/d</td>
<td>199</td>
<td>200</td>
</tr>
<tr>
<td>No pigs alive per litter</td>
<td>9.7</td>
<td>7.5</td>
</tr>
<tr>
<td>% Mortality</td>
<td>15</td>
<td>20</td>
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</table>

*Treatments: B, Basal diet + No injection; B + I, Basal diet + Injection of d-α-tocopherol; D, Dietary supplementation + No injection; and D + I, Dietary supplementation + Injection of d-α-tocopherol.

bEffect of parity (P < .001).

dEffect of parity (P < .05).

eEffect of diet (P < .01).

bEffect of parity (P < .01).

effect of diet (P < .001).

effect of parity (P < .01).

effect of diet (P < .05).

effect of parity (P < .01).

Diet x injection interaction (P < .05).
Table 3. Effect of treatment and parity on serum enzyme activity of sows

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B+I</td>
</tr>
<tr>
<td>GSH Px activity, units/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding</td>
<td>1.45</td>
<td>1.48</td>
</tr>
<tr>
<td>D 110 gestation</td>
<td>.98</td>
<td>1.13</td>
</tr>
<tr>
<td>Farrowing</td>
<td>.81</td>
<td>.97</td>
</tr>
<tr>
<td>D 14 lactation</td>
<td>.96</td>
<td>1.09</td>
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<tr>
<td>D 21 lactation</td>
<td>1.07</td>
<td>1.13</td>
</tr>
<tr>
<td>LDH activity, units/mL</td>
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<td></td>
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<tr>
<td>Breeding</td>
<td>143</td>
<td>159</td>
</tr>
<tr>
<td>D 110 gestation</td>
<td>131</td>
<td>165</td>
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<tr>
<td>Farrowing</td>
<td>220</td>
<td>204</td>
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<tr>
<td>D 14 lactation</td>
<td>190</td>
<td>145</td>
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<tr>
<td>D 21 lactation</td>
<td>222</td>
<td>180</td>
</tr>
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</table>

*Treatments: B, Basal diet + No injection; B + I, Basal diet + Injection of d-α-tocopherol; D, Dietary supplementation + No injection; and D + I, Dietary supplementation + Injection of d-α-tocopherol.

*Glutathione peroxidase. One unit of activity was defined as the amount of enzyme that will convert 1 μmol of NADPH per min at pH 7.0 and 20°C.

*Lactic acid dehydrogenase. One unit of activity was defined as the amount of enzyme per mL of serum that will decrease optical density .001 units*min⁻¹*cm⁻¹ of light path at 340 nm and 20°C.

*Effect of stage (P < .001).

*Effect of parity (P < .001).

*Effect of parity (P < .01).

*Effect of parity (P < .05).
Table 4. Effect of treatment and parity on α-tocopherol concentration of sows (mg/L)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B+I</td>
</tr>
<tr>
<td>Serum&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding</td>
<td>1.20</td>
<td>1.25</td>
</tr>
<tr>
<td>D 110 gestation</td>
<td>.91</td>
<td>1.04</td>
</tr>
<tr>
<td>Farrowing</td>
<td>.75</td>
<td>1.23</td>
</tr>
<tr>
<td>D 14 lactation</td>
<td>1.25</td>
<td>1.49</td>
</tr>
<tr>
<td>D 21 lactation</td>
<td>1.30</td>
<td>1.53</td>
</tr>
<tr>
<td>Milk&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth (colostrum)</td>
<td>3.16</td>
<td>7.03</td>
</tr>
<tr>
<td>D 14 lactation</td>
<td>1.14</td>
<td>1.75</td>
</tr>
<tr>
<td>D 21 lactation</td>
<td>1.17</td>
<td>1.41</td>
</tr>
</tbody>
</table>

<sup>a</sup>Treatments: B, Basal diet + No injection; B + I, Basal diet + Injection of d-α-tocopherol; D, Dietary supplementation + No injection; and D + I, Dietary supplementation + Injection of d-α-tocopherol.

<sup>b</sup>Effect of stage (P < .01).

<sup>c</sup>Parity x stage interaction (P < .001).

<sup>d</sup>Effect of stage (P < .001).

<sup>e</sup>Treatment x stage interaction (P < .001).

<sup>f</sup>Effect of diet (P < .05).

<sup>g</sup>Effect of parity (P < .01).

<sup>h</sup>Effect of diet (P < .01).

<sup>i</sup>Effect of parity (P < .01).

<sup>j</sup>Effect of injection (P < .01).

<sup>k</sup>Effect of parity (P < .001).

<sup>l</sup>Effect of parity (P < .05).

<sup>m</sup>Diet x injection interaction (P < .05).
Table 5. Effect of treatment and parity on enzyme activity of pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment*</th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B + I</td>
<td>D</td>
<td>D + I</td>
<td>SE</td>
<td>First</td>
<td>Second</td>
<td>Third</td>
</tr>
<tr>
<td>Serum GSH Px activity, units/mL^bde</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.02</td>
<td>.18</td>
<td>.23</td>
<td>.16</td>
</tr>
<tr>
<td>Birth</td>
<td>.18</td>
<td>.19</td>
<td>.21</td>
<td>.17</td>
<td></td>
<td>.18</td>
<td>.23</td>
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<tr>
<td>D 14 lactation</td>
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<td>.45</td>
<td>.49</td>
<td>.46</td>
<td>.03</td>
<td>.37</td>
<td>.50</td>
<td>.53</td>
</tr>
<tr>
<td>D 21 lactation</td>
<td>.51</td>
<td>.50</td>
<td>.50</td>
<td>.57</td>
<td>.03</td>
<td>.50</td>
<td>.55</td>
<td>.50</td>
</tr>
<tr>
<td>Serum LDH activity, units/mL^cde</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.03</td>
<td>311</td>
<td>195</td>
<td>220</td>
</tr>
<tr>
<td>Birth</td>
<td>294</td>
<td>215</td>
<td>244</td>
<td>216</td>
<td>41</td>
<td>313</td>
<td>338</td>
<td>317</td>
</tr>
<tr>
<td>D 14 lactation</td>
<td>333</td>
<td>329</td>
<td>312</td>
<td>315</td>
<td>19</td>
<td>313</td>
<td>338</td>
<td>317</td>
</tr>
<tr>
<td>D 21 lactation</td>
<td>364</td>
<td>353</td>
<td>362</td>
<td>319</td>
<td>19</td>
<td>400</td>
<td>347</td>
<td>301</td>
</tr>
<tr>
<td>Liver GSH Px activity, units/g^ef</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.9</td>
<td>10.2</td>
<td>13.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Birth</td>
<td>12.2</td>
<td>11.5</td>
<td>10.7</td>
<td>10.6</td>
<td>.9</td>
<td>10.2</td>
<td>13.0</td>
<td>10.6</td>
</tr>
<tr>
<td>D 21 lactation</td>
<td>20.4</td>
<td>19.3</td>
<td>19.9</td>
<td>19.4</td>
<td>.9</td>
<td>16.3</td>
<td>20.5</td>
<td>22.4</td>
</tr>
</tbody>
</table>

*Treatments: B, Basal diet + No injection; B + I, Basal diet + Injection of d-α-tocopherol; D, Dietary supplementation + No injection; and D + I, Dietary supplementation + Injection of d-α-tocopherol.

^Glutathione peroxidase. One unit of activity was defined as the amount of enzyme that will convert 1 μmol of NADPH per min at pH 7.0 and 20°C.

^Lactic acid dehydrogenase. One unit of activity was defined as the amount of enzyme per mL of serum that will decrease optical density .001 units*min^-1*cm^-1 of light path at 340 nm and 20°C.

^Effect of stage (P < .001).

^Parity x stage interaction (P < .01).

^Parity x stage interaction (P < .001).

^Parity x stage interaction (P < .05).

^Effect of parity (P < .01).

^Effect of parity (P < .001).

^Effect of parity (P < .05).
Table 6. Effect of treatment and parity on α-tocopherol concentration of pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatmenta</th>
<th></th>
<th></th>
<th></th>
<th>Parity</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B+I</td>
<td>D</td>
<td>D+I</td>
<td>SE</td>
<td>First</td>
<td>Second</td>
<td>Third</td>
</tr>
<tr>
<td>Birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum, mg/L</td>
<td>.63</td>
<td>.27</td>
<td>1.26</td>
<td>.54</td>
<td>.18b</td>
<td>.61</td>
<td>.85</td>
<td>.57</td>
</tr>
<tr>
<td>Liver, mg/kg</td>
<td>1.39</td>
<td>.43</td>
<td>3.38</td>
<td>2.38</td>
<td>.90b</td>
<td>2.19</td>
<td>2.30</td>
<td>1.20</td>
</tr>
<tr>
<td>Kidney, mg/kg</td>
<td>.26</td>
<td>.12</td>
<td>.61</td>
<td>.47</td>
<td>.10d</td>
<td>.44</td>
<td>.26</td>
<td>.39</td>
</tr>
<tr>
<td>Heart, mg/kg</td>
<td>.28</td>
<td>.19</td>
<td>.74</td>
<td>.63</td>
<td>.10e</td>
<td>.45</td>
<td>.38</td>
<td>.54</td>
</tr>
<tr>
<td>Psoas muscle, mg/kg</td>
<td>.02</td>
<td>NDb</td>
<td>.14</td>
<td>.06</td>
<td>.03d</td>
<td>.02</td>
<td>.07</td>
<td>.07</td>
</tr>
<tr>
<td>Longissimus muscle, mg/kg</td>
<td>ND</td>
<td>ND</td>
<td>.13</td>
<td>.05</td>
<td>.03e</td>
<td>.01</td>
<td>.05</td>
<td>.08</td>
</tr>
<tr>
<td>D 14 lactation</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum, mg/L</td>
<td>3.60</td>
<td>4.78</td>
<td>4.40</td>
<td>5.36</td>
<td>.53</td>
<td>4.56</td>
<td>4.34</td>
<td>4.71</td>
</tr>
<tr>
<td>D 21 lactation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum, mg/L</td>
<td>3.69</td>
<td>4.92</td>
<td>6.22</td>
<td>5.24</td>
<td>.50g</td>
<td>5.90</td>
<td>5.00</td>
<td>4.16</td>
</tr>
<tr>
<td>Liver, mg/kg</td>
<td>4.64</td>
<td>5.75</td>
<td>9.51</td>
<td>9.11</td>
<td>.72e</td>
<td>10.88</td>
<td>7.74</td>
<td>3.14</td>
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<tr>
<td>Kidney, mg/kg</td>
<td>2.89</td>
<td>4.09</td>
<td>6.28</td>
<td>6.94</td>
<td>.32ce</td>
<td>5.16</td>
<td>4.40</td>
<td>5.60</td>
</tr>
<tr>
<td>Heart, mg/kg</td>
<td>4.12</td>
<td>5.50</td>
<td>8.55</td>
<td>8.68</td>
<td>.34e</td>
<td>7.13</td>
<td>5.73</td>
<td>7.28</td>
</tr>
<tr>
<td>Psoas muscle, mg/kg</td>
<td>2.84</td>
<td>4.00</td>
<td>6.46</td>
<td>6.37</td>
<td>.34e</td>
<td>4.54</td>
<td>4.50</td>
<td>5.71</td>
</tr>
<tr>
<td>Longissimus muscle, mg/kg</td>
<td>1.92</td>
<td>2.83</td>
<td>4.62</td>
<td>4.73</td>
<td>.23e</td>
<td>3.13</td>
<td>3.40</td>
<td>4.04</td>
</tr>
</tbody>
</table>

*Treatments: B, Basal diet + No injection; B + I, Basal diet + Injection of d-α-tocopherol; D, Dietary supplementation + No injection; and D + I, Dietary supplementation + Injection of d-α-tocopherol.

bEffect of diet (P < .05).

cEffect of injection (P < .05).

dEffect of diet (P < .01).

eEffect of diet (P < .001).

fEffect of parity (P < .05).

gDiet x injection interaction (P < .05).

bND = none detected (< .01 mg/kg).
Table 7. Effect of treatment and parity on organ weights (g) of pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatmenta</th>
<th></th>
<th></th>
<th>SE</th>
<th>Parity</th>
<th></th>
<th></th>
<th></th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B+I</td>
<td>D</td>
<td>D+I</td>
<td>SE</td>
<td>first</td>
<td>Second</td>
<td>Third</td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>29</td>
<td>30</td>
<td>37</td>
<td>30</td>
<td>2</td>
<td>31</td>
<td>31</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.8</td>
<td>8.9</td>
<td>11.2</td>
<td>9.2</td>
<td>.6</td>
<td>9.5</td>
<td>9.7</td>
<td>9.3</td>
<td>.5</td>
</tr>
<tr>
<td>Heart</td>
<td>8.9</td>
<td>9.2</td>
<td>11.1</td>
<td>8.6</td>
<td>.5</td>
<td>9.5</td>
<td>9.7</td>
<td>9.2</td>
<td>.5</td>
</tr>
<tr>
<td>Psoas muscle</td>
<td>4.3</td>
<td>4.5</td>
<td>5.2</td>
<td>4.4</td>
<td>.3</td>
<td>3.7</td>
<td>4.9</td>
<td>5.1</td>
<td>.3b</td>
</tr>
<tr>
<td>Longissimus muscle</td>
<td>7.6</td>
<td>8.4</td>
<td>9.9</td>
<td>7.3</td>
<td>.6d</td>
<td>7.2</td>
<td>8.5</td>
<td>9.1</td>
<td>.5c</td>
</tr>
<tr>
<td>D 21 lactation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>129</td>
<td>140</td>
<td>133</td>
<td>139</td>
<td>10</td>
<td>136</td>
<td>136</td>
<td>134</td>
<td>9</td>
</tr>
<tr>
<td>Kidney</td>
<td>37</td>
<td>36</td>
<td>37</td>
<td>35</td>
<td>3</td>
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<td>Heart</td>
<td>31</td>
<td>32</td>
<td>31</td>
<td>29</td>
<td>3</td>
<td>32</td>
<td>30</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>Psoas muscle</td>
<td>24</td>
<td>25</td>
<td>25</td>
<td>24</td>
<td>2</td>
<td>22</td>
<td>25</td>
<td>26</td>
<td>2</td>
</tr>
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<td>Longissimus muscle</td>
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<td>59</td>
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<td>58</td>
<td>5</td>
<td>56</td>
<td>55</td>
<td>57</td>
<td>4</td>
</tr>
</tbody>
</table>

aTreatments: B, Basal diet + No injection; B + I, Basal diet + Injection of d-α-tocopherol; D, Dietary supplementation + No injection; and D + I, Dietary supplementation + Injection of d-α-tocopherol.

bEffect of parity (P < .01).

cEffect of parity (P < .05).

dDiet x injection interaction (P < .05).
Table 8. Effect of treatment and parity on organ α-tocopherol content (μg) of pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment( ^a )</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B + I</td>
</tr>
<tr>
<td>Birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Heart</td>
<td>3.3</td>
<td>1.6</td>
</tr>
<tr>
<td>D 21 lactation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>600</td>
<td>770</td>
</tr>
<tr>
<td>Kidney</td>
<td>105</td>
<td>146</td>
</tr>
<tr>
<td>Heart</td>
<td>130</td>
<td>180</td>
</tr>
</tbody>
</table>

\(^a\)Treatments: B, Basal diet + No injection; B + I, Basal diet + Injection of d-α-tocopherol; D, Dietary supplementation + No injection; and D + I, Dietary supplementation + Injection of d-α-tocopherol.

\(^b\)Effect of diet (\(P < .05\)).

\(^c\)Effect of diet (\(P < .001\)).

\(^d\)Effect of parity (\(P < .001\)).

\(^e\)Effect of parity (\(P < .05\)).
used as a response criterion, 11 IU of vitamin E per kg of diet during gestation and 22 IU/kg during lactation may be adequate. Moreover, dietary and injectable tocopherol supplementation of the sows can improve the tocopherol status of sows and their offspring.
ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my major professor Dr. Richard C. Ewan for his guidance, help, and positive criticism.

To Dr. L. E. Evans, Dr. J. L. Sell, Dr. R. E. Serfass, and Dr. C. R. Youngs for serving on my advisory committee.

To my fellow graduate students, faculty, farm crew, and laboratory technicians for their support and assistance during my study at Iowa State University.

To the "Instituto Nacional de Investigaciones Agrarias" of Spain for financial support.

To Dr. Santiago Martin Rillo for his encouragement and guidance.

Greatest appreciation is due to my family, Carmen, Pedro, Lola and Pilar.

Thank you.