8-1994

The effect of vitamin E in plasma and lipid peroxidation in newborn pigs

Sung min Choi

To the Graduate Council:

I am submitting herewith a thesis written by Sung min Choi entitled "The effect of vitamin E in plasma and lipid peroxidation in newborn pigs." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

J.P. Hitchcock, Major Professor

We have read this thesis and recommend its acceptance:

Jim Miller, Ralph Hall

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Sung Min Choi entitled "The Effect of Vitamin E on Plasma Iron and Lipid Peroxidation in Newborn Pigs." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

John P. Hitchcock

J. P. Hitchcock, Major Professor

We have read this thesis
and recommend its acceptance:

James K. Miller

Ralph F. Hall

Accepted for the Council:

Lee Minkel

Associate Vice Chancellor
and Dean of The Graduate School

THE EFFECT OF VITAMIN E ON PLASMA IRON AND LIPID PEROXIDATION IN NEWBORN PIGS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Sung Min Choi

August 1994

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to the many individuals who have contributed to the successful completion of this manuscript:

To Dr. John P. Hitchcock for serving as major professor. His support, friendship, guidance, and encouragement throughout my graduate program.

To Dr. Jim Miller for serving on the graduate committee, reviewing the manuscript and many times of discussion that we have engaged in.

To Dr. Ralph Hall for serving on the graduate committee, reviewing the manuscript. His friendship and explanation about things that I have wondered about.

To Dr. Arnold M. Saxton for his assistance in analyzing data.

To Sun Tan Tan and Linda Miller for their friendship and assistance in the laboratory so many ways.

To my parents, brothers, and sisters for their love and guidance throughout my life and their mental, moral and financial support.

And finally to my wife, Jill. Her love, patience, and financial support. Never lost smile and faith in me from the first day we met.

ABSTRACT

Three experiments were conducted to determine the effect of injected alpha-tocopherol on plasma iron and lipid peroxidation in the newborn pig and the optimum level of vitamin E and iron for practical application and optimum performance in the commercial swine industry.

In the first experiment 48 newborn cross-bred pigs were used in a 2*4 factorial arrangement with 8 pigs coming from each of 6 litters. The treatments consisted of 2 levels of iron: 0 or 100 mg as gleptoferrin at 3 days of age and four alpha-tocopherol levels: 0, 300, 600 and 900 IU.

The second (32 pigs) and third (48 pigs) experiments utilized 80 newborn cross-bred pigs in a 2*4 factorial arrangement with 4 pigs per treatment in experiment 2 and 6 pigs per treatment for experiment 3. The treatments consisted of two levels of iron: 100 or 200 mg as gleptoferrin at 3 days of age and four alpha-tocopherol injections: 0, 300, 600 and 900 IU. Results obtained from experiment 1 indicated that iron injections of 100 mg significantly increased blood hematocrit and hemoglobin levels on wk1, wk2, and wk3 when compared to no iron injections. Alpha-tocopherol injected at 300, 600 and 900 IU significantly increased plasma α-tocopherol concentration on wk1, wk2, and wk3. There were

more significant changes at an early age (wk1 and wk2) than wk3 on plasma α-tocopherol concentration. Iron injection of 100 mg significantly increased plasma iron concentration on wk1 and wk3 when compared to no iron injection. Pigs injected with 100 mg of iron had lower plasma lipid peroxidation levels on wk1, wk2, and wk3 when compared to uninjected pigs. This result suggests that iron injection of 100 mg did not cause a tremendous oxidative stress and damage to the newborn pig. Plasma iron was increased by an injection of 100 mg of iron at 3 days of age but lipid peroxidation levels were lower. Thus, the injection of 100 mg of iron must not provide a serious free radical challenge to the young newborn pig. Iron injections of 100 mg did not affect the weight gain at 21 days and weaning. Pigs treated with vitamin E 900 IU injections tended to be heavier than other levels of vitamin E treated baby pigs at day 21 and at weaning. Partial correlation coefficients (-.44) between 21 day's weight and Vit.E on wk3 suggest that heavier baby pigs had lower Vit.E concentration in their plasma. Wk1 tiobarbituric reactive substances (TBA) and wt of pig at day 21 and weaning wt had significant negative correlations. Vit.E injections were positively highly correlated with wk1 TBA values and wk2 Vit.E. Wk1 TBA, wk2 TBA (.017), and wk3 TBA (.033) were highly positive correlated with each other. Plasma iron was not correlated with wk2 TBA (.69), or wk3 TBA (.41). These results suggest that iron injection of 100 mg did not result in a

elevated plasma iron concentration that would cause free radical damage in the newborn pig.

The results obtained from experiments 2 and 3 indicated that there was a significant increase when higher injections of α-tocopherol were given to have higher plasma α-tocopherol concentrations during the experimental period. Lipid peroxidation levels were significantly decreased with vitamin E injections of 300, 600, and 900 IU on wk2 and wk3 when compared to no vitamin E injections. There was a tendency for Vit. E injections to decrease lipid peroxidation levels on wk1. Iron injections of 100 or 200 mg did not increased free radical stress. As observed in experiment 1, increasing the amount of iron injected from 0 to 100 mg did not result in increased lipid peroxidation. There was a trend for Vit.E injections to increase weights at 21 days and weaning. However, the birth weights of groups 600 and 900 IU Vit.E could have influenced the 21 day and weaning weight, since they were heavier at birth. Weight at birth, and day 21, and vitamin E were negatively highly correlated -0.37 and -0.34, with wk1 Vit.E, respectively. Partial correlation coefficients (-.35) between 21 day weight and Vit.E on wk3 indicated that heavier baby pigs had lower Vit.E concentration in the plasma. Wk1, wk2 and wk3 TBA and wt of the pig at day 21 had high negative correlations -.36, -.40,and -.33, respectively. This indicated that larger pigs had lower TBA values at all 3 measurement times. Plasma iron was not significantly

correlated with TBA at any measurement period. In addition, plasma vitamin E and TBA at all 3 measurement periods were not significantly correlated in these 2 experiments.

Increasing the amount of iron injected from 100 to 200 mg did not result in increased lipid peroxidation. Thus, the injection of 200 mg of iron must not provide a significant free radical challenge to the newborn pig. Based on wk2 and wk3 TBA values, this suggests that a 300 IU injection of Vit.E could be a marginal recommendation volume without severe oxidative damage with 100 or 200 mg iron.

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

CHAPTER 1

INTRODUCTION and LITERATURE REVIEW

In the newborn, particularly the preterm newborn, the protective system of the animal body may not be fully active, in part because the nutritional sources of antioxidant vitamins and necessary cofactors for the protective enzymes are insufficient (Gerdin et al.,1985).

Meyer et al.(1981) demonstrated that plasma tocopherol levels decreased in the first week postweaning and remained lower than at weaning for 5 weeks postweaning. Mahan and Moxon (1980) reported that nursing piglets had higher plasma tocopherol levels that decreased after weaning in pigs not receiving supplemental vitamin E.

Loudenslager et al.(1986) found that the serum tocopherol levels of pigs from dams not supplemented with vitamin E were 28 % lower than the serum tocopherol levels of pigs from dam's fed vitamin E supplemented diets. Either natural feed ingredients do not contain an adequate amount of natural tocopherols or young pigs are unable to absorb adequate amounts of natural tocopherol to maintain serum tocopherol at preweaning levels.

Parenteral administration of iron(Fe) to nursing pigs is a common practice in the swine industry to prevent anemia (Miller,1981). However, there are some toxic effects including

waxy muscle degeneration and transudations into the pericardium and thoracic cavities after Fe injection to the nursing piglets. Death losses, after considering the majority of the litter, occurred 8 to 12 hours after the administration of a commonly accepted form and amount of parenteral Fe (Tollerz,1973). Tollerz and Lannex (1964) have suggested that the toxic effects of Fe are concomitant with vitamin E and selenium deficiency in the sow's diet.

Cook et al.(1981) have reported that iron toxicosis from parenteral Fe-dextran has been difficult to produce in baby pigs in the United States even when the dam's diet contains low vitamin E and Selenium. But when pigs are fed massive doses of Fe it happened.

Moore (1992) reported that vitamin E injections may help to decrease the cellular and tissue damage caused by iron injection in newborn pigs. Addition of vitamin E at 900 IU increased ($P<.05$) plasma concentration of Fe at days 4, 8, 12, and 16. Vitamin E decreased ($P<.05$) lipid peroxidation (LP) at days 2, 4, 8, 12, and 16 in plasma and in liver. Addition of vitamin E increased ($P<.05$) liver Fe concentration. Fe decreased ($P<.05$) plasma vitamin E concentration. Fe increased ($P<.05$) LP in plasma at days 2, 4, 8, and 12 in liver.

Therefore, this study's objectives were (1) to determine the effect of injected alpha-tocopherol on plasma iron and lipid peroxidation in newborn pigs (2) to determine the optimum level of vitamin E and iron for practical application

and optimum performance in the swine industry.

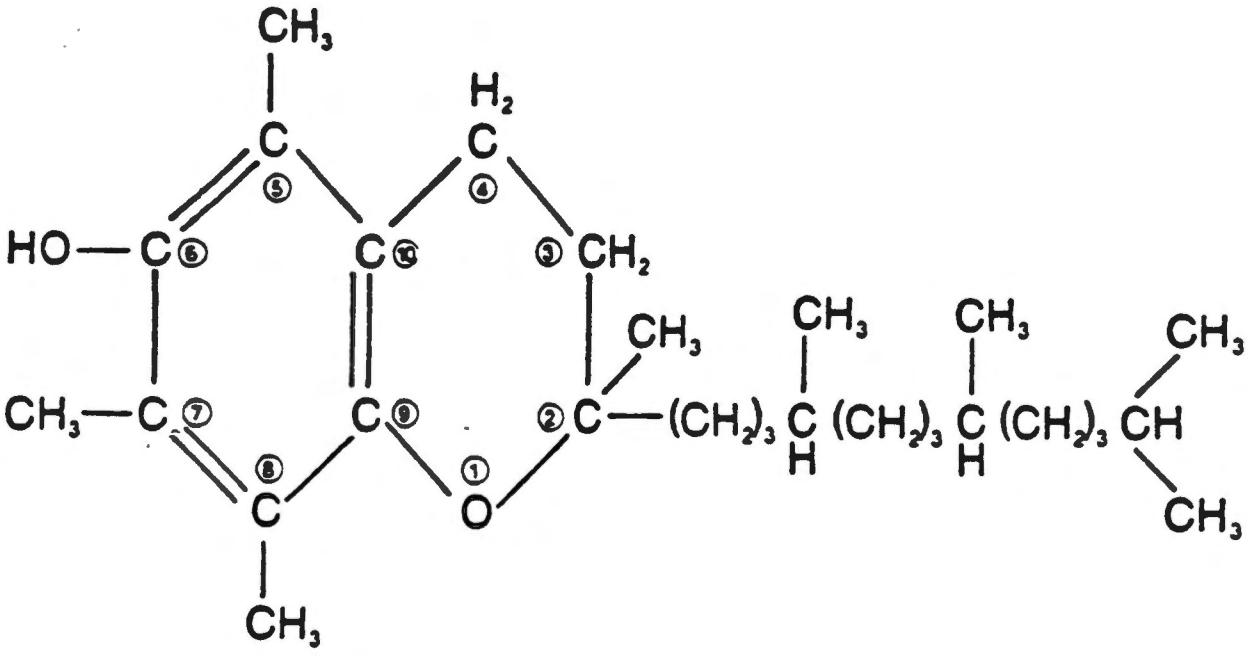
Chemistry of Vitamin E

There are eight forms of vitamin E in nature: four tocopherols (α,β,γ and σ) and four tocotrienols(α,β,γ and σ). The difference between α, β, γ and σ are due to replacement of methyl groups on the aromatic ring of the compound. Tocopherols and tocotrienols have different unsaturated positions on the ring side chain.

The tocopherols are pale yellow, viscous oils at room temperature, some of which crystallize at low temperature.The tocopherols are freely soluble in lipid solvents but insoluble in water. In the absence of air, these are stable to heat treatment up to about 200 degrees but readily oxidized in the presence of air. They darken gradually when exposed in the light (Schudel et al.,1972). D-alpha tocopherol's detailed structure and chemical name are presented in Figure 1.

Absorption and Metabolism of Vitamin E

Various studies have indicated that much less vitamin E is absorbed, or at least retained, in the body than is vitamin A. Vitamin E recovered from a test dose was found to range from 65 to 80% in human, rabbit, and hen, although in



Chemical Name:	2,5,7,8-Tetramethyl-2(4',8',12-trimethyl-tridecyl)-6-chromanol
IUPAC Name:	2R, 4'R, 8'R-alpha tocopherol
Trivial Name:	RRR-alpha-tocopherol
Common Names:	d-alpha-tocopherol,Natural vit.E
Molecular Weight:	430.9
Empirical formular:	$C_{29}H_{50}O_2$

Figure 1. D-alpha-Tocopherol

chicks it was reported at about 25%.

It is not known what proportion that fecal vitamin E represents of unabsorbed tocopherol and how much may come via secretion in the bile. The latter usually has a tocopherol content similar to that of blood plasma.

Absorption also is related to fat digestion and is facilitated by bile and pancreatic lipase. Whether presented as the free alcohol or as esters, most vitamin E is absorbed as the alcohol. Esters are largely hydrolyzed in the gut wall, and the free alcohol enters the intestinal lacteals and is transported via lymph to the general circulation (McDowell,1989).

Vitamin E in the plasma is attached mainly to lipoproteins in the globulin fraction. Rates and amounts of absorption of the various tocopherols and tocotrienols are in the same general order of magnitude as their biological potencies. Alpha-tocopherol is absorbed best, with γ-tocopherol absorption 85% that of α forms, but with rapid excretion. Thus , non-α-tocopherol forms tend to be discriminated against, and one can generally assume that most of the vitamin E activity within plasma and other animal tissues is α-tocopherol (Ullrey,1981). In humans, whose natural diet contains a high percentage of non-α forms, blood serum tocopherols identified consisted of about 87% α-, 11% γ-,and 2% β-tocopherol.

Tocopherols pass through placental membranes and also the

mammary gland. Thus the diet of the female influences the store of the young at birth and the amount obtained from mother's milk. However, less than 2% of dietary vitamin E is transfered from feed to milk (McDowell,1989). Van Sauer et al. (1989) reported placental transfer of vitamin E is inefficient, so the newborn is dependent on colostrum for vitamin E.

Biochemical Function as a Membrane Antioxidant

Vitamin E is regarded as the major lipid-soluble antioxidant, preventing oxidative attack of membrane lipids and other membrane-associated compounds.

Vitamin E has a number of different functions. One of the most important functions is its role as an intercellular and intracellular antioxidant. It prevents oxidation of unsaturated lipid materials within cells, thus protecting fats within the cell membrane from breaking down. If lipid hydroperoxides are allowed to form in the absence of adequate tocopherols, direct cellular tissue damage can result, in which peroxidation of lipids destroys structural integrity of the cells and causes metabolic derangements. Morphological damage to the muscle is common in cases of vitamin E and selenium deficiency, and dystrophic tissue membranes may leak creatine and transaminases into plasma (McDowell,1989).

Vitamin E reacts or functions as a chain-breaking

antioxidant, so neutralizing free radicals and preventing oxidation of lipids within membranes. At least one important function of vitamin E is to prevent production of free radicals at the initial stage. Consequences of lipid peroxidation include perturbation of membrane microarchitecture, inhibition of membrane enzyme activity, and accumulation of reaction products(e.g., lipofuscin associated with the brown spot of aging) that are not readily degraded to harmless metabolic debris (Ullrey,1981).

The more active the cell (e.g., the cells of skeletal and involuntary muscles),the greater is the inflow of lipids that contribute to the energy supply and the greater is the risk of tissue damage if vitamin E is limiting. This antioxidant property also ensures erythrocyte stability and maintenance of capillary blood vessel integrity.

Antioxidant properties of the vitamin explain earlier observations, dating back to 1926, that large intakes of cod liver oil, which is high in unsaturated fatty acids but low in vitamin E, caused muscular dystrophy in various herbivora that did not occur when hydrogenated oil was fed. It is clear that highly unsaturated fatty acids in the diet increase vitamin E requirements (Maynard and Loosli, 1979).

Alpha-tocopherol may be involved in the formation of structural components of biological membranes, thus exerting a unique influence on architecture of membrane phospholipids(Ullrey, 1982).

Large doses of vitamin E protected chicks against Escherichia coli with increased phagocytosis and antibody production (Tengerdy and Brown, 1977).

When pigs were fed minced colon from swine with dysentery or with pure culture of Treponema hyodysenteriae, vitamin E and selenium supplementation to a deficient diet increased resistance to the disease (Teige et al., 1978).

Calves receiving 125 IU of vitamin E daily were able to maximize their immune responses compared to calves receiving low dietary vitamin E (Reddy et al., 1987).

The initiating process by a primary radical $R^{\cdot}$ leads to lipid radicals $L^{\cdot}$, which then add oxygen in a diffusion-limited reaction. The resulting lipid peroxyl radicals continue a chain process.

$$R^{\cdot} \longrightarrow L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$$

$$LOO^{\cdot} + LH \longrightarrow L^{\cdot} + LOOH$$

Chain breaking antioxidants shorten the length of the chain reaction by trapping the peroxyl radical $LOO^{\cdot}$. Vitamin E has been shown to be a very effective scavenger of these radicals, whereas it does not react at comparable rates with carbon-centered radicals (Burton and Ingold.,1981).

Ascorbate can act as an antioxidant by scavenging radicals in the aqueous phase of the cell or by restoring reduced vitamin E, but it also includes a pro-oxidant effect,

commonly found in the presence of transition metal ions.

Vitamin C is the first plasma antioxidant to be depleted when lipid peroxidation is induced in vitro (Frei et al.,1988).

Alpha-tocopherol is an essential, lipid-soluble nutrient and a membrane constituent in cardic muscle (Janero and Barrnett,1981).

Antioxidant activity was assessed by measuring parameters of lipid peroxidation including thiobarbituric acid (TBA) reactants, lipid peroxides, and pentane production. Alpha-tocopherol is an active antioxidant but alpha-tocopheryl acetate is not an effective antioxidant, because alpha-tocopheryl acetate is not effective in decreasing pentane and TBA reactant concentrations in the in vitro oxidant stress systems (Knight et al., 1987).

Wispe et al., (1986) have reported that a single injection of alpha-tocopherol in newborn rabbits decreased lung, liver, and blood TBA reactants and expired pantane in newborn rabbits exposed to either room air or hyperoxia. However, in these animals, alpha-tocopherol administration increased liver, but not lung lipid peroxides (LOOH).

Deficiency of Vitamin E

Vitamin E deficiency has been shown to increase iron toxicity in both animals and infants (Table 1.). Iron

compounds are generally used for the prevention against iron deficiency anemia in pigs. These compounds have caused sudden deaths with myodegeneration in vitamin E deficient pigs (Lannex et al.,1962; Williams et al.,1975). Vitamin E deficiency symptoms are presented in Table 1.

Microangiopathy of pigs(MAP,mulberry heart disease} is characterized by acute heart failure and sudden cardiac death (SCD) associated with myocardial and endothelial cell damage. Cardiovascular damage can be induced experimentally in selenium(Se) and vitamin E deficient pigs fed on oxidized fat (Grant.1961, Van Vleet and Ferrans.,1977). MAP of pigs can be regarded as an animal model for oxidative damage. The pig is a suitable model for studies of human cardiovascular disease (Van Vleet and Ferrans,1977).

Dove and Ewan .(1990) suggested that addition of Cu (250 ppm) as $CuSO_4$ for growth performance increased the loss of natural tocopherols from the diet and decreased serum tocopherols. Pigs fed diets containing 250 ppm Cu may need supplemental tocopherol to maintain proper serum tocopherol levels.

Duthie et al.(1989) have indicated that the stress-susceptible pig has an antioxidant abnormality which can be partially compensated for by increasing the dietary intake of vitamin E. Vitamin E supplements may reduce the incidence of stress-related mortalities among stress-susceptible pigs.

TABLE 1. Effects of Vitamin E Deficiency

Pathology	Effected tissue	Species
Necrotizing myopathy	Skeletal muscle	Pig, rat, horse, chicken, lamb, catfish, duck
	Heart	Pig, rat, calf, goat, deer
Necrosis	Liver	Pig, rat, chicken
Fetal death and resorption	Placenta	Pig, rat, cow, chicken
Anemia	Erythrocytes	Pig, rat, catfish
Hemolysis	Erythrocytes	Chicken, rat, premature human infant
Gastric ulceration	Stomach	Pig
Steatitis (lipofuscin accumulation)	Adipose tissue	Pig, rat, cat, mouse, mink
Depigmentation	Incisors	Rat

(Newberne and Conner, 1989; Machlin, 1991)

A decline in use of pasture for pigs and an increase in artificial drying of grains have resulted in a lowering of vitamin E intake and an increase in the occurence of deficiency symptoms. Also grains low in selenium increase the need for vitamin E (Wahlstrom et al.,1991).

Nutritional Requirements of Vitamin E

Vitamin E requirements are extremely difficult to determine because of the interrelationship with other dietary factors, therefore, its requirement is dependent on dietary levels of polyunsaturated fatty acids (PUFA), antioxidants, sulfur amino acids and selenium. The requirement may be increased with increasing levels of PUFA, oxidizing agents, vitamin A, carotenoids, and trace minerals and decreased with increasing levels of fat-soluble antioxidant, sulfur amino acids, and selenium (McDowell, 1989). Adequate diets containing sufficient cystine and methionine and containing a minimum of PUFA, have vitamin E requirements that appear to be low (McDowell,1989).

The levels of PUFA found in unsaturated oils such as cod liver oil, corn oil, soybean oil, sunflower oil, and linseed oil, all increased vitamin E requirements. This is especially true if these oils are allowed to undergo oxidative rancidity in the diet or are in the process of peroxidation when consumed by the animal. If they become completely rancid

before ingestion, the only damage is the destruction of vitamin E present in the oil and in the feed containing the rancidifying oil. But if they are undergoing active oxidative rancidity at the time of consumption, they apparently cause destruction of body stores of vitamin E as well (Scott et al.,1982).

The amount of vitamin E required per gram of PUFA is dependent on experimental conditions, species differences, levels and kinds of PUFA, and test used. Nevertheless, for a number of species, 0.6 IU of vitamin E per gram of PUFA is inadequate and 1.0 IU is a realistic minimum.

A combination of stress of infection and presence of oxidized fat in swine diets was reported to exaggerate vitamin E needs still further (Teige et al., 1978). These researchers reported that supplements of 100 IU vitamin E and 0.1 ppm selenium did not entirely prevent deficiency lesions in weanling pigs afflicted with dysentery and fed 3% cod liver oil.

Determination of the vitamin E requirement is more complicated because the body has a fairly large ability to store both vitamin E and selenium. Sows maintained on a diet deficient in vitamin E and selenium produced normal piglets during the first reproductive cycle of the deficiency and clinical deficiency signs occured only after five such cycles (Glienke and Ewan,1974).

A number of the studies to establish requirements for

both nutrients have underestimated the requirements by failing to account for their augmentation of both body stores as well as experimental dietary concentrations.

The lean genotype pigs might require different levels of certain vitamins or other nutrients, including vitamin E. Even rations supplemented with vitamin E will not guarantee that pigs will be getting enough of the vitamin E (Krauss,1993). Vitamin E requirements for animals are presented in Table 2.

Interactions with Unsaturated Fatty Acids

Phospholipids are important structural components in membranes, and they are natural targets of lipid peroxidation (Kappus,1985).

Begin et al., (1985) have indicated that lipid peroxidation increased as a result of the increase in dietary PUFA supply. The extent of lipid peroxidation may be fatty acid specific in the cancer cell, and the potency of a given PUFA in killing cancer cells has correlated with the extent of lipid peroxidation of the PUFA substrate incorporated in the cells and was independent of its ability to synthesize eicosanoids. Consequently, cancer cells are thought to be killed as a result of an elevation of toxic peroxidation products generated by the PUFAs.

TABLE 2. Vitamin E Requirements for Animals.

Species	Requirements (IU/kg diet)
Swine	
Prewean (1 - 5 kg)	16
Early weaner (5-10 kg)	16
Starter (10 - 20 kg)	11
Grower (20 - 50 kg)	11
Finisher (50 - 110 kg)	11
Breeding gilts, sows, and boars	11
Turkeys	
starting and growing	30
Breeding	40
Rat	30
Mouse	30
Chickens	
Starting	30
Growing	10
Laying	10
Breeding	30
Cattle	
Young	20
Finishing	10
Breeding	20
Horses	
Young	20
Breeding	20
Fish	
Catfish	100
Trout	30

Machlin (1991)

Iritani et al.,(1980) have suggested that polyunsaturated fatty acid(PUFA) is essential for the growth of the body and source of essential fatty acid.

Horwitt (1952) has reported that PUFA reduced plasma cholesterol and increased high density lipoprotein (HDL) thus reducing the likelihood heart disease.

Alpha-tocopherol content of selected feeds are presented in Table 3.

Metabolism and Toxicity of Iron

Iron is required for red blood cell formation (hemoglobin), myoglobin, and a number of iron containing enzymes found in the body. The baby pig is born with a limited supply of iron. Sow's milk is low in iron and since the baby pig's diet consists solely of milk, iron deficiency anemia is a common problem in the baby pig. An intramuscular injection of 100-200 mg iron from iron dextran or gleptoferron is commonly given at one to three days of age to prevent anemia (Rea et al,1991).

Over 90 percent of the iron required during the first few weeks of life is for hemoglobin synthesis (Braude et al.,1962).

Table 3. Alpha-Tocopherol Content of Feeds (ppm)[a,b]

Source	Mean	Range
Alfalfa meal, dehydrated 17 % protein	73	28-121
Alfalfa meal, sun cured 13 % protein	41	18-61
Alfalfa hay	53	23-102
Barley, whole	36	22-43
Corn, whole	20	11-35
Cottonseed meal	9	2-16
Eggs	11	8-12
Fat, animal	8	2-16
Fish meal, herring	17	8-13
Lard	12	2-30
Linseed meal	8	3-10
Meat and bonemeal	1	1-2
Milo	12	10-16
Oats, whole	20	18-24
Poultry by-products meal	2	1-4
Soybean meal, solvent process	3	1-5
Wheat, whole	11	3-15
Wheat, bran	17	15-19

[a] Adapted from Bauernfeind (1980) and Ullrey (1981)
[b] When only alpha-tocopherol concentration of a feed is available, total tocopherols can be calculated by multiplying the value by 1.2 to account for the other tocopherols present.

Puls (1988) reported that pigs are born with adult hemoglobin and liver iron levels that drop to 70% of initial value by day 3 with resulting anemia if an injectable supplement is not given. Inadequate dietary protein diminishes serum Fe. Excessive Fe can interfere with the immuune response system.

There is a pathological expansion of total body iron stores, due mainly to an inappropriate increase in absorption of dietary iron (Gordeuk et al.,1987; Powell et al.,1980).

The liver is the major recipient of excess absorbed iron in the human, and after several years of high tissue iron concentration, portal fibrosis and eventually cirrhosis develop (Milder et al.,1980).

Iron has been shown to accelerate lipid peroxidation and free radical formation which promotes oxidative damage of cellular membranes (Heys and Dormandy, 1981).

Clinical,epidemiological and experimental studies support the hypothesis that myocardial iron overload plays a role in the etiopatheogenesis of human cardiovascular diseases (Buja and Roberts, 1971; Sullivan, 1986). Increased heart disease risk is associated with the progressive accumulation of stored iron (Sullivan, 1986).

The antioxidant status of the rat liver microsomes from iron-loaded rats decreased steadily as the time of the incubation increased. This was in contrast with the control

microsomes which displayed a delay in the onset of consumption of alpha-tocopherol and subsequently showed a slower rate of decline on prolonged incubation. After 5 minute of incubation(at 37°C) with the exogenous iron stress the alpha-tocopherol levels had decreased to 64% and 34% of the original levels in the control and iron-loaded microsomes, respectively. In the microsomes from the rats in which iron overload was induced in vivo, the depletion of 40% of the total alpha-tocopherol was enough to promote the complete abolition of the lag phase in lipid peroxidation on subsequent exogenous iron stress and suggests the formation of lipid hydroperoxides in vivo (Rice-Evans and McCarthy, 1989).

Bacon et al., (1986) have discovered that there are marked reductions in the plasma and hepatic alpha-tocopherol concentrations in rats receving diets supplemented with carbonyl iron. For example, in rats that were maintained on a 3.0 - 3.3% carbonyl iron-supplemented diet for up to 3 weeks, there was a 65 - 75% reduction in both plasma and hepatic alpha-tocopherol levels.

It became clear that all animals have an extremely limited capacity to excrete iron, the iron absorption and iron balance are largely controlled by the intestinal mucosa, and that a specific iron-protein compound, transferrin, exists in the blood serum and serves as the carrier for iron, while the iron-protein compound, ferritin, functions in both iron absorption and iron storage. Characteristic changes in the

levels of serum iron and of storage iron occur in iron deficiency and in various pathological conditions, so that determination of these levels in the animal can be used as an aid in the diagnosis and detection of nutritional and metabolic disorders involving iron (Underwood, 1966).

Intercellular Iron Transport

The pathways between the uptake of iron at a cell membrane and its incorporation into ferritin or into heme are unknown, but it has often been assumed that there must be a " labile pool " of iron which allows transfer between membranes and proteins. Such a labile pool may consist of iron chelated by amino acids, sugars, nucleotides, etc, (Worwood,1977).

Functions of the Transit Pool

The intercelluar iron pool which is presented in Figure 2 is very important. It is the focal point of intercellular iron metabolism and its interaction with extracellular transferrin and thus similar transit pools in other tissues provide a pool of exchangeable iron which allows an equilibrium to be established between the various metabolic processes in the cell but also between different organs in the body. The equilibrium between the transit pool in the small

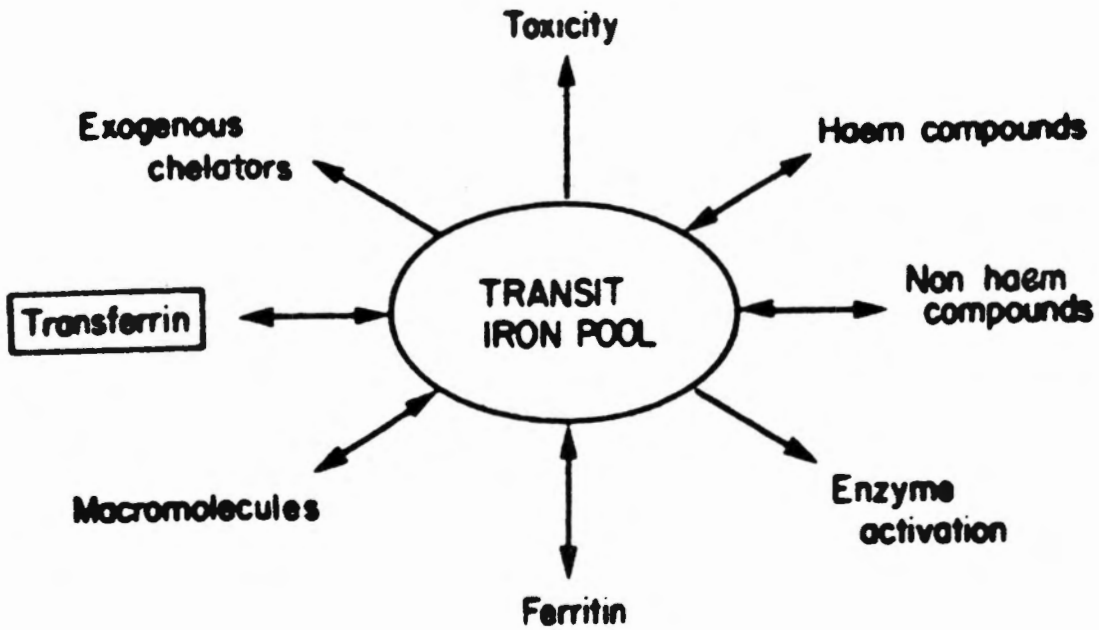


Figure 2. Transit Iron Pool

intestinal equilibrium and the pools in the rest of the body probably play a major role in the regulation of iron absorption (Cavill et al.,1975).

Enlargement of the transit pool beyond normal metabolic

needs stimulates the synthesis of ferritin and increases the availability of iron for uptake by transferrin(Lipschitz et al.,1971).

Wills (1969) has demonstrated that the role of this reactive iron pool in lipid peroxidation is probably of major importance in iron toxicity found in overload states.

Storage of Iron

Although iron stored as ferritin and hemosiderin is accessible, under normal circumstances only about 1 mg of iron (0.1%) enters and leaves the storage compartment daily in man, demobilization of large amounts of storage iron is a relatively slow process, which may take several days or weeks.

Free Radicals

Iron is mainly transported in plasma bound to transferrin and stored intracellularly bound to ferritin. However, non-protein-bound iron ("free iron") is found when the binding capacity of transferrin is exceeded or ferritin-bound iron is released after reduction of iron by superoxide radical or ascorbic acid (Boyer and McCleary., 1987).

Free iron can react with peroxides to form highly active hydroxyl radicals (Fenton Reaction) which damage lipids, proteins,sugars, and DNA. This mechanism is believed to be

important in the pathophysiology of thalassaemia (Halliwell and Gutteridge. 1989), malaria, and ischaemic reperfusion injury (McCord. 1985).

The major chain-initiating events are the formation of alkoxyl radicals by Fe^{2+} - catalyzed reductive cleavage of phospholipid hydroperoxides (eq 1), the reduction of superoxide-derived hydrogen peroxide to hydroxyl radicals by Fe^{2+} (eq 2,the fenton reaction), and the generation of other oxidants by reactions of Fe^{2+}, Fe^{3+}, and superoxide (Minotti and Aust., 1989).

$$LOOH + Fe^{2+} \longrightarrow LO\cdot + OH^- + Fe^{3+} \quad (1)$$

$$H_2O_2 + Fe^{2+} \longrightarrow HO\cdot + OH^- + Fe^{3+} \quad (2)$$

Oxidation in this system probably is initiated largely via reaction 1, which is favored by the levels of lipid hydroperoxide (approximately 0.1 - 0.2 %) present in the phospholipid used (Liebler et al., 1986).

Graf et al.,(1984) demonstrated that deferoxamine is a very effective iron chelator that can make the metal completely inaccessible for Fenton chemistry.

The nearly complete protection from peroxide damage with deferoxamine strongly suggests iron involvement. This implies that the Fenton reaction is required and therefore it is reasonable to postulate that hydroxyl radicals are mediating the damage. The source of the iron is unclear. While no iron was added to the solution, it is a common contaminant of

commercially available compounds (Halliwell and Gutteridge,1985; Wong et al.,1981).

Carpenter (1965) reported that brain tissue, due to its high rate of oxygen consumption and high phospholipid content with polyunsaturated fatty acids, is particularly susceptible to peroxide agents, free radical generating compounds, and lipid peroxidation.

Radicals, lipid peroxidation products, can be identified in principle by means of at least four methods : (1) direct resistration using ESR-spectroscopy techniques; (2) measuring the chemiluminescence of electronically excited products formed by recombination of lipid radicals; (3) spin-trapping method and (4) craft polymerization method.

Lipid Peroxidation

Jaeschke (1991) have suggested that a reductive stress, i.e. excessive NADH formation, can cause mobilization of trace amounts of iron, effectively by reducing iron in solution and initiating lipid peroxidation under certain circumstances, and that it may be relevant for the development of alcoholic liver disease and ischemia-reperfusion injury. A potential for a NADH-induced iron mobilization certainly exists during ethanol metabolism as well as hypoxia and ischemia. However, the excess NADH formation and iron formation alone may not be sufficient to cause cell damage.

Schaich and Borg (1988) have reported that the solubility of iron complexes in the lipid phase of membranes is a critical determinant of their catalytic effectiveness in initiating lipid peroxidation.

Burdon et al.,(1987) suggested that decreased protein synthesis has been related to increased lipid peroxidation in Hela cells, but no conclusive evidence for the relationship between the two phenomena has been reported.

Meydani et al.,(1988) have suggested that in an in vitro system there is a differential susceptibility between brain regions to lipid peroxidation that varies with the peroxidative agent used. In F334 rats, cerebellum and brain stem are more susceptible to lipid peroxidation than other brain regions. This susceptibility is partially affected by the alpha-tocopherol contents of their regions, which can be manipulated by dietary vitamin E, but not Se.

Enzymatic or nonenzymatic initiation of peroxidation of fatty acids residues of phospholipids, taking place at the expense of the interaction with the oxygen radicals, results in the formation not of one but several stereospecific products, over a wide range of different compounds (Vliegenthart and Veldink.,1982).

It is unlikely that the products of free-radical peroxidation of phospholipid are the only damaging agents. Something like "extra costs for the production" polyenoic phospholipids necessary for the normal functioning of the cell

and the activated oxygen species inevitably interact to form products responsible for the biomembrane damage (Kagan 1988).

Ildiko et al., (1988) have shown that ascorbate is a promoter rather than an inhibitor of lipid peroxidation. Under same conditions, ascorbate can also inhibit lipid peroxidation by terminating peroxidative chains as well as by regenerating vitamin E.

Determination of End-Product Lipid Peroxidation

Choi and Tappel (1969) have proposed that the method of quantitative evaluation of the intensity of LPO process is based on the identification of flourescent compounds accumulating in the tissue of animals or plant.

Evans et al.,(1967) have shown that volatile hydrocarbons, in the exhaled air, are formed during LPO as a result of a continuous free-radical transformation of unsaturated fatty acid lipid residues.

The method of assessing LPO intensity at the level of the entire organism, is based on quantitative determination of the endogenous ethane or pentane content in the air exhaled by animals and humans.

Thiobarbituric Acid (TBA) - Reactive Products of LPO

The determination of TBA was based on the assumption that

the reaction of TBA with LPO products resulted in a colored adduct with absorption minimum in the red range of the visual spectrum at 532nm (Pryor,1978).

Sinnhuber et al.,(1958) have suggested that the formation of chromogen, which is itself the condensation product of two TBA molecules, with one molecule of malonyl dialdehyde (MDA), caused the colored end product to result by the reaction of TBA with LPO products.

Raharjo et al.,(1991) suggested that the use of a C_{18} cartridge to filter samples improved the detection limit from 2 nmol MDA/ml to 40 pmol MDA/ml of filtrate for ground beef extracts.

Brief Review of Moore (1992) Research

Moore (1992) injected neonatal pigs with 0 or 900 IU of vitamin E (D-alpha tocopherol, 300 IU/mL) and 0, 100 and 400mg of iron (gleptoferron, 200 mg/mL) in 3*2 factorial arrangement with 6 pigs from each of 8 litters. During the 16 days after injection, vitamin E increased ($P<.05$) concentrations of Fe but decreased ($P<.05$) lipid peroxidation in plasma and liver. Injected Fe decreased ($P<.05$) plasma vitamin E concentration but increased ($P<.05$) lipid peroxidation in plasma and liver. Iron and vitamin E in combination increased hematological parameters.

CHAPTER 2

MATERIALS AND METHODS

Experiments, Animals, and Treatments

EXPERIMENT 1

Previous research had demonstrated that a vitamin E injection of 900 IU had significant effects on newborn pigs when compared to no vitamin E injection (Moore, 1992). The objective of this research was to confirm previous research and determine the effect of intermediate levels of vitamin E on plasma iron and lipid peroxidation in newborn pigs.

Animals and Treatments

This experiment utilized 48 newborn cross-bred pigs from Blount farm with 8 pigs from each of six litters. Baby pigs were ear notched at 3 days of age and housed in the farrowing crates with their mothers for the duration of the experiment. Sows were fed a corn soybean meal based lactation diet which met or exceeded NRC(1988) recommendations. Weights of baby pigs were measured at birth, 21 days, and weaning. Experimental treatments for experiment 1 are presented in Table 4.

Table 4. Experimental Treatments for Experiment 1

Treatment	Iron (mg)[a]	Vit.E(IU)[b]
1.	0	0
2.	0	300
3.	0	600
4.	0	900
5.	100	0
6.	100	300
7.	100	600
8.	100	900

[a]Iron as gleptoferron, 200 mg/mL. Single injection.

[b]Vitamin E as D-α-tocopherol. 300 IU/mL. Single injection.

EXPERIMENT 2

The objective of this experiment was to determine the optimum combination of iron and vitamin E levels for newborn pigs in confinement facilities. Iron levels of 100 or 200 mg in combination with 0, 300, 600 or 900 IU injections of vitamin E were utilized in this experiment and are presented in Table 5. Variables measured were hematocrit and hemoglobin in the whole blood, iron, vitamin E, and lipid peroxidation levels in the plasma. Body weights at birth, 21 days and at weaning were collected.

Animals and Treatments

This experiment was conducted utilizing 32 cross-bred newborn pigs from Blount farm. Eight newborn pigs coming from each of four litters were ear notched after 3 days of age and housed in farrowing crates with their mother for the duration of the experiment. Sows were fed a corn soybean meal based lactation diet which met or exceeded NRC (1988) recommendations.

Table 5. Experimental Treatments for Experiments 2 and 3

Treatment	Iron (mg)[a]	Vitamin E (IU)[b]
1.	100	0
2.	100	300
3.	100	600
4.	100	900
5.	200	0
6.	200	300
7.	200	600
8.	200	900

[a]Iron as gleptoferron, 200 mg/mL. Single injection.

[b]Vitamin E as D-α-tocopherol. 300 IU/mL. Single injection.

EXPERIMENT 3

Less than a desirable number of newborn pigs were available in experiment 2. Thus experiment 3 was a repeat of experiment 2 with the same treatment combination levels used for iron and vitamin E as were utilized in experiment 2 (Table 5).

Animals and Treatments

This experiment utilized 48 newborn cross-bred pigs from Blount farm with 8 pigs coming from each of six litters. Baby pigs were ear notched at 3 days of age and housed in the farrowing crates with their mothers for the duration of the experiment. Sows were fed a corn soybean meal based lactation diet which met or exceeded NRC (1988) recommendation. Weight of baby pigs were measured at birth, 21 days, and weaning.

Procedure and Analysis of Samples

Baby pigs (3 - 6 days old) on the experiment were given treatment injections subcutaneously in the neck area. The treatments consisted of 2 iron levels of 0 or 100mg iron as gleptoferron (200mg/mL) in experiment 1 and 100 or 200 mg iron in experiment 2 and 3 and four vitamin E (d-α-tocopherol) levels: 0, 300, 600, or 900 IU (300 IU/mL). The experiment was

a 2*4 factorial arrangement with four animals per treatment in experiment 2 and six animals per treatment in experiment 3. The animals were bled via anterior vena cava puncture at 7, 14 and 21 days after injection. The whole blood samples collected from the pigs were heparinized with sodium heparin to prevent coagulation and subjected to further analysis. The blood was collected and placed in a light tight-box and covered by aluminum foil to prevent loss of vitamin E activity due to UV electromagnetic radiation. Blood hemoglobin was determined by the method of Crosby et al.(1954) and hematocrits were determined by the microhematocrit method of McGovern et al. (1955). Plasma was obtained by centrifugation of the whole blood at an RCF (relative centrifugal force) of 3090 g's for 10 minutes and removal of the plasma to a seperate tube for storage. The plasma was then stored at -45°C until analyses were conducted. All handling and analysis of plasma were carried out under a safelight (non-UV) to prevent loss of α-tocopherol.

Analysis of α-tocopherol in plasma utilized the procedures described by Moore (1992) in Appendix Table C. Lipid peroxidation analysis was carried out utilizing a modified procedure by Yaggi (1987) for blood plasma in Appendix Table D. All α-tocopherol analyses were conducted utilizing a Waters High Performance Liquid Chromatography (HPLC) system including a fluorescent detector, 712 WISP autosampler, Waters 510 and 6000A HPLC pumps, Waters data

module and a μbonda-pac C_{18} 10 μ column. Iron in plasma was analyzed with an atomic absorption spectrophotometer as described in Appendix Table E.

Statistical Analysis of Data

The data were analyzed by the general linear models (GLM) procedure of the Statistical Analysis Syatem (SAS) (Barr et al.,1979). T tests were used to seperate means . The GLM procedure is a multivariate regression analysis, determining differences by significant F test.

The p.S.E. was calculated by adding all the LSmean standard errors together and dividing by the number of treatment combinations of iron and vitamin E. LSmeans were used because of the unbalanced data.

The independent variables were iron injection and α-tocopherol concentration and iron*α-tocopherol interaction. The dependent variables were hematocrit, hemoglobin, lipid peroxidation of plasma, plasma concentration of α-tocopherol, iron concentration in plasma and body weights at birth, 21 days and at weaning.

Data from experiment 2 and experiment 3 were combined and analyzed together since both experiments had identical designs and levels of injection. This was determined to be advantageous because of the low number of pigs available for use in experiment 2.

CHAPTER 3

RESULTS AND DISCUSSION

Experiment 1

Hematocrit concentrations and hemoglobin concentrations are presented in Tables 6 and 7. Iron injections of 100 mg significantly increased blood hematocrit and hemoglobin levels on wk1, wk2, and wk3 when compared to no iron injections. Vitamin E injections of 300, 600, and 900 IU numerically increased blood hematocrit and hemoglobin concentrations on wk1 and wk3 with the exception of Vit.E 600 IU on wk3. The overall vitamin E effect was not significant.

The concentrations of α-tocopherol in plasma are presented in Table 8. Alpha-tocopherol injected at 300, 600 and 900 IU significantly increased plasma α-tocopherol concentration on wk1, wk2, and wk3. This is in obvious agreement with results obtained by Kayden and Traber (1986) who has reported that alpha-tocopherol concentration in plasma was directly correlated with alpha-tocopherol injection in rats. Iron injection of 100 mg did not change α-tocopherol concentration on wk1, wk2, and wk3. Alpha-tocopherol concentrations in the plasma were higher on wk1 than wk2 and continued to decrease at wk3. There were more significant changes in early age (wk1 and wk2) than wk3 on plasma α-

Table 6 Effect of Iron and Vitamin E on Blood Hematocrit Concentration (%PCV) (Exp.1)

		VITAMIN E(IU)					Anova	Prob.		pSE
	Fe(mg)	0	300	600	900	MEAN	Fe	VIT.E	Fe*VIT.E	
No.	Pigs	12	12	12	12					
WK1	0	16.0^{A}	18.7^{B}	18.5^{B}	19.3^{B}	18.2^{c}	0.0001	0.4482	0.0481	0.83
	100	26.4	24.8	25.1	26.1	25.6^{d}				
MEAN		21.2	21.8	21.8	22.7					
WK2	0	16.0^{A}	20.6^{B}	16.9^{A}	19.2^{B}	18.2^{c}	0.0001	0.0653	0.0222	0.85
	100	27.7	27.1	26.6	26.6	27.0^{d}				
MEAN		21.9^{A}	23.9^{B}	21.7^{A}	22.9^{AB}					
WK3	0	16.3	22.2	16.7	19.6	18.7^{c}	0.0001	0.1736	0.1583	1.46
	100	26.6	25.8	25.6	26.6	26.2^{d}				
MEAN		21.5	24.0	21.2	23.1					

^{A,B} Means in the same row with different superscripts differ significantly.
^{c,d} Means in the same column with different superscripts differ significantly.
pSE is the average std error (df=36) for Fe*Vit.E interaction.

TABLE 7 Effect of Iron and Vitamin E on Blood Hemoglobin Concentration (g/dl) (Exp.1)

		VITAMIN E(IU)					Anova Prob.			pSE
	Fe(mg)	0	300	600	900	MEAN	Fe	VIT.E	Fe*VIT.E	
No.	Pigs	12	12	12	12					
WK1	0	5.9	6.7	6.5	6.5	6.4^{c}	0.0001	0.6022	0.5352	0.39
	100	8.7	8.8	8.2	8.8	8.6^{d}				
MEAN		7.3	7.7	7.4	7.7					
WK2	0	5.6	7.0	6.0	6.7	6.3^{c}	0.0001	0.6404	0.1571	0.54
	100	10.7	9.7	9.4	9.8	9.9^{d}				
MEAN		8.1	8.4	7.7	8.3					
WK3	0	5.8^{B}	8.6^{A}	6.3^{B}	8.1^{A}	7.2^{c}	0.0001	0.1639	0.0009	0.54
	100	11.1	10.4	11.7	10.5	10.9^{d}				
MEAN		8.4	9.5	8.9	9.3					

^{A,B} Means in the same row with different superscripts differ significantly.
^{c,d} Means in the same column with different superscripts differ significantly.
pSE is the average standard error (df=36) for Fe*Vit.E interaction.

TABLE 8 Effect of Iron and Vitamin E on Plasma Vitamin E Concentration (μg/ml)(Exp. 1)

		VITAMIN E(IU)					Anova Prob.			pSE
	Fe(mg)	0	300	600	900	MEAN	Fe	VIT.E	Fe*VIT.E	
No.	Pigs	12	12	12	12					
	0	1.2	4.0	5.1	5.9	4.1				
WK1							0.6209	0.0088	0.7987	1.20
	100	1.9	4.2	4.4	7.3	4.5				
MEAN		1.6[A]	4.1[B]	4.8[B]	6.6[B]					
	0	1.6	1.7	3.1	3.1	2.4				
WK2							0.7200	0.0014	0.4470	0.38
	100	1.2	2.5	3.1	3.2	2.5				
MEAN		1.4[A]	2.1[A]	3.1[B]	3.2[B]					
	0	0.7	1.2	2.1	1.8	1.4				
WK3							0.7783	0.0015	0.6692	0.33
	100	0.4	1.2	2.1	2.3	1.5				
MEAN		0.6[A]	1.2[B]	2.1[C]	2.0[C]					

[A,B,C] Means in the same row with different superscripts differ significantly.
pSE is the average standard error (df=36) for Fe*Vit.E interaction.

tocopherol concentration. This is in obvious agreement with results obtained by Moore (1992) who reported that alpha-tocopherol concentration in plasma was directly correlated with alpha-tocopherol injection in baby pigs.

The concentrations of plasma iron are presented in Table 9. Iron injection of 100 mg significantly increased plasma iron concentration on wk1 and wk3 when compared to no iron injection. This is in obvious agreement with Rea et al,. (1991) who reported that iron injections of 100 - 200 mg was desirable for newborn pigs. Vitamin E injections of 600 IU significantly reduced plasma iron concentration on wk1. Overall, when vitamin E injections were increased the iron levels tended to decrease. The physiological explanation for this observation is not immediately apparent. Vitamin E 300 IU injections without iron resulted in higher iron concentration than no vitamin E injections on wk1 and wk2 and at wk3 were numerically higher. This is a contradictory result to that of Moore (1992) who reported that plasma iron concentration was significantly increased by an alpha-tocopherol injection concentration of 900 IU on days 1, 4, and 16.

Plasma lipid peroxidation levels are presented in Table 10. There were no significantly different plasma lipid peroxidation levels between iron treatments. When Vit. E injections were increased lipid peroxidation levels were decreased on wk1 and wk2 without iron injections when compared to no Vit.E injection. Iron injections of 100 mg resulted in

Table 9 Effect of Iron and Vitamin E on Plasma Iron Concentration (μg/g) (Exp.1)

		VITAMIN E (IU)					Anova Prob.			pSE
	Fe(mg)	0	300	600	900		Fe	VIT.E	Fe*Vit.E	
No.	Pigs	12	12	12	12					
WK1	0	0.59^{AB}	0.89^{B}	0.41^{A}	0.35^{A}	0.56^{c}	0.0001	0.0335	0.0506	0.14
	100	1.83^{A}	1.35^{B}	1.15^{B}	1.32^{B}	1.41^{d}				
MEAN		1.21^{A}	1.12^{A}	0.78^{B}	0.83^{AB}					
WK2	0	0.70^{B}	1.63^{A}	0.41^{B}	0.53^{B}	0.82	0.2823	0.1371	0.0573	0.24
	100	1.05	0.97	0.99	0.99	0.99				
MEAN		0.88	1.30	0.70	0.74					
WK3	0	0.73	1.02	0.53	0.73	0.76^{c}	0.0168	0.7630	0.4045	0.17
	100	1.13	1.01	1.10	0.99	1.06^{d}				
MEAN		0.93	1.02	0.81	0.86					

A,B Means in the same row with different superscripts differ.
c,d means in the same column with different superscripts differ.
pSE is the average standard error (df=36) for Fe*Vit.E interaction.

Table 10 Effect of Iron and Vitamin E on Plasma Lipid Peroxidation (thiobarbituric reactive substances) (nmol/ml) (Exp.1)

		VITAMIN E (IU)					Anova Prob.			pSE
	Fe(mg)	0	300	600	900	MEAN	Fe	VIT.E	Fe*VIT.E	
No.	Pigs	12	12	12	12					
WK1	0	63.7	63.3	55.3	59.0	60.3	0.0940	0.8556	0.6948	6.27
	100	51.5	56.5	55.8	48.0	52.9				
MEAN		57.6	59.9	55.5	53.5					
WK2	0	57.1	49.6	50.7	50.3	51.9	0.2094	0.9279	0.3712	6.24
	100	39.2	46.1	52.9	48.3	46.5				
MEAN		48.2	47.9	51.8	49.2					
WK3	0	43.7	39.5	44.9	45.7	43.4	0.1101	0.2205	0.3183	4.28
	100	33.7	35.8	48.6	36.7	38.7				
MEAN		38.7	37.7	46.7	41.2					

pSE is the average standard error (df=36) for Fe*Vit.E interaction.

lower plasma lipid peroxidation levels on wk1, wk2, and wk3 when compared to no iron injections. This result suggest that iron injection of 100 mg did not cause oxidative stress or damage to the newborn pig. This result was unexpected and contrary to the hypothesis that increased iron should result in increased lipid peroxidation. Plasma iron was increased by an injection of 100 mg of iron at each sampling time but lipid peroxidation levels were lower. Thus, the injection of 100 mg of iron must not provide a serious free radical challenge to the young newborn pig.

Effects of iron and vitamin E on weights are presented in Table 11. Iron injections of 100 mg did not affect the weight gain at 21 days and weaning. Pigs treated with vitamin E 900 IU injections tended to be heavier than other levels of vitamin E treated baby pigs at days 21 and weaning weight.

Partial correlation coefficients and probability levels for weights and vitamin E, iron, and TBA are presented in Table 12. Weight at birth, day 21 and weaning had a negative high correlation -0.52, -0.76, and -0.57 with wk1 Vit.E, respectively. Partial correlation coefficients (-.44) between 21 day weight and Vit.E on wk3 suggest that heavier baby pigs had lower Vit.E concentrations in their plasma. Wk1 TBA and wt of pig at day 21 and weaning wt had a significant negative correlation. Wk3 TBA and weaning wt had a high negative correlation coefficient (-.48). These results suggest that bigger baby pigs need to be injected with an adjusted volume

Table 11 Effect of Iron and Vitamin E on Weight (lbs)(Exp.1)

		VITAMIN E (IU)				MEAN	ANOVA PROB.			pSE
	Fe(mg)	0	300	600	900		Fe	VIT.E	Fe*VIT.E	
No.	Pigs	12	12	12	12					
WT0	0	3.8	3.6	3.9	4.2	3.9	0.6195	0.7210	0.9712	0.37
	100	3.6	3.7	3.7	4.0	3.7				
MEAN		3.7	3.6	3.8	4.1					
WT21	0	13.0	10.7	13.1	14.2	12.8	0.5558	0.3664	0.5435	1.21
	100	12.6	12.2	11.2	13.2	12.3				
MEAN		12.8	11.5	12.2	13.7					
WNWT	0	21.9	20.1	20.6	26.0	22.1	0.1628	0.1839	0.3689	1.79
	100	19.7	21.6	19.1	21.3	20.4				
MEAN		20.8	20.8	19.8	23.7					

pSE is the average standard error (df=36) for Fe* VIT.E interaction

Table 12 Partial Correlation Coefficients and Probability Levels for Weights and Vitamin E, Iron and TBA(Exp.1)

	VIT.E WK1	VIT.E WK2	VIT.E WK3
WT 0	0.0186 (-0.52)	0.2749 (-0.26)	0.1083 (-0.37)
WT 21	0.0001 (-0.76)	0.0845 (-0.40)	0.0499 (-0.44)
WN WT	0.0087 (-0.57)	0.3906 (-0.20)	0.2382 (-0.28)

	TBA WK1	TBA WK2	TBA WK3
WT 0	0.2471 (-0.27)	0.4436 (0.19)	0.3237 (0.23)
WT 21	0.0032 (-0.63)	0.2211 (-0.29)	0.1102 (-0.37)
WN WT	0.0143 (-0.54)	0.0966 (-0.38)	0.0341 (-0.48)

	Iron WK1	Iron WK2	Iron WK3
WT 0	0.1094 (0.36)	0.6386 (0.11)	0.6580 (0.11)
WT 21	0.0010 (0.68)	0.4644 (0.17)	0.7476 (-0.08)
WN WT	0.0073 (0.58)	0.2794 (0.25)	0.7815 (-0.07)

Numbers in ( ) are partial correlation coefficients

of vitamin E to maintain higher plasma levels of vitamin E and lower levels of TBA. Plasma iron was positively highly correlated with day 21 and weaning weight at 7 days. This result suggests that iron injections of 100 mg were beneficial for early weight gain and through until weaning.

Partial correlation coefficients between Vit.E, TBA, and Iron are presented in Table 13. Vit.E injections were positively highly correlated with wk1 TBA values and wk2 Vit.E. Wk1 TBA, wk2 TBA (.017), and wk3 TBA (.033) were highly positively correlated with each other. Plasma iron was not correlated with wk2 TBA (.69), or wk3 TBA (.41). These results suggest that iron injection of 100 mg did not result in elevated plasma iron concentrations that would cause free radical damage in the newborn pig.

Experiments 2 and 3

The effect of vitamin E and iron on blood hematocrit concentrations are presented in Table 14. Hematocrit concentrations were not significantly changed by α-tocopherol injections of 300, 600, and 900 IU when compared to no injection. Iron injections of 200 mg significantly changed wk2 and wk3 hematocrit concentrations when compared to 100 mg iron injections. There were no differences in hematocrit concentration among α-tocopherol treatment levels on wk3. Iron injections of 100 and 200 mg did not significantly change

Table 13 Partial Correlation Coefficients between Vitamin E, TBA, and Iron (Exp.1)

	VIT.E1	TBA1	Fe1	VIT.E2	TBA2	Fe2	VIT.E3	TBA3	Fe3
VIT.E1	.	.68	-.39	.52	.37	.06	.56	.19	-.14
	.	.001	.087	.02	.11	.80	.10	.41	.54
TBA1		.	-.23	.31	.53	-.11	.40	.48	-.32
		.	.33	.19	.017	.64	.08	.033	.17
Fe1			.	-.03	-.09	.087	.10	-.20	-.10
			.	.19	.69	.71	.67	.41	.69
VIT.E2				.	.13	.26	.51	.14	.04
				.	.59	.28	.02	.55	.87
TBA2					.	-.33	.27	.81	.01
					.	.15	.24	.0001	.96
Fe2						.	-.26	-.46	.03
						.	.27	.039	.89
VIT.E3							.	.25	-.03
							.	.28	.89
TBA3								.	-.007
								.	.97
Fe3									.
									.

If prob.<.05 significantly correlated.
Vit.E1 means week 1 Vit.E.

TABLE 14 Effect of Iron and Vitamin E on Blood Hematocrit Concentration (%PCV) (Exp.2+3)

		VITAMIN E(IU)					Anova Prob.			pSE
	Fe(mg)	0	300	600	900	MEAN	Fe	VIT.E	Fe*VIT.E	
No.	Pigs	21	22	17	18					
WK1	100	31.8	33.0	33.8	33.0	32.9	0.1095	0.5908	0.6130	1.69
	200	36.2	32.5	35.7	35.3	34.9				
MEAN		34.0	32.8	34.8	34.2					
WK2	100	32.3	35.2	33.9	33.0	33.7^{C}	0.0001	0.9737	0.2536	1.13
	200	38.1	36.3	37.8	38.7	37.7^{D}				
MEAN		35.4	35.8	35.9	35.9					
WK3	100	32.8	33.3	34.6	33.6	33.6^{C}	0.0001	0.0897	0.3079	1.16
	200	39.5^{A}	35.3^{B}	39.0^{A}	37.3^{AB}	37.8^{D}				
MEAN		36.1	34.3	36.8	35.4					

^{A,B} Means in the same row with different superscripts differ significantly.
^{C,D} Means in the same column with different superscripts differ significantly.
pSE is the average standard error (df=77) for Fe*Vit.E interaction.

hematocrit concentration on wk1. Iron injections of 200 mg resulted in a desirable increase in hematocrit concentration at wk2 and wk3 in the newborn pigs when compared to injections of 100 mg.

Hemoglobin data are presented in Table 15. Hemoglobin concentration was not changed by vitamin E injections of 300, 600, and 900 IU when compared to no injection on wk1, wk2, and wk3. There were significant differences in hemoglobin concentration with 200 mg iron treatments on wk2, and wk3. However, hemoglobin concentration was not increased by 200 mg iron injections when compared to 100 mg iron injections on wk1. Iron injections are essential to prevent baby pig anemia. This is in agreement with Lannex et al.,(1962) that iron compounds are generally used for the prevention of iron deficiency anemia in pigs. These results are typical of normal hematology of pigs that received 100 - 200 mg of iron.

The concentrations of α-tocopherol in plasma are presented in Table 16. Alpha-tocopherol injection at 300, 600, and 900 IU significantly increased plasma alpha-tocopherol concentrations on wk1, wk2, and wk3 when compared to no injection. Alpha-tocopherol injection of 900 IU resulted in a significantly higher plasma α-tocopherol concentration when compared to 0, 300, and 600 IU on wk1 , wk2, and wk3. Higher injections of α-tocopherol significantly increased plasma α-tocopherol concentration during the experimental period. This is in apparent agreement with results obtained by Moore (1992)

Table 15 Effect of Iron and Vitamin E on Blood Hemoglobin Concentration (g/dl)(Exp. 2+3)

Fe(mg)		VITAMIN E(IU) 0	300	600	900	MEAN	Anova Prob. Fe	VIT.E	Fe*VIT.E	pSE
No.	Pigs	21	22	17	18					
WK1	100	8.9	8.8	9.4	9.2	9.0	0.1222	0.5335	0.7768	0.48
	200	10.0	9.1	9.6	9.8	9.6				
MEAN		9.4	9.0	9.5	9.4					
WK2	100	9.7	10.1	9.7	9.1	9.6^{A}	0.0001	0.5688	0.6707	0.42
	200	11.0	11.1	11.0	11.2	11.1^{B}				
MEAN		10.3	10.6	10.4	10.1					
WK3	100	9.8	10.4	10.5	10.2	10.3^{A}	0.0001	0.8141	0.5543	0.49
	200	12.2	11.3	12.0	11.7	11.8^{B}				
MEAN		11.0	10.8	11.3	11.0					

pSE is the average standard error (df=77) for Fe*Vit.E interaction.
^{A,B} Means in the same row with different superscripts differ significantly.

Table 16 Effect of Iron and Vitamin E on Plasma Vitamin E Concentration (μg/ml) (Exp. 2+3)

		VITAMIN E(IU)					Anova Prob.			pSE
	Fe(mg)	0	300	600	900	MEAN	Fe	VIT.E	Fe*VIT.E	
No.	Pigs	21	22	17	18					
WK1	100	0.9	1.8	3.1	3.4	2.9	0.3712	0.0001	0.1540	0.46
	200	0.8^{C}	2.0^{BC}	2.7^{AB}	4.9^{A}	2.6				
MEAN		0.8^{D}	1.9^{C}	2.9^{B}	4.1^{A}					
WK2	100	0.4^{D}	0.9^{C}	1.5^{B}	1.9^{A}	1.2	0.0869	0.0001	0.8486	0.15
	200	0.7^{C}	1.2^{B}	1.5^{B}	2.0^{A}	1.4				
MEAN		0.5^{D}	1.1^{C}	1.5^{B}	2.0^{A}					
WK3	100	1.1^{A}	1.2^{A}	1.2^{A}	1.6^{B}	1.3	0.1543	0.0001	0.2107	0.15
	200	0.6^{C}	1.0^{B}	1.3^{AB}	1.6^{A}	1.1				
MEAN		0.9^{C}	1.1^{B}	1.2^{B}	1.6^{A}					

^{A,B,C,D} Means in the same row with different superscripts differ significantly.
pSE is the average std error (df=77) for Fe*Vit.E interaction.

who demonstrated that vitamin E injected at 900 IU significantly increased plasma alpha-tocopherol concentration. Iron injections of 100 and 200 mg did not significantly change plasma α-tocopherol levels on wk1, wk2, and wk3. Iron injection of 100 or 200 mg did not decrease plasma Vit.E levels in the newborn pig.

The concentrations of plasma iron are presented in Table 17. Plasma iron concentration was significantly changed by iron injections of 200 mg on wk1, wk2, and wk3 when compared to 100 mg injections. Vitamin E injections of 300, 600, and 900 IU did not change plasma iron levels on wk1, wk2, and wk3. When Vit.E injection levels were increased, plasma iron concentration did not increase on wk2 and wk3. These results suggest that when 200 mg iron were injected, baby pigs did use 200 mg iron more efficiently than 100 mg iron. Plasma iron levels measured in these experiments were not in agreement with the finding of Moore (1992) who reported that plasma iron concentration was significantly increased by an alpha-tocopherol injection concentration of 900 IU.

Plasma lipid peroxidation values are presented in Table 18. Iron injections of 200 mg did not significantly change blood lipid peroxidation levels on wk1 and wk2, but tended to be lower wk3 when compared to 100 mg iron injections. Lipid peroxidation levels were significantly decreased with vitamin E injections of 300, 600, and 900 IU on wk2 and wk3 when compared to no vitamin E injections. There was a tendency for

Table 17 Effect of Iron and Vitamin E on Plasma Iron Concentration (μg/g)(Exp. 2+3)

		VITAMIN E (IU)					Anova Prob.			pSE
	Fe(mg)	0	300	600	900	MEAN	Fe	VIT.E	VIT.E*Fe	
No.	Pigs	21	22	17	18					
WK1	100	1.87	1.50	1.30	1.36	1.51[A]	0.0168	0.4883	0.2935	0.24
	200	1.70	2.27	1.87	1.88	1.93[B]				
MEAN		1.78	1.89	1.59	1.62					
WK2	100	1.27	1.28	1.23	1.22	1.25[A]	0.0001	0.8016	0.8105	0.15
	200	1.56	1.75	1.66	1.81	1.70[B]				
MEAN		1.41	1.51	1.44	1.51					
WK3	100	1.11	0.93	1.19	1.19	1.11[A]	0.0001	0.8722	0.6227	0.14
	200	1.58	1.73	1.66	1.62	1.65[B]				
MEAN		1.35	1.33	1.43	1.41					

[A,B] Means in the same column with different superscripts differ.
pSE is the average std error (df=77) for Fe*Vit.E interaction.

Table 18 Effect of Iron and Vitamin E on Plasma Lipid Peroxidation (thiobarbituric reactive substance) (nmol/ml) conc. (Exp. 2+3)

		VITAMIN E (IU)					Anova Prob.			pSE
	Fe(mg)	0	300	600	900	MEAN	Fe	VIT.E	Fe*VIT.E	
No.	Pigs	21	22	17	18					
WK1	100	53.1^{A}	46.5^{AB}	40.5^{B}	45.1^{AB}	46.3	0.1063	0.0300	0.4049	3.15
	200	44.1^{B}	47.0^{A}	40.2^{B}	38.8^{B}	42.5				
MEAN		48.6^{A}	46.8^{AB}	40.3^{C}	42.0^{BC}					
WK2	100	53.1^{A}	41.4^{B}	38.7^{B}	37.7^{B}	42.7	0.2948	0.0147	0.2023	3.2
	200	41.3	41.0	40.0	38.7	40.2				
MEAN		47.2^{A}	41.2^{B}	39.3^{B}	38.2^{B}					
WK3	100	36.7^{A}	30.5^{B}	29.6^{B}	27.5^{B}	31.0^{D}	0.0677	0.0050	0.5905	2.03
	200	30.6	29.2	26.4	26.9	28.3^{E}				
MEAN		33.7^{A}	29.8^{B}	28.0^{B}	27.2^{B}					

pSE is the average standard error (df=77) for Fe*Vit.E interaction.
^{A,B,C} Means in the same row with different superscripts significantly different.
^{D,E} Means in the same column with different superscripts significantly different.

Vit. E injections to decrease lipid peroxidation levels on wk1. Iron injections of 100 or 200 mg did not increase free radical stress. The overall effect of vitamin E suggests that a 300 IU injection of Vit.E could be a marginal recommendation volume without severe oxidative damage with 100 or 200 mg iron. However, the vitamin E injection of 300 IU with 200 mg iron significantly increased plasma lipid peroxidation levels when compared to no iron injection at wk1. This finding was not expected but was found to be related to a number of smaller pigs on this treatment. Further study of this group revealed more lighter pigs than in the other groups. Thus, as noted in experiment 1, pigs with small body weights had increased lipid peroxidation levels. As observed in experiment 1, increasing the amount of iron injected from 100 to 200 mg did not result in increased lipid peroxidation. Thus, the injection of 200 mg of iron must not provide a significant free radical challenge to the newborn pig. These results prompted us to carefully and intensively study the results of Moore (1992) who had indicated that higher levels of injected iron resulted in increased levels of lipid peroxidation products in plasma. This careful study of this previous research revealed that 100 mg iron injected did not result in a significant increase in plasma lipid peroxidation levels at most ages measured but that injection of 400 mg of iron did cause significantly increased levels of plasma lipid peroxidation. Thus in all three experiments reported here, the

provision of 100 or 200 mg of iron has not caused a significant increase in plasma lipid peroxidation. We would postulate that these levels of iron administration did not provide a significant free radical challenge to the pig. Further research in the future will be required to ascertain at what level of iron administration above 200 mg, the pig is significantly challenged by Fe free radicals to cause an increase in TBA. This research and the research of Moore (1992) would suggest that somewhere between 200 mg and 400 mg of injected iron there is a break point at which increased iron does cause an increase in plasma lipid peroxidation.

Effects of iron and vitamin E on weights are presented in Table 19. Iron injections of 200 mg did not change weight gain at 21 days and weaning. Vitamin E injections of 300, 600, and 900 IU had no effect on the weight of pigs at 21 days and weaning. There was a tendency for Vit.E injections to increase weights at 21 days and weaning. However, the birth weights of groups 600 and 900 IU Vit.E could have influenced the 21 day and weaning weight, since they were heavier at birth.

Partial correlation coefficients for weights and vitamin E, iron, and TBA are presented in Table 20. Weight at birth, and days 21, and vitamin E were negatively highly correlated -0.37 and -0.34, with wk1 Vit.E, respectively. Partial correlation coefficients (-.35) between 21 day weight and Vit.E on wk3 indicated that heavier baby pigs had lower Vit.E concentration in the plasma. Wk1, wk2 and wk3 TBA and wt of

Table 19 Effect of Iron and Vitamin E on Weight (lbs) (Exp. 2+3)

		VITAMIN E (IU)				MEAN	ANOVA PROB.			pSE
	Fe(mg)	0	300	600	900		Fe	VIT.E	Fe*Vit.E	
No.	Pigs	21	22	17	18					
WT0	100	4.3	3.9	4.7	4.7	4.4	0.5224	0.2028	0.3078	0.23
	200	4.4	4.6	4.5	4.6	4.5				
MEAN		4.4	4.2	4.6	4.6					
WT21	100	13.3	13.3	14.6	14.9	14.0	0.9120	0.6178	0.3938	0.76
	200	13.8	14.5	14.4	13.3	14.0				
MEAN		13.5	13.9	14.5	14.1					
WNWT	100	21.4	22.5	24.1	24.7	22.9	0.9827	0.4036	0.1740	1.05
	200	23.5	23.0	23.2	21.9	22.9				
MEAN		22.0	22.8	23.6	23.3					

pSE is the average standard error (df=77) for Fe*VIT.E interaction

Table 20 Partial Correlation Coefficients for Weight and Vitamin E, Iron, TBA.(Exp.2+3)

	VIT.E WK1	VIT.E WK2	VIT.E WK3
WT 0	0.0047 (-0.37)	0.2554 (-0.16)	0.6521 (-0.06)
WT 21	0.0128 (-0.34)	0.0641 (-0.26)	0.0118 (-0.35)
WNWT	0.2083 (-0.18)	0.4178 (-0.11)	0.0468 (-0.28)

	TBA WK1	TBA WK2	TBA WK3
WT0	0.7888 (0.38)	0.8832 (-0.02)	0.8713 (0.02)
WT 21	0.0083 (-0.36)	0.0034 (-0.40)	0.0170 (-0.33)
WN WT	0.0749 (-0.25)	0.0099 (-0.35)	0.0404 (-0.29)

	IRON WK1	IRON WK2	IRON WK3
WT 0	0.1530 (-0.20)	0.1034 (0.23)	0.8010 (0.04)
WT 21	0.0617 (-0.26)	0.6304 (0.07)	0.4612 (-0.10)
WNWT	0.3293 (-0.14)	0.3883 (0.12)	0.6596 (-0.06)

Numbers in ( ) are partial correlation coefficients.

the pig at day 21 had high negative correlations -.36, -.40,and -.33,respectively. This indicated that larger pigs had lower TBA values at all 3 measurement times. Plasma iron levels approached being significantly negativly correlated to 21 day weight at wk1.

Partial correlation coefficients between Vit.E, TBA, and Iron are presented in Table 21. Plasma wk1 Vit.E was positively highly correlated with wk2 Vit.E. The values for wk1 TBA, wk2 TBA (.0001), and wk3 TBA (.08) were highly positively correlated with each other. Plasma iron at wk1 was highly correlated with wk2 Fe (.009) and wk3 Fe (.002). These results suggest that plasma iron was not significantly correlated with TBA at any measurement period. In addition, plasma vitamin E and TBA at all 3 measurement periods were not significantly correlated in these 2 experiments.

Table 21 Partial Correlation Coefficients between Vitamin E, TBA, and Iron (Exp.2+3)

	VIT.E1	TBA1	Fe1	VIT.E2	TBA2	Fe2	VIT.E3	TBA3	Fe3
VIT.E1	.	-.10	.03	.48	-.12	-.06	.08	-.06	-.03
	.	.48	.80	.0003	.41	.68	.56	.69	.85
TBA1		.	-.03	.07	.51	-.11	.16	.24	.04
		.	.84	.63	.0001	.42	.27	.08	.77
Fe1			.	-.007	-.15	.36	.09	-.17	.42
			.	.96	.29	.009	.50	.23	.002
VIT.E2				.	-.18	-.05	.08	-.02	-.02
				.	.20	.72	.56	.91	.90
TBA2					.	-.09	.15	.51	-.03
					.	.54	.28	.0001	.85
IRON2						.	.05	-.23	.22
						.	.71	.10	.11
VIT.E3							.	.29	.08
							.	.04	.58
TBA3								.	-.17
								.	.23
Fe3									.
									.

If prob.<.05 significantly correlated.

CHAPTER 4

GENERAL DISCUSSION

Three experiments were conducted during a 10 month period to determine the effects of injected alpha-tocopherol on plasma iron and lipid peroxidation in the newborn pig and to determine the optimum level of Vit.E and iron for practical application and optimum performance in the commercial swine industry.

These experiments were focused on which levels of iron injection were optimum without causing severe oxidative damage by free radicals. If iron injections caused oxidative stress how much alpha-tocopherol was optimum for newborn pigs and would be economical.

Results of these experiments indicate that iron injections were essential for hemoglobin synthesis to prevent anemia caused by confinement facilities and low placental transfer from the sow to the newborn pig. Moore (1992) reported that a 400 mg iron injection significantly increased lipid peroxidation in the newborn pig. However, results from these experiments suggest that iron injections of 100 or 200 mg could be a recommended volume without severe oxidative stress by iron induced free radicals. Results from experiment 1 had more variation which may have influenced the results because of a low number of pigs (48) compared to experiment 2

and 3 together (80) pigs. Lipid peroxidation levels, in general, were not affected by Vit.E injections of 300, 600 and 900 IU but significant decreases were observed when compared to no Vit. E injection. These results suggest that a 300 IU Vit.E injection with 100 or 200 mg iron is adequate to prevent free radical oxidative stress and allow acceptable performance in the swine industry. These experiments have demonstrated that administration of 100 or 200 mg of iron was inadequate to cause a significant increase in plasma lipid peroxidation products. Thus, further research will be required to determine the point between 200 and 400 mg of injected iron at which iron free radical oxidative stress increases plasma lipid peroxidation products. Injection of vitamin E resulted in increased plasma vitamin E levels and decreased plasma TBA values with increasing level of injected vitamin E (Exp. 2 and 3). Under the conditions of these experiments, it appeared that levels of vitamin E injection above 300 IU were no more effective than the 300 IU vitamin E level. The negative relationships observed in these experiments between weight and vitamin E and TBA indicate that in future research animal weight may need to be used as a continuous independent covariate in the analyses of data such as these or that vitamin E and TBA may need to be expressed on a total body or total blood volume basis since small pigs tended to have higher values when vitamin E and TBA were expressed in units of concentration where as larger or faster growing pigs had

lower levels of plasma vitamin E and TBA. We also observed that especially in determining TBA that there were large relative differences between litters as to the average TBA for the whole litter. In these experiments, each experimental treatment combination was administered to at least 1 pig in each litter such that all treatments were represented in each litter to help control this variation due to litter or the sow or other causes. Further research might need to consider looking at change in TBA level after injection of the treatments until the next measurement period and analyze and express TBA in terms of change per fixed period of time.

These experiments have contributed important information to our understanding of the relationships between injected iron and vitamin E and their effects on free radical peroxidation, lipid peroxidation, plasma vitamin E, iron and TBA in the neonatal pig.

CHAPTER 5

SUMMARY

Three experiments were conducted to determine the effect of injected alpha-tocopherol on plasma iron and lipid peroxidation in the newborn pig and the optimum level of vitamin E and iron for practical application and optimum performance in the commercial swine industry.

In the first experiment 48 newborn cross-bred pigs were used in a 2*4 factorial arrangement with 8 pigs coming from each of 6 litters. The treatments consisted of 2 injections of iron: 0 and 100 mg as gleptoferron and four alpha-tocopherol levels: 0, 300, 600 and 900 IU.

The second (32 pigs) and third (48 pigs) experiments utilized 80 newborn cross-bred pigs in a 2*4 factorial arrangement with 4 pigs per treatment in experiment 2 and 6 pigs per treatment for experiment 3. The treatments consisted of two injections of iron: 100 and 200 mg as gleptoferron and four alpha-tocopherol injections: 0, 300, 600 and 900 IU.

Results obtained from experiment 1 indicated that iron injections of 100 mg significantly increased blood hematocrit and hemoglobin levels on wk1, wk2, and wk3 when compared to no iron injections. Alpha-tocopherol injected at 300, 600 and 900 IU significantly increased plasma α-tocopherol concentration

on wk1, wk2, and wk3. There were more significant changes at an early age (wk1 and wk2) than wk3 on plasma α-tocopherol concentration. Greater changes at an earlier age, were probably related to rapid clearance of alpha-tocopherol from plasma, at 3 wk. Most of the injected alpha-tocopherol had cleared so differences were less evident. Iron injection of 100 mg significantly increased plasma iron concentration on wk1 and wk3 when compared to no iron injection. Pigs injected with Fe at 100 mg had lower plasma lipid peroxidation levels on wk1, wk2, and wk3 when compared to no iron injections. This result suggested that iron injection of 100 mg did not cause an oxidative stress and damage to the newborn pig. Plasma iron was increased at each sampling time by the injection of 100 mg of iron but lipid peroxidation levels were lower. Thus, the injection of 100 mg of iron must not provide a serious free radical challenge to the young newborn pig. Iron injections of 100 mg did not affect the weight gain at 21 days and weaning. Pigs treated with vitamin E 900 IU injections tended to be heavier than pigs treated with other levels of vitamin E treated baby pigs at days 21 and at weaning. Partial correlation coefficients (-.44) between 21 day weight and Vit.E on wk3 suggest that heavier baby pigs had lower Vit.E concentration in their plasma. Wk1 TBA and wt of pig at day 21 and weaning wt had significant negative correlations. Vit.E injections were positively highly correlated with wk1 TBA values and wk2 Vit.E. Wk1 TBA, wk2 TBA (.017), and wk3 TBA

(.033) were highly positively correlated each other. Plasma iron was not correlated with wk2 TBA (.69), or wk3 TBA (.41). These results suggest that iron injection of 100 mg did not result in an elevated plasma iron concentration that would cause free radical damage in the newborn pig.

The results obtained from experiments 2 and 3 indicated that there was a significant increase in plasma alpha-tocopherol concentration when higher injections of α-tocopherol were given during the experimental period. Lipid peroxidation levels were significantly decreased with vitamin E injections of 300, 600, and 900 IU on wk2 and wk3 when compared to no vitamin E injections. There was a tendency for Vit. E injections to decrease lipid peroxidation levels on wk1. Iron injections of 100 or 200 mg did not increased free radical stress. As observed in experiment 1, increasing the amount of iron injected from 100 to 200 mg did not result in increased lipid peroxidation. There was a tendency for Vit.E injections to increase weights at 21 days and weaning. However, the birth weights of groups 600 and 900 IU Vit.E could have influenced the 21 day and weaning weight, since they were heavier at birth. Weight at birth, and day 21, and vitamin E were negatively highly correlated -0.37 and -0.34, with wk1 Vit.E, respectively. Partial correlation coefficients (-.35) between 21 day weight and Vit.E on wk3 indicated that heavier baby pigs had lower Vit.E concentration in the plasma. Wk1, wk2 and wk3 TBA and wt of the pig at day 21 wt had high

negative correlations -.36, -.40,and -.33,respectively. This indicated that larger pigs had lower TBA values at all 3 measurement times. Plasma iron was not significantly correlated with TBA at any measurement period. In addition, plasma vitamin E and TBA at all 3 measurement periods were not significantly correlated in these 2 experiments.

Increasing the amount of iron injected from 100 to 200 mg did not result in increased lipid peroxidation. Thus, the injection of 200 mg of iron appears not to provide a significant free radical challenge to the newborn pig. Based on wk2 and wk3 TBA values, this suggests that a 300 IU injection of Vit.E could be a marginal recommendation volume without severe oxidative damage with 100 or 200 mg iron.

LITERATURE CITED

LITERATURE CITED

Bacon, B.R., R. O'Neal, G.M. Brittenham, and C.H. Park. 1986. Effects of vitamin E deficiency on hepatic mitochondrial oxidative metabolism in experimental dietary iron overload(abstract). Hepatology 5:950.

Barr, A. J., J. P. Said, and G. T. Helwig. 1979. SAS Users Guide. SAS Institute Corporation, Raleigh, NC.

Bauernfeind, J. C. 1980. Alpha-tocopherol in Foods. In L. J. Machlin (ed.) Vitamin E: A Comprehensive Treatise. pp. 99-168. Marcel Dekker, New York.

Begin, M. E., G. Ells, and D. F. Horrobin. 1985. Polyunsaturated fatty acid induced cytotoxicity against tumor cells and its relationship to lipid peroxidation. J Natl. Cancer Inst. 80:188.

Boyer, R.F., and C.J. McCleary. 1987. Superoxide iron and a primary reductant in ascorbate-mediated ferritin iron release. Free Rad. Biol. Med. 3:389.

Braude, R., A.G. Chamberlain, M. Kotarbinska, and K.G. Mitchell, 1962. The metabolism of iron in piglets given labelled iron either orally or by injection. Brit. J. Nutrition 16:427.

Burdon, R.H., V. Gill, and C. Rice-Evans. 1987. Free radicals: Oxidant stress and drug action. pg. 347-361. Richelieu press, London.

Burton, G.W., and K.U. Ingold. 1981. J. Am. Chem. Soc. 103:6472.

Buja, L.M., and W.C. Roberts. 1971. Iron in the heart. Etiology and clinical significance. Am. J. Med. 51: 209.

Carpenter, C.S. 1965. A histochemical study of oxidative enzymes in the nervous system of vitamin E deficient rats. Neurology 15:328.

Cavill, I., M. Worwood, and A. Jacobs. 1975. Internal regulation of iron absorption. Nature(Lond.) 256: 328.

Choi, K.S., and A.L. Tappel. 1969. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. Biochemistry 8:2827.

Conner, J. July 1993. How much vitamin E is enough. Swine practitioner. pg:4-7.

Cook, R.W., E.R. Miller, C.K. Whitehair, P.K. Ku, and R. Michel. 1981. The tolerance to iron injections in vit.E-selenium deficient pigs. Michigan Agri. Exp. Res. Rep. 437:94.

Cprilipko, l.L., C.N. Orlov, and V.E. Kagan. 1983. Accumulation of gaseous lipid peroxidation products in the expired air in children during hyperbaric oxidation (in Russia). Bull. Exp.Biol. Med., 96: 1367.

Crosby, W.H., J.I. Munn., and F.W. Furth. 1954. Standardizing a method for clinical hemoglobinometry. U.S. Armed Forces M. J. 5:693.

Dove, C.R., and R.C. Ewan. 1990. Effect of excess dietary copper, iron or zinc on the tocopherol and selenium status of growing pigs. J. Anim. Sci. 68:2407.

Duthie, G.G., J.R.Arther, F. Nicol, and M. Walker. 1989. Biochemistry division, the Rowett Research Institute,Aberdin AB2 9SB. Research in Veterinary Science. 46:226.

Evans, C.D., G.R. List, A. Dolev, D.G. McConnel,and R.L.Hoffman. 1967. Pentane from thermal decomposition of lipoxidase-derived products. Lipids 2:432.

Frei, B., R. Stocker, and B.N. Ames. 1988. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natl Acad Sci 1988:9748.

Gerdin, E., O. Tyden, and U.J. Erickson . 1985. The development of antioxidant enzymatic defense in the prenatal rat lung: activities of superoxide dismutase, glutathione peroxide and catalase. Pediatr Res. 19: 687.

Glienke, L.R., and R.C. Ewan. 1974. Selenium in the nutrition of the young pig. J. Anim. Sci. 39:975(Abstr.).

Grant, C.A. 1961. Morphological and actiological studies of dietetic microangiopathy in pigs (mulberry heart). Acta Vet Scand 2(suppl 3):1.

Gordeuk, V.R., B.R. Bacon, and G.M. Brittenham. 1987. Iron-overload:causes and consequences. Annu. Rev. Nutr. 7:485.

Graf, E., J.R. Mahoney, R.G. Bryant, and J.W. Eator. 1984. Iron-catalized hydroxyl radical formation. Stringent requirement for free iron cordination site. J. Biol. Chem. 259:3620.

Halliwell, B., and J.M.C. Gutteridge. 1985. Free radicals in biology and medicine. Oxford: Clarendon press.

Halliwell, B., and J.M.C. Gutteridge. 1989. Free radicals in biology and medicine. 2 Ed. Oxford: Oxford University Press.

Heys, A.D., and T.L. Dormandy. 1981. Lipid peroxidation in iron-overloaded spleens. Clin. Sci. 60:295.

Horwitt, M.K. 1952. In modern nutrition and health and disease, Chapter 5, Section D. Vitamin E, 5th Ed by Goodhart, R. and M.E. Shils. Phialdelphia, Lea and Febiger.

Ildiko, F., and J.M. Marx. 1988. Lipid peroxidation of rabbit small intestinal microvillus membrane vesicles by iron complexes. Biochemica et Biophysica acta 96:96.

Iritani, N., E. Fukuda, and Y. Kitamura. 1980. Effect of corn oil feeding on lipid peroxidation in rats. J. Nutr. 110:924.

Jaeschke H., C. Kleinwaechter, and A. Wendel. 1991. NADH-Dependent reductive stress and ferritin bound iron in allyl alcohol-induced lipid peroxidation in vivo: The protective effect of vitamin E. Chem. Biol. Interactions, 81:57.

Janero, D. R., and R.Barrnett. 1981. Cellular and thylakoid membrane phospholipids of Chlamydomonas reinhardtii 137. J.Lipid Res. 22:1126.

Kagan, V. E. 1988. Lipid peroxidation in biomembranes. CRP press.

Kappus, H. 1985. Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. In: Sies H. ed. Oxidative stress. New York Academy Press, 1985:273.

Kayden, H.J., and M.G. Traber. 1986. Clinical, nutritional and biochemical consequences of apolipoprotein-beta deficiency. Adv. Exp. Med. Biol. 201:67.

Knight,M., J. Cook, J. Wispe, and R. J. Roberts. 1987. Comparison of the antioxidant properties of alpha-tocopherol and alpha-tocopheryl acetate in newborn rabbit lung. Dev. Pharmacol. Ther. 10:245.

Krauss, K. July 1993. Do your clients' pigs get enough vitamin E ? Swine practitioner. pg. 6.

Lannex, N., P. Lundberg, and G. Tollerz. 1964. Lowered resistance to iron in vitamin E deficient piglets and mice. Nature 195:1006.

Liebler, D.C., D. S. Kling, and D. J. Reed. 1986. Antioxidant protection of phospholipid bilayers by alpha-tocopherol. Control of alpha-tocopherol status and lipid peroxidation by ascorbic acid and glutathione. J. Biol. Chem.261:12114.

Lipschitz, D.A., M. O. Simon, S. R. Lynch, J. Dugard, T. H. Bothwell, and R. W. Charlton. 1971. Some factors affecting the release of iron from reticuloendothelial cells. Br. J. Haematol. 21: 289.

Loudenslager, M.J., P.K. Ku, P.A. Whetter, D.E. Ullrey, C.K. Whitehair, H.D. Stowe and E.R. Miller. 1986. Importance of diet of dam and colostrum to the biological antioxidant status and parenteral iron tolerance of the pig. J. Anim. Sci. 63:1905.

Machlin. 1991. Vitamin E. In: L. J. Machlin (ed.) Handbook of Vitamins. pp.99-184. Marcel Dekker. New York.

Mahan, D.C. and A.L. Moxon. 1980. Effect of dietary selenium and injectable vit.E-selenium for weanling swine. Nutr. Rep. Int. 21:829.

Maynard, L.A., and J. K. Loosli. 1979. Animal Nutrition. 7th Ed., pg:283-355. Tocopheryl acetate in new born rabbit lung.

McCord J.M. 1985. Oxygen-deprived free radicals in postischmic tissue injury. N. Engl. J. Med. 312:159.

McDowell, L. S. 1989. Vitamin E in Animal Nutrition. Pg.97-98.

McGovern, J.J., A.R. Jones, and A.G. Steinberg. 1955. The hematocrit of capillary blood. N. Engl. J. Med. 253: 308.

Meydani, M., J. B. Macauley, and J. B. Blumberg. 1988. Effect of dietary vitamin E and selenium on susceptibility of brain regions to lipid peroxidation. Lipids Vol.23 No.5.

Meyer, W.R., D.C. Mahan, and M.L. Moxon. 1981. Value of dietary selenium and Vit.E for weanling swine as measured by performance and tissue selenium and glutathione activities. J. Anim. Sci. 52:302.

Milder, M.S., J. D. Cook, and S. Stray. 1980. Indiopathic hemochromatosis, an intern report. Medicine 59:34.

Miller, E.R. 1981. Iron. Animal. Nutr. Health 36(5):p.14.

Minotti, G., and S.D. Aust. 1989. The role of iron in oxygen radical mediated lipid peroxidation. Chem.-Biol. Interact. 71:1.

Moore, J. 1992. Effects of dietary pharmacological concentrations of alpha-tocopherol, ascorbic acid and iron on lipid peroxidation, iron tissue distribution, hematological, immunological, performance, and tissue parameters of young rats and pigs (PhD. Dissertation). University of Tennessee.

Nafstad I. 1971. The vitamin E deficiency syndrome in pigs. Light and electron microscopic studies on myocardial vascular injury. Vet. Pathol. 8:239.

Newberne, P. M., and M. W. Conner. 1989. Tie vitamins. pp.796-833. In: J. J. Kaneko (ed.) Clinical Biochemistry of Domestic Animals. Academic Press, San Diego, CA.

NRC. 1988. Nutrient requirements of domestic animals: Nutrient requirements of swine. 9th Ed. National Academy of Sciences- National Research Council, Washington, D.C.

Powell, L. W., M.L. Bassett, and J. W. Hallyday. 1980 Homocromatosis update. Gastroenterology. 78:374.

Pryor, W.A., 1978. The formation of free radicals and the consequences of their reactions in vivo. Photochem. Photobiol. 28:787.

Puls, R. 1988. Mineral levels in animal health, iron, pigs: tissue levels. Can Vet J. 29(7):568.

Raharjo, S., J.N. Sofos, J.A. Maga, K.G.D. Allen, and G.R. Schmidt. 1991. Improved limit of detection and specificity of the thiobarbituric acid method for measuring lipid peroxidation in ground beef. J. of Anim. Sci. 69.(Supp. 1):348.

Rea, J. C., D. E. Ullrey, E. Poe, Jr., and S. Pollman. 1991. Minerals for swine in TN pork industry handbook.

Reddy, P.G., J. L. Morril, H.C. Minocha, and J. S. Stvenson. 1987. Vitamin E is immunostimulatory in calves. J. Dairy Sci. 70: 993.

Rice-Evans C., and P. McCarthy. 1989. Iron overload and predisposition of cells to antioxidant consumption and peroxidative damage. Free Radical Res Commun. 1989 7(3-6):307.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.

Schaich, K.M., and D. C. Borg. 1988. Fenton reactions in lipid phases. Lipids 23:570.

Schudel, P., H. Mayer, and O, Isler. 1972. The vitamins: Chemistry second edition Vol. V pg. 192.

Scott, M.L., M. C. Nesheim, and R. J. Young. 1982. Nutrition of the chicken. pg.119-276.

Sinnhuber, R.S., and T. C. Yu. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Res. 23:626.

Sullivan, J. L. 1986. Sex, iron, and heart disease. Lancet II:1162.

Teige, J., F. Saxegaard, and A. Froslie. 1978. Influence of diet on experimental swine dysentery. 2. Effects of a vitamin E and selenium deficient diet supplemented with 3 % cod liver oil, Vit.E or selenium. Acta Vet.Scand. 19:133.

Tengerdy, R.P., and J. C. Brown. 1977. Effect of vitamin E and A on humoral immunity and phagocytosis in E. coli infected chicken. Poult. Sci. 56,957.

Tollerz, G. 1973. Vit.E, selenium (and some related compounds) and tolerance towards iron in piglets. Acta Agri. Scand. Suppl. 19:84.

Tollerz, G, and N. Lannex. 1964. Protection against iron toxicity in vit.E-deficient piglets and mice by vit.E and synthetic antioxidants. Nature 201:846.

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

Ullrey, D.E. 1982. Vitamin E for swine alpha-tocopherol. Distill. Feed Conf., Distill.Feed Res. Counc. Cincinati 37:81.

Underwood, E.J. 1966. The mineral nutrition of livestock. Chap.6, Iron.

Van Vleet, F. J., and V. J. Ferrans. 1977. Ultrastructure of hyaline microthrombi in myocardial capillaries of pigs with spontaneous mulberry heart disease. Am. J. Vet. Res. 38:2077.

Vliegenthart, J.F.G., and G.A. Veldink. 1982. Lipoxygenases, in free radicals in biology. Vol.5, Pryor, W.A., Ed., Academic press, New York, p.29.

Wahlstrom, R. C., L. J. Kortan, and W. B. Thomas. 1991. Vitamins for swine. Tennessee pork industry handbook.

Williams, M.L., R. J. Skott, P. L. O'Neal, and F. A. Oski. 1975. Role of dietary iron and fats on vitamin E deficiency. New Engl. J. Med. 292:887.

Wills, E. D. 1969. Lipid peroxide formation in microsomes. The role of non-heme iron. Biochem. J. 113:325.

Wispe, J. R., M. Knight, and R. J. Roberts. 1986. Lipid peroxidation in newborn rabbits: Effect of oxygen, lipid emulsion, and vitamin. Pediatr. Res. June; 20(6): 505.

Wong, S. F., B. Halliwell, R. Richmond, and W. R. Skowroneck. 1981. The role of superoxide and hydroxyl radicals in the degradation of hyaluronic acid induced by metal ions and by ascorbic acid. J. Inorg Biochem. 14:127.

Worwood, M. 1977. The clinical biochemistry of iron in iron excess aberrations of iron and porphyrin metabolism. Semin-Hematol. 14(1):3.

Yaggi, K. 1987. Assay for serum lipid peroxide level and its clinical significance. Lipid peroxide in biology and medicine. p:223.

APPENDICES

APPENDIX A. MICRO HEMATOCRIT

PROCEDURE

1. Fill capillary hematocrit tube two-thirds full with whole blood.
2. Seal one end of the hematocrit tube with clay.
3. Place the hematocrit tubes in the grooves of the centrifuge head, with the sealed end away from the center of the centrifuge.
4. Centrifuge for five minutes.
5. Remove the hematocrit tubes from the centrifuge.
6. Place the centrifuged capillary tube in the groove of the plastic indicator so that the bottom of column of red cells coincides with the black line on the plastic indicator.
7. Rotate the bottom plate so that the 100 percent line is directly beneath the red line on the plastic indicator; then hold the bottom plate in this position. Now, using the finger hole, rotate the top plate so that the spiral line intersects the capillary tube at the plasma-air interface.
8. Rotate both discs together until the spiral line intersects the capillary tube at the red cell-white cell interface.
9. The cell volume in percent is read from the point on the scale directly beneath the red line of the plastic indicator.

PRINCIPLE

Whole blood is centrifuged for packed red blood cell volume.The red blood cells are measured and expressed as a percent of the whole blood volume.

REAGENTS AND EQUIPMENT

1. Capillary hematocrit tubes
2. Clay, to seal one end of the hematocrit tubes
3. Centrifuge, 11,500 to 15,500 revolutions per minute (RPM), with an appropriate head for the capillary hematocrit tubes
4. Micro hematocrit reader
5. Whole blood with an anticoagulant

APPENDIX B. HEMOGLOBIN PROCEDURE

PROCEDURE

1. Obtain whole heparinized blood.
2. Gently invert blood tube 4 times.
3. Place rubber tubing on the end of the sahli pipette and pull blood in to the pipette until the blood rises approximately 1 cm above the fill line.
4. Remove the pipet from the blood tube and wipe on a clean tissue.
5. Gently tap the tip of the pipet on the end of the finger until the blood is even with the fill line.
6. Place the sahli pipette in the drabkins tube and by blowing, evacuate the blood into the tube.
7. Mix and allow to stand for 15 minutes.
8. Put a sample of the drabkin solution in the spectrophotometer and zero the instrument.
9. Place a sample of the blood + drabkins into the spectrophotometer and read.
10. In order to get a hemoglobin value multiply the absorbance reading by the standard factor value. The answer will be in g/100ml.

Example of calculation:

Avg. reading * standard factor = Hb g/100ml
Ex. 0.321 * 37.49 = 12.03 g/100ml

MATERIALS

Spectrophotometer set at 540 nm
Drabkin solution - 5 ml per tube
Sahli pipette - 20 cmm (0.02ml)
Drabkin factor-standard
Test tubes- to hold at least 5 ml of solution
Rubber tubing with mouth piece
Hemoglobin standard

APPENDIX C. VITAMIN E EXTRACTION METHOD FOR PLASMA

1. Place 0.5 ml plasma into 4 ml polypropylene tube.
2. Add 0.5 ml isopropyl alcohol (HPLC grade).
3. Vortex 15 sec. - allow to sit 15 min.
4. Add 2.5 ml hexane (HPLC grade) - shake for 10 min.
5. Vortex 30 sec.
6. Allow to sit for 1 min.
7. Centrifuge 20 min @ 1000 g's at 5 °C.
8. Remove organic layer into 4 ml polypropylene tube.
9. Evaporate under N_2 - use hood.
10. Add 0.5 ml MEOH - shake for 5 min.
11. Allow to sit for 30 min.
12. Vortex for 10 sec.
13. Filter (.2 micron) into 1.5 ml capacity snap-cap polypropylene centrifuge vials. Use 1 cc syringe.
14. Inject 10 - 50 micro liters into HPLC system.

EQUIPMENT

1. Polypropylene tubes with caps (4 ml)
2. Reagent grade hexane (HPLC grade)
3. Reagent grade isopropyl alcohol (HPLC grade)
4. Nitrogen gas (filtered)
5. Methyl alcohol (HPLC grade)
6. HPLC system
7. Mobile phase: 100% methyl alcohol
8. Column: Microbonda Pak C_{18} (Waters)
9. Fluorescence detection: 295 nm excitation 330 nm emission

APPENDIX D. LIPID PEROXIDATION

Thiobarbituric acid plasma

1. Pipette 50 μl of plasma into a polypropylene tube(12 ml).
2. Add 4.0 ml of 12/N H_2SO_4.
3. Shake gently for 5 min.
4. Add 0.5 ml of 10 % phosphotungstic acid and vortex for 5 sec.
5. Allow the mixture to stand at room temperature for 10 min.
6. Centrifuge the mixture for 15 min.
7. Discard the supernatant (pour off easily).
8. Add 4.0 ml of distilled water to the sediment.
9. To this mixture add 1.0 ml of TBA (2- thiobarbituric acid) reagent (a mixture of equal volumes of 0.67 % TBA aqueous solution and glacial acetic acid).
10. Vortex for 15 sec. until the sediment is mixed well with water and TBA reagent.
11. Heat the mixture at 95 °C for 60 min. in a water bath. Loosely cap the tube.
12. Cool the tube with cool tap water.
13. Add 5.0 ml of n-butanol and shake horizontally and vigorously for 15 min.
14. Allow to stand at room temperature for 10 min.
15. Centrifuge the mixture at 1000 g for 30 min. at 0°C.
16. Remove the n-butanol layer and measure absorbance at 530-537 nm on a spectrophotometer.

* For the standard reading : put 50 μl of standard solution into 12 ml polypropylene tube then use steps 8 - 16. Various concentrations from 0 to 250μl can be used to establish linearity of standard curve and be adapted to concentration range of samples.

METHOD OF CALCULATION

$$\text{Plasma LP} = \frac{f}{F} * 20 = \text{nmol/ml}$$

f = sample absorption

F = standard absorption

METHODS TO MAKE REAGENTS (lipid peroxidation)

12/N H_2SO_4

To make 1 liter: 336 mls (28 * 12) of H_2SO_4 into 1 liter of distilled water: put water about 500 ml, into 1 liter beaker, slowly add 12/ N sulfuric acid, add remaining water, wait at least 2-3 hours until this mixture cools down and add more water to make 1 liter.

10 % PHOSPHOTUNGSTIC ACID

To make 500 ml : Weigh 50 g of phosphotungstic acid put into 450 ml distilled water, wait for 20 min. then add 50 ml distilled water.

TBA REAGENT

To make 1 liter: Prepare 3.35 g of 2 thiobarbituric acid then add 500 ml distilled water (to faciliate dissolving of the TBA, use small amount of heat but do not boil the mixture). After dissolution and cooling, add 500 ml glacial acetic acid under a hood. Stir and mix reagent for 5 minutes. Wrap flask with aluminum foil and store in reduced light. Should be made new daily and do not use if made more than 2 days prior to use.

STANDARD

To make 1 liter of 0.01 mmol tetramethoxypropane standard solution: (A) Prepare .01 M tetramethoxypropane (0.82 ml) and put this into 500 ml volumetric flask then add H_2O to make 500 ml of solution.
(B) Take 1 ml of (A) into 1 liter volumetric flask then add H_2O to make 1 liter of solution.

Tetramethoxypropane: Molecular Weight (146.2 g)

Fifty (50) microliters of this standard solution B contains .5 nmole of tetramethoxypropane.

APPENDIX E. SERUM IRON AND TOTAL IRON-BINDING CAPACITY BY ATOMIC ABSORPTION SPECTROPHOTOMETER

Procedure

Serum Iron

1. Place 1 ml serum in a 12*75 mm glass tube.
2. Add 1 ml of 20 % TCA, cap tube loosely, mix, and heat in a water bath at 90°C for 15 min.
3. Cool, centrifuge, and determine the iron level in the supernatant by atomic absorption spectrophotometry, comparing with standards to calculate the iron content.

Comment: If ample serum is available, use 2 ml of serum and 2 ml of TCA.

Total Iron-Binding Capacity

1. Place 2.0 ml of serum in a 12*75 glass tube.
2. Add 2.0 ml of ferric chloride solution (500 μg of ferric iron per 100 ml). Mix and let stand 5 min.
3. Add 200 mg of magnesium carbonate and mix four times during a 30-min period. Centrifuge.
4. Remove 2.0 ml of the supernatant liquid and transfer to another clean plastic tube. Add 2.0 ml of TCA, cap lightly, and heat in a water bath at 90°C for 15 min.
5. Cool, centrifuge, and determine the iron content of the supernatant by atomic absorption spectrophotometry, calculating the iron content by comparison with stnadards.

The result, so determined, should be multiplied by two (2.0) to obtain the actual value. This factor takes into account the dilution inherent in addition of the saturated ferric chloride in Step 2.

VITA

Sung Min Choi was born August 2, 1963 in Chunbuk, Korea. He is the son of the Rev. Byung Doo Choi. He attended elementary and secondary education in Chunbuk, Korea. In March 1983, he entered the Chunbuk National University, Korea and graduated with a Bachelor of Science in Animal Science in February 1990. During that time he served military service for three years. In Spring 1992, he decided to study in America for his Master of Science degree at the University of Tennessee, Knoxville.

He received his Master of Science degree in Animal Science in August of 1994.