V. DISCUSSION AND SUMMARY

Discussion

The effect of vitamin E on humoral immune response

Aujeszky's disease (AD) can cause significant damage to swine production in Thailand. So that AD vaccine is programmed on most pig farms. And determination of AD antibody titer can be achieved by serum-virus neutralization test (SN test) which is more accurate than other methods (Stewart *et al.*, 1978).This is the reason for using AD titer as a measure in this study. The results in this part showed three interesting topics for consideration as the follows:

First, the piglets had detectable AD titers which were considerably higher at the beginning before AD vaccine was given. This was because the piglets were born from vaccinated sows and they had received passive antibodies through colostrum and their mother's milk (Wittmann *et al.*,1979). Besides, mean titers among groups were significantly different. These results were from random group of the subjected pigs. In fact, all pigs should have been tested and grouped by graded titer to equalize means among treatments. But the experiment had been planned to start immediately after weaning and the laboratory for SN-test was far from the field experiment, in Bangkok. So that all collected samples were tested later. However, a considerable numbers of pigs and available statistic design made the results acceptable. Additionally, Wittmann *et al.*, (1979) have reported that considerable high passive AD antibodies did not impaired antibody response against virus inactivated AD vaccine.

Secondly, the results showed considerably lower titer through out the 28 days trial which may have caused the effect of treatment to be non-significant. The

lower AD titers were probably due to vaccine attenuated virus type which was used in this study. These findings were similar to those from McFerran *et al.*, (1979). The former study reported that mean AD titer of pigs which had attenuated vaccine by SN assay were less than 2 at seven days after vaccination and 2-4 after the fourteenth day. And the titers from pigs receiving inactivated vaccine developed antibody titers two times higher. Interestingly, all pigs showed good response following field virus challenge. Inactivated vaccine resulted in higher titer. Pigs given attenuated vaccines showed significant protection from death after experimental virus infection although they developed lower titers. This indicated the nature of AD titer and the results from this study were neither vaccination nor SN test errors.

Finally, the results showed slight antibody response after first vaccination. Because all mean AD titer on the seventh day were lower than that on the first day except in treatment four. After the secondary vaccination, antibody response could be observed especially in treatment two. These findings were consistent with Wright *et al.*, (1984). It can be explained that high passive antibodies decreased primary humoral response in vaccinated pigs. Pigs born from sows challenged with field virus had the highest passive antibodies but responded with the lowest active titers. It was clear, that piglets from unvaccinated sows born with no passive AD titer showed hypothesized antibody response. This suggested that maternal antibodies could neutralize partial attenuated virus in vaccine but not completely because all piglets developed secondary antibody response after being challenged with field virus. This was similar to the response in the control pigs. These results are probably due to the fact that the reaction of passive antibodies and attenuated viruses inversely increased cell mediated immune response.

The present results were similar to Wright *et al.*, (1984) that primary antibody response seem to be hinted. However, the mean AD titers reached levels which were not significantly different from group to group ($P > 0.05$). During this phase, significant vitamin E treatment effect on AD antibody response was observed. It was noticed that dietary vitamin E concentration of more than 75 mg/kg showed higher antibody response than that of 35 mg/kg, control. A vitamin E level of 75 mg/kg produced the optimal AD titer response in this experimental condition. Additional effect was not found in higher vitamin E levels. It was noticed that results varied widely which was probably caused by other factors.

Effect of vitamin E on cellular immunity

In vitro lymphocyte proliferation is the standard procedure in the study of cellular immunity for years. Because the test indicates the ability of separated lymphocyte in proliferate response to activators in the same manners as *in vivo* expansion of cellular immune reaction. Activators can be designed to indicate function of cell population of interests. In this study mitogen PHA and Con A known as T-cell activator and PWM for B-cell clone were used. These mitogens specifically bound to sugar residues on cell surface glycoproteins as well as TCR and CD3 molecules resulting in activated lymphocytes undergoing cell expansion. The results of incorporated ^{3}H-thymidine into new blast cell showed as Δ cpm reflecting the degree of cell proliferation.

In this study mean Δ cpm of all mitogen stimulation tests slightly changed with non-aged correlation in each ten day test. The results indicated maturation of piglet cellular immunity (Hoskinson *et al.*, 1990 and Becke *et al.*, 1993). Over all Δ cpm data, effect of vitamin E treatments were not found to be significant. But

most of all mean Δ cpm tend to increase following higher dietary vitamin E concentrations with non-statistical significance. These manifestations may be explained by the evidence that dietary vitamin E levels widely differed in each treatment but mean plasma vitamin E of each group had slight differences. Additionally, it must be less than those in cellular level. In fact, vitamin E level in separated lymphocytes was not investigated in this study. It can be expected to have less difference among the vitamin E treatments and to be lower than in plasma.

Activated lymphocytes produce significant reactive oxygen species. This is necessary for signal transduction and gene expression but it is potential harmful in excess amount (Palmer *et al.*, 1997). This evidence implies that higher vitamin E or other antioxidants are needed during cell proliferation. In this study, separated lymphocytes were in resting state and may take up nearly the same amount of vitamin E because cell transportation of vitamin E in all groups is determined by genetic mechanism *via* tocopherol binding protein and their receptors. So that without additional of exogenous vitamin E in lymphocyte cultures, the effect of vitamin E on proliferate abilities may have depended on original cellular vitamin E saturation.

The effect of vitamin E on immunity has been prominently proposed in stabilizing membrane of immune cells and scavenging free radicals and other lipid peroxide intermediates. These were previously called suppressive factors existing in blood circulation. Lessard *et al.*, (1992) has reported that autologous serum from vitamin E deficiency pigs inhibited mitogen induced lymphocyte proliferation. But the deterioration was restored when fetal calf serum was supplemented in cell cultures or experimental pigs were fed 33 IU per kg vitamin E. In the present results, vitamin E level in the control diet was 30 mg/kg on average and fetal calf

serum was supplemented in cell cultures. It may be these two points causing the results showing no difference. This finding indicated that the lowest vitamin E treatment was adequate to maintain normal cellular function *in vitro*. In this condition, without immuno-suppressive factors the effect of vitamin E on cellular immunity was found but not significantly.

Additionally, on the second week of experiment 2, younger weaned pigs around the experiment cages showed symptoms of swine fever disease due to natural infection; some pigs were eventually died including two pigs from the control group and one pig from treatment 2which possibly due to low vitamin E and immune status.This finding suggested that more than 132 mg/kg vitamin E supplementation (treatment3 and 4) may decrease the death of pigs suffering from natural infection. However, it needs more evidences and systematic experiments to verify this hypothesis.

Because many factors alter immune systems, immuno modulating effect of vitamin E depend on the relationship between the vitamin and those factors. For instance lipid peroxidation suppress immune functions while vitamin E scavenge oxidation reactions. It was reported that LP-BM5 retrovirus infection in mice develops extensive lipid peroxidation and causes murine immuno-deficiency syndrome(AIDS) (Liang *et al.*, 1997). This evidence is most likely associated with declined vitamin E status. Pacht *et al.*, (1997) have found that vitamin E status significantly decreased in HIV-positive subjects in both early and long-term investigations in human. Not only vitamin E but also reduced glutathione concentration decreased by half within one week following influenza challenge. (Meydani *et al.*, 1995) Additionally, malondialdehyde (MDA) level and reduced homocysteine in plasma increased in common variable immunodeficiency (CVI) subjects. This indicated a high degree of lipid oxidation. Not surprisingly, vitamin

E and beta-carotene status in those subjects were almost at deficiency level (Aukrust *et al.,* 1997). Another reactive oxygen intermediates, PGE_2 produced by activated macrophage and monocytes has been found to suppress T cell proliferation *in vitro*. It was proposed that vitamin E decreases PGE_2 production by decreasing cyclooxygenase enzyme activity and inhibiting phospholipase A_2 activity (Meydani *et al.,* 1995). In conclusion, vitamin E improves immune function *via* regulations of the eicosanoid production.(Gu *et al.,* 1995)

Meydani *et al.,* (1995) have reviewed that age-associated dysregulation of immune response and oxidative stress similarly resulted in suppression of IL-2 production, protein tyrosine phosphorylation, reduced intracellular calcium mobilization, low DNA binding activity of nuclear transcription factors NFAT and NF-*K*B and increased binding activity of AP-1. Franci *et al.,* (1996) demonstrated that hyperthermal stress, at 42 ^{o}C for one hour affected lymphocyte culture in many ways; increase of superoxide anion and decline of PWM-induce lymphocyte proliferation, immunoglobulin production, and IL-2 synthesis. Supplementation of vitamin E can restore these detrimental results but not of vitamin C. The mechanisms by which vitamin E regulates these evidences have not been well understood. McCarty, (1997) proposed that vitamin E increased interleukin-2 activity by restoring the down regulatory effect of cAMP which associates with the age-related decline in immune response.

The direct effects of vitamin E on immune cells have also been stated. It was found that vitamin E treatment has an effect on the increase in size of CD4 and CD8 positive T-cells and expansion of CD5 positive B-cell. However, the effects vary on ages and lymphocyte subpopulation (Brohee *et al.,* 1995 and Harbrige, 1996).

The effect of vitamin E on serum or plasma α-tocopherol concentration

Serum or plasma α-tocopherol levels of all pigs were not significantly different on the initial day, which ranged from 1.25 to 1.48 μg per ml in serum and 1.51 to 1.79 μg per ml in plasma. These concentrations were considered as a normal level and slightly higher than 1.35 μg/ml, the initial level at weaning reported by Chung *et al.*, (1992). The results were consistent with that of pigs received dietary vitamin E 15 IU/kg for 10 days which was considered low but above deficiency, 0.5 μg/ml (Hoppe, 1991). Pigs are born with very low vitamin E status, 0.62 mg/ml because of poor ability of the vitamin to cross the placenta. Therefore, vitamin E status of piglets mainly affected by the vitamin concentration in their mothers' milk (Hidiroglou *et al.*, 1993). Overall the present results showed that blood α-tocopherol was significantly elevated following increase dietary vitamin E levels and the course of administration. This evidence has shown after day 14. In experiment 2, plasma α-tocopherol concentrations on day 10 of all treatments significantly declined. Except for serum of pigs in treatment 4 receiving total dietary vitamin E 244 mg/kg remained nearly the initial concentration. These findings suggested that pigs especially needed vitamin E during one week after weaning. Although in experiment 1, serum α-tocopherol levels did not decrease after weaning, they also did not respond to increase of vitamin E levels. It has been known that pigs suffering from many sources of stress extensively generate reactive oxygen species, and require high amounts of vitamin E to maintain unchanged vitamin E status. However, the decrease of vitamin E status failed to alter *in vitro* lymphocyte proliferation in this study. These finding suggested that plasma vitamin E concentration may have not reflected the vitamin concentration in immune cells.

The effect of vitamin E on average daily gain and feed conversion ratio

The effect of vitamin E treatments on ADG and FCR was not found in the present study. Some investigators have also reported similar results (Duthie *et al.*, 1987, Bonnette *et al.*, 1990, and Mohan *et al.*, 1990). It may have been that the vitamin E level of 30 mg/kg in control ration was enough to maintain normal growth in this experimental conditions. However, it has been well known that a potential stress and infection associating with imbalance of animal nutrition decrease growth performance (Spurlock *et al.*, 1997). In those poor conditions, vitamin E may be more beneficial. Additionally, vitamin E may improve growth by strengthening epithelium cells of digestive tracts resulting in an increase nutrient digestibility and absorption.

Summary

- Basically, weaned pig ration contained on average 30 mg/kg vitamin E.
- The additive effect of vitamin E on antibody response significantly showed in treatment 2 concentrating 75 mg/kg vitamin E especially during secondary antibody response.
- ^{3}H-thymidine incorporation of mitogen induced lymphocyte proliferation showed a trend of increase Δ cmp following higher vitamin E levels.
- Serum or plasma α-tocopherol increase following increase of dietary vitamin E levels and course of vitamin administration.
- ADG and FCR were better in pigs receiving approximately 110 mg/kg vitamin E.

- Dietary vitamin E level of 75 to 110 significantly improved AD titer response and tended to better cellular immune response and growth performance, ADG and FCR.

Further investigation

From this study, it is interesting to investigate vitamin E concentration in lymphocytes or the other immune-cells both in resting and activating stages. Additionally, the relationship between vitamin E levels in lymphocytes and serum may explain a slight response of lymphocyte proliferation to higher levels of vitamin E supplementation. In this study the effect of vitamin E on a specific antibody response was determined which is only one pathway of the immune systems. An investigation of the whole body reactions may show the greater significant effects. To determine a total gramma globulin may be better than the specific antibodies in further study. Because the effect of vitamin E depends on its antioxidant properties, the other antioxidant systems and the degrees of lipid oxidation should be also determined to verify the results and explain in a wide extent.