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The absorption and metabolism of vitamin E in swine

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The absorption and metabolism of vitamin E in swine

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GENERAL LITERATURE REVIEW

History of Vitamin E

In the early 1920s, vitamins A, B (thiamine) and C were known to exist and identification of vitamin D was almost complete (Mason, 1977). It had been previously demonstrated that vitamins A and B were essential for normal growth and development in the rat. Yet, when rats were fed semipurified diets supplemented with all known minerals and vitamins, growth was normal but reproduction was frequently affected (Osborne and Mendel, 1919; Mattill and Conklin, 1920). A series of experiments were then conducted to determine whether reproduction required different nutrients than those required for normal growth. Evans and Bishop (1922) found that when rats were fed a semipurified diet, known to produce superior growth and vigor, strange fetal resorption occurred during gestation. But all the early phases of pregnancy were normal. The diet, developed by Osborne and Mendel (1919), consisted of casein, starch, lard, butterfat, salts and brewer's yeast. Evans and Bishop concluded that the diet was missing "factor X", an unknown dietary substance needed for normal reproduction.

After numerous natural feedstuffs were tested, it was found that lettuce, when added to Osborne and Mendel's semipurified diet, prevented rat fetal resorptions. Later it was found that only the fatty component of lettuce leaves,

chorophyll-rich green oil, contained "factor X" (Evans, 1962). Studies using other feedstuffs found that wheat germ oil also contained the factor.

The early hypothesis was that the embryo's requirement for vitamins A and D, at the time the only fat-soluble vitamins known, were not met. It was later demonstrated that rats when fed diets that contained cod liver oil, a source high in vitamins A and D, fetal resorption increased. Evans and Bishop concluded that another fat-soluble dietary vitamin must be responsible for the reproductive failure (Mason, 1977).

Reproductive problems are not limited to the female rat. Testicular atrophy, described in numerous reports, could be prevented but not cured by "factor X" (Osborne and Mendel, 1919; Mattill and Stone, 1923; Mattill et al., 1924; Mason, 1925). Based on these early reports, this new fat-soluble dietary factor was often referred to as the "antisterility factor X". But a few years later, Sure (1924) and Evans (1925) proposed the name vitamin E, the next serial alphabetical designation.

In the following years scientists discovered that a deficiency of vitamin E caused other physiological disease conditions. Evans and Burr (1928) reported paralysis in the suckling young of low-vitamin E mother rats. Pappenheimer and Goettsch (1931) described a "nutritional

encephalomalacia" in chicks fed a semipurified diet, but they did not know the cause. The diet was similar to that of Evans and Burr (1928). A "nutritional muscular dystrophy" was found in guinea pigs and rabbits fed a natural food diet treated with ferric chloride in ether (Goettsch and Pappenheimer, 1931). Waddell and Steenbock (1928) had earlier demonstrated that vitamin E in feed can be destroyed by a ferric chloride treatment. Madsen et al. (1933) reported that cod liver oil when added to the synthetic or natural diets of guinea pigs, rabbits and goats, produced symptoms of nutritional muscular dystrophy. Olcott and Emerson (1937) concluded that the function of vitamin E depends on its ability as a lipid antioxidant. The biological functions of vitamin E continued to be investigated over the next 20 years.

The early vitamin E research was hampered by the lack of a potent source of vitamin E and by long unreliable bioassays for the determination of vitamin E in feedstuffs (Mason, 1977). In the 1930s, major breakthroughs were made in the chemical composition and analysis of vitamin E. Evans et al. (1936) isolated an alcohol from wheat germ oil that had considerable vitamin E-like activity. They suggested the structural formula of $C_{29}H_{50}O_2$ and proposed the name "alpha-tocopherol". Tocopherol is from the Greek words "tokos" (offspring), "pherein" (to bear) and "-ol" (alcohol).

Two other tocopherols, beta and gamma, were isolated from vegetable oils the following year (Emerson et al., 1937). A year later Fernholz (1938) published the structural formula for alpha-tocopherol and Karrer et al. (1938) synthesized the compound. Emmerie and Engel (1938) described a ferric chloride-dipyridyl method for determining vitamin E content in foods. This method was later applied to blood and tissues.

Isolation of other compounds with tocopherol-like activity continued for the next 30 years. A fourth tocopherol, delta, was isolated in 1947 (Stern et al., 1947). Four tocotrienols, containing an unsaturated side chain, were also isolated from a variety of vegetable oils: alpha (Pennock et al., 1964), beta (Green et al., 1960), gamma (Pennock et al., 1964) and delta (Pennock et al., 1964; Whittle et al., 1966). Thus, there are 8 structurally similar naturally occurring compounds. These 8 natural compounds, along with several synthetic compounds that contain tocopherol-like biological activity, make up the generic term "vitamin E".

The Structure of Vitamin E

In the years following the discovery (Evans and Bishop, 1922) and publication of the structural formula (Fernholz, 1938) of alpha-tocopherol, great amount of time and energy

was spent on elucidating the structure and chemistry of numerous compounds with vitamin E-like activity. The backbone structure of tocopherol is tocol (Figure 1). Tocol, 2-methyl 2(4′, 8′, 12′ trimethlytridecyl) chroman 6-ol, consists of a 16 carbon side chain (phytol) linked to a 2 methyl, 6 chromanol aromatic ring. In the tocol structure, R1, R2, and R3 are hydrogen (IUPAC-IUB Commission on Biochemical Nomenclature (CBN), 1974). The present numbering system for the tocol structure was proposed by Karrer et al. (1939).

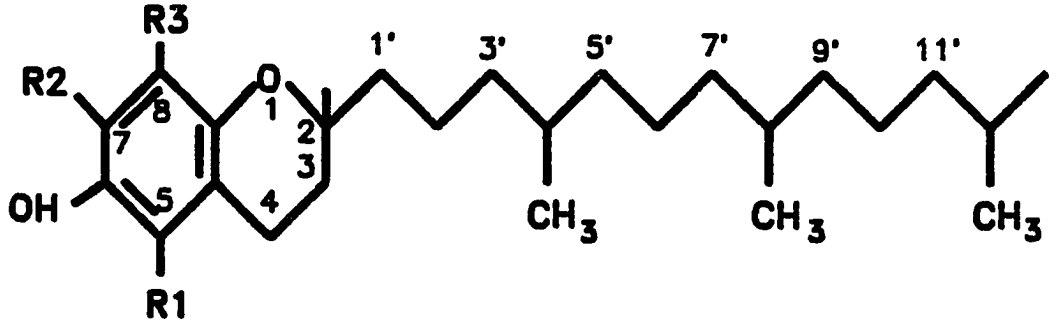

Figure 1. Tocol structure.

Tocopherol is the generic descriptor for all mono-, di- and trimethyltocols. The initial hypothesis was that tocol derivatives, alpha, beta, gamma, and delta, only differed by the number and position of the methyl groups on the benzeniod ring of the chromanol structure (Table 1). This hypothesis

proved to be incorrect when other tocol derivatives, tocotrienols, were discovered (Pennock et al., 1964; Whittle et al., 1966).

Table 1. Position of methyl groups in vitamin E isomers

Position[a]	Tocopherols	Tocotrienols
R1, R2, R3	Alpha	Alpha
R1, R3	Beta	Beta
R2, R3	Gamma	Gamma
R3	Delta	Delta

[a]Refer to Figure 1.

Tocotrienols have the same ring structure as tocopherols but contain an unsaturated side chain and double bonds between carbons 3′ and 4′, 7′ and 8′, and 11′ and 12′. Biopotency of the tocol isomers differs by both the number and position of methyl groups, and degree of saturation of the side chain.

The full chemical name of alpha-tocopherol is 2, 5, 7, 8 -tetramethyl-2-(4′, 8′, 12′-trimethyl-tridecyl) chroman-6-ol, whereas the full chemical name for alpha tocotrienol is 2, 5, 7, 8-tetramethyl-2-(4′, 8′, 12′ - trimethyl-trideca-3′, 7′, 11′-trienyl) chroman-6-ol (IUNS Committee on Nomenclature, 1978). The structure of alpha-tocopherol indicates that

there are three centers of asymmetry: at carbons -2, -4′ and -8′. Tocotrienols contain only one center of asymmetry at C-2, but contain two sites of geometrical isomerism: position C-3′ and C-7′ (Machlin, 1984). Natural alpha- (Mayer et al., 1963), beta- and gamma- (Isler et al., 1962) tocopherols were shown to have a 2R, 4′R, 8′R configuration. This 2R, 4′R, 8′R configuration, the only form found in nature, is often referred to by the trivial prefixes RRR or [d]. Natural tocotrienols have been assigned a 2R, 3′-trans, 7′-trans configuration (Schudel et al., 1963; Mayer et al., 1967).

Whereas all natural tocopherols have a R-configuration at the C-2 position, many structural isomers of RRR-tocopherol have been synthesized. The isomer 2-epi alpha-tocopherol, 2S, 4′R, 8′R tocopherol, is epimeric only at the C-2 position. The semi-synthetic 2-ambo-alpha-tocopherol is produced from natural phytol and is a mixture of 2R, 4′R, 8′R and 2S, 4′R, 8′R tocopherols. But, not necessarily in equimolar proportions. The totally synthetic isomer, all-rac- or [dl]-alpha-tocopherol, is a condensation product of trimethylhydroquinone and racemic isophytol. The result is a totally synthetic mixture of the 4 possible enantiomeric pairs of the 8 diastereoisomers (Ullrey, 1981). Vitamin E nomenclature has a controversial history and this literature review was intended to provide

only a brief review of the subject. More extensive reviews on this topic can be found elsewhere (IUPAC-IUB Committee on Biological Nomenclature, 1974; IUNS Committee on Nomenclature, 1978).

Within plants, tocopherols are found as a free alcohol and are associated with the lipid portions of green leaves and seeds. The biosynthesis of tocopherols within plants occurs in stages. The tocotrienol structure is synthesized and then the chromanol ring is methylated. Tocopherol is produced when the side chain is hydrogenated (Pennock et al., 1964). Animals can not synthesize tocopherol, therefore, they must acquire their tocopherol requirements through the consumption of plant foliage, seeds or animal tissue products. In animals, tocopherol is associated with lipid portion of cell and cell component membranes.

As a free alcohol, tocopherols are relatively unstable due to the ease of oxidation of the hydroxyl group at position C-6. Thus, tocopherols can be easily oxidized by light, oxygen, heat, alkali and trace minerals (Cu and Fe). Tocopherol stability can be increased in heat and alkali by the removal of oxygen. Pure natural tocopherol, a slightly viscous, pale yellow oil, is insoluble in water but freely soluble in fat, oil and fat solvents.

The oxidative stability of tocopherol can be increased by the acylation of the free phenolic hydroxyl at position

C-6 (Ullrey, 1981). Many current supplemental forms of tocopherol are ester derivatives. The principal types being the acetate or hydrogen succinate ester of RRR-alpha-tocopherol, and acetate ester of all-rac-alpha-tocopherol (Ames, 1979). Both acetate, CH_3COO^-, and succinate, $HOOCCH_2CH_2COO^-$, react with the hydroxyl group at position C-6 on the tocopherol structure. Ester derivatives are relatively unaffected by oxygen and ultraviolet light. In pure form, acetate esters are light yellow, nearly odorless, clear viscous oil, whereas succinate esters are white, nearly odorless powders. The feed industry commonly uses beadlets or powders containing tocopheryl acetate incorporated in oil or in an emulsified form. Gelatin and sugar, gum acacia, soygrits or dextrin are commonly used as carriers (Ullrey, 1981).

Standardization of Vitamin E

In the 1930s and 1940s, many new compounds with vitamin E-like activity were discovered or produced. And there was a general agreement throughout the world that a standard for vitamin E needed to be established. A standard would allow for the comparison among the various forms of vitamin E. The history of the vitamin E standard was marred with confusion and disagreement. In 1947, the World Health Organization (WHO) Expert Committee on Biological Standardization approved

an International Standard for vitamin E, as proposed by the Department of Biological Standards in 1941. The standard, an International Unit (IU), was defined as the average amount of orally administered vitamin required to prevent fetal gestation resorption in vitamin E deprived rats. An IU was equal to 1.0 mg of [dl]-alpha-tocopheryl acetate. The standard was based on data using the synthetic compound racemic alpha-tocopheryl acetate. This compound was synthesized by condensation of trimethyl hydroquinone and phytol bromide. The phytol was isolated from natural sources. Later, this product was labeled as [dl]-alpha-tocopheryl acetate (Diplock, 1985).

Advances in chemistry led to the chemical synthesis of authentic racemic isophytol and this was used in the synthesis of another compound, also called [dl]-alpha-tocopheryl acetate. But the stereochemistry of these two compounds was different. The latter compound, all-rac-alpha-tocopheryl acetate, was a non-equimolar mixture of the 8 distereoisomers. The IU was actually set to the compound 2-ambo-alpha-tocopheryl acetate, an unequal mixture of RRR- and SRR-alpha-tocopheryl acetate. The confusion existed because the standard was established using 2-ambo-alpha-tocopheryl acetate but was reported in the literature as all-rac- or [dl]-alpha-tocopheryl acetate. Once the initial supply of 2-ambo-alpha-tocopheryl acetate

was exhausted, it was impossible to exactly reproduce the initial mixture.

In 1957, the WHO Expert Committee on Biological Standards reported that the International Standard for vitamin E ceased to exist; the IU is still in use in the United States and Canada (WHO Expert Committee on Biological Standardization, 1957; Diplock, 1985). In 1962, the Animal National Research Council (ANRC) introduced the ANRC Vitamin E Reference, a gelatin beaded preparation of 2-ambo-alpha-tocopheryl acetate. But, the reference was labeled [dl]-alpha-tocopheryl acetate (Matterson, 1962). The discrepancy on a vitamin E standard increased. The U.S. Pharmacopeia and National Formulary (1980) attempted to resolve the confusion with weight/unit relationships for various vitamin compounds by using USP units (Table 2).

Table 2. USP units per mg of vitamin E compound[a]

Compound	USP unit
All-rac-alpha-tocopheryl acetate, [dl]	1.00
All-rac-alpha-tocopherol, [dl]	1.10
RRR-alpha-tocopherol acetate, [d]	1.36
RRR-alpha-tocopherol, [d]	1.49
All-rac-alpha-tocopheryl acid succinate, [dl]	0.89
RRR-alpha-tocopheryl acid succinate, [d]	1.21

[a]U.S. Pharmacopeia and National Formulary, 1980.

But these units are numerically equal to the IU that was adopted from Harris and Ludwig's (1949a, b) work. Ames (1979) concluded that because the standard was set to 2-ambo-alpha-tocopheryl acetate but reported as all-rac-tocopheryl acetate, the USP unit bioequivalences were invalid. But, Weiser and Vecchi (1981) reported that the previously established biopotencies were considered to be valid.

In an attempt to reduce the confusion on the lack of a biological standard for vitamin E and possibly reintroduce an international standard, an informal meeting was held in 1982 at WHO. The meeting ended in agreement that there was a need for a reference standard for vitamin E activity. But no decision was made as to the relative biological activity of RRR-alpha-tocopheryl acetate and all-rac-alpha-tocopheryl acetate, or whether the reference be biological or chemical. The committee concluded that international reference materials are unnecessary, and it is not appropriate for the committee to designate fixed biological relationships between various forms of alpha-tocopherol. Also, the assessment of therapeutic claims made with respect to particular forms of alpha-tocopherol is the responsibility of individual nation control authorities (WHO Expert Committee on Biological Standardization, 1983).

Structure and Biological Activity

The main components of the tocol structure must be considered when discussing the biological activity of tocopherols (refer to previous section). The hydroxyl group at position C-6 plays an important role in the biological function of vitamin E. The hydrogen of the hydroxyl group is donated to a free radical, stabilizes the radical and prevents propagation. A tocopherol radical, formed when hydrogen is donated from position C-6, can be regenerated to tocopherol (Diplock, 1985). Tocopherol quinone, the next product formed in the metabolism of tocopherol, can not be converted to tocopherol and is not active. Replacing the hydroxyl group with an ether or allophanate results in a loss of vitamin E-like activity. Esterification or substitution of an amino group at the position C-6 does not alter the biological activity (Kasparek, 1980).

The number and position of methyl groups on the chromanol ring also influences the biological activity. The fully methylated RRR-alpha-tocopherol has the highest biological activity and is the most predominant isomer found in blood and tissues. The loss of methyl group(s) reduces the biological activity (Table 3). Gamma-tocopherol has considerably less activity than alpha-tocopherol; however, the concentration of gamma-tocopherol in the diet is two to three times that of alpha-tocopherol. The in vitro

antioxidant activity of tocopherols is the opposite of in vivo activity. This effect may be due to differences between isomers in tissue retention. Synthetic 5, 7-dimethyltocol has about 60% of the biological activity of RRR-alpha-tocopherol, whereas 5- or 7-methyltocol has no activity (Kasparek, 1980). Differences in number and position of methyl groups on chromanol ring of tocotrienols has a similar effect on biological activity as that observed in tocopherols.

Table 3. Biological activity of tocopherols and tocotrienols

Compound	Position of methyl group	Bio-activity[a]
Alpha-tocopherol	R1, R2, R3	100
Beta-tocopherol	R1, R3	40-50
Gamma-tocopherol	R2, R3	1-11
Delta-tocopherol	R3	1
Alpha-tocotrienol	R1, R2, R3	29
Beta-tocotrienol	R1, R3	5

[a]Machlin, 1984: rat fetal resorption assay.

The isoprenoid side chain of tocopherols facilitates the incorporation and retention in biological membranes (Niki et al., 1985) and, therefore, has an important role in the biological activity. Adding a double bond, shortening,

lengthening, or eliminating the side chain of tocopherols reduces the biological activity. Tocotrienols contain an unsaturated side chain, and have lower biological activity than their tocopherol counterparts (Kasparek, 1980).

The three centers of asymmetry in the tocol structure also influence the biological activity of tocopherols. Weiser and Vecchi (1982) used a rat fetal resorption assay to compare the eight stereoisomers of tocopherol. They concluded that the configuration at position C-2 had the most influence on biological activity. At position C-2 the S-configuration had lower activity than the R-configuration. A similar trend was seen at position C-4'; the R-configuration had higher activity than S-configuration. The configuration at position C-8' had only a minor influence on biological activity (Table 4). Machlin et al. (1982) concluded that only the configuration at position C-2 is important in biological activity. Weiser and Vecchi (1982) also reported a synergistic action between isomers when one or more isomers were present. A difference of 31% was found between the measured biological activity of individual isomers and the calculated biological activity of individual isomers. The lower biological activity of non-RRR-alpha-tocopherol isomers may indicate that animal systems prefer natural (RRR-) alpha-tocopherol.

Table 4. The effect of stereoisomerism on biological activity

Configuration			
C-2	C-4′	C-8′	Bio-activity, %[a]
R	R	R	100
R	R	S	90
R	S	S	73
S	S	S	60
R	S	R	57
S	R	S	37
S	R	R	31
S	S	R	21

[a]Weiser and Vecchi, 1982.

Biological membranes in animals may act as chiral discriminators, retaining RRR-alpha-tocopherol more efficiently than other tocopherols (Burton and Ingold, 1986).

Vitamin E Assays

The two major assay methods for tocopherol assessment are chemical and biological. Chemical assays are used for the qualitative and quantitative analysis tocopherols. Biological assays allow for the measurement of the biological activity of tocopherols and tocopherol-like compounds. There are a large number of procedures available today for the extraction and separation of tocopherols from synthetic and biological materials. Most procedures involve solvent extraction and chromatographic separation. Recent reviews have extensively covered the available procedures (Desai, 1980; Parrish, 1980; Machlin, 1984; Diplock, 1985).

Chemical Assay

The three common methods for tocopherol detection in chemical assays are: spectrophotometric, colorimetric, or spectrofluorometric detection. Spectrophotometric detection uses the measurement of light absorption at the wavelength of maximum absorption for the tocopherol under investigation. Concentration can be determined directly from the extinction coefficient of the respective tocopherol. In the classical method of Emmerie and Engel (1938), tocopherols reduce ferric iron to ferrous iron stoichiometrically in an ethanolic solution. The ferrous ions form a red complex with 2, 2'-dipirdyl, and are measured spectrophotometrically at 520 nm. The detection of tocopherols by the colorimetric method is sensitive and easily performed, but is non-specific.

Spectrofluorometry is the most sensitive method for tocopherol detection. The usage of this method increased when Duggan (1959) discovered that tocopherols have their own natural fluorescence. Fluorometric detection is simple, fast, very sensitive and accurate. Few biological substances interfere with tocopherol fluorescence. Fluorometric methods typically use an excitation wavelength of 295 nm and an emission wavelength of 340 nm (Machlin, 1984).

The recent advances in chromatography, especially high performance liquid chromatography (HPLC), has greatly increased the speed and accuracy of tocopherol analysis.

With minimal amount of cleanup time, tocopherol isomers can be separated easily by a HPLC system. A number of recently published reviews have extensively covered chromatographic techniques used in tocopherol analysis (Buttriss and Diplock, 1984; Ball, 1988; DeLeenheer et al., 1988).

Bioassay

Bioassays are the only true measure of the biological activity of tocopherols. In bioassays, tocopherol or compounds containing tocopherol-like activity are supplemented in attempt to reverse the symptoms of a vitamin E deficiency. Three types of bioassays are routinely used: rat fetal resorption or testicular atrophy, erythrocyte hemolysis, and the occurrence of nutritional muscular dystrophy symptoms. The fetal resorption test was the first method used for the assessment of vitamin E activity. Before the start of the test, rats are fed a tocopherol-free diet for 3 to 4 months. The rats are test-mated to fertile males to ensure they are vitamin E deficient. If no living fetuses are detected on day 20 of gestation, depletion is deemed adequate and the experiment is started. The rats are then mated and during days 5 to 9 of pregnancy graded levels of a tocopherol standard, commonly RRR-alpha-tocopherol, and unknown test substance are administered. On day 19 of gestation, the rats are killed and the uteri are examined for

living fetuses and implantation sites of resorbed fetuses. The test substance is compared to the tocopherol standard. This assay is tedious, time consuming and requires numerous replications. But the test is still regarded as an accurate reference point in assessing biological activity (Machlin, 1984; Diplock, 1985).

The hemolysis of blood cells serves as another bioassay to assess the biopotency of tocopherols. But this test is not a direct measure of in vivo tocopherol biopotency. The hemolysis test measures the in vitro hemolytic effect of an oxidizing agent, usually dialuric acid or hydrogen peroxide, to assess the protective effect of tocopherol against these agents. Erythrocytes from deficient rats, fed a tocopherol deficient diet for 3 to 4 weeks will show no spontaneous hemolysis in vitro. The addition of dialuric acid to vitamin E deficient rat blood will cause greater than 95% hemolysis of erythrocytes. In the test, once a vitamin E deficient state in the rat is obtained, graded levels of RRR-alpha-tocopherol and test substance are orally administered. Forty hours after the dose, the hemolysis test is conducted and the percentage of red blood cell hemolysis is calculated (Friedman et al., 1958).

Nutritional muscular dystrophy is a common vitamin E deficiency symptom in many animal species. Both the time of onset of creatinuria and the extent of muscular degeneration

lesions have been used as indirect measures of tocopherol status. Tocopherols and test compounds are evaluated on their effectiveness to alleviate nutritional muscular dystrophy symptoms.

Absorption

Vitamin E absorption is dependent upon normal lipid digestion and absorption. The efficiency of tocopherol absorption, therefore, is related to normal pancreatic function, bile secretion, micelle formation and penetration across intestinal membrane (Bjorneboe et al., 1990). Dietary tocopherol is released from associated proteins by proteolytic enzymes and(or) acidity in the stomach. Tocopherols then flow into the small intestine with other digesta where they are dissolved in fat globules which are produced from dietary lipids. These fat globules are referred to as the oily phase (Weber, 1981, 1984).

The oily phase, by the action of bile and pancreatic juice, is transformed into a micellar phase. Both pancreatic juice and bile are required for maximal lipid absorption; if one or both are lacking, lipid and fat-soluble vitamin absorption will decrease. Vitamin E absorption is substantially decreased in patients with lipid malabsorption disorders. Pancreatic lipases hydrolyze triglycerides into long chain fatty acids and monoglycerides. Bile salts exert

a detergent action, are cofactors for enzymes and are involved in the formation of mixed micelles. The formation of micelles is necessary for normal lipid absorption. Mixed micelles contain monoglycerides, long chain fatty acids, phospholipids, cholesterol, bile salts and fat-soluble vitamins. Tocopherol within mixed micelles is mostly in the alcohol form. Tocopherol absorption is enhanced by the presence of dietary lipids, especially medium chain fatty acids. But, polyunsaturated fatty acids (Gallo-Torres et al., 1971) and retenoic acid (Berri et al., 1981) have been shown to reduce tocopherol absorption.

The first step in the uptake of fat-soluble vitamins is the passage through the unstirred water layer and the lipid membrane of the absorptive cells to finally arrive at the brush border. Micelles collide with the cell membrane and the components of the mixed micelles, except bile salts, penetrate the cell membrane and eventually reach the cytoplasm. The uptake of tocopherol from the gut is thought to be a non-saturable diffusion process, not carrier mediated. The movement of tocopherol through the epithelial cell is not well understood and is thought to require several stages (Gallo-Torres, 1980; Diplock, 1985).

Eventually, tocopherol in the cell is released into the lymphatic system within chylomicrons. Mammals transport most of the absorbed tocopherol from the gut circulation via the

lymphatic system, however, a small amount may be absorbed and transported through the portal system. In birds, fat-soluble vitamins are extensively absorbed though the portal system. The major site of tocopherol absorption in mammals is the medial segment of the small intestine; no tocopherol is absorbed in the large intestine (Gallo-Torres, 1980).

Hydrolysis of tocopherol esters is not necessary for absorption, but hydrolysis improves absorption (Gallo-Torres, 1980). Esters are hydrolyzed to free alcohol in the intestinal lumen by pancreatic lipases (Diplock, 1985) and(or) carboxylic ester hydrolase (Lombardo and Guy, 1980; Mathias et al., 1981a). There is limited evidence of ester hydrolysis inside the enterocyte of the rat by a mucosal esterase (Mathias et al., 1981b).

The absorption efficiency of tocopherols and tocopherol esters is relatively poor. Most experiments have used alpha-tocopheryl acetate to measure absorption efficiency while few studies have used alpha-tocopherol. Losowsky (1979) concluded that on average only 33% of the dietary vitamin E is absorbed into the body. Gallo-Torres (1980) reported that absorption efficiency is somewhat influenced by experimental protocol and that 20 to 40% of oral intake is absorbed. Dietary constituents, minerals and lipids, may cause autoxidation of alpha-tocopherol in the gastrointestinal tract and decrease the percentage of

absorption (Burton et al., 1988).

The mode of administration affects absorption efficiency. An oral dose of tocopherol as an aqueous-miscible emulsion is absorbed twice as well as tocopherol in an oily form (Schmandke and Schmidt, 1965). The vitamin E status, deficient vs non-deficient (Burton et al., 1988), nutritional status, fasting vs non-fasting, and percent fat in diet, does not affect the percent of absorption of dl-alpha-tocopherol (Kelleher et al., 1972). As the dose of tocopherol increases, the efficiency of absorption decreases (Traber et al., 1986).

The rate and amount of absorption of tocopherols and tocotrienols are in the same general order of magnitude as their biopotencies (Ullrey, 1981). Alpha- and gamma-tocopherol are absorbed equally as well (Traber et al., 1986), but there may be a preferential secretion of gamma-tocopherol into bile (Traber and Kayden, 1989).

Transport

Within the mucosal cell, lipids and fat-soluble vitamins accumulate into fat droplets inside the smooth endoplasmic reticulum (SER). Vitamin A is re-esterified in the SER, however, vitamin E does not have to be re-esterified for transport to take place. The fat droplets are released by the SER and taken up by the Golgi complex. The Golgi complex

encloses lipids and fat-soluble vitamins with apoproteins to form triglyceride rich lipoproteins, chylomicrons and very low density lipoproteins (VLDL). Encased within lipoproteins, lipids and fat-soluble vitamins can be transported in the aqueous medium of the extracellular spaces. Lipoproteins are released into the lateral intercellular spaces and eventually reach the general circulation through the intestinal lymphatics (Weber, 1981).

Vitamin E circulates in lymph and blood bound non-specifically to lipoproteins. No specific plasma transport protein for vitamin E has been identified. Lipoproteins are commonly classified by their flotation density: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Lipoproteins are composed of a hydrophobic core, contains triacylglycerols and cholesterol esters, and an amphipathic surface, contains unesterified cholesterol, phospholipids and apolipoproteins. Chylomicrons and VLDL are synthesized in the intestines; and the liver synthesizes VLDL and HDL. High density lipoproteins and LDLs are formed in the plasma. A majority of the tocopherol in chylomicron/VLDL is contained within the core but some can be found at the surface. Each lipoprotein fraction contains tocopherol with the majority in the beta-lipoprotein fraction (Machlin, 1984).

In the general circulation, tocopherol contained within chylomicrons and VLDLs is rapidly exchanged with other lipoproteins. The specific lipoprotein fraction that transports the majority of the tocopherol, varies by species and nutritional state. Tocopherol is also transported within erythrocyte membranes and they contain about 20% of the amount found in plasma (Chow, 1975). Erythrocytes preferentially retain RRR-tocopherol over SRR-tocopherol and secrete SRR-tocopherol into plasma (Cheng et al., 1987). There is a rapid exchange of tocopherol between plasma and erythrocytes; 25% turnover per hour (Kayden and Bjornson, 1972). A high correlation can be found between total serum lipid concentration and serum tocopherol concentration (Gallo-Torres, 1980). Tocopherol concentration in blood is affected by dose of tocopherol, concentration of lipids and lipoproteins in blood, rate of tocopherol removal by tissues, and tissue retention of tocopherol. Tocopherol is also found in blood platelets and may serve as a sensitive indicator of tocopherol status (Lehmann et al., 1988).

The half-life of radiolabeled tocopherol in general circulation is about 12 minutes (Bjorneboe et al., 1987), whereas the half-life of chylomicrons ranges from 5 to 15 minutes. Plasma alpha-tocopherol is cleared by the liver in association with chylomicron remnants (Bjorneboe et al., 1990). Chylomicron remnants are formed from the action of

lipoprotein lipase on chylomicrons. Parenchymal cells within the liver contain the majority of hepatic tocopherol (Bjorneboe et al., 1986). The liver secretes tocopherol into the circulatory system via VLDL. Alpha-tocopherol-quinone is the primary hepatic oxidation product of alpha-tocopherol (Bjorneboe et al., 1990).

Tissue Deposition

Tocopherol is rapidly taken up by all tissues and is stored in an unmodified, non-esterified form. Vitamin E will accumulate in all tissues over time (Machlin and Gabriel, 1982). Tissue uptake of tocopherol differs from that of other vitamins; there is no deposition threshold in any tissue except the liver. Two mechanisms have been recognized to deliver tocopherol to tissues. Lipoprotein lipase hydrolyzes the triglycerides carried within chylomicrons and VLDL, and may play a role in tocopherol transfer to tissues (Traber et al., 1985). A low density lipoprotein receptor mechanism that delivers LDL to cells may also deliver tocopherol (Traber and Kayden, 1984). Adipose tissue, liver and muscles are the major storage sites for tocopherol. The amount of tocopherol in tissues is directly related to the logarithm of tocopherol intake (Gallo-Torres, 1980).

Tissue Depletion

Depletion of body stores of vitamin E varies among species and by tissue. After the removal of vitamin E from the diet, plasma and liver are rapidly depleted of tocopherol. Cardiac and skeletal muscle tocopherol are depleted more slowly (Gallo-Torres, 1980). Tocopherol within adipose tissue is depleted very slowly or negligibly. Tocopherol deposited in adipose tissue may not be bioavailable (Machlin et al., 1979) and may be one of the largest non-exchangable pools of tocopherol in humans and lower animals (Gallo-Torres, 1980). But, swine may be able to draw upon vitamin E stored in adipose tissue (Jensen et al., 1990). Berri (1972) reported that tissue other than adipose tissue contains a labile pool and a fixed pool of tocopherol. The fixed pool is retained for long periods. Gamma-tocopherol is taken up by tissues but is eliminated from tissues at a faster rate than alpha-tocopherol (Peak and Berri, 1971). Thus, the methyl group at position C-5 may also be important in tissue retention of tocopherols (Gallo-Torres, 1980).

Metabolism and Excretion

Tocopherol metabolism remains unclear and the full pathway is yet to be proven. A major problem is the difficulty to distinguish between tocopherol derivatives that

arise as true metabolites in vivo and those that arise as artifacts of the isolation procedure (Diplock, 1985). Numerous oxidation products of tocopherol can be produced in vitro, depending on the type of solvent used in the procedure. But, whether any or all of these products occur in vivo is yet to be resolved.

It is generally accepted that the first stage of vitamin E metabolism is the opening of the chromanol ring in the oxidative conversion of tocopherol to a tocopheroxy-radical (TR). The TR can then be converted to tocopherol-quinone (TQ). The conversion of tocopherol to TR is reversible, whereas the conversion of TR to TQ is not (Figure 2). Tocopherol-quinone can be biologically reduced to tocopherol-hydroquinone (THQ); this reaction is also reversible.

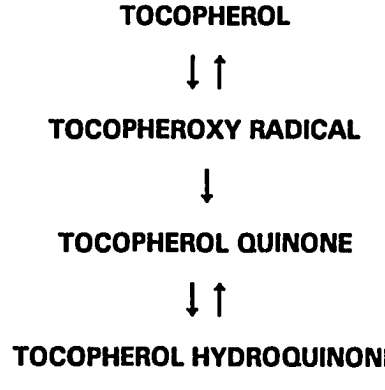

Figure 2. Metabolites of tocopherol

Tocopherol-quinone and THQ have little vitamin E-like activity. The liver conjugates TQ and it is excreted in bile. Within the kidney TQ is reduced, conjugated, and the side chain is oxidatively degraded to form a conjugate of tocopheronic acid (Figure 3). This product is excreted in urine. Tocopheronic acid can also be converted to tocopheronolactone and is also excreted in urine (Olson, 1981; Weber, 1981).

Also, tocopheronic acid and tocopheronolactone can be conjugated with glucuronic acid and excreted in urine as water soluble detoxification products of tocopherol. These products have no vitamin E-like activity. Radioactivity was found associated with tocopheronic acid and tocopheronolactone in the urine of rabbits and humans dosed with radiolabeled tocopherol. These metabolites are commonly referred to as Simon's metabolites (Simon et al., 1956). The formation mechanism of these urine metabolites is not known and, at a physiological dose, represents a minor excretion pathway for tocopherol. Tocopherol quinone can also be biologically reduced to a dimer; the structure is not well defined. The dimer can alternatively be converted into a trimer (Figure 4). Dimer and trimer oxidation products have been reported in the literature but it is not clear whether these products are present in mammalian tissue under physiological conditions (Gallo-Torres, 1980).

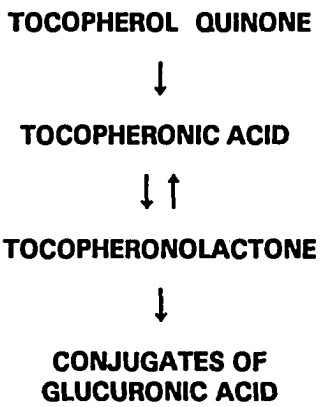

Figure 3. Urinary metabolites of tocopherol-quinone

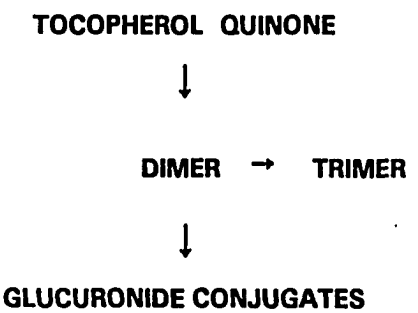

Figure 4. Fecal metabolites of tocopherol-quinone

There has been some evidence of the occurrence of tocopherol metabolites (TQ, THQ, dimer, trimer, and more polar products) in tissues (Machlin, 1984). Oxidation of tocopherol within the gastrointesinal tract may add metabolites to the feces.

The major excretion route for tocopherol and its metabolites is through the feces. Urinary excretion represents a minor pathway, while 10 to 75 % of a dose can be recovered from feces. The amount recovered depends on the biological preparation and analytical methods used. The possible sources of fecal tocopherol include incomplete absorption, secretion from intestinal cells into the lumen, desquamation of intestinal epithelium and excretion in bile (Diplock, 1985). Little free alpha-tocopherol appeared in bile after a dose of ^{3}H-alpha-tocopherol or ^{3}H-alpha-tocopheryl acetate. But a large amount of radioactivity was found associated with an unknown tocopherol metabolite, thought to be a conjugate of glucuronic acid that differed from Simon's metabolites (Gallo-Torres, 1980). Ingold et al. (1987) reported that natural undeuterated tocopherol was found in the feces of rats months after being fed a diet with deuterated tocopherol as the only source of vitamin E. They concluded that tocopherol was returning to the gut from body tissues. The source of the undeuterated tocopherol may be plasma, lymph or bile.

Vitamin E Requirement for Swine

General

A nutrient requirement may be defined as the minimum level required to prevent deficiency symptoms and still allow for adequate performance, provided sufficient amounts of all other nutrients are provided. But, the requirement level may not be adequate for maximum performance, since most requirement levels do not include a surplus. Vitamin intake can be expressed as marginal, requirement, optimum, or excess. A marginal level of vitamin intake is lower than the requirement and may predispose the animal to a deficiency. A requirement level prevents deficiency symptoms from occurring but it may not provide adequate amounts of vitamin for optimum growth. An optimum level of intake allows the animal to reach its genetic potential for growth. A vitamin provided in excess is still safe in most instances but may prove to be toxic for some vitamins. An excess amount of vitamins, however, may not be economical (McDowell, 1989).

When recommending levels of dietary vitamin E supplementation, one must consider several factors that may influence the concentration of vitamin E in the ration and(or) affect the ability of the animal to utilize the vitamin. Hence, the level of vitamin E supplementation may need to be adjusted accordingly (McDowell, 1989). Ullrey (1981) and NRC (1988) have published comprehensive reviews of

recent research on establishing the tocopherol requirement for swine. Rather than repeating the data, the following sections will concentrate on factors that may influence the vitamin E requirement for swine.

Role of Selenium

Selenium (Se) has an important interrelationship with vitamin E. It is impossible to determine the requirement for vitamin E without considering the Se level in the diet. Selenium delays the onset of some vitamin E deficiency symptoms, and vitamin E and Se have a close relationship within the cell. Selenium is known to reduce tocopherol requirements in 3 ways: 1) preserves the integrity of the pancreas in the chick and allows normal fat digestion, 2) reduces the amount of tocopherol required to maintain membrane integrity through glutathione peroxidase, and 3) aids in retention of tocopherol in plasma (McDowell, 1989). The content of Se in feedstuffs is known to vary by geographic region. Selenium sensitive diseases are more prevalent in areas of low soil Se availability (Ullrey, 1981). Body stores of both tocopherol and Se complicate the determination of the tocopherol requirement. Many studies may have underestimated the vitamin E tocopherol requirement because of the tocopherol contribution from body stores (McDowell, 1989).

Reliable Data

Natural vitamin E is associated with the lipid fraction of green leaves and seeds of plants. The vitamin E content in plants is known to vary by species, variety, weather conditions, and stage of maturity. Variations in nutrient content of 10 to 30% can be expected in feedstuffs (United States - Canadian Tables on Feed Composition, 1982). The major swine feedstuffs, corn and soybean meal, may not contain as much natural vitamin E as previously thought. Early data were based on reducing properties of tocopherol and tocotrienols, without the separation of different isomers or other reducing substances. Data based on this type of assay tend to overestimate the tocopherol content and give limited data on the biological availability of the substances recorded. But, recent technological advances have lead to the development of HPLC assays that allow for complete separation of tocopherol isomers and a more accurate assessment of vitamin E content. Tocopherol isomers vary in biological activity (refer to previous section) and typically only alpha-tocopherol, the most potent isomer, is routinely assayed.

Stability

Natural tocopherols act as antioxidants in biological samples and in feedstuffs. Thus, many substances, when added

to diets, will oxidize tocopherol and reduce the tocopherol content. Some of the factors that promote tocopherol destruction are oxygen, heat, light, moisture, pH, and trace minerals. And, when feed enters the gastrointestinal tract, tocopherol oxidation may occur because of interactions with pH, minerals and microorganisms (Borenstein et al., 1988). Copper and iron will increase natural tocopherol destruction in stored feed (Dove, 1988). High salt levels in diets collect moisture and reduce tocopherol stability. Moisture increases the oxidative effects of these factors (McDowell, 1989).

Any process that exposes feedstuffs to oxygen can be expected to cause large losses of natural tocopherol. Grinding, bleaching, milling and storage can cause considerable tocopherol loss. Forage crops dried by exposure to light and air can rapidly lose tocopherol. Dehydrated alfalfa stored for 12 weeks at 32^{o}C resulted in a 65% loss of the alpha-tocopherol (Livingston et al., 1968). Corn artificially dried for 54 minutes at 107^{o}C lost 41% of the alpha-tocopherol content (Adams, 1973). High moisture corn preserved with organic acids caused a large loss of tocopherol (Young et al., 1975).

The amount and type of fat present in the diet will affect vitamin E content and absorption. Diets low in fat may reduce lipid absorption and thus reduce fat-soluble

vitamin absorption. Polyunsaturated fatty acids (PUFA) have been shown to reduce the vitamin E content in feed and body tissues (McDowell, 1989). Animals fed PUFA require additional vitamin E; the amount depends on the source of fat, degree of unsaturation and storage conditions of the feed (Chow and Draper, 1974). It has been recommended that diets be supplemented with 3 mg of tocopherol per gram of PUFA (Putnam and Comben, 1987). The addition of fat-soluble antioxidants will spare tocopherol and will alleviate some vitamin E/Se deficiency symptoms (McDowell, 1989).

Methods of Swine Production

Changes in the methods of swine production have directly affected the vitamin E requirements of swine. The move from pasture production to confinement rearing has lead to a reduction in natural vitamin E intake. Young, lush forage is a good source of natural vitamin E, whereas a typical corn-soybean meal diet has a relatively low vitamin E content. Solvent extraction method of producing soybean meal removes much of the vitamin E with the oil.

Stress from disease, environment and(or) management practices may also increase the requirement for vitamin E. Sudden deaths associated with vitamin E deficiency are frequently seen in weaned pigs after the commingling of litters; physical exertion may also be involved (Ullrey,

1981). Advances in genetics and the use of repartitioning agents have increased the potential for production and may have increased nutrient requirements. Mycotoxins that cause intestinal disturbances such as vomiting, diarrhea and intestinal bleeding may cause reduced absorption of tocopherol (McDowell, 1989). Any disease or parasite that affects the gastrointestinal tract may reduce tocopherol absorption. Animals with fat malabsorption diseases will have reduced absorption of vitamin E. Vitamin levels adequate for growth and reproduction may not be adequate for normal immune function. Pigs fed diets containing 110 IU/kg of vitamin E had greater amount of primary anti-*Escherichia coli* serum antibodies than pigs fed diets containing 22 IU/kg of vitamin E (Ellis and Vorhies, 1976).

Any factor or practice that reduces feed intake may cause the subsequent decrease in vitamin intake and failure to meet the vitamin E requirement. It may be necessary to include vitamin E at a higher concentrations to ensure adequate tocopherol intake. Gestating sows may require a higher concentration of vitamin E per kg than growing/finishing pigs. Also, a high energy diet may require additional vitamin E per kg. High ambient temperature may reduce intake and require the vitamin E concentration to be adjusted to meet the daily tocopherol requirement.

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EXPLANATION OF DISSERTATION FORMAT

The following sections are written as papers for submission to the Journal of Animal Science and follow the style and form of the Journal of Animal Science. The research reported was conducted by John F. Less under the supervision of Dr. R. C. Ewan. Mr. Less was responsible for the completion of all aspects of the experiments described.

SECTION 1. EFFECT OF GENETIC BACKGROUND ON VITAMIN E AND SELENIUM STATUS IN WEANLING SWINE

Abstract

Blood samples were obtained from weanling pigs within two swine herds to evaluate the effects of genetic background on vitamin E and selenium status. Serum was analyzed for alpha-tocopherol (ALPHA), lactic acid dehydrogenase (LDH) and glutathione peroxidase (GSH-Px) activity. All pigs within their respective herd were reared under common environment, nutrition and management. In Herd 1, at weaning (6 to 7 wk) one male and one female pig from each of 10 randomly selected litters within a genetic line were bled. Nineteen genetic lines were sampled in Herd 1. A wide variation was detetcted in serum ALPHA concentration ($P<.001$), GSH-Px ($P<.001$) and LDH ($P<.01$) activity among genetic lines. Within Herd 1, mean serum ALPHA ranged from .251 to .775 mg/liter, and a sex x genetic line interaction was seen in serum ALPHA. Purebreds ranked by serum ALPHA were Landrace>Hampshire>Yorkshire>Chester White>Duroc. Duroc and Duroc crosses tended to have lower serum ALPHA concentration than Landrace, Hampshire and their crosses. Mean serum GSH-Px activity in Herd 1 ranged from .464 to .877 units/ml. Ranking of purebreds by serum GSH-Px activity were

Landrace>Hampshire>Chester White>Duroc>Yorkshire. Hampshire, Landrace and their crosses tended to have higher serum GSH-Px activity than Yorkshire, Duroc and their crosses. Serum LDH activity ranged from 332 to 480 units/ml. Purebreds ranked by serum LDH activity were Duroc>Hampshire>Landrace>Yorkshire>Chester White. In Herd 2, six genetic lines were sampled. At weaning (23 to 32 d), two male and two female pigs from each of five randomly selected litters within a genetic line were bled. Sows were fed ad libitum and pigs did not have access to creepfeed. A variation ($P<.02$) was detected in serum ALPHA among genetic lines in Herd 2, and mean serum ALPHA ranged from .525 to .963 mg/liter. The crossbred line HD-YL had greater serum ALPHA than all other lines. No differences were found in serum enzyme activity among genetic lines in Herd 2. The variation in serum ALPHA, GSH-Px and LDH activity among genetic lines suggests that there are differences in vitamin E and selenium status among genetic lines of swine.

Key words: Swine, Genetic Variation, Vitamin E, GSH-Px, LDH

Introduction

There continues to be reports of vitamin E and Selenium (Se) responsive conditions in confinement reared pigs fed diets adequately supplemented with vitamin E and Se.

Genetics, environment, dietary constituents, and diseases may influence the vitamin E and(or) Se requirement of swine and predispose some pigs to a deficiency. The ability to respond adequately to stressors, environment and diseases varies greatly among breeds of swine (Hoppe, 1990).

Pigs that died from spontaneous mulberry heart disease had lower levels of alpha-tocopherol in heart and liver tissue than healthy pigs fed the same vitamin E adequate diet (Rice and Kennedy, 1989). Stowe and Miller (1985) reported wide variations in serum vitamin E and Se in growing pigs raised under similar environment and fed the same ration. Some aspects of vitamin E or Se metabolism in swine may be genetically influenced. The hypothesis that genetics has an influence on vitamin E and(or) Se metabolism has been suggested previously (Jorgensen et al., 1977; Atroshi et al., 1981; Bendich et al., 1983).

No previous report has compared the vitamin E and Se status among a large number of genetic lines reared under common conditions. Thus, the objective of this study was to determine whether genetic background has an effect on the vitamin E and Se status of swine.

Materials and Methods

General

Vitamin E and Se status were based on serum alpha-tocopherol (ALPHA) concentration, lactate dehydrogenase

(LDH) and glutathione peroxidase (GSH-Px) activity. Within two swine herds, blood samples were obtained from orbital sinus of pigs at weaning and serum was separated for analysis. Serum was analyzed for tocopherols (Cort et al., 1983), LDH (Amador et al., 1963) and GSH-Px activity (Paglia and Valentine, 1967). Diets were analyzed for vitamin E (Cort et al., 1983) and Se (Olson et al., 1975) concentration. Pigs within each herd were reared under common conditions of housing, environment, nutrition and management. But, each herd was under different management practices.

Herd 1

Pigs from 19 genetic lines (Table 1) were bled when weaned at 6 to 7 wk of age. The genetic lines sampled included six purebred lines, two crossbred lines, three purebred Duroc lines selected for limb-soundness and three stress-sensitive lines. Stress-sensitivity was identified in parents by Halothane testing.

From each genetic line, 10 litters were randomly selected and from these litters one male and one female pig were bled. The unavailability of adequate numbers of litters in some genetic lines resulted in an unequal number of samples per line. A total of 453 pigs from 194 litters were sampled. Sows were fed a pelleted corn-soybean meal diet ad

libitum and pigs had access to creep feed at 3 wk of age.

Herd 2

Pigs from six genetic lines (Table 2) were bled when weaned at 23 to 32 d of age. The genetic lines sampled included two purebred lines and four crossbred lines. From each genetic line, five litters were randomly selected and within each litter two male and two female pigs were bled. The unavailability of adequate numbers of litters in some genetic lines resulted in an unequal number of samples per line. A total of 107 pigs from 28 litters were sampled. Sows were fed a corn-soybean meal diet ad libitum and pigs did not have access to creep feed.

Statistics

Data were statistically analyzed by least-squares analysis of variance as performed by GLM procedure of SAS (1985). The model included sex, genetic line and sex x genetic line. The residual was the error term for sex and genetic line; and litter(genetic line) was the error term for genetic line. Mean separation was performed by Duncan's Multiple Range Test. Data are reported as least-square means.

Results

Feed

Analyzed vitamin E and Se concentration of experimental diets can be seen in Table 3. Alpha-tocopheryl acetate was detected in both diets from Herd 1; none was detected in sow feed of Herd 2. All diets contained adequate levels of Se.

Herd 1

A wide variation ($P<.001$) was detected in serum ALPHA concentration among genetic lines (Table 4). Mean serum ALPHA ranged from .251 to .775 mg/liter, with a herd average of .476 mg/liter. Serum ALPHA concentration did not vary ($P>.10$) by sex but there was a sex x genetic line ($P<.01$) interaction (Table 5).

Purebred Land had higher ($P<.05$) serum ALPHA than all other purebreds. Hamp had higher ($P<.05$) serum ALPHA than Chester, York and Duroc. Land x Hamp cross had higher ($P<.05$) serum ALPHA than Hamp x Land cross. No difference was detected in serum ALPHA among the lines HL, LH, HL x HL, or LH x LH. Land-X and York-X had greater ($P<.05$) serum ALPHA than Duroc-X. Serum ALPHA concentrations did not differ ($P>.10$) between Land-X, York-X and Hamp-X. Within the soundness lines, DD-LOW had greater ($P<.05$) serum ALPHA than DD-HIGH and DD-CONTROL. Serum ALPHA concentration did not differ among the three stress-sensitive lines.

Serum gamma-tocopherol (GAMMA) varied (P<.0001) among genetic lines; mean GAMMA ranged from .003 to .085 mg/liter (Table 6). Pigs with high serum ALPHA concentration tended to have high serum GAMMA concentration (R =.66).

Serum GSH-Px activity varied (P<.001) among genetic lines and mean activity ranged from .464 to .877 units/ml (Table 7). The herd average was .704 units/ml. Purebred York had lower (P<.05) serum GSH-Px activity than all other purebred lines. Serum GSH-Px activity did not differ (P>.10) among Land, Hamp, and Chester or among the crossbred lines. In the soundness lines, DD-LOW had greater (P<.05) serum GSH-Px activity than DD-HIGH. GSH-Px activity was lower (P<.05) in STR-C than in D-STR and STR-(+) lines.

Serum LDH activity varied (P<.01) among genetic lines and mean serum LDH activity ranged from 332 to 480 units/ml (Table 8). The herd average was 397 units/ml. Purebred Chester had lower (P<.05) serum LDH activity than all other purebreds. The crossbred line LH had higher (P<.05) LDH activity than HL, HL x HL or LH x LH. No difference in serum LDH activity was detected within the soundness or stress-sensitive lines.

Herd 2

Serum ALPHA concentration varied (P<.02) among genetic lines and mean serum ALPHA ranged from .525 to .963 mg/liter (Table 9). The herd average was .730 mg/liter. Sex had no

effect (P>.10) on serum ALPHA concentration. The genetic line HD-YL had greater (P<.05) serum ALPHA concentration than all the other lines. Serum GAMMA varied (P<.02) by genetic line and the herd average was .0276 mg/liter. As similar to serum ALPHA, the genetic line HD-YL had greater (P<.05) serum GAMMA than the other lines.

Although there was variation in serum GSH-Px and LDH activity among genetic lines, the difference were not significant (Table 10). The mean serum GSH-Px activity ranged from .530 to .758 units/ml and the mean serum LDH activity ranged from 394 to 561 units/ml. Herd 2 had an average GSH-Px activity of .649 units/ml and an average LDH activity of 464 units/ml.

Discussion

The blood level of vitamin E is the balance between the rate of absorption from the gastrointestinal tract and the uptake, retention and release from peripheral tissues. The vitamin E level in plasma is dependent on age, previous vitamin E nutrition and blood lipid concentration. Alpha-tocopherol is the most potent and predominate isomer in the blood. And, there is a high correlation between dietary vitamin E and plasma level of ALPHA, and between plasma ALPHA and liver ALPHA (Wiss et al., 1962; Simensen et al., 1979). Thus, plasma ALPHA level can be used as an indicator of

vitamin E status (Ullrey, 1981).

The weanling pig serum ALPHA-tocopherol concentrations in this experiment were similar to other trials at our station (R. C. Ewan, personal communication), but were somewhat lower than what has been reported by other stations (Young et al., 1977; Stowe and Miller, 1985; Loudenslager et al., 1986). Although pigs within a farm were reared under common environment and management, serum vitamin E levels varied among genetic lines. Mean serum ALPHA concentration varied by .524 mg/liter in Herd 1 and by .438 mg/liter in Herd 2. These results are supported by Stowe and Miller (1985) who reported a wide range in serum vitamin E concentrations among age-matched, commonly reared and fed growing pigs. The exact genetic background of the pigs sampled was not reported. In another trial, Stowe and Miller suggested that there are differences in vitamin E/Se metabolism between pigs selected as hypo- or hyperselenemic.

A genetic influence on vitamin E status has been reported in other species. Maplesden et al. (1960) reported that plasma vitamin E differed among breeds of cattle, especially at high levels of vitamin E intake. The breeds compared included Angus, Shorthorn and Hereford. Vitamin E levels in tissues of spontaneously hypertensive rats were lower than that of Wister-Kyoto rats (Bendich et al., 1983). The authors suggest that the differences may be caused by

defects in vitamin E absorption, transport or membrane uptake and retention. Janero and Burghardt (1988) found that the vitamin E concentration in myocardial membranes of spontaneously hypertensive rats was threefold less than normotensive rats. Thakker et al. (1987) suggested that vitamin bioavailability in humans differs by ethnic background. Underwood et al. (1970) reported no ethnic differences were seen in human liver ALPHA concentration.

There are limited data on whether sex has an influence on tocopherol status. Weglicki et al. (1969) reported that female rats had higher vitamin E concentration in some tissues than male rats. Wei Wo and Draper (1975) found no sex difference in serum vitamin E concentration of Alaskan Eskimos. Human females have been reported to have slightly higher plasma vitamin E levels than males but the difference was not significant (Behrens et al., 1982). In children, ages 6 mo to 15 yr, Morita et al. (1989) found no difference in serum ALPHA between the sexes. The effect of sex on serum vitamin E concentration has either not been reported or not addressed in previous trials using nursing (Malm et al., 1976; Loudenslager et al., 1986) or growing pigs (Young et al., 1977; Stowe and Miller, 1985). In the present experiment, no sex difference was detected in serum ALPHA, but in Herd 1 a sex x genetic line interaction was seen.

Intake of colostrum and milk is important to the

biological antioxidant status of the young pig (Loudenslager et al., 1986). Colostrum contains a higher level of vitamin E than milk and both are dependent on gestation and lactation diets (Nielsen et al., 1973; Malm et al., 1976; Loudenslager et al., 1986). Nursing pigs rely on milk vitamin E to maintain their serum vitamin E levels (Stowe and Miller, 1985) because vitamin E is inefficiently transferred across the placenta in the pig. Differences in milk production among breeds may affect the serum vitamin E level of pigs at weaning. Meyer et al. (1981) reported that plasma vitamin E was high in nursing pigs but decreased after weaning.

It is generally accepted that white breeds (Land, York and Chester) have superior maternal characteristics to colored breeds (Duroc, Hamp, Berkshire and Poland China) which are known for growth and carcass traits. Shurson et al. (1985) reported that purebred Duroc pigs are more lethargic at birth than purebred Yorks. Subsequently, the duroc pigs provided a poor suckling stimulus to the sow and milk production decreased. This may explain some of the difference seen in serum ALPHA concentration. Certain genetic lines may have more energetic pigs than other lines, thus stimulating more milk production and mammary transfer of ALPHA. The influence of this hypothesis on serum ALPHA concentration at weaning is yet to be determined.

Rice and Kennedy (1989) concluded that some pigs are

genetically predisposed to suboptimal levels of ALPHA in myocardial subcellular membranes; this was in spite of apparently adequate dietary vitamin E intake. These finding were from the analysis of pig myocardium of pigs which died from diagnosed cases of mulberry heart disease. The pigs may have a higher rate of lipid peroxidation in myocardial subcellular membrane, therefore they have a higher vitamin E requirement than control pigs. It might be expected that the DD-LOW line with its poor feet and limb structure may require more vitamin E (lower plasma ALPHA) than high or control line, however, this was not the case.

Duthie et al. (1987) reported no difference in serum ALPHA between halothane reactor and non-reactor pigs. But in a majority of tissues, reactors had greater levels of ALPHA than non-reactors. The authors hypothesized that the higher ALPHA in tissues of reactors may be due to a greater uptake of ALPHA in response to an increased free radical load, or through blockage of ALPHA degradation pathway within muscles, or by increased rate of fat oxidation in muscle. In Herd 1, all stress-senstive lines had similar levels of serum ALPHA. The non-reactors in the Duthie et al. (1987) study were heterozyous for the halothane gene, which would be comparable to the STR-C line in the present study. Perhaps serum ALPHA is not an appropriate indicator of vitamin E status in stress-sensitive lines.

Selenium is an essential component of the enzyme GSH-Px (Rotruck et al., 1973) and there is a high correlation between plasma GSH-Px activity and plasma Se (Ewan, 1976; Chavez, 1979; Meyer et al., 1981; Adkins and Ewan, 1984) in pigs. Thus, plasma or serum GSH-Px activity is commonly used as an indicator of Se status in swine. Selenium efficiently passes across the placenta in pigs (Young et al., 1977; Van Saun et al., 1989) and is efficiently transferred in colostrum and milk (Mahan et al., 1975). Colostrum has higher Se concentration than milk and the Se content of both is diet dependent (Rasmussen, 1974; Loudenslager et al., 1986).

There have been numerous reports in the literature suggesting that genetic background has an influence on serum Se and(or) serum GSH-Px activity. Jorgensen et al. (1977) reported that red blood cell GSH-Px activity varied more among litters than among pigs within litters and suggested some genetic influence. Jensen et al. (1979) found a similar litter effect in Se-related variables. Stowe and Miller (1985) reported large variations in serum Se in age matched, commonly reared and fed growing pigs.

In pigs reared under similar conditions, Wegger et al. (1982) reported that RBC GSH-Px activity varied more between litters than among pigs within a litter, and found no sex-linked differences. In randomly selected slaughter house

pigs of the same breed, wide variations were found in the amount of liver and kidney GSH-Px activity (Wegger et al., 1980). There may be differences in Se status within lines of the same breed. Sankari (1985) also found wide variations in plasma GSH-Px activity in 4 wk old piglets from within the same area and within farms.

Pigs found hypo- or hyperselenemic at a young age retained their respective status throughout life. Hyposelenemic pigs absorbed less Se via gut than hyperselenemic, suggesting a genetic difference between the groups. In addition, matings between gilts and boars of like-status produced progeny of that same status (Stowe and Miller, 1985). Jorgensen and Wegger (1979) reported that pigs consuming an adequately Se supplemented diet and pigs with higher plasma GSH-Px activity were less susceptible to diseases than pigs with low GSH-Px activity. Schanus et al. (1981) reported that stress-susceptible pigs have a deficiency in the enzyme GSH-Px. Other studies have found no difference in GSH-Px activity between Halothane reactors and non-reactors (O'Brien, 1985; Duthie et al., 1989). These reports support the hypothesis that there may be genetic variation in Se status of swine.

There have been reports of genetic variation in Se status of sheep (Tucker et al., 1980; Atroshi et al., 1981; Sandholm et al., 1983). Also, research has suggested a

genetic influence in other plasma enzymes: arylesterase (Kubek et al., 1969), adenosine deaminase (Widar and Ansay, 1975) and alkaline phosphatase (Kierek-Jaszczuk et al., 1978).

The enzyme LDH is released from damaged cells and serum LDH activity is commonly used as a general indicator of tissue and membrane damage. Serum LDH activity may be useful in diagnosing nutritional muscular dystrophy (Paulson et al., 1968). Elevated serum LDH activity has been shown in pigs fed a vitamin E/Se deficient diet (Ewan and Wastell, 1970). The serum LDH activity was consistent with other studies from our station (Dove, 1988) and suggest that the pigs sampled were not vitamin E/Se deficient.

Implications

Some genetic lines of swine may have higher vitamin E and(or) Se requirements due to a genetically impaired ability to absorb, retain or metabolize vitamin E and Se.

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Table 1. Genetic lines of swine sampled in Herd 1

Genetic Line	Description[a]
Chester	Purebred Chester White
Duroc	Purebred Duroc (Parent)
York	Purebred Yorkshire
Hamp	Purebred Hampshire
Land	Purebred Landrace
LH	Land x Hamp
HL	Hamp x Land
HL x HL	Hamp-Land x Hamp-Land
LH x LH	Land-Hamp x Land-Hamp
Duroc-X	Duroc x Maternal line[b]
Hamp-X	Hamp x Maternal line
York-X	York x Maternal line
Land-X	Land x Maternal line
DD-CONTROL	Duroc (Control limb score)[c]
DD-HIGH	Duroc (High limb score)
DD-LOW	Duroc (Low limb score)
STR-(+)	Parents positive for stress gene[d]
STR-C	Parents carriers of stress gene
D-STR	Duroc x STR-C

[a]Sire x Dam.

[b]White maternal line (Chester x York x Land).

[c]Pigs selected by limb-soundness.

[d]Stress gene identified by Halothane test (Chester x York x Pietrain).

Table 2. Genetic lines of swine sampled in Herd 2

Genetic line	Description[a]
York	Purebred Yorkshire
Land	Purebred Landrace
LY	Land x York
YL	York x Land
HD-H	Hamp-Duroc x Hamp
HD-YL	Hamp-Duroc x YL

[a]Sire x Dam.

Table 3. Analyzed vitamin E and selenium concentration of diets

Diet	Acetate[a] mg/kg	Alpha[b] mg/kg	Se, ppm
Herd 1:			
Sow feed	8.9	3.2	.311
Creep feed	17.4	1.7	.748
Herd 2:			
Sow feed	ND[c]	7.6	

[a]Acetate = alpha-tocopheryl acetate.

[b]Alpha = alpha-tocopherol.

[c]ND = none detected.

Table 4. Serum alpha-tocopherol concentration in genetic lines of swine (Herd 1)

Genetic Line	Alpha-tocopherol, mg/liter[a,b]
DD-CONTROL	.251 c
DD-HIGH	.297 cd
Duroc	.302 cd
HL	.402 de
York	.403 de
Duroc-X	.425 e
STR-C	.426 e
HL x HL	.439 e
Chester	.448 ef
DD-LOW	.461 ef
STR-(+)	.495 efg
Hamp-X	.512 efg
D-STR	.513 efg
LH x LH	.526 efgh
York-X	.566 fgh
Hamp	.600 gh
Land-X	.616 gh
LH	.643 h
Land	.775 i

[a]Least-squares means.

[b]Effect of genetic line (P<.001).

[c,d,e,f,g,h,i]Means with different superscripts differ (P<.05).

Table 5. Serum alpha-tocopherol concentration by sex in genetic lines of swine (Herd 1)

Genetic Line	Alpha-tocopherol, mg/liter[a,b]	
	Male	Female
Duroc	.308	.296
York	.384	.422
Chester	.452	.443
Hamp	.649	.551
Land	.706	.844
Duroc-X	.421	.428
Hamp-X	.577	.447
York-X	.603	.529
Land-X	.712	.519
HL	.436	.368
HL x HL	.352	.526
LH x LH	.452	.601
LH	.742	.545
DD-CONTROL	.245	.257
DD-HIGH	.332	.262
DD-LOW	.434	.489
STR-C	.467	.386
STR-(+)	.465	.525
D-STR	.470	.556

[a]Least-squares means.

[b]Genetic line x sex interaction (P<.01).

Table 6. Serum gamma-tocopherol concentration in genetic lines of swine (Herd 1)

Genetic Line	Gamma-tocopherol, mg/liter[a,b]	
DD-CONTROL	.003	c
STR-C	.005	cd
Duroc	.011	cd
Chester	.014	cd
STR-(+)	.015	cde
DD-HIGH	.016	cde
York	.019	cde
D-STR	.023	cdef
Hamp-X	.023	cdef
Land-X	.025	cdefg
York-X	.026	cdefgh
Duroc-X	.029	defghi
HL	.038	efghi
LH	.043	fghij
HL x HL	.047	ghij
DD-LOW	.048	hij
Hamp	.051	ij
LH x LH	.062	j
Land	.085	k

[a]Least-squares means.

[b]Effect of genetic line (P<.001).

c,d,e,f,g,h,i,j,k Means with different superscripts differ (P<.05).

Table 7. Serum glutathione peroxidase activity in genetic lines of swine (Herd 1)

Genetic Line	Activity, units/ml[a,b,c]
York	.464 d
STR-C	.498 d
D-STR	.619 e
York-X	.644 ef
Duroc-X	.674 efg
Duroc	.677 efg
STR-(+)	.679 efg
DD-HIGH	.707 fgh
Hamp-X	.720 fgh
LH x LH	.722 fghi
Chester	.733 fghi
Land-X	.737 fghi
HL	.747 ghi
DD-CONTROL	.775 hij
HL x HL	.800 hij
Hamp	.811 hij
Land	.814 ij
LH	.826 j
DD-LOW	.877

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

[b]Least-squares means.

[c]Effect of genetic line ($P<.001$).

[d,e,f,g,h,i,j]Means with different superscripts differ ($P<.05$).

Table 8. Serum lactic acid dehydrogenase activity in genetic lines of swine (Herd 1)

Genetic Line	Activity, units/ml[a,b,c]
Chester	332 d
DD-HIGH	344 d
DD-LOW	353 d
Hamp-X	361 de
DD-CONTROL	372 def
Duroc-X	375 defg
York-X	377 defgh
STR-C	401 efghi
Land-X	404 efghi
D-STR	405 efghi
York	413 fghi
Land	414 fghi
Hamp	417 fghi
Duroc	423 ghi
HL x HL	425 hi
LH x LH	428 i
STR-(+)	429 i
HL	441 ij
LH	480 j

[a]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.

[b]Least-squares means.

[c]Effect of genetic line ($P<.01$).

[d,e,f,g,h,i,j]Means with different superscripts differ ($P<.05$).

Table 9. Serum tocopherol concentration in genetic lines of swine (Herd 2)

Genetic Line	N[b]	Lit[c]	Tocopherol, mg/liter[a] ALPHA[d]	GAMMA[e]
LY	20	5	.525 [f]	.015 [g]
YL	19	5	.675 [g]	.016 [g]
Land	20	5	.717 [g]	.018 [g]
York	18	5	.732 [g]	.005 [f]
HD-H	12	3	.734 [g]	.030 [g]
HD-YL	20	5	.963 [h]	.077 [h]

[a]Least-squares means.

[b]Number of pigs.

[c]Number of litters.

[d]Effect of Genetic Line ($P<.02$).

[e]Effect of Genetic Line ($P<.01$).

[f,g,h]Within columns means with different superscripts differ ($P<.05$).

Table 10. Serum enzyme activity in genetic lines of swine (Herd 2)

Genetic Line	N[a]	Lit[b]	Activity, units/ml	
			GSH-Px[c]	LDH[d]
York	18	5	.530	485
YL	19	5	.542	394
Land	20	5	.656	561
LY	20	5	.664	409
HD-H	12	3	.711	473
HD-YL	20	5	.758	461

[a]Number of pigs.

[b]Number of litters.

[c]GSH-Px = Glutathione peroxidase. One unit of activity was defined as the amount of enzyme that will convert 1 umol of NADPH per min at pH 7.0 and 20°C.

[d]LDH = 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.

SECTION 2. THE APPARENT DIGESTION OF D-ALPHA-TOCOPHERYL SUCCINATE AND DL-ALPHA-TOCOPHERYL ACETATE IN GROWING SWINE: A SHORT TERM STUDY

Abstract

Nine 20 to 40 kg pigs were used in a 13 d balance trial to evaluate the bioavailability of dl-alpha-tocopheryl acetate (dl-ACE) and d-alpha-tocopheryl succinate (d-SUCC). For 5 wk before the start of the trial, pigs were individually penned and limit fed a corn-soybean meal diet that contained 2% tallow, 214 ppm copper, and no supplemental vitamin E or selenium (BASAL diet). Serum alpha-tocopherol (ALPHA), lactic acid dehydrogenase (LDH) and glutathione peroxidase (GSH-Px) activity were monitored during this period. Before the trial, pigs were blocked (three pigs/block) by pretrial serum ALPHA concentration. Within block, pigs were randomly allotted to treatments: 1) BASAL, 2) BASAL + 58 IU/kg dl-ACE, or 3) BASAL + 58 IU/kg d-SUCC. All diets were fed to maintain body wt (BW), i.e., block one and two were fed 1% BW/d, and block three was fed 1.4% BW/d. On d 0 treatments were started and total feces were collected twice daily for the entire trial. Blood was collected on d 0, 4, 8 and 13. Serum was analyzed for tocopherols, LDH and GSH-Px activity. Tocopherol esters and isomers were analyzed by HLPC. For the overall trial mean, d 0 to 13, pigs fed

diets that contained dl-ACE or d-SUCC esters had higher serum ALPHA ($P<.06$) than pigs fed the BASAL diet. Over the 13 d trial, serum ALPHA linearly increased in pigs fed dl-ACE ($P<.04$) and d-SUCC ($P<.01$). No difference was detected in serum ALPHA between dl-ACE and d-SUCC at any time in the trial. Serum enzymes did not differ among treatments. During d 6 to 13, pigs fed dl-ACE or d-SUCC more efficiently digested ALPHA ($P<.01$) and had higher apparent vitamin E balance ($P<.01$) than pigs fed BASAL. Both dl-ACE and d-SUCC were efficiently hydrolyzed and effective in raising the serum concentration of ALPHA. And, at a low level of feed intake, there was no biodiscrimination in hydrolysis or digestion between dl-ACE and d-SUCC.

Key Words: Swine, Tocopherol, Tocopheryl Acetate, Tocopheryl Succinate.

Introduction

Within plants, tocopherols are found as free alcohols and are associated with the lipid portions of green leaves and seeds. Because animals can not synthesize vitamin E, they must acquire their requirement through the consumption of plant foliage, seeds or animal tissue products. In the natural form, a free alcohol, tocopherols are relatively unstable due to their antioxidant properties. The oxidation of tocopherols is accelerated by light, oxygen, heat, alkalines, trace minerals, storage, mechanical and chemical

processing. Oxidation destroys the biological activity of tocopherols.

The oxidative stability of tocopherols can be increased by the acylation of the free phenolic hydroxyl in position C-6 (Ullrey, 1981). Many of the current forms of supplemental vitamin E are ester derivatives of alpha-tocopherol (ALPHA); the principal types being an acetate or succinate ester. These ester derivatives have no antioxidant activity, and thus, are relatively unaffected by oxidative factors.

The digestibility of vitamin E in rats and humans has been reported to range from 10 to 75% (Gallo-Torres, 1980). But little information has been reported on the digestion of vitamin E esters in swine.

Thus, the objectives of this experiment were to: 1) measure the bioavailability of dl-alpha-tocopheryl acetate (dl-ACE) and d-alpha-tocopheryl succinate (d-SUCC) in swine by serum ALPHA concentration and the fecal excretion method, and 2) monitor the vitamin E and selenium (Se) status through the analysis of serum activity of lactic acid dehydrogenase (LDH) and glutathione peroxidase (GSH-Px).

Materials and Methods

Pretrial

Twelve pigs from three vitamin E/Se deficient females were weaned, penned four/pen and fed ad libitum a starter diet until pigs weighed 20 kg. The pigs were then fed a grower diet (BASAL diet) from 20 kg to the start of the trial. Both starter and grower diets contained supplemental copper but no supplemental vitamin E or Se. The diets were stored at least 14 d before feeding (Table 1). The diets were formulated to meet or exceed all NRC (1988) requirements, except for vitamin E and Se. Three pigs were removed from the trial during the pretrial period. Serum tocopherol, LDH and GSH-Px activity were monitored throughout the pretrial period. One week before the start of the trial, pigs were moved into metabolism pens as an adjustment period.

Design

Nine 20 to 40 kg pigs were assigned to three blocks by their pretrial serum alpha-tocopherol (ALPHA) concentration (Table 2). Within each block (three pigs/block), pigs were randomly allotted to treatments: 1) BASAL, 2) BASAL + 58 IU/kg dl-ACE, or 3) BASAL + 58 IU/kg d-SUCC. Throughout the 13 d balance trial, all diets were fed to maintain body wt (BW) in two equal feedings. Blocks one and two were fed 1% BW/d and block three was fed 1.4% BW/d. On d 0, treatments

were started and total feces were collected twice daily throughout the trial. Blood was collected on d 0, 4, 8 and 13. Feces, feed and serum were frozen at -20°C until analyzed for vitamin E.

Serum Analysis

Serum tocopherols were determined by high performance liquid chromatography (HPLC) with fluoroscence detection; 294 nm excitation wavelength and 323 nm emission wavelength (Cort et al., 1983). The mobile phase was 3.5% tetrahydrofuran (vol/vol) in HPLC-grade hexane and a flow rate of approximately 2.0 ml/min. Serum (2 ml) was deproteinized with redistilled absolute ethanol (3 ml) and tocopherols were extracted with hexane (1 ml). The hexane was injected directly into HPLC. Tocopherol isomers and alpha-tocopheryl acetate were identified and quantitated by comparison of retention times and peak areas to vitamin E standards. Serum LDH (Amador et al., 1963) and GSH-Px activity (Paglia and Valentine, 1967) were determined within 24 h of blood collection.

Feed and Feces Analysis

Selenium was determined by the method of Olson et al. (1975). Tocopherol isomers and alpha-tocopheryl acetate in feed and feces were extracted with acetone in a soxhlet

extractor, evaporated to dryness and redissolved in hexane (Cort et al., 1983). Hexane was injected directly into the HPLC for separation and quantitation of tocopherols. The chromatographic conditions and detection settings were the same as described for serum tocopherol analysis. Feed samples were ground through a 1.0 mm screen; fecal samples were not ground and were analyzed on a wet-basis.

The separation and quantitation of alpha-tocopheryl succinate were achieved by HPLC analysis with fluoroscence detection. The fluorometer settings were 284 nm excitation wavelength and 306 nm emission wavelength. Feed samples were ground through a 1.0 mm screen; fecal samples were not ground and were analyzed on a wet-basis. Approximately .5 g of sample was weighed into a tared 16 x 120 mm screw top culture tube and 3.0 ml of HPLC-grade hexane and 30 ul of acetic acid were added. The tube was capped with a teflon lined lid and vortexed for 30 s. The sample was placed in a closed cabinet and allowed to sit over night at room temperature. The following day the sample was centrifuged and 25 ul of the hexane phase was injected directly into the HPLC.

A normal phase HPLC system, equipped with a time gradient program, was used to elute the strongly retained ester. A two-solvent gradient was used: solvent A (mobile phase) was 3.5% tetrahydrofuran (vol/vol) in HPLC-grade hexane and solvent B (gradient solvent) was 20%

tetrahydrofuran and .5% acetic acid (vol/vol) in HPLC-grade hexane. The flow rate was approximately 2.0 ml/min. The total program time was 37.0 min, which allowed for total HPLC analysis time of 40.0 min/sample. A concave curve gradient of solvent B, increasing from 0 to 70% of solvent B, was run from 2.0 to 17.0 min. A linear reverse gradient proceeded from 17.01 to 20.0 min, and 100% of solvent A was run from 20.01 to 37.0 min. This gradient program allowed for good separation and resolution alpha-tocopheryl succinate and eluted alpha-tocopheryl succinate from the column within 16 to 20 min.

Calculations

Apparent digestibility data were reported for d 6 to 13, and by periods 1 to 4, i.e., period 1 = d 0 to 4, period 2 = d 5 to 7, period 3 = d 8 to 10 and period 4 = d 11 to 13. Apparent digestion coefficients (ADC) were calculated using the following equation: [(intake - excretion)/intake)] x 100. The ALPHA and gamma-tocopherol (GAMMA) values used in intake calculations were the average of all experimental diets. The amount of ALPHA produced from the hydrolysis of the esters was determined and taken into account in ALPHA ADC calculations. Apparent vitamin E balance (E-BAL) was calculated by using the sum of vitamin E activity (ALPHA + GAMMA + ester) for feed and feces components and using them

in the ADC equation.

Statistics

Data were statistically analyzed by least-squares analysis of variance as performed by the GLM procedure of SAS (1985). The dependent variables were ester and ALPHA digestion coefficients, and E-BAL. The model included treatment, period or day, and block. Treatment x block was the error term for treatment, period x block was the error term for period, and period x day was the error term for day. Contrasts were used to test treatment differences within digestion coefficients and periods. Data are reported as least-square means.

Results

Feed

Analyzed vitamin E and Se concentration of experimental diets can be seen in Table 3. The analyzed values for alpha-tocopheryl acetate and alpha-tocopheryl succinate were 83 and 105% of the calculated values, respectively.

Serum

For the entire 13 d trial, pigs fed diets containing esters had higher serum ALPHA ($P<.06$) than pigs fed the BASAL diet (Table 4). Serum ALPHA linearly increased from d 0 to

13 in pigs fed dl-ACE (P<.04) and d-SUCC (P<.01, Figure 1). No difference was detected in serum ALPHA between dl-ACE and d-SUCC at any point of the trial. Pigs fed diets supplemented with esters had higher serum ALPHA on d 4, 8 (P<.05) and 13 (P<.01) than pigs fed the BASAL diet.

Serum GAMMA, LDH and GSH-Px activity did not differ among treatments and remained relatively stable throughout the trial (Table 4 and 5).

Apparent Digestion

Day 6 to 13 During the period of d 6 to 13, both dl-ACE and d-SUCC were efficiently digested, 87 and 88% respectively, as measured by fecal excretion of unhydrolyzed ester (Table 6). Pigs fed diets supplemented with dl-ACE or d-SUCC more efficiently (P<.01) digested ALPHA and had greater (P<.01) E-BAL than pigs fed the BASAL diet. Pigs fed d-SUCC tended to digest ALPHA more efficiently than pigs fed dl-ACE, but the difference was not significant.

Periods 1 to 4 During the periods of 1 to 4, the apparent digestibility of ester decreased in pigs fed dl-ACE (linear P<.01, Table 7). No difference was detected in ADC of ester between dl-ACE and d-SUCC during any of the four periods. ALPHA digestibility linearly decreased from period 1 to 4 in pigs fed dl-ACE (P<.01) and d-SUCC (P<.001). The

ADC of ALPHA did not differ between dl-ACE and d-SUCC treatments in any of the four periods. Pigs fed diets supplemented with dl-ACE or d-SUCC had a higher ADC of ALPHA than pigs fed BASAL in period 1 ($P<.001$), 2 and 3 ($P<.01$) and 4 ($P<.05$). Over the course of the trial, pigs fed BASAL had a negative ADC of ALPHA and no consistent trend was detected.

Apparent E-BAL linearly ($P<.01$) decreased in pigs fed dl-ACE and d-SUCC over the 4 periods. No difference was detected in E-BAL between dl-ACE and d-SUCC in any period. Within periods, pigs fed diets supplemented with dl-ACE or d-SUCC had higher E-BAL than pigs fed BASAL in period 1 ($P<.001$), 2 ($P<.01$), 3 and 4 ($P<.05$).

Discussion

The lack of difference between dl-ACE and d-SUCC in serum ALPHA concentration suggests that digestion of both esters was similar. Blood concentration of tocopherol is affected by dose of tocopherol, level of lipids and lipoproteins in blood, rate of tocopherol removal by tissues, and tissue retention of tocopherol which is dependent on previous dietary tocopherol level (Gallo-Torres, 1980).

Vitamin E supplementation had no effect on serum enzymes at any point during the trial. Diets were not supplemented with Se; therefore Se from the feedstuffs was adequate to prevent the occurrence of a vitamin E/Se deficiency even when

dietary vitamin E is low. Malm et al. (1976) and Young et al. (1977) reported that the addition of vitamin E to a swine diet increased plasma Se in swine but did not cause a subsequent increase in serum GSH-Px activity. The slight rise in LDH activity on d 13 in pigs fed BASAL reflects the very high LDH activity in one pig (1015 units/ml). This pig may have been vitamin E/Se deficient and exhibited vitamin E/Se deficiency symptoms.

The results from the present trial indicate that both dl-ACE and d-SUCC were efficiently hydrolyzed as measured by excretion of unhydrolyzed ester. The high apparent digestibilities of the esters suggest that hydrolysis was not affected by the addition of acetate or succinate to ALPHA or by the form of ALPHA (d vs dl). Both acetate (CH_3COO^-) and succinate ($HOOCCH_2CH_2COO^-$) react with the hydroxyl group at position C-6 on the tocopherol structure. Naturally (d) derived vitamin E has a 2R, 4'R, 8'R configuration while totally synthetic vitamin E (dl) is a mixture of the 4 possible enantiomeric pairs of the 8 diastereoisomers (Ullrey, 1981). Biodiscrimination at the level of the gut was reported by Ingold et al. (1987). Ingold and coworkers reported that the hydrolysis of RRR-alpha-tocopheryl acetate was more complete than SRR-alpha-tocopheryl acetate. Chirality of active enzymes in pancreatic juice and(or) bile salts may cause biodiscrimination at the level of the gut

(Gallo-Torres, 1980; Lombardo and Guy, 1980). But, in the present trial there was no evidence that would suggest there was discrimination in hydrolysis between dl-ACE and d-SUCC.

The ALPHA produced from the hydrolysis of vitamin E esters was extensively digested, indicated by the high ADC of ALPHA in pigs fed dl-ACE and d-SUCC. Pigs fed d-SUCC had a slightly higher ALPHA digestion coefficient than pigs fed dl-ACE. This may suggest that ALPHA produced from the hydrolysis of d-SUCC is more efficiently digested than the ALPHA produced from the hydrolysis of dl-ACE. Scott (1965) reported that the chick digests l-ALPHA as efficiently as d-ALPHA. Weber et al. (1964) concluded that l-ALPHA was absorbed more efficiently than d-ALPHA but that the l-form was excreted more rapidly.

The major excretion route for vitamin E and its metabolites is through the feces; urinary excretion represents a minor pathway. But the measurement of fecal excretion of tocopherols tends to underestimate digestion due to the contribution of endogenous vitamin E. In rats, vitamin E metabolites are commonly found in bile but little free ALPHA can be detected (Gallo-Torres, 1980). Lee-Kim et al. (1988) reported that enterohepatic circulation of vitamin E in rats is minimal. The amount of ALPHA returning to the gut via enterohepatic recirculation in swine still remains to be determined.

The high apparent digestibilities of ester and ALPHA found in the present trial are not supported by other reports in the literature. Simon et al. (1956) reported that rabbits excreted 90% of a dose of d-alpha-tocopheryl succinate in feces in the 6 d following the dose. Kelleher et al. (1972a) used the fecal excretion method and reported that rats absorb 60 to 75% of a dose of dl-ALPHA. A patient with steatorrhea excreted 56% (absorbed 44%) of a dl-alpha-tocopheryl acetate dose (Berri and Poukka Evarts, 1972).

By measuring unabsorbed radioactivity, Davies et al. (1971) concluded that 58% of dl-ALPHA was absorbed. Kelleher and Losowsky (1970) used the fecal excretion method and reported that patients with steatorrhea absorbed 59% of a dose of dl-ALPHA (range 31-83%) while non-steatorrhea patients absorbed 72% (range 51-86%). MacMahon et al. (1971) used two methods to study the absorption of dl-ALPHA in rats. The fecal excretion method revealed that 60% of a dose was absorbed, but simultaneous collection of lymph accounted for only 42% of the dose. Vitamin E status, deficient vs non-deficient (Burton et al., 1988), nutritional status, fasting vs non-fasting or percent fat in diet, does not affect the percent absorption of dl-ALPHA (Kelleher et al., 1972b).

The fact that the pigs were on very low feed intake may have affected gut motility and influenced the results. A

problem encountered during the trial was the inconsistent amount of feces excreted each day. This problem can be minimized by pooling samples over multiple days. Gallo-Torres (1980) stated that fecal excretion of vitamin E is influenced by intestinal motility and enterohepatic circulation.

Dietary minerals and lipids may accelerate the oxidation of natural vitamin E in the gastrointestinal tract and decrease the amount absorbed (Burton et al., 1988). Oxidation of ALPHA within the gastrointestinal tract may cause the digestion of ALPHA to be overestimated.

Pigs fed diets supplemented with esters had higher E-BAL than pigs fed BASAL. Vitamin E-BAL takes into account the major sources of vitamin E in the diet: ALPHA, GAMMA and supplemented esters. This may be a more appropriate method for assessing vitamin E status than plasma ALPHA concentration or the apparent digestibility of individual forms of vitamin E.

Implications

Both dl-ACE and d-SUCC were effective in raising the serum concentration of ALPHA in swine. And, there was no evidence to suggest that biodiscrimination occurred in the hydrolysis or digestion between dl-ACE or d-SUCC.

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Table 1. Composition of diets[a]

Ingredient	Starter	Grower[b]
	%	
Corn, yellow dent	49.41	77.38
Soybean meal, 48.5%	29.84	17.70
Dried whey	15.00	
Beef tallow	2.00	2.00
Calcium carbonate	.71	.91
Dicalcium phosphate	1.10	.86
Salt, iodized	.25	.50
L-lysine	.20	
DL-methionine	.10	
Antibiotic[c]	.25	.05
Mineral premix[d]	.10	.05
Vitamin premix[e]	1.00	.50
Copper sulfate	.05	.05
Calculated Analysis		
NE, kcal/kg	2,100	2,211
Protein, %	21.17	15.78
Lysine, %	1.41	.74
Vitamin A, IU/kg	7,898	7,493
Copper, ppm	230	214

[a]Stored approximately 14 d before feeding.

[b]Grower = BASAL diet.

[c]At .25% of diet, contributed the following per kilogram of diet: 100 mg chlortetracycline, 55 mg penicillin and 110 mg sulfamethazine.

[d]At .10% of diet, contributed the following per kilogram of diet: 200 mg Zn, 100 mg Fe, 11 mg Cu, 55 mg Mn and 1.5 mg I.

[e]At 1% of diet, contributed the following per kilogram of diet: 4,400 IU vitamin A palmitate, 1,100 IU vitamin D_2, 6.6 mg riboflavin, 17.6 mg d-pantothenic acid, 33 mg niacin and 22 ug vitamin B_{12}, .44 mg santoquin.

Table 2. Pretrial period

	Pig	Alpha[a]	Wt, kg	TRT	Feed, g/d
Block 1:	1930	.4357	40.9	d-SUCC	364
	1933	.7831	32.1	BASAL	364
	1931	.6465	34.8	dl-ACE	364
Block 2:	1934	.3327	36.7	d-SUCC	364
	1932	.2865	35.7	BASAL	364
	1935	.2062	31.6	dl-ACE	364
Block 3:	3291	.1502	23.3	d-SUCC	318
	3337	.1212	23.0	BASAL	318
	3376	.1277	21.9	dl-ACE	318

[a]Serum alpha-tocopherol (mg/liter) at time of allotment.

Table 3. Vitamin E and selenium concentration of treatment diets

Item	Treatment BASAL	d-SUCC	dl-ACE	Avg[a]
Alpha[b]	2.55	1.58	2.21	2.11
Gamma[c]	12.79	10.77	12.55	12.04
Acetate[d]	ND[e]	ND	48.04	
Succinate[f]	ND	50.48	ND	
Se, ppm	.232	.203	.225	

[a]Average values for ALPHA and GAMMA were used in apparent digestion calculations.

[b]Alpha-tocopherol, mg/kg.

[c]Gamma-tocopherol, mg/kg.

[d]Alpha-tocopheryl acetate, mg/kg.

[e]ND = none detected.

[f]Alpha-tocopheryl succinate, mg/kg.

Table 4. Effect of vitamin E supplementation on serum tocopherols

Day	Treatment		
	BASAL	d-SUCC	dl-ACE
	Alpha-tocopherol, mg/liter		
0	.508	.434[a]	.399[b]
4[c]	.514	.858	.922
8[c]	.528	1.162	1.191
13[d]	.534	1.324	1.308
0-13[e]	.521	.945	.955
	Gamma-tocopherol, mg/liter		
0	.104	.039	.037
4	.100	.038	.032
8	.071	.031	.026
13	.067	.022	.014
0-13	.085	.032	.027

[a]Linear effect of time (P<.01).

[b]Linear effect of time (P<.04).

[c]Supplement > BASAL (P<.05).

[d]Supplement > BASAL (P<.01).

[e]Supplement > BASAL (P<.06).

Table 5. Effect of vitamin E supplementation on serum enzyme activity

Day	Treatment		
	BASAL	d-SUCC	dl-ACE
	LDH activity, units/ml[a]		
0	219	208	223
4	215	217	213
8	223	222	230
13	486	191	211
0-13	285	210	219
	GSH-Px activity, units/ml[b]		
0	1.380	1.271	1.412
4	1.429	1.291	1.317
8	1.408	1.229	1.244
13	1.404	1.292	1.340
0-13	1.405	1.271	1.328

[a]LDH = 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$\cdot min^{-1} \cdot cm^{-1}$ of light path at 340 nm and 20°C.

[b]GSH-Px = Glutathione peroxidase. One unit of activity was defined as the amount of enzyme that will convert 1 umol of NADPH per min at pH 7.0 and 20°C.

Table 6. Apparent digestion coefficients and vitamin E balance during day 6 to 13

		Apparent digestion[a]			
TRT	Units	Ester[b]	ALPHA[b]	GAMMA	E-BAL[bc]
BASAL	%	ND[d]	-50	41	-17
d-SUCC	%	88	87	46	75
dl-ACE	%	87	72	34	69

[a]The SE of mean for ester, alpha, gamma, and E-BAL were 1.0, 10.8, 5.7 and 8.5, respectively.

[b]Supplement > BASAL ($P<.01$).

[c]E-BAL = Apparent vitamin E balance, % = Intake ((Ester + Alpha + Gamma) - Excretion(Ester + Alpha + Gamma)/Intake(Ester + Alpha + Gamma)) x 100.

[d]ND = none detected.

Table 7. Apparent digestion coefficients (ADC) and vitamin E balance during periods 1 to 4

Period	SE	Treatment BASAL	d-SUCC	dl-ACE
		ADC of ester, %		
1	1.3	ND[a]	98.2	97.6[b]
2	1.5	ND	95.7	89.3
3	1.5	ND	83.0	86.2
4	1.5	ND	89.3	87.9
		ADC of ALPHA, %		
1[c,f]	14.5	-35.3	93.9[d]	89.6[e]
2[f]	16.7	-18.0	90.2	76.9
3[f]	16.7	-24.4	85.1	69.2
4[g]	16.7	-97.6	86.7	72.6
		Apparent vitamin E balance, %		
1[c,f]	11.5	-8.1	90.4[e]	88.4[e]
2[g]	13.5	7.7	84.4	73.4
3[g]	13.5	2.6	68.7	66.9
4[g]	13.5	-53.3	76.1	70.6

[a]ND = none detected.

[b]Linear (P<.02) and quadratic (P<.06) effect of period.

[c]Supplement > BASAL (P<.001).

[d]Linear (P<.001) effect of period.

[e]Linear (P<.01) effect of period.

[f]Supplement > BASAL (P<.01).

[g]Supplement > BASAL (P<.05).

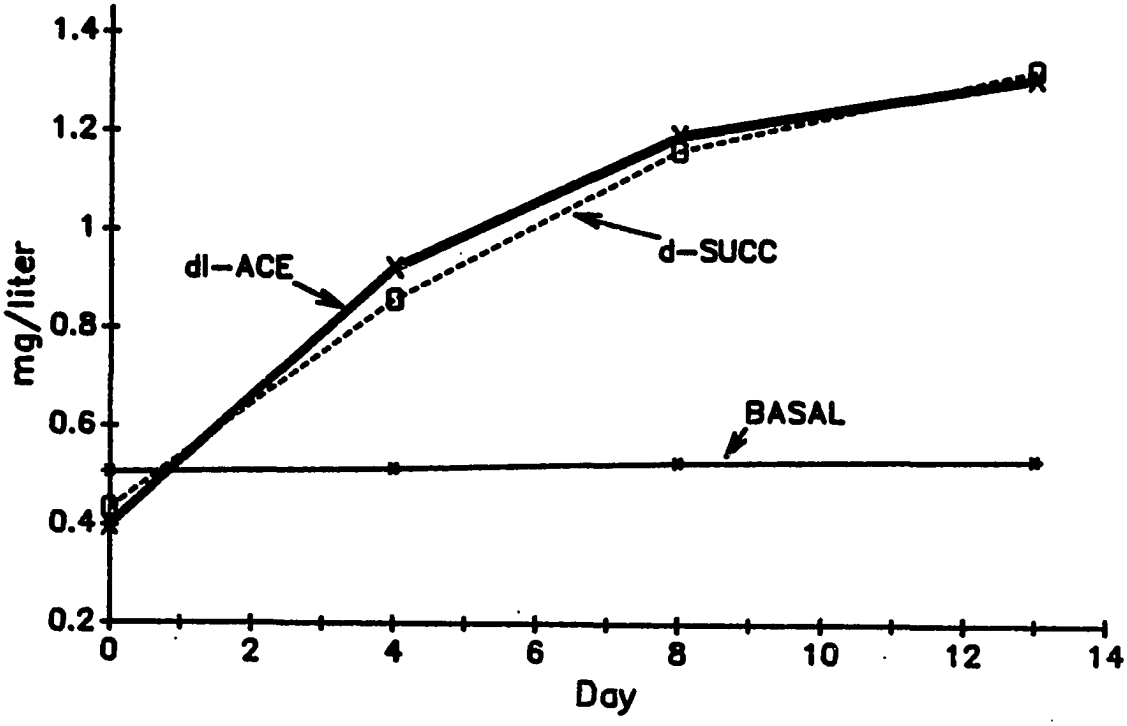

Figure 1. Effect of vitamin E supplementation on serum alpha-tocopherol

SECTION 3. THE BIOAVAILABILITY OF D-ALPHA-TOCOPHERYL ACID SUCCINATE AND DL-ALPHA-TOCOPHERYL ACETATE IN GROWING SWINE: A LONG TERM STUDY

Abstract

Sixteen 20 to 30 kg pigs were used to evaluate the bioavailability of dl-alpha-tocopheryl acetate (dl-ACE) and d-alpha-tocopheryl succinate (d-SUCC). From weaning to the start of the trial pigs were fed corn-soybean meal diets containing no supplemental vitamin E or selenium. Pigs were randomly assigned to treatments (trt): 1) CONTROL, 2) BASAL, 3) BASAL + 22 IU/kg dl-ACE, or 4) BASAL + 22 IU/kg d-SUCC. The CONTROL pigs were killed and tissues samples taken. The remaining pigs were used in a 28 d balance trial in which pigs were fed at 3% body wt/d; feed intake was adjusted weekly. Total feces were collected daily and blood samples were taken weekly. Tocopherol isomers and esters were separated by HPLC and detected by fluorescence. Before and after the trial pigs in trt 3 and 4 were given an oral 500 IU dose of ester. For 6 d following the dose, plasma samples were taken and total feces were collected. After completion of the second dose test the remaining pigs were killed and tissue samples taken. Pigs fed diets containing dl-ACE or d-SUCC had higher plasma alpha-tocopherol (ALPHA, $P<.05$) than

pigs fed BASAL diet for the overall trial mean. Tissue ALPHA was higher (P<.001) in pigs fed esters than in tissues of pigs fed BASAL or the CONTROL pigs. Within trt, tissue ALPHA varied (P<.01) among tissues; internal organs tended to have higher ALPHA than muscle, plasma or bile. For the overall trial mean, pigs fed dl-ACE had a higher (P<.01) apparent digestion coefficient (ADC) of ester and lower ADC of ALPHA (P<.01) than pigs fed d-SUCC. After a 500 IU dose, plasma ALPHA decreased (P<.01) faster in pigs fed dl-ACE than in pigs fed d-SUCC. But, area under plasma ALPHA curve was similar for both esters. There was evidence to suggest that there was a difference in the pattern of digestion between dl-ACE and d-SUCC. dl-ACE was hydrolyzed more efficiently than d-SUCC but d-SUCC resulted in a slower, more sustained increase in plasma ALPHA.

Key Words: Swine, Tocopherol, Tocopheryl Acetate, Tocopheryl Succinate

Introduction

Natural tocopherols, as an alcohol in plants and forages, are subject to oxidative destruction from a variety of factors. Oxidative losses can virtually deplete feedstuffs of all natural tocopherols. Thus, diets may have to be supplemented with vitamin E to meet the requirements of the animal. Supplemental forms of vitamin E are typically

the acetate or succinate derivatives of alpha-tocopherol. These ester derivatives have no antioxidant activity and are relatively unaffected by oxidative factors.

The apparent digestion of vitamin E has been reported to range from 10 to 75% (Gallo-Torres, 1980). But little information has been reported on the apparent digestion of vitamin E esters in swine.

The objectives of this experiment were to measure the bioavailability of dl-alpha-tocopheryl acetate (dl-ACE) and d-alpha-tocopheryl acid succinate (d-SUCC) in swine by: 1) monitoring the effects of ester supplementation on serum and tissue alpha-tocopherol (ALPHA) concentration, 2) measuring the apparent digestion of vitamin E esters by fecal excretion method, and 3) measuring the digestion of a 500 IU oral dose of vitamin E ester by plasma concentration and fecal excretion.

Materials and Methods

Pretrial

Weanling pigs from four crossbred litters (six pigs/litter) were put in raised 1.2 x 1.2 m nursery pens and fed a dried whey, corn and soybean meal starter diet (Table 1) ad libitum for 4 wk. The diet contained 230 ppm copper, no supplemental vitamin E or selenium (Se), and was stored 14 d at room temperature before feeding. After 4 wk, pigs were

placed in individual pens and fed a grower diet (Table 1) at 3% body wt (BW)/d in two equal feedings. The grower diet contained 214 ppm copper, no supplemental vitamin E or Se and was stored 14 d before feeding. The diet was formulated to meet or exceed all NRC (1988) requirements, except for vitamin E and Se. During the pretrial period, serum tocopherols, LDH and GSH-Px activity were monitored.

After 2 wk on restricted intake, four pigs from each litter were selected by weight and serum ALPHA. Within each litter, pigs were randomly assigned to one of four treatments (trt): 1) CONTROL, 2) BASAL, 3) BASAL + 22 IU/kg dl-ACE, or 4) BASAL + 22 IU/kg d-SUCC. The CONTROL pigs were killed and tissue samples taken. Tissue samples were frozen at -20^{o}C until analyzed for tocopherols. The remaining pigs were surgically implanted with an indwelling venous femoral catheter using standard surgical procedures and principles for aseptic surgery as described by Gay and Heavner (1986). Anesthesia was induced with ketamine hydrochloride (20 mg/kg) and maintained with 2 to 5% halothane in oxygen. Postoperatively, wounds and body temperature were monitored daily and antibiotics were given as indicated. Pigs were allowed 5 to 7 d to recover from surgery before starting the trial.

Dose test

Pigs assigned to the dl-ACE and d-SUCC trt were orally given a 500 IU dose of their respective ester. The oral dose test was given twice: before and after the 28 d trial. The dl-ACE was dissolved in ethanol, and d-SUCC was in powder form. The esters were mixed with 200 g of the BASAL diet and fed at the AM feeding. Pigs on BASAL trt received 200 g of the BASAL diet. Plasma samples were taken at 0, 2, 4, 6, 8, 10, 12, 24 h, and then periodically for 6 d. Pigs were fed 100 g of BASAL diet at each sampling time during the first 12 h. Otherwise, plasma samples were taken during the AM feeding. Total feces were collected for 6 d. The BASAL diet was fed to all trt for the remainder of the test. Plasma and fecal samples were frozen at -20°C until analyzed for vitamin E.

Trial

The pigs in trt 2, 3 and 4 were used in a 28 d balance trial. Seven days following the first oral dose, d 0, trt were started and total feces were collected twice daily until the end of trial. Pigs were fed at 3% BW/d in two equal feedings, and feed intake was adjusted weekly. Diets were stored 14 d at room temperature before the addition of the esters. Blood was collected weekly and plasma was separated and analyzed for tocopherol isomers, LDH and GSH-Px activity. Feces, feed and plasma were frozen at -20°C until analyzed

for vitamin E.

Serum/Plasma analysis

Serum/plasma tocopherols were determined by high performance liquid chromatography (HPLC) with fluoroscence detection as described in Section 2 herein. The HPLC system used could not distinguish between d- and l-forms of ALPHA. Serum LDH (Amador et al., 1963) and GSH-Px (Paglia and Valentine, 1967) activity were determined within 24 h of blood collection.

Tissue analysis

Tissues were analyzed for tocopherols by the homogenization of tissue (2 to 3 g) in 10 ml (wt/vol) of phosphate-EDTA-buffer (pH 7.0) and extracted with hexane (1 ml) as described for serum. The chromatographic conditions were the same as described for serum.

Feed analysis

Selenium was determined by the method of Olson et al. (1975). Tocopherol isomers and alpha-tocopheryl acetate in feed and feces were extracted with hexane, then separated and quantitated by HPLC by a modified method of Ingold et al. (1987). The chromatographic conditions and detection settings were the same as described for serum tocopherol

analysis. Feed samples were ground through a 1.0 mm screen. Fecal samples were not ground and were analyzed on a wet-basis. Approximately .3 to .5 g of sample was weighed into a tared 16 x 120 mm screw top culture tube and 3.0 ml of .1 M sodium dodecyl sulfate (SDS) solution with 2% ascorbic acid (wt/vol) was added. The tube was capped with a teflon lined lid, vortexed and heated at 60°C for 45 to 60 min. Four milliliters of distilled ethanol were added and the mixture was heated at 60°C for 1 h. Tocopherols were extracted with hexane; the hexane phase was injected directly into the HPLC. The procedure was modified for feed by adding ethanol first and then deionized water. Deionized water was substituted for the SDS solution.

The separation and quantitation of alpha-tocopheryl succinate was achieved by HPLC analysis with fluoroscence detection. The fluorometer settings were 284 nm excitation wavelength and 306 nm emission wavelength. A normal phase HPLC system, equipped with a time gradient program, was used to elude to the ester. A two-solvent gradient was utilized: solvent A (mobile phase) was 3.5% tetrahydrofuran (vol/vol) in HPLC grade hexane, and solvent B (gradient solvent) was 20% tetrahydrofuran and .5% acetic acid (vol/vol) in HPLC grade hexane. The sample was injected at time = 0 and a concave curve gradient of solvent B was run from .5 to 16.0 min. The gradient increased solvent B from 0 to 70%. A

linear reverse gradient of solvent B, 70 to 0%, proceeded from 16.01 to 21.0 min. The mobile phase, 100% of solvent A, was run for 11 min to requilibrate the column. The total program time was 32.0 min which allowed for a total run time of 35.0 min/sample. The flow rate was set at approximately 2.0 ml/min. The time gradient program efficiently eluted alpha-tocopheryl succinate at approximately 18.0 min with good separation and resolution.

Calculations

Apparent digestibility coefficients (ADC) and apparent vitamin E balance were calculated as described in Section 2 herein. Pharmacokinetic parameters were estimated from graphs of individual animal plasma ALPHA concentration curve. Area under plasma ALPHA concentration curves (AUC) for each animal was calculated by the trapezoidal rule (Koch-Weser, 1974; Welling, 1986).

Statistics

Data were statistically analyzed by least-squares analysis of variance as performed by the GLM procedure of SAS (1985). The dependent variables were ADC of ester and ALPHA, and vitamin E balance. The model included treatment and time. Contrasts were used to test for treatment differences within digestion parameters and within periods. Regression analyses were performed on serum and digestion parameters to

determine the effects of time. Data are reported as least-square means.

Results

Feed Analysis

The analyzed concentration of ALPHA and gamma-tocopherol (GAMMA) remained stable throughout the trial (Table 2). The concentration of dl-ACE tended to decrease as the trial progressed.

Plasma Parameters

Pigs fed diets supplemented with either dl-ACE or d-SUCC had higher ($P<.05$) plasma ALPHA concentration than pigs fed the BASAL diet for the overall mean of the 28 d trial (Table 3). Plasma ALPHA was not different between dl-ACE and d-SUCC at any point of the trial (Figure 1). Plasma GAMMA was very low and sometimes not detectable, and no trt differences were detected (data not shown). Plasma LDH and GSH-Px activity did not differ among treatments at any point of the trial (Table 4). From d 0 to 28, plasma LDH activity linearly decreased in pigs fed BASAL ($P<.05$), dl-ACE and d-SUCC ($P<.001$). No trend could be detected in plasma GSH-Px activity.

Tissue Tocopherol

No difference was detected between the trt groups, control and BASAL, or between dl-ACE and d-SUCC; therefore, data were pooled into non-supplemented and supplemented groups (Table 5). In all tissues sampled, tissue ALPHA concentration was higher ($P<.001$) in pigs fed esters than in pigs fed the BASAL diet or the CONTROL pigs. Within trt, internal organs tended to have higher ALPHA concentration than muscles, plasma or bile. In pigs fed supplemented diets, the pancreas and heart had higher ($P<.05$) ALPHA concentration than all other tissues. Tissue GAMMA was very low or not detectable in the tissues sampled (Table 6). Data were also pooled into non-supplemented and supplemented groups. Tissue GAMMA followed a trend similar to that of tissue ALPHA; internal organs tended to have higher GAMMA concentrations than muscles, bile or plasma. Pigs fed diets supplemented with esters had higher ($P<.05$) GAMMA concentration in liver, pancreas and spleen than pigs fed non-supplemented diets.

Digestion of ester

During wk 1, both dl-ACE and d-SUCC were efficiently absorbed, as measured by fecal excretion of unhydrolyzed ester (Table 7). Within d 3, pigs fed dl-ACE digested the ester more efficiently than pigs fed d-SUCC ($P<.01$). The

apparent digestion coefficient (ADC) of ester decreased (linear P<.01 and quadratic P<.01) in pigs fed dl-ACE or d-SUCC during d 1 to 7.

For the overall mean of wk 1 to 4, pigs fed dl-ACE had greater (P<.01) ADC of ester than pigs fed d-SUCC. In individual weeks, pigs fed dl-ACE tended to digest the ester more efficiently than pigs fed d-SUCC, but the difference was only significant in wk 3 (P<.05). Ester digestibility decreased from wk 1 to 4 in pigs fed d-SUCC (linear P<.01 and quadratic P<.01). An effect of week (P<.05) was detected in pigs fed dl-ACE, but no trend was seen. For the overall trial mean of wk 1 to 4, a week x treatment interaction (P<.01) was detected.

Digestion of ALPHA

Within periods of wk 1, pigs fed diets supplemented with esters digested ALPHA more efficiently than pigs fed BASAL in periods 1, 2, 3, 4 (P<.001) and 5 (P<.01, Table 8). In period 5, pigs fed d-SUCC had a higher (P<.05) ADC of ALPHA than pigs fed dl-ACE. The digestibility of ALPHA decreased during wk 1 in pigs fed d-SUCC (linear P<.01 and quadratic P<.01) and in pigs fed dl-ACE (linear P<.05).

Pigs fed diets supplemented with esters had greater ALPHA digestion coefficients in wk 1 (P<.001), 2, 3, and 4 (P<.05), and overall trial mean (P<.01) than pigs fed the

BASAL diet. Although in individual weeks pigs fed d-SUCC tended to digest ALPHA more efficiently than pigs fed dl-ACE, the difference was only significant in wk 1 ($P<.05$) and in the overall trial mean ($P<.01$). ALPHA digestibility decreased in pigs fed d-SUCC (linear $P<.05$ and quadratic $P<.05$) from wk 1 to 4. An effect of week ($P<.05$) was detected in pigs fed dl-ACE but no trend could be established.

Apparent Vitamin E Balance

Within period 1, pigs fed d-SUCC had greater ($P<.05$) vitamin E balance than pigs fed dl-ACE (Table 9). Pigs fed dl-ACE or d-SUCC had higher vitamin E balance than pigs fed BASAL in periods 1 ($P<.001$), 2, 3 and 5 ($P<.01$). From period 1 to 5 in wk 1, vitamin E balance decreased in pigs fed d-SUCC (linear $P<.01$ and quadratic $P<.01$) and dl-ACE (linear $P<.01$ and quadratic $P<.05$).

Pigs fed diets supplemented with esters had greater ($P<.001$) vitamin E balance than pigs fed BASAL in wk 1 and the overall trial mean. No trt differences were detected in wk 2, 3 or 4. Vitamin E balance from wk 1 to 4 decreased (linear $P<.01$ and quadratic $P<.01$) in pigs fed d-SUCC. An effect of week ($P<.05$) was seen in pigs fed dl-ACE, but no trend could be established.

Digestion of oral dose tests

Ester Both dl-ACE and d-SUCC were efficiently absorbed in Dose Test 1, 84 and 76% respectively (Table 10). No trt difference was detected in Dose Test 1, but in Dose Test 2, dl-ACE was more efficiently absorbed than d-SUCC ($P<.001$).

ALPHA In both dose tests, pigs fed diets supplemented with esters had digested ALPHA more efficiently than pigs fed BASAL ($P<.001$). No difference was detected in either dose test between dl-ACE and d-SUCC.

Vitamin E balance Pigs fed diets supplemented with dl-ACE or d-SUCC had greater vitamin E balance than pigs fed BASAL in Dose Test 1 ($P<.001$) and 2 ($P<.05$). In Dose Test 2, pigs fed dl-ACE had higher ($P<.01$) vitamin E balance than pigs fed d-SUCC.

Plasma tocopherol: Oral dose tests

Dose Test 1 Pigs fed supplemented diets had greater plasma ALPHA concentration than pigs fed BASAL during h 4 to 168 (Figure 2). Plasma ALPHA was higher ($P<.001$) in pigs fed dl-ACE in h 4 to 10 than pigs fed d-SUCC. But, plasma ALPHA was greater in pigs fed d-SUCC in h 96, 144 ($P<.05$), and 168 ($P<.01$) than in pigs fed dl-ACE. Time of plasma ALPHA maximum (*Tmax*) was earlier ($P<.001$) and terminal plasma ALPHA

(*CT*) was lower (P<.05) in pigs fed dl-ACE than in pigs fed d-SUCC (Table 11). The ratio of terminal to initial plasma ALPHA was higher (P<.05) in pigs fed d-SUCC than in pigs fed dl-ACE. The elimination of ALPHA (*Cmax/CT*) was faster (P<.01) in pigs fed dl-ACE than in pigs fed d-SUCC. There was no difference in area under plasma concentration curve (AUC) between dl-ACE and d-SUCC.

Dose Test 2 Plasma ALPHA concentration curves were similar to that of Dose Test 1 (Figure 3). Plasma ALPHA was higher (P<.05) in pigs fed dl-ACE during h 4 to 12 than pigs fed d-SUCC. But, plasma ALPHA tended to be greater in pigs fed d-SUCC during h 96 to 144 than in pigs fed dl-ACE, however, the difference was not significant. Pigs fed dl-ACE had higher (P<.01) ALPHA *Cmax* than pigs fed d-SUCC (Table 12). The *Cmax/CT* was higher (P<.01) in pigs fed dl-ACE than in pigs fed d-SUCC. There was no difference in AUC between dl-ACE and d-SUCC.

Discussion

Vitamin E supplementation had no effect on plasma ALPHA during the 28 d balance trial. Plasma ALPHA in pigs fed dl-ACE or d-SUCC increased the first week of the trial then decreased in the remaining 3 wk of the trial. The loss of supplemented esters in the diet (Table 2) may account for the decrease in plasma ALPHA seen in the later week of the trial.

The higher plasma ALPHA on d 28 of pigs fed d-SUCC suggests that there was biodiscrimination between the two esters. But, the more extensive loss of dl-ACE from the diet may explain the slight difference in plasma ALPHA between dl-ACE and d-SUCC rather than biodiscrimination between these two forms of vitamin E.

Treatment had no effect on plasma enzyme activity at any point of the trial. Diets were not supplemented with Se, which suggests that dietary Se was adequate to meet the Se requirement. The decline in plasma LDH activity and adequate GSH-Px activity indicates that pigs fully recovered from surgery and the vitamin E/Se status was adequate.

The plasma ALPHA concentration curves suggest that dl-ACE is hydrolyzed faster than d-SUCC; indicated by the earlier time of maximum plasma ALPHA. But, the higher *Cmax/CT* of ALPHA in pigs fed dl-ACE suggests that dl-ALPHA, or l-ALPHA, is eliminated from the plasma faster than d-ALPHA. The lack of a trt difference in AUC suggests that both esters were absorbed to the same extent.

Tissue ALPHA was higher in all tissues of pigs supplemented with esters than CONTROLS or BASAL, indicating that all tissues accumulate the vitamin. Organs tended to have higher ALPHA than muscles and plasma suggesting tissues have different vitamin E metabolic rates or tissue uptake. Tissues of pigs fed d-SUCC tended to have higher ALPHA than

tissues of pigs fed dl-ACE. This nonsignificant difference may be from the lower levels of dl-ACE in diet rather then from biodiscrimination at the gut or tissue. But, tissues were collected after the second oral dose of ester and this may have influenced the results. Tissue GAMMA concentration was lower than tissue ALPHA in all tissues sampled, suggesting that tissues prefer ALPHA over GAMMA. Cheng et al. (1987) reported that human erythrocytes preferentially retain RRR-ALPHA over SRR-ALPHA. It has been reported that tissues preferentially uptake RRR-ALPHA over SRR-ALPHA (Ingold et al., 1987). Peak and Berri (1971) reported that GAMMA is taken up by tissues but is eliminated from tissues at a faster rate than ALPHA. The methyl group at C-5 may be important in tissue retention of tocopherols (Gallo-Torres, 1980).

Both dl-ACE and d-SUCC were efficiently hydrolyzed, 48 to 74% absorbed, as measured by excretion of unhydrolyzed ester. There was evidence which suggested biodiscrimination had occurred at the level of the gut; dl-ACE was digested more efficiently than d-SUCC for overall trial mean of wk 1 to 4. Biodiscrimination at the level of the gut was reported by Ingold et al. (1987). Ingold and coworkers concluded that the hydrolysis of RRR-alpha-tocopheryl acetate was more complete than SRR-alpha-tocopheryl acetate. Chirality of active enzymes in pancreatic juice and(or) bile salts may

cause biodiscrimination within the gut (Gallo-Torres, 1980; Lombardo and Guy, 1980).

The high digestion coefficients of esters in Dose Test 1 suggest that a pharmacological dose is absorbed as efficiently as a physiological dose. The low ADC of d-SUCC in oral Dose Test 2 may have been from an error in the measurement of the dose or fecal output of ester.

The ALPHA produced from the hydrolysis of vitamin E esters was extensively digested in pigs fed dl-ACE or d-SUCC as indicated by high ADC of ALPHA. The higher digestion coefficient of ALPHA for overall trial means in pigs fed d-SUCC suggests that there was biodiscrimination between the two forms of ALPHA. The d-form of ALPHA is absorbed more efficiently than the dl-form. Weber et al. (1964) concluded that in rats, l-ALPHA was absorbed more efficiently than d-ALPHA, but that the l-form was excreted more rapidly. Scott (1965) reported that the chick absorbs l-ALPHA as efficiently as d-ALPHA.

Pigs fed BASAL had a relatively low and inconsistent ADC of ALPHA throughout the trial. This was somewhat influenced by the low number of animals in the later stages of the trial. The high digestibility of ALPHA in the dose tests suggests that a pharmacological dose of ALPHA is digested as efficiently as a physiological dose.

The digestion of vitamin E is reported to be relatively

incomplete; the experimental protocol influences the results. Most vitamin E digestibility work has concentrated on the digestion of dl-ACE and dl-ALPHA in rats and humans. Few studies have used swine as a model. A reveiw of the literature reveals a wide range of digestibilities for vitamin E, 10 to 86% (Simon et al., 1956; Blomstrand and Forsgren, 1968; Gallo-Torres, 1970; Kelleher and Losowsky, 1970; Davies et al., 1971; Berri and Poukka Evarts, 1972; Kelleher et al., 1972; MacMahon, 1971; Mathias et al., 1981).

Traber et al., (1986) reported that the digestibility of ALPHA and GAMMA is not different, but there may be a preferential secretion of GAMMA into bile (Traber and Kayden, 1989). McMurry and Rice (1982) concluded that beta-, gamma- and delta-tocopherols and tocotrienols are not absorbed or retained in plasma or tissues in swine.

The fact that the pigs were on low feed intake (3% BW/d) may have affected gut motility and influenced the results. The apparent digestibility of ester in this trial was somewhat lower than results obtained at very low feed intake (Less and Ewan, 1988). This suggests that amount of feed intake affects the efficiency of ester hydrolysis.

The major excretion route for vitamin E and its metabolites is through the feces; urinary excretion represents a minor pathway. The fecal excretion method tends to underestimate absorption due to the contribution of

endogenous vitamin E. Vitamin E metabolites are commonly found in bile while little free ALPHA is found (Gallo-Torres, 1980). In the present trial, the bile and plasma contained similar amounts of ALPHA. The HPLC chromatograph of bile was very clean and had an appearance similar to that of a plasma sample; very few compounds with tocopherol-like fluoroscence were detected. Some ALPHA is returning to the gut via the bile, but the amount and role it has in vitamin E metabolism is yet to be determined. A normal 40 to 60 kg pig secretes approximately 1.5 to 2 liter/24 h (1 $ml^{-1} \cdot h^{-1}$ kg BW, Dr. C. Lumen, personal communication, Department of Nutritional Physiology, Iowa State University). At this amount of secretion bile would have added .315 mg of ALPHA in 24 h: 10 to 15% of ALPHA excretion. But in the gut, ALPHA within bile may be degraded, excreted or reabsorbed.

Implications

At a low level of feed intake (3% BW/d), there was evidence to suggest that biodiscrimination had occurred between dl-ACE and d-SUCC in hydrolysis and digestion. dl-ACE was hydrolyzed more efficiently than d-SUCC but d-SUCC resulted in a slower, more sustained increase in plasma ALPHA.

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Table 1. Diet composition[a]

Ingredient	Starter	Grower[b]
	%	
Corn, yellow dent	48.35	72.65
Soybean meal, 48.5%	30.00	21.10
Dried whey	15.00	
Soybean oil	3.00	3.00
Calcium carbonate	.90	.90
Dicalcium phosphate	1.10	1.25
Salt, iodized	.25	.50
Antibiotic[c]	.25	.05
Mineral premix[d]	.10	.05
Vitamin premix[e]	1.00	.50
Copper sulfate	.05	.05
Calculated Analysis		
NE, kcal/kg	2,135	2,218
Protein, %	20.90	17.00
Lysine, %	1.22	.84
Vitamin A, IU/kg	7,826	7,172
Copper, ppm	230	215

[a]Stored approximately 14 d prior to feeding.

[b]Grower diet = BASAL diet.

[c]At .25% of diet, contributed the following per kilogram of diet: 100 mg chlortetracycline, 55 mg penicillin and 110 mg sulfamethazine.

[d]At .10% of diet, contributed the following per kilogram of diet: 200 mg Zn, 100 mg Fe, 11 mg Cu, 55 mg Mn and 1.5 mg I.

[e]At 1% of diet, contributed the following per kilogram of diet: 4,400 IU vitamin A palmitate, 1,100 IU vitamin D_2, 6.6 mg riboflavin, 17.6 mg d-pantothenic acid, 33 mg niacin and 22 ug vitamin B_{12}, .44 mg santoquin.

Table 2. Analyzed vitamin E and selenium concentration of diets

TRT	Day	Vitamin E				Se, ppm
		Alpha[a]	Gamma[b]	Ace[c]	Succ[d]	
BASAL:	0	1.75	5.54	NA[e]	NA	
	7	1.59	4.46	NA	NA	
	14	1.77	5.23	NA	NA	
	22	1.80	5.72	NA	NA	
	Avg	1.73	5.24	NA	NA	.312
d-SUCC:	0	1.91	5.03	NA	20.41	
	7	1.75	4.08	NA	18.16	
	14	1.90	5.77	NA	16.73	
	22	2.21	6.72	NA	19.04	
	Avg	1.94	5.40	NA	18.41	.266
dl-ACE:	0	1.69	5.04	15.28	NA	
	7	1.78	4.02	14.15	NA	
	14	1.79	5.19	10.62	NA	
	22	1.85	5.67	8.72	NA	
	Avg	1.78	4.98	12.19	NA	.329
Total avg		1.82	5.21	NA	NA	

[a]Alpha = Alpha-tocopherol, mg/kg.

[b]Gamma = Gamma-tocopherol, mg/kg.

[c]Ace = Alpha-tocopheryl acetate, mg/kg.

[d]Succ = Alpha-tocopheryl succinate, mg/kg.

[e]NA = not applicable.

Table 3. Effect of treatment on serum alpha-tocopherol (mg/liter)

Day	Treatment		
	BASAL	d-SUCC	dl-ACE
0	.129	.132	.141
7	.118	.270	.247
13	.135	.245	.177
20	.106	.196	.118
28	.093	.202	.140
0-28[a]	.116	.209	.165

[a]Supplement > BASAL ($P<.05$).

Table 4. Effect of treatment on serum enzyme activity

Day	Treatment		
	BASAL	d-SUCC	dl-ACE
	LDH activity, units/ml[a]		
0	264[b]	219[c]	252[c]
7	205	168	208
13	172	154	171
20	177	148	161
28	166	136	155
	GSH-Px activity, units/ml[d]		
0	.845	.849	.991
7	.995	1.010	.968
13	1.021	.868	.936
20	.848	.812	.837
28	1.056	1.033	1.108

[a]LDH = 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$\cdot min^{-1} \cdot cm^{-1}$ of light path at 340 nm and 20°C.

[b]Linear effect of day ($P<.05$).

[c]Linear effect of day ($P<.001$).

[d]GSH-Px = Glutathione peroxidase. One unit of activity was defined as the amount of enzyme that will convert 1 umol of NADPH per min at pH 7.0 and 20°C.

Table 5. Effect of treatment on tissue alpha-tocopherol concentration (mg/kg wet tissue)

Tissue	Treatment	
	Non-supplement	Supplement
Bile[a]	.064 [b]	.375[b]
Ham[a]	.053 [b]	.438 [b]
Longissimus muscle[a]	.035 [b]	.461 [b]
Plasma[ac]	.080 [b]	.434 [b]
Liver[a]	.126 [bd]	.993 [d]
Kidney[a]	.077 [b]	1.080 [d]
Spleen[a]	.177 [bd]	1.473 [de]
Lung[a]	.170 [bd]	1.676 [e]
Pancreas[a]	.134 [bd]	2.140 [f]
Heart[a]	.248 [d]	2.210 [f]

[a]Supplement > Non-supplement (P<.001).

[b]Plasma is in mg/liter.

[c,d,e,f]Means within columns with different superscripts differ (P<.05).

Table 6. Effect of treatment on tissue gamma-tocopherol concentration (mg/kg wet tissue)

Tissue	Treatment	
	Non-supplement	Supplement
Ham	.001 [a]	.000 [a]
Longissimus muscle	.001 [a]	.000 [a]
Plasma[b]	.001 [a]	.000 [a]
Bile	.007 [a]	.015 [ac]
Kidney	.030 [acd]	.017 [ac]
Lung	.016 [ac]	.053 [cd]
Liver[e]	.027 [acd]	.084 [d]
Heart	.066 [d]	.091 [fg]
Pancreas[e]	.052 [cd]	.103 [fg]
Spleen[e]	.051 [cd]	.133 [fg]

[a,c,d,f,g]Means within columns with different superscripts differ (P<.05).

[b]Plasma is in mg/liter.

[e]Supplement > Non-supplement (P<.05).

Table 7. Effect of vitamin E supplementation on the apparent digestion coefficient of ester (%)

Period	Day	Treatment		
		BASAL	d-SUCC	dl-ACE
1[a]	1	NA[b]	100.0[c]	100.0[c]
2[a]	2	NA	71.2	72.9
3[ad]	3	NA	57.8	74.3
4[a]	4	NA	59.6	69.8
5[a]	5-7	NA	62.0	70.5
Week				
1[a]		NA	67.1[c]	74.8[e]
2[ag]		NA	59.7	61.5
3[a]		NA	48.2	66.5
4[adg]		NA	64.4	62.8
1-4		NA	59.8	66.4

[a]Supplement > BASAL (P<.001).

[b]NA = not applicable.

[c]Linear (P<.01) and quadratic (P<.01) effect of time.

[d]dl-ACE > d-SUCC (P<.01).

[e]Effect of week (P<.05).

[f]dl-ACE > d-SUCC (P<.05).

[g]Week x Treatment interaction (P<.01).

Table 8. Effect of vitamin E supplementation on the apparent digestion coefficient of ALPHA (%)

Period	Day	Treatment		
		BASAL	d-SUCC	dl-ACE
1[a]	1	3.1	89.4[b]	79.8[c]
2[a]	2	-2.8	78.2	59.5
3[a]	3	-28.7	76.4	61.0
4[a]	4	8.3	81.5	51.6
5[de]	5-7	11.7	83.2	56.8
Week				
1[ae]		3.3	82.9[f]	60.5[g]
2[h]		11.4	79.4	49.9
3[h]		31.0	73.2	63.7
4[h]		32.4	83.1	57.1
1-4[ai]		19.5	79.6	57.8

[a]Supplement > BASAL (P<.001).

[b]Linear (P<.01) and quadratic (P<.01) effect of time.

[c]Linear (P<.05) effect of time.

[d]Supplement > BASAL (P<.001).

[e]d-SUCC > dl-ACE (P<.05).

[f]Linear (P<.05) and quadratic (P<.05) effect of time.

[g]Effect of week (P<.05).

[h]Supplement > BASAL (P<.05).

[i]d-SUCC > dl-ACE (P<.001).

Table 9. Effect of vitamin E supplementation on apparent vitamin E balance (%)

Period	Day	Treatment BASAL	Treatment d-SUCC	Treatment dl-ACE
1[ab]	1	5.9	86.8[c]	74.5[d]
2[e]	2	-0.3	56.7	42.8
3[e]	3	-23.8	45.6	46.0
4	4	9.9	50.6	36.7
5[e]	5-7	13.5	53.5	40.8
Week				
1[a]		5.7	56.7[c]	45.4[f]
2		13.7	49.3	31.5
3		31.5	38.8	44.5
4		32.9	55.3	38.9
1-4[a]		20.9	50.0	40.1

[a]Supplement > BASAL (P<.001).

[b]d-SUCC > dl-ACE (P<.05).

[c]Linear (P<.01) and quadratic (P<.01) effect of time.

[d]Linear (P<.01) and quadratic (P<.05) effect of time.

[e]Supplement > BASAL (P<.01).

[f]Effect of week (P<.05).

Table 10. The digestibility of an oral 500 IU dose of vitamin E ester

Dose Test	Treatment BASAL	d-SUCC	dl-ACE
	Apparent digestion coefficient of ester, %		
1	NA[a]	76.1	84.1
2[b]	NA	44.3	87.1
	Apparent digestion coefficient of ALPHA, %		
1[c]	-6.3	96.0	84.1
2[c]	32.3	93.5	85.8
	Apparent digestion coefficient of GAMMA, %		
1	9.5	15.3	4.6
2[d]	38.2	62.6	62.9
	Apparent vitamin E balance, %		
1[c]	-2.8	73.5	70.8
2[de]	33.6	45.0	75.1

[a]NA = not applicable.

[b]dl-ACE > d-SUCC (P<.001).

[c]Supplement > BASAL (P<.001).

[d]Supplement > BASAL (P<.05).

[e]dl-ACE > d-SUCC (P<.01).

Table 11. Plasma ALPHA pharmacokinetic data after a 500 IU oral dose of vitamin E ester (Dose Test 1)

Parameter	Treatment d-SUCC	SE	dl-ACE	SE
C_{max}[a]	1.45	.29	2.21	.25
C_o[b]	.07	.02	.06	.01
C_T[cd]	.33	.05	.16	.04
T_{max}[ef]	24.7	.7	6.0	.7
AUC[g]	128.0	28.5	105.0	24.7
C_{max}/C_o	22.6	6.5	43.4	5.6
C_T/C_o[d]	5.2	.4	3.0	.4
C_{max}/C_T[h]	4.4	1.6	14.7	1.4

[a]Maximum plasma concentration, mg/liter.

[b]Initial plasma concentration, mg/liter.

[c]Terminal plasma concentration, mg/liter.

[d]d-SUCC > dl-ACE ($P<.05$).

[e]Time at plasma maximum, hour.

[f]d-SUCC > dl-ACE ($P<.001$).

[g]Area under plasma ALPHA concentration curve.

[h]dl-ACE > d-SUCC ($P<.01$).

Table 12. Plasma ALPHA pharmacokinetic data after a 500 IU oral dose of vitamin E ester (Dose Test 2)

Parameter	Treatment d-SUCC	Treatment dl-ACE	SE
Cmax[ab]	.98	1.82	.13
Co[c]	.15	.09	.02
CT[d]	.49	.35	.06
Tmax[e]	29.1	5.3	8.0
AUC[f]	98.6	103.9	18.0
Cmax/Co	6.7	30.2	9.9
CT/Co	3.4	5.1	1.3
Cmax/CT[g]	2.0	5.6	.7

[a]Maximum plasma concentration, mg/liter.

[b]dl-ACE > d-SUCC ($P<.01$).

[c]Initial plasma concentration, mg/liter.

[d]Terminal plasma concentration, mg/liter.

[e]Time at plasma maximum, hour.

[f]Area under plasma ALPHA concentration curve.

[g]dl-ACE > d-SUCC ($P<.05$).

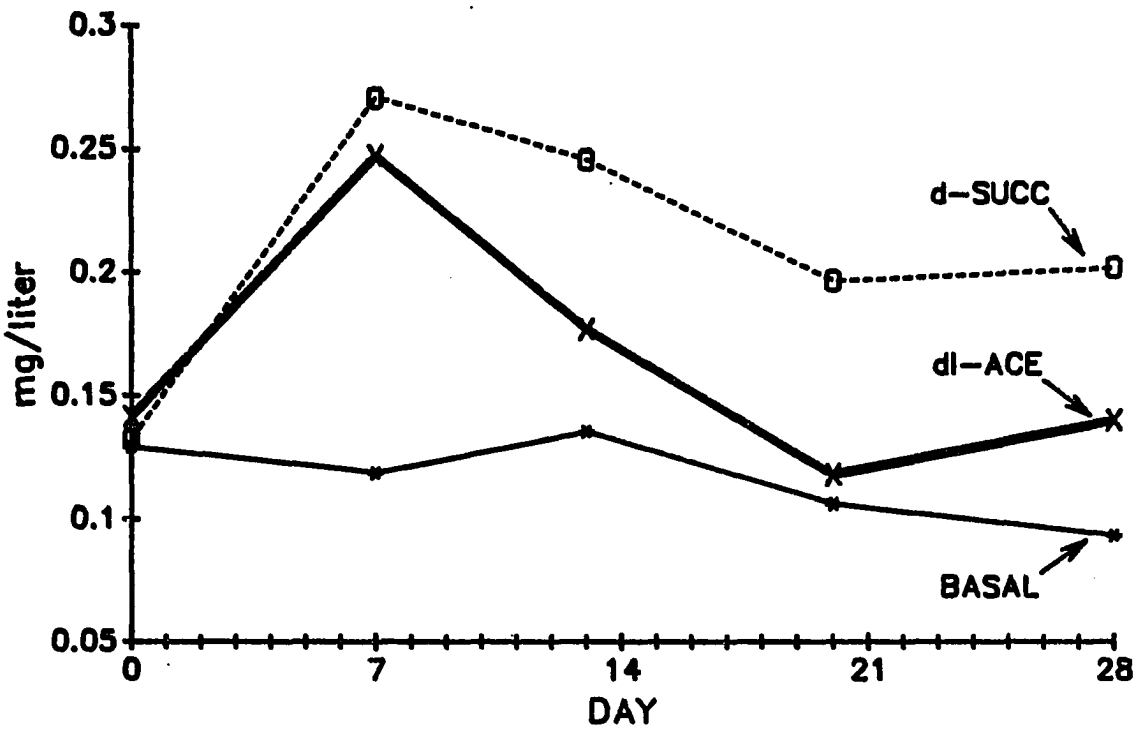

Figure 1. Effect of vitamin E supplementation on plasma alpha-tocopherol

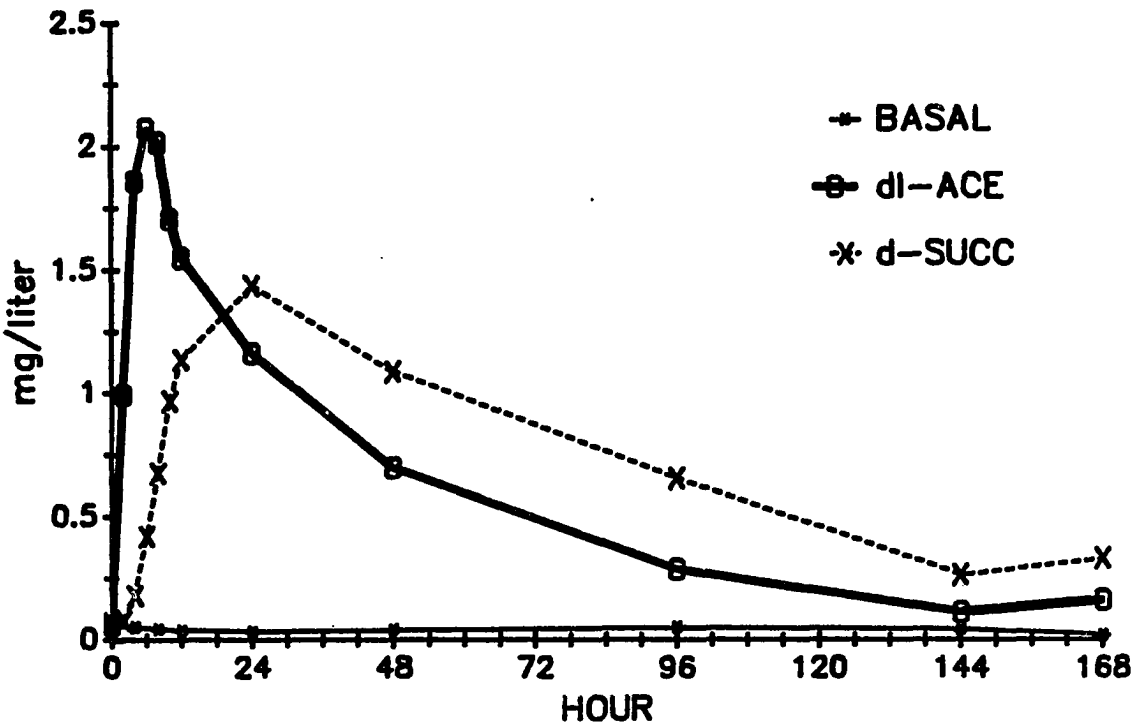

Figure 2. Effect of an oral 500 IU dose vitamin E ester on plasma alpha-tocopherol (Dose Test 1)

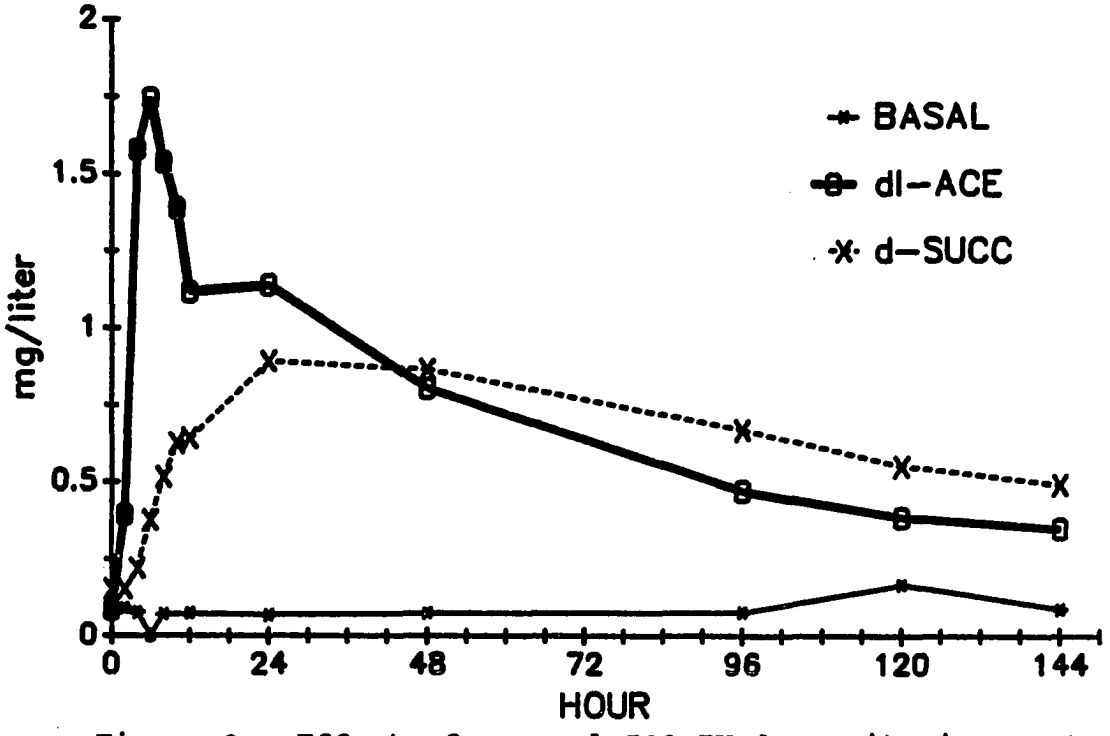

Figure 3. Effect of an oral 500 IU dose vitamin E ester on plasma alpha-tocopherol (Dose Test 2)

SUMMARY AND DISCUSSION

The continued occurrence of vitamin E/Se deficiencies in confinement reared pigs fed diets adequately supplemented with vitamin E and Se suggests that E/Se requirement is being compromised. Genetics, environment, dietary constituents, and disease may predispose animals to a deficiency.

Blood samples obtained from two swine herds gave evidence that suggests vitamin E/Se status varies by genetic background. A wide variation was detected in serum ALPHA concentration, glutathione peroxidase (GSH-Px) and lactic acid dehydrogenase (LDH) activity among genetic lines. Within a herd, pigs were of similar age and reared under common environment, nutrition and management. No previous reports have compared the vitamin E and Se status among a large number of genetic lines reared under similar environment and fed the same diet.

Vitamin E/Se status is commonly measured by analysis of blood concentrations of tocopherols, GSH-Px and LDH activity. Alpha-tocopherol (ALPHA) is the most potent and predominate isomer in the blood and tissues, is typically the only isomer assayed. Selenium is an essential component of GSH-Px (Rotruck et al., 1973) and in pigs there is a high correlation between plasma GSH-Px activity and plasma Se (Ewan, 1976; Chavez, 1979). Both vitamin E and Se are involved in the body antioxidant defense system. Serum LDH

activity can be used as a general indicator of tissue and membrane damage and elevated serum LDH activity has been shown in pigs fed a vitamin E/Se deficient diet (Ewan and Wastell, 1970). Serum data indicated that pigs from the two herds were in adequate vitamin E/Se status, showed no symptoms of vitamin E/Se deficiency.

Other reports have suggested that genetics influence vitamin E/Se status: swine (Jensen et al., 1979; Stowe and Miller, 1985; Sankari, 1985), cattle (Maplesden et al., 1960), rats (Bendich et al., 1983), sheep (Sandholm et al., 1983) and humans (Thakker et al., 1987).

The blood level of vitamin E is the balance between the rate of absorption from the gastrointestinal tract, and the uptake, retention and release from peripheral tissues. Intake of colostrum and milk are important to the vitamin E/Se status of the young pig (Loudenslager et al., 1986). Nursing pigs rely on milk vitamin E to maintain their serum vitamin E levels (Stowe and Miller, 1985). Milk contains a lower level of vitamin E and Se than colostrum and both are dependent on gestation and lactation diets (Rasmussen, 1974; Malm et al., 1976). Differences in vitamin E/Se status among genetic lines could be contributed to: milk concentration of ALPHA and Se, amount of milk production may or differences in vitamin E/Se digestion and metabolism.

Swine diets are commonly supplemented with vitamin E

because oxidative losses can deplete feedstuffs of natural tocopherols. The supplemental forms of vitamin E are typically derivatives of ALPHA, the principal types being an acetate and succinate esters. Vitamin E esters have no antioxidant activity and are relatively unaffected by oxidative factors. But, must be hydrolyzed in the intestinal lumen before absorption can take place. Vitamin E absorption is dependent upon normal lipid digestion and absorption. The uptake of tocopherol from the gut is thought to be a non-saturable diffusion process which is not carrier mediated (Gallo-Torres, 1980).

Limited data can be found on the apparent digestibility of vitamin E esters in swine. A study in which pigs were fed diets at 1.0 to 1.4% body wt/day indicated that dl-alpha-tocopheryl acetate (dl-ACE) and d-alpha-tocopheryl succinate (d-SUCC) were efficiently digested and there was no difference between the esters. The lack of a treatment difference in digestion between the esters suggests that at low level of feed intake there was no discrimination between esters in hydrolysis.

The tendency for ALPHA produced from the hydrolysis of d-SUCC to be more efficiently digested than ALPHA produced from dl-ACE suggests that ALPHA digestion was affected by the type of ALPHA in the ester (dl-form < d-form). A low level of feed intake there was no evidence of biodiscrimination

between dl-ACE and d-SUCC in hydrolysis, but ALPHA produce from d-SUCC was digested more efficiently than ALPHA from dl-ACE.

In a following study, using pigs fed at 3% body wt/day, there was evidence of biodiscrimination between dl-ACE and d-SUCC. The hydrolysis of dl-ACE was more efficient than d-SUCC, however, the ALPHA produced from the hydrolysis of d-SUCC was more efficiently digested than ALPHA produced from dl-ACE. These results support the hypothesis that the d-form is more efficiently absorbed than dl-form. But, the of feed intake may have affected gut motility and influenced the fecal excretion of vitamin E.

Fecal excretion is the major excretion route for vitamin E and its metabolites. In both trials, absorption efficiency was estimated by fecal excretion method. But, the fecal excretion method tends to underestimate digestion due to the contribution of endogenous vitamin E. The amount of endogenous vitamin E in swine needs to be investigated.

Pharmacokinetic data collected from pigs fed a 500 IU oral dose of dl-ACE or d-SUCC also supports the balance trial data; there was biodiscrimination in digestion between the two forms of vitamin E esters. The plasma ALPHA maximum was obtained faster in pigs fed dl-ACE than pigs fed d-SUCC, suggesting that dl-ACE was hydrolyzed faster than d-SUCC. But, dividing plasma ALPHA maximum by the terminal plasma

ALPHA indicates that plasma ALPHA was eliminated faster in pigs fed dl-ACE than in pigs fed d-SUCC. Non-d-ALPHA forms may be eliminated from the blood more quickly than d-ALPHA.

The area under plasma ALPHA concentration curve was not effected by type of ester; both dl-ACE and d-SUCC were digested to the same extent. The comparison of the apparent digestion of dl-ACE and d-SUCC in the 4 week trial to the digestion of a 500 IU oral dose, indicates that a large dose was digested as efficiently as a physiological dose..

Data on the digestion of vitamin E in swine is lacking. More studies are needed to investigate the effects of environment, feed intake and dietary consistuents have on vitamin E digestion.

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