II. LITTERATURE REVIEW

II.1 Vitamin E

II.1.1 Chemistry

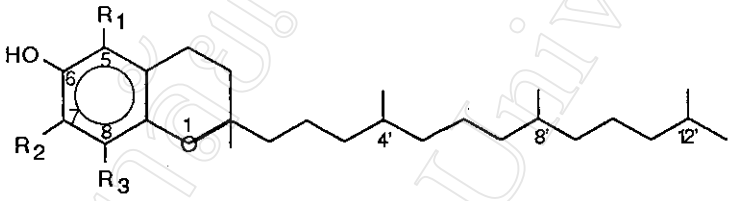
Vitamin E is a generic name giving to a group of eight structurally related tocopherol and tocotrienol compounds. They have relative biological activities of α-tocopherol, the most biologically active form of vitamin E (Combs, 1993). Hence, they similarly have the basic structure named "tocol". Tocol consists of two parts, a chromanol nucleus and a phytyl side-chain as shown in figure 1.

The difference between tocopherols and tocotrienols is the degree of saturation of their side chains; tocotrienols have double bounds but tocolpherols do not. The additions of methyl groups available on the 5,7 and 8 carbon's position of the benzene ring derive α, β, γ or δ-tocopherol or -tocotrienol isomers, a relative forms of the vitamin. Activities of vitamin E depend on hydroxyl group on the 6 carbon's position, methyl groups on phenol ring and side chain. Therefore the more modification there is of α-tocopherol structure, the less biological activity shows. The physical properties of the tocopherols are shown in table 1. Vitamin E is well known as a "biological antioxidant" which means it can potentially inhibit a harmful lipid peroxidation reaction, which normally occur in an animal 's body. Lipid peroxidation is triggered by oxygen species produced naturally by various vital metabolic processes in cells. Polyunsaturated fatty acids (PUPAs) are more susceptible to peroxidation due to their double bounds. Without a powerful antioxidant system, cell membrane primarily consisting of lipid components is prone to undergo oxidation bringing about membrane destruction. Like other antioxidants, vitamin E can inhibit oxidation by donating the hydrogen on its C-6

position hydroxyl group. But vitamin E is considered to have a predominant role in membrane protection because its phytyl side-chain integrated with lipid portion can achieve perfect lipid peroxidation inhibition. Although vitamin E can inhibit oxidation reaction, its natural forms are sensitive to be inactivated by oxygen accelerated by UV light, heat, high pH and some elements (Macrae, 1993 and Dove *et al*., 1991). Therefore, vitamin E is generally sold in markets in an esterified form as its C-6 hydroxyl group is temporally protected to make it be stable.

Chemical structures of the vitamin E group:

Tocophero

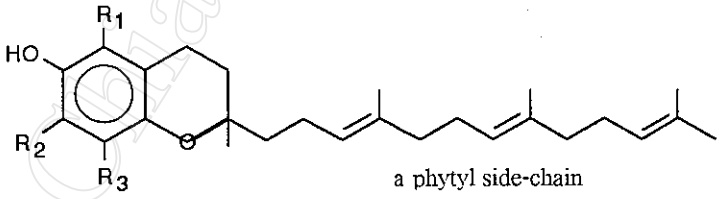


a chromanol nucleus

a phytyl side-chain

vitamer	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3
tocol	H	H	H

Tocotrienols



a chromanol nucleus

a phytyl side-chain

vitamer	R_1	R_2	R_3
α-tocotrienol	CH_3	CH_3	CH_3
β-tocotrienol	CH_3	H	CH_3
γ-tocotrienol	H	CH_3	CH_3
δ-tocotrienol	H	H	CH_3
tocotrienol	H	H	H

Figure 1. Chemical structures of the vitamin E group From Combs (1993)

Table 1 Physical properties of tocopherols

	α-T	β-T	γ-T	δ-T	α-Tacetate
Formula	$C_{29}H_{50}O_2$	$C_{28}H_{48}O_2$	$C_{28}H_{48}O_2$	$C_{27}H_{46}O_2$	$C_{31}H_{52}O_3$
Molecular weight	430.69	416.66	416.66	402.62	472.73
Boiling point (°C,0.1 atm)	210-220	200-210	200-210		184
Absorption maximum in ethanol	294 nm	297 nm	298 nm	298 nm	285.5 nm
Extinction coefficient($E^{1\%}$)in ethanol	71.0	86.4	92.8	91.2	43.6
Optical rotation(α546 in ethanol, *RRR*-isomers)	+0.32°	+2.9°	+3.2°	+3.4°	+3.2°

Macrae *et al.*, (1993)

II.1.2 Source

Vegetable oils such as wheat germ, sunflower seed and cottonseed are naturally rich in vitamin E. The other sources of vitamin E are shown in table 2. Unlike green plants, animals can neither synthesize nor store large amount of vitamin E. Although natural vitamin E consists of different ratios of the eight isomers, α-tocopherol is predominant form. Vitamin E sold on markets is manufactured from either chemical synthesis named "all-recemic-tocopherol" or vegetable oil extraction called "natural identical (*R,R,R*)-α-tocopherol. More complicated than extraction, the synthetic vitamin is produced by chemical reactions called acid-catalyzed condensation of trimethyl hydroquinone (TMHQ) with isophytol. In fact many steps are involved in preparations of TMHQ and isophytol (Ottaway, 1993). Yet this method has advantages in mass production. Although the products come from two different processes, they have the same vitamin E activity and finally undergo esterification such as acetate and succinate esters, forms of stable vitamin E for commercial purposes.

Table 2. Tocopherol content of refined vegetable oils, (mg/ 100 g oil)

Oil	α-T	β-T	γ-T	δ-T	α-T-3	β-T-3	γ-T-3	Vit.E activity[a]
Coconut	0.35	0	0.17	0.35	1.29	0.10	1.32	0.78
Corn	14.26	0.38	64.9	2.75	0.58	0	0	21.1
Cotton seed	35.26	0	29.98	0	0	0	0	38.26
Olive	11.92	0	0.72	0	0	0	0	11.99
Palm	18.32	0	0	0	11.46	0	5.75	21.82
Peanut	11.62	0	12.98	0.33	0	0	0	12.92
Rapeseed	17.65	0	27.04	0.04	0	0	0	20.35
Safflower seed	34.05	0	3.5	0.49	0	0	0	34.4
Soyabean	10.99	0	62.4	20.4	0	0	0	17.43
Sunflower seed	59.5	0	3.54	0	0	0	0	59.85
Wheatgerm	149.44	81.19	0	0	0	0	0	183.0

α-T = α-tocopherol; β-T = β-tocopherol; γ-T = γ-tocopherol; δ-T = δ-tocopherol; α-T-3 = α-tocotrienol; β-T-3 = β-tocotrienol; γ-T-3 = γ-tocotrienol.

[a] total vitamin E activity = α-tocopherol + 0.4(β-tocopherol) + 0.1(γ-tocopherol) + 0.01 (δ-tocopherol) + 0.3(α-tocotrienol) + 0.05(β-tocotrienol) + 0.01(γ-tocotrienol).

From Ottaway (1991)

II.1.3 Transportation and Metabolism of vitamin E

Digestion of ingested vitamin E involves bile salts and pancreatic juice. Depending on lipid, vitamin E is absorbed with chylomicron, dispensed lipid in bile salts and gastric juices. Therefore, defects in genes causing abnormality of lipid absorption and metabolism associate with vitamin deficiency although the

diets contain adequate vitamin E (Rice *et al.*, 1989). Maximal absorption of vitamin E takes place in middle of the small intestine. Micelle attached α-tocopherol or free alcohol forms of vitamin E to pass the lumen of the gut throughout lymphatic ducts. In the case of esterified vitamin E, such as acetate or succinate esters, being ingested; it is hydrolyzed by duodenal mucosal esterase before absorption. Even by injection route, vitamin E esters are locally hydrolyzed by esterases present in plasma and various tissues (Gallo-Torres, 1971). Hidiroglou *et al.*, (1988) and Chung *et al.*, (1992) reported that dietary *d*-α-tocopherol supplementation produced more efficacy and bioavailability than *dl*-α-tocopheryl acetate. That is probably due to incomplete hydrolization of esterases. Liver is the primary target site of vitamin E metabolism because α-tocopherol levels rapidly increased within 24 hours responding to vitamin E administration (Hidiroglou, 1987). From the liver, α-tocopherol is selectively released together with low density lipoprotein (LDL) or very high density lipoprotein (HLDL) therefore the predominant form of plasma vitamin E is α-tocopherol (Hoppe *et al.*, 1991). Then, it is transported to other tissues. Serum, liver and platelet are temporal pools of vitamin E, which rapidly respond to the vitamin intake (Njeru *et al.*, 1994). In contrast, adipose and skeletal muscles are permanent pools, which are not temporally affected by low serum status (Jensen, 1990). The α-tocopherol uptake of cells is by two possible pathways. First, it is incorporated *via* LDL receptors on cell membrane. Some studies reported that LDL receptors on ovarian tissues, lymphocytes and buccal cells promote increase of vitamin E uptake by cells from serum (Aten *et al.*, 1994). Another proposed that α-tocopherol is disassociated from carriers by lipase and transferred into cells by re-associating fatty acid and lipid binding protein. Tocopherol is transported across cytoplasm within cell to organelles such as mitochondria plasma membrane. In these environments,

lipophillic compounds as tocopherol need protein carriers recently known as tocopherol-binding protein (TBPs). It was found that TBPs preferably bind the α-form and have specific target sites (Dutla-Roy *et al.*, 1994). Metabolite of vitamin E is tocopheryl radical. It can be reversed to an active form by the help of reduced glutathione (GSH) or vitamin C. In minor cases, α-tocopherol radical is oxidized to tocopheryl quinone and further reduced to α-tocopheryl hydroquinone or other oxidation product. This inactive vitamin E is finally excreted with bile salts in faeces.

II.1.4 Vitamin E determination

Vitamin E analysis involves two procedures, extraction and detection. After preparing according to their nature, samples are chemically treated. This process is known as alkaline saponification to free vitamin E from bounded substance. Then it is extracted in organic solvents such as n-hexane and petroleum ether in which vitamin E becomes more soluble. Since naturally free vitamin E is susceptible to air oxidation, antioxidants such as vitamin C or BHT are added in extractant (A.O.A.C., 1990). Vitamin E extract is spontaneously contaminated by some fat-soluble reducing agent. Removing this interference makes the analysis more sensitivity. However, the development of chromatography technique results in the solution of interference problems and the vitamin E isomers are separated in quantify. In detection mode, many techniques are alternatively used depending on analytical scales and availability of equipment.

II.1.5 Vitamin E requirement of weaned pigs

In general, recommended level of vitamin E which potentially prevents weaned pigs from vitamin E and selenium deficiency syndromes range from 10-30

mg/kg diet (Ullrey, 1981 and Jensen *et al.*, 1988). This is a rather wide range because many factors compromise the requirements such as pre-weaning vitamin E status (Mahan, 1994), other food antioxidants, stress and dietary PUFAs. Potential stress and high unsaturated fat increases vitamin E requirement. In contrast, good vitamin E status and food rich in antioxidants minimize the need for vitamin E. It has been noticed that plasma vitamin E rapidly decreases following weaning and then gradually increases when receiving high amount of vitamin E and aging (Mayer *et al.*, 1981). These indicate intensive demands and inefficient vitamin E absorbtions. Moreover, vitamin E supplementation for particular purposes is considerably different. Machlin (1993) has reviewed vitamin E supplement 20-100 mg/kg feed increase E. *coli* resistance and at a level of 20 IU per day better recovery from *Treponima hydysenteria*e in pigs. It is likely that the effective dose tends to relate to types of infections.

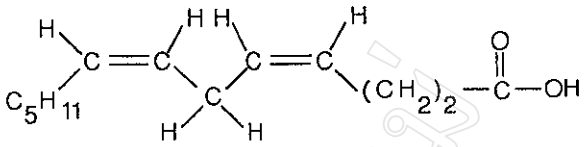
II.2.1 Lipid peroxidation

Lipid peroxidation is potentially harmful to living cells causing membrane destruction. Free radicals or highly reactive oxygen-containing molecules are initiators due to their unstabilities. They initially react with polyunsaturated fatty acids (PUFAS) which have the methylene ($-CH_2$) groups between two double bounds, illustrated in figure 2. These groups are chemically susceptible to oxidation and can induce further chain reaction called "autoxidation" (Macrae *et al.*, 1993). The autoxidation follows three steps, initiation, propagation and termination. In the initial step, PUFAs are attracted with free radicals producing lipid radical. Rapidly reacting with other lipid molecules, lipid radicals undergo propagation step. Without antioxidants such as vitamin E, the chain reaction occurs continuously until peroxyl radicals react with each another to produce a stable product known as

the termination step. The autoxidation is illustrated in figure 2. Vitamin E inhibits the autoxidation by donating its hydrogen atoms making lipid radicals inactive.

Although free radicals have been known for their potentially harmful toxicity, they have also been recently recognized as necessary substance for biological processes. An importance of free radical generation was to break down the vicious cycle, which naturally develops from damaged mitochondria. But excessive free radicals can simultaneously damage more mitochondria (Amemiya, 1987). It was found that vitamin E can potentially break the vicious cycle before it promotes the production of radicals (Isliker *et al.*, 1997).

L H is a PUFA with a structure like linolenic acid:



Initiation

Radicals formed in normal metabolism or from certain types of toxins $\longrightarrow$ $R^{\bullet}$

$R^{\bullet} + LH \longrightarrow RH + L^{\bullet}$

Lipid radical

Propagation

$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$

Lipid radical — Lipid peroxyl radical

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

Lipid hydroperoxide

Termination

No antioxidant present:

$2\,LOO^{\bullet} \longrightarrow LOOL + O_2$

Vitamin E present:

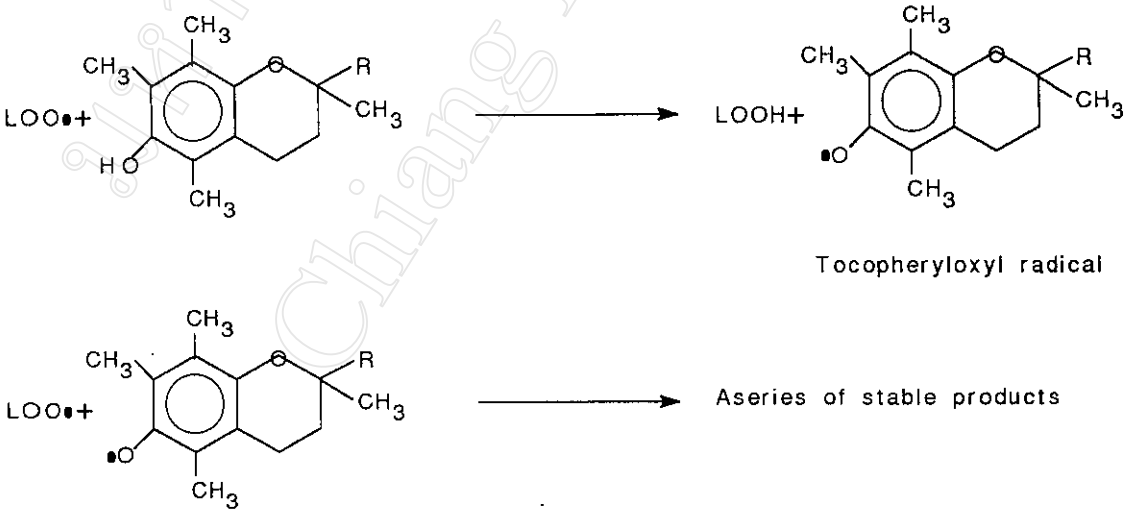
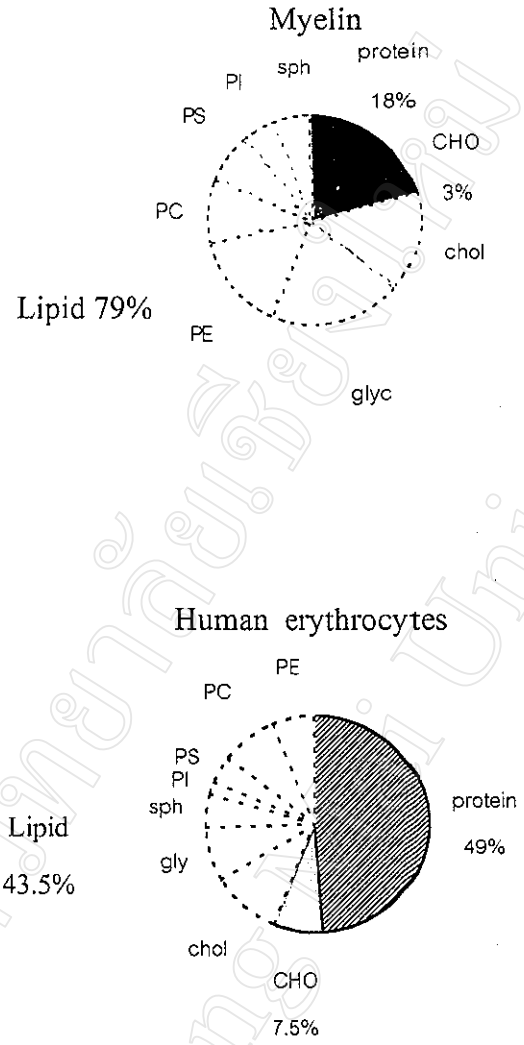


Figure 2 Lipid peroxidation chain reaction

From Macrae *et al.*, 1993

II.2.2 Lipid bilayer of cell membrane

Membranes are boundaries of living cells. They are composed of large amounts of lipid and protein and small amount of carbohydrate. Lipid plays an important role forming a bilayer around cells. To create membrane bilayer, phospholipids associate their hydrophobic parts in opposite directions, acting as the inner and outer surfaces (Plummer, 1989). Thus protein and carbohydrate can integrate with these lipid bilayer structures. Types and functions of cells determine proportions of the membrane 's components. Examples of the composition of some membranes are shown in figure 3. Membranes act as cell barriers which selectively in-out transport cell vital agents such as oxygen and nutrients. Moreover, there are various molecules embedded on cell membrane (SaalmÜller *et al.,* 1994). These involve a number of vital and survival processes inside the body. For example, each immune cells possess a variety of cell surface antigens (CDs), immunoglubulins (Igs) and histocompatibility complex (MHC) e.g. These molecules act as receptors, signal transductors and antigen recognitors. If cell membranes are changed, these molecules and their functions are affected.



Abbreviations

CHO = carbohydrate

PE = phosphatidyl ethanolamine PC = phosphatidyl choline

PS = phosphatidyl serine PI = phosphatidyl inositol and phosphatidic acid

PG = phosphatidyl glycerol sph = sphingomyelin

gly = glycolipids chol = cholesterol

card = cardiolipin (diphosphatidyl glycerol)

Figure 3 The chemical composition of some membranes

From Plummer, (1989)

II.2.3. Vitamin E protect lipid oxidation

High dietary fat especially PUFAs causes an increased rate of lipid peroxidation. Rats receiving 20% fat rations increased pentane production, accumulation of malondihyde in the live and in vitro spontaneous red cell lysis. (Backingham, 1985) These significantly high values indicated intensive lipid peroxidation. Fatty acid, like linoleic acid and some lipoproteins can easily react with oxygen radicals. These reactive products probably attract with cell membranes resulting in changes of cell permeability measured by rate of albumin passing cell membrane (Henning *et al.*, 1989). They also cause cells to lose integrity. Vitamin E is considered as a particular antioxidant protecting lipid peroxidation. Low density lipoproteins (LDLs) separated from vitamin E supplemented subjects showed significant resistance against in vitro copper-induce oxidation (Dieber-Rotheneder, 1991) Also directly incubating vitamin E and endothelial cells decreased cell injury from oxidized LDLs (Kuzuya *et al.*, 1991). In addition, supplementation of 160 mg per kg vitamin E retarded iron-induced lipid peroxidation of pig muscle cells (Monahan, 1990).

II .2.4. Lipid peroxidation influence immunity

High dietary fat, especially PUFAs has been reported as having suppressive effects on immunity in animals (Maki *et al.*, 1992). Fish oil consumption which is rich in PUFAs decreased interleukin-1 production of peripheral blood monocyte, interleukin-2 production of T-lymphocyte and lymphocyte proliferation induced by mitogens. Moreover dietary fish oil increased mortality in mice challenged with *Salmonella typhimunium* (Rosenberg, 1992). The mechanism by which fish oil inhibits immune functions is unclear. However, it has been reported that stimulated

peripheral blood mononuclear (PBMCs) produced reactive oxygen species. These reactive substances can oxidize normal low density lipoproteins (LDL) and very low density lipoproteins (VLDL). These oxidized lipoproteins were sufficient to inhibit DNA synthesis and gamma interferon releasing in concanavalin A stimulated PBMCs (Kasiske *et al.*, 1991). Besides, oxygen species altered lymphocyte cytotoxic functions. (Grever *et al.*, 1980) Although oxygen species failed to cause cell death, they significantly inhibited cytotoxic activities of lymphocytes both antibody and nonantibody mediated pathways. Therefore preventing these suppressive effects by supplementation of adequate vitamin E seems to be beneficial. Yet high concentration of vitamin E can effectively inhibit superoxide (O_2^-) production from macrophages. The mechanisms by which vitamin E decreased O_2^- production acted through inhibition of protein kinase C (Sakamoto, 1989). However, Okano *et al.*, (1990) have reported that oral vitamin E supplementation failed to impair *in vivo* superoxide production in polymorphonuclear cells (PMNs) because vitamin E concentration in PMNs cannot reach the level which effectively inhibit O_2^- production.

II.3 An over view of the immune system

This is the background of the immune system that may help to follow the effect of vitamin E on immune responses. (Abbas *et al.*, 1994 and Gershwin *et al.*, 1995) The immune system is self defense mechanism mechanism of the host against pathogens and tumors. Basically, it can be classified as innate immunity and acquired immunity. In innate immunity, responsible cells and mediators react nonspecifically with antigen, a general term for pathogens and substances causing immune reactions. These systems function immediately at the site of invasion so called "first line defenses". Cells and mediators which are involved in these

functions are skin, mucous membrane, phagocytes, natural killer cells (NK cells), cytokines , tumor necrosis factors and complement. On the other hand, an acquired immunity acts specifically and develops following antigen stimulation. This line of defense recognizes antigens previously encounter and immediately attracts them. Repeated encounter of the same antigen, acquired immunity become more effective. However, the two immune systems work together to perfect antigen elimination. Cells active in this line are T-and B-lymphocytes. Acquired immunity or specific immunity can be subdivided into humoral and cellular immunity according to their different routes of functions.

Humoral immunity is identified as function by antibodies which are produced from plasma cells or activated B - cells. In fact, antibodies function with other immune pathways to obtain more powerful activities such as providing opsonization, complement fixation. An antibody is a glycoprotein molecule which is bounded on the surface of B-cells or released in blood circulation. Consisting of two binding ends, antibodies specifically bind counterpart antigens to inactivate them and trigger immune defenses. Antibodies produced from each clone, B-cell population propagating from the same original cell, have identical binding sites. And each clone can have different classes of antibodies such as IgM, IgD, IgG, IgA and IgE to serve optimize functions. Antibodies are products of immunoglobulin genes. Those genes are unique in their ability to recombine gene fragments resulting in numerous gene patterns and consequently producing a diversity of antibody specific binding sites. Therefor, this diversity enables antibodies to react with any antigens possibly existing in nature. However some B-cell clones which are never activated by antigens disappear ; this is called "clonal selection".

B-cells can be activated in several ways. One is initiated when surface antibodies bind antigens. This drives resting B-cells into cell cycles in which numbers of intracellular secondary messengers are systematically produced and function. Entire antibody-bound antigen is phagocytosed inside B-cells, consequently associated on MHC class II. This antigen-antibody association is presented to restricted $CD4^+$ helper T cells. Then, helper T-cells directly signal on the surface membrane of B-cells or produce cytokine to react with B-cell receptors. The result is that B-cell undergoing proliferation and differentiation to produce large amount of specific antibodies and recognize that antigen.

Cellular immunity functions by T lymphocytes. The cell which play an important role are helper T-cell and cytotoxic T-cell which possess CD4 and CD8 antigen on their surface membranes, respectively. However, some species, for example swine has a unique T-cell which show both CD4 and CD8 simultaneously. These so called $CD4^+$ $CD8^+$ T-cells can have both helper and cytotoxic functions (SallmÜller *et al.*, 1994). Yet their unique roles are not known. Both helper and cytotoxic T cells have T cell receptors (TCR) and CD3 antigen. Behaving like an antibody on B cell membrane, TCRs are responsible for antigen recognition associated with MHC molecules. CD4 molecules determine antigen recognition with MHC class II. Whereas CD8 involve the recognition with MHC class I. $CD8^+$ T-cell recognize and lysis infected or tumor cells. But $CD4^+$ T-cells functions are more complicated. In activating phases, following recognition $CD4^+$ cells produce growth-promoting cytokine and their receptors to obtain significant activation. Action of various cytokine such as IL-6 and IL-4 lead to proliferation and differentiation of specific T cells. Then, effector phase functions in cooperation with other immune cells such as B-cells, macrophage and inflammatory leukocytes to eliminate antigens.

II.3.2 Vitamin E and natural immunity

Relationships between vitamin E and natural immunity have been reported in pigs and other animal species. Wuryastuti (1993) has found that vitamin E deficiency in pigs at 90 days significantly decreased phagocytic and microbiocidal abilities to ingest yeast cells. Besides, this impairment was increased in additive selenium inadequacy. It was suggested that oxidative damage of responsible cell's membranes retarded production and response of chemotoxis substances. In other species, Sakamoto *et al.,* (1990) has reported that intraperitonial injection of 5 mg per head vitamin E significantly enhanced numbers of macrophages by increase T-kininogen levels in plasma indicating significant activated macrophages. Moreover, colony-stimulating factors (IL-1) and chemotactic factors (IL-6) were noticeably increased. Similar result showed the additive effect of vitamin E on interferon-C-reactive protein, B-lysins, lysozymes and serum transferins. Weekly oral supplementation of 2800 mg vitamin E per calf resulted in more effective abilities of calf serum to inhibit viral replication in vitro. This was accomplished without specific antibody advantage (Reddy *et al.,* 1986). Vitamin E also increased neutrophil functions, antibody dependent cell cytotoxic and phagocytosis against *Staphylococus aureus* (Eicher-Pruiett *et al.,* 1992). However, the mechanism of vitamin E regulating non-specific immunities has not been clear.

II.3.3 Vitamin E and humoral immunity

Humoral immunity directly functions by B-cells and their specific antibodies. The effect of vitamin E on humoral immunity may be investigated by measuring specific antibody productions or numbers of B-cells. However, the numbers of B-cells slightly change in the resting state. Besides, only specific B-cells proliferate in response to a specific antigen entering. So that concentration of specific antibodies following antigen or vaccine immunization is a popular measure. Tengerdy *et al.*, (1973) has found that mice supplemented 60-180 mg/kg vitamin E increased antibody titer following sheep red blood cell challenge. The effect was seen more clearly on primary antibody response than on the secondary and the results are more significant on IgG than IgM. But, Reddy *et al.*, (1986) and Hidiroglou *et al.*, (1992) have reported that high dose of vitamin E had the additive effects on IgM but not IgG. Similar results have reported that high doses of vitamin E, 150-300 international unit (IU)/kg feed improved antibody response and phagocytic activity to E *coli* challenge (Bains, 1994). Yet the roles by which vitamin E regulate humoral immunity is not clear. It has been proposed that vitamin E activates cooperation of immune cells such as T-cells, macrophages and plasma cells at site of innoculation. This hypothesis results from the use of 20-30% vitamin E replacing mineral oil in viral-antigen vaccine. The results showed rapid, higher antibody response in vitamin E treated chickens (Franchini *et al.*, 1995). In the experiment in which 50% vitamin E replaced Freund's incomplete adjuvant indicated vitamin E significantly reduced swelling at site of vaccine injection. Similar treatments produced high serum IgM titers and better peak and persistency of IgG titers against *E. coli* as compared with administration of Freud's adjuvant alone. Moreover some clinical signs such as rectal temperature following LPS

antigen challenge were also relieved by vitamin E indicating its well balanced effects during inflammation. It was also noted that vitamin E treatments improved humoral immunity by increasing B-cell population (Babinszky *et al.*, 1991). In weaned pigs, supplementation of 220 IU/kg vitamin E resulted in higher hemagglutination titer against SRBC. Immunization (Peplowski, 1981). In addition, vitamin E influencing humor immune response by altering cortisol concentration has been illustrated. (Reddy *et al.*, 1987) Calves receiving 125 IU vitamin E per day had higher specific antibody titers than controls given no vitamin E. Those results were significant at week 21 following antibovine herpes-virus type I immunization. It was noticed that plasma cortisol reduced inversely correlated with plasma vitamin E and antibody titers increased.

II.3.4 Vitamin E and cellular immunity

It is known that vitamin E stabilizes cell membrane and increases its integrity in which various surface antigens integrate. Including immune cells, these important molecules act as receptors which trigger extension of very complex, effective immune responses. So far, the direct effect of vitamin E on immune regulation is not known. At least, there was clear evidence that vitamin E deficiency decreased T and B cells proliferation in guinea pigs and rats (Bendich *et al.*, 1984 and Eskew *et al.*, 1985). Moreover, it has been proposed that vitamin E influences enzyme, protein and DNA synthesis or alters lipid radical. The products from prostanoid and cortisol metabolic pathways are believed to be potential immuno-suppressive factors.

Many studies have shown the relationship between vitamin E supplement and improved cellular immunity. Tanaka *et al.*, (1979) has hypothesized that vitamin E directly affects helper T cell functions by activating B-cell to produce

antibodies. In this study, rats were treated with different levels of vitamin E and immunized firstly with sheep red blood cells and then repeatedly with TNP-HRBC. The results showed that antibody titers against TNP and HRBC increased following elevating vitamin E doses. The effect which was significant on anti-TNP indicated that vitamin E amplified the cooperation between TNP-specific T cells and responsible B cells. Other supportive paper reported that experimental removal Ia+ accessory cells from spleen cell culture impaired proliferation responding to Con A. Yet this impairment was not found in cultures directly supplemented with vitamin E. The results indicated that vitamin E activated functions of Ia$^-$ cell population (Corwin, 1991). It was interpreted that vitamin E enabled microtubular which extensively required vitamin E to complete cell's division.

However, Lessard *et al.*, (1991) showed that vitamin E did not have successive effect on either T or B cells. Because lymphocytes separated from vitamin E deficiency pigs produced normal function in mitogen induced cell proliferation. But those measures were inhibited by suppressive factors existing in serum of pigs receiving inadequate vitamin E. It was hypothesized that the suppressive factors were free radicals generated intensively without natural antioxidant. This was proved by the study of the effect of vitamin E and dietary fat sources on sows and their piglet's immunity (Nemec *et al.*, 1994). The results showed that fish oil supplement rich in PUFAs negatively affected sow's cellular immune functions and developments of cellular immunity in her piglets. And the negative effects were restored with the addition of high levels of vitamin E.

Additionally, it was reported that increased vitamin E levels in human PBMCs correlated with changing interluekin and prostaglandin E-2 production as well as the delayed-type hypersensitivity skin test (Bendich *et al.*, 1990).

Objectives of study

- To investigate an additive effect of higher vitamin E supplementation both on humoral and cellular immune response
- To evaluate an optimal vitamin E level which improve weaned pig immune response
- To study the effect of vitamin E on production performances, average daily gain and feed conversion ratio