Egg production, egg fertility and hatchability are among the most important economic traits for chicken breeder farms. Failure of health maintenance may cause great economic loss. Although mass vaccination against Newcastle disease (ND), infectious bursal disease (IBD) and infectious bronchitis (IB) has been practiced throughout the commercial poultry industry, outbreaks of those diseases still occur occasionally. Hence, vaccination responses and enhancement of immune system are matters of great concern.

Vitamin E is primarily known as an antioxidant in reducing cellular free radical damage. In addition, vitamin E may affect the development and maintenance of immunocompetence through multiple functions by acting directly on the immune cell or by indirectly altering metabolic and endocrine parameters, which in turn influence immune function (Gershwin et al., 1985). Vitamin E supplementation in mammals has been shown to increase production of interleukin (IL)-2, leading to enhanced proliferation of T cells, and reduce production of prostaglandin E, a T-cell suppressive factor, as a result of a decreased peroxynitrite formation (Meydani et al., 2005).

The National Research Council (1994) recommended levels of vitamin E for breeder chickens are assessed ultimately in terms of reproductive performance, the allowance for optimal immunocompetence is not included. Dietary vitamin E was found to improve reproduction and antioxidant capability of breeder chickens (Lin et al., 2004; Lin et al., 2005a, b), but very few information is currently available on the effect of vitamin E supplement on the immune response. Considering vitamin E content in the ingredients, irrespective of degradation during storage, corn-soybean meal diet without supplemental vitamin E is supposed to meet or exceed the NRC (1994) recommended level of vitamin E.

Previous reports on effects of vitamin E on immune response of chickens were few and not consistent (Marsh et al., 1986; Ritcher et al., 1985, 1986; Lohakare et al., 2005). The purpose of this study, thus, was to evaluate whether long-term feeding of corn-soybean meal diet without supplemental vitamin E might impair immunity and whether supplementation of vitamin E enhances immunologic response to sheep red blood cell (SRBC) and different commercial vaccines in breeder chickens.

**MATERIALS AND METHODS**

**Experimental animals**

Experiment 1 was conducted using two-way cross (line 7x11) female Taiwan native breeder chicks, and two-way cross (line 9x12) male Taiwan native breeder chicks were employed for experiment 2 (Livestock Research Institute, Council of Agriculture, Hsinhua, Tainan 712, Taiwan).
Three hundred day-old female chicks in Experiment 1, and 90 day-old male chicks in Experiment 2 were reared in concrete floor pens padded with rice hull litter in an open-sided growing house till 16 weeks and 22 weeks of age, respectively. At 16 weeks and 22 weeks of age, birds from the flock were randomly allotted to five treatment groups and moved into the open-sided cage breeder house. Each bird was housed in an individual cage measuring $36 \times 25 \times 39$ cm for females and $47 \times 32 \times 61$ cm for males. Each treatment group, containing 60 pullets in Experiment 1, and 18 cockerels in Experiment 2, was equally separated into three experimental units (replicates). Units were randomly distributed to minimize the cage effect. One feeder trough was used for a unit (replicate) of 20 birds for females and 6 birds for males. Feed and water were supplied ad libitum. All experimental procedures were approved by the Laboratory Animal Management Committee of the Livestock Research Institute.

### Diets and experimental design

Based on the guidelines suggested by NRC (1994) and the Extension Booklet for Taiwan Native Chicken (1995), basal corn-soybean meal diets (calculated vitamin E contents have been shown in Table 1) were formulated to meet the nutrient requirements for Taiwan native breeder chickens during different periods, except that vitamin E was omitted from the vitamin premix (Table 1). The premixes were stored in airtight container at -20°C until being mixed with the diets. All diets were prepared biweekly and provided in meal form. To minimize vitamin E interactions with other nutrients, selenium, synthetic sulfur amino acids or oils were not added to the basal diets (Lin et al., 1989; Levander 1992), because the base ingredient composition was considered sufficient to meet the minimum dietary requirements of native chickens. Birds from each of the five treatment groups were fed the basal diet supplemented with five graded levels of vitamin E (all-rac-α-tocopherol acetate) (Roche, F. Hoffmann-La Roche Ltd, 4002 Basel, Switzerland), respectively as described in Table 2.

### Vaccination program and antigen challenge

Vaccinations were performed under standard vaccination programs implemented at the Livestock Research Institute for Taiwan native breeder chickens. All birds were vaccinated with Marek’s disease (MD), Newcastle disease (ND), infectious bursal disease (IBD) and infectious bronchitis (IB) vaccines (Fort Dodge Laboratories, Iowa 50616, USA) at different ages as described in Table 2. Killed vaccines were administered by intramuscular injection and live vaccines were administered by eye drop method. Vaccines were handled according to the recommendations of the manufacturer. All birds in the flock received the same dose of vaccine at the same time.

### Table 1. Composition of basal diets and weeks fed in experiment 1 and 2

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Growing period (Exp. 1 and 2)</th>
<th>Laying period (Exp. 1)</th>
<th>Mature period (Exp. 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn (%)</td>
<td>64.6</td>
<td>65.1</td>
<td>70.9</td>
</tr>
<tr>
<td>Soybean meal (43.5%) (%)</td>
<td>25.0</td>
<td>25.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Fish meal (64%) (%)</td>
<td>3.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Wheat bran (%)</td>
<td>4.5</td>
<td>6.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Dicalcium phosphate (%)</td>
<td>1.2</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Calcium carbonate (%)</td>
<td>1.2</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Salt (%)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin premix (1) (%)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mineral premix (2) (%)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Total (%)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

1 Provided per kilogram of diet: vitamin A (retinyl acetate), 1.9 mg; cholecalciferol, 27.5 µg; thiamine, 1.1 mg; riboflavin, 4.4 mg; pyridoxine 2.2 mg; vitamin B12, 6.6 µg; menadione, 1.1 mg; biotin 0.11 mg; folic acid, 0.55 mg; niacin 44 mg; pantothenic acid, 12 mg.

2 Provided per kilogram of diet: iron, 64 mg; copper, 12 mg; manganese, 64 mg; cobalt, 0.2 mg; zinc, 40 mg; iodine, 0.68 mg.
different ages in Experiment 2 were not reused to avoid secondary response. After 7 days, blood samples were collected and sera from each sample were stored at -20°C for subsequent measurement of antibody titer to SRBC and the vaccines.

Cell-mediated response was measured by reaction to phytohaemagglutinin-P (PHA-P) at 36 weeks of age in Experiment 1. Twelve birds from each treatment group were randomly chosen and intradermally injected with 0.1 ml PHA-P (Sigma chemical Co., St. Louis, MO 63178) at left wattle. Besides, the other wattle (right) was also injected with the same volume of PBS as control. The accumulation of lymphocytes which stand for cell-mediated immune response will reflect in the swelling of injected skin.

Measurement of humoral immune response

Antibody response to SRBC: Sheep red blood cells were used as T-dependent antigen to quantify the antibody response. The antibody assay procedures described by Munns and Lamont (1991) were followed. The agglutination titer was expressed as the log2 of the highest titer giving 50% agglutination.

Antibody titer to NDV was measured using haemagglutination inhibition (HI) technique. Twentyfive µl of serum containing antibody was serially diluted into 96-well ground bottom plate with phosphate buffer saline (PBS). The same volume of virus antigen was added to react and bind with the antibody. Addition of 2% red blood cell (RBC) solution in each well will show the ability of ND virus left to agglutinate with RBC. If there was enough antibody bound to virus during the incubation period, haemagglutination would be inhibited completely. The titers were expressed as log2 of the reciprocal of the last serum dilution showing haemagglutination inhibition.

Antibody titer to IBDV and IBV were determined by ELISA kits (IDEXX Inc., Westbrook, ME 04092) (Snyder et al., 1984), according to manufacturer’s instructions. Twenty µl of 100-fold buffer diluted serum from each chicken were used and read at 650 nm in a microplate reader.

Cell-mediated immune response

The PHA-P skin test of chicken was considered to be a thymus-dependent response using swelling of wattle caused by PHA-P injection (Goto et al., 1978). Wattle thickness was measured using a pressure-sensitive meter. Cell-mediated immune response (wattle index) was calculated as the difference between the PHA-P and PBS (control) injected sites.

Wattle response to PHA-P, in millimeters, was calculated as follows:

\[
\text{PHA-P value} = (\text{post PHA-P inj.-pre PHA-P inj.}) - (\text{post PBS inj.-pre PBS inj.})
\]

Where,

- post PHA-P inj. = thickness of left wattle 24 h after injection of 0.1 ml PHA-P;
- pre PHA-P inj. = thickness of left wattle before injection;
- post PBS inj. = thickness of right wattle 24 h after injection of 0.1 ml PBS;
- pre PBS inj. = thickness of right wattle before injection.

Weight gain and packed cell volume (PCV)

Weight gain and PCV were used as indicators to evaluate nutritional status in chickens. Body weight and weight gain for each female and male bird were recorded every 2 to 3 weeks beginning at the age of 17 and 23 weeks, respectively. Hematocrits were measured at 31 weeks of age in Experiment 1 and 27 and 31 weeks of age in Experiment 2. Blood samples were collected into haperinized
VITAMIN E ON IMMUNE RESPONSE OF CHICKENS

Random samples for determination of antibody titers etc. were from individual birds (experimental unit) (n = 12) of different treatments. Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute, 1996) for the effects of treatment, age and their interactions. Statements of statistical significance were based on p<0.05. Least-squares means of the five treatments were compared by Tukey’s honestly significant difference (HSD) test.

RESULTS

Weight gain

Pullets fed diets supplemented with different levels (0 to 160 mg/kg) of vitamin E during laying period (Experiment 1) exhibited no significant difference in average body weight at 17, 26 and 35 weeks of age, or in weight gain from 17 to 26 weeks of age (Table 3). However, pullets fed 160 mg/kg supplemental vitamin E had significantly higher (p<0.01) body weigh gain during 26 to 35 weeks of age than those fed 0 and 40 mg/kg vitamin E. Cockerels fed different levels (0 to 160 mg/kg) of supplemental vitamin E during mature period (Experiment 2) showed no significant

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### Table 3. Effect of supplemental vitamin E on body weight (BW) and body weight gain (BWG) of Taiwan native breeder chickens

<table>
<thead>
<tr>
<th>Variables</th>
<th>Wks post-adding Vit. E</th>
<th>Added vitamin E (mg/kg)</th>
<th>SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 20 40 80 120 160</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 17</td>
<td>0 1,382 1,399 1,386 1,371 19.1 0.89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 26</td>
<td>9 1,534 - 1,577 1,588 1,636 1,668 1,624 30.6 0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 35</td>
<td>18 - 1,572 1,587 1,624 31.8 0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWG g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 17 to 26</td>
<td>0 9 153 - 158 180 193 160 17.1 0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 26 to 35</td>
<td>9 18 37.6 abc 31.1 c 67.6 abc 89.0 ab 97.3 a 14.5 &lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Effects of supplemental vitamin E on antibody titers in response to sheep red blood cell (SRBC) and packed cell volume (PCV) of Taiwan Native breeder cockerels (Experiment 2) (n =12)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age (wks)</th>
<th>Wks post-adding vit. E</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>-</th>
<th>Age x 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRBC</td>
<td>27 31</td>
<td>4 8</td>
<td>3.59</td>
<td>4.68</td>
<td>4.08</td>
<td>3.82</td>
<td>4.10</td>
<td>4.05</td>
<td></td>
</tr>
<tr>
<td>PCV (%)</td>
<td>27 31</td>
<td>4 8</td>
<td>4.02 ab 4.93a 4.20b 4.00b 3.98a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diets x 3</td>
<td>40.8 38.8 39.9 40.9 41.5 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Statistical analysis

Random samples for determination of antibody titers etc. were from individual birds (experimental unit) (n = 12) of different treatments. Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute, 1996) for the effects of treatment, age and their interactions. Statements of statistical significance were based on p<0.05. Least-squares means of the five treatments were compared by Tukey’s honestly significant difference (HSD) test.
Table 5. Effects of supplemental vitamin E on antibody titers in response to Newcastle disease (ND), infectious bursal disease (IBD), and infectious bronchitis (IB) vaccine of Taiwan Native breeder cockerels (Experiment 2) (n = 12)

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Age (wks)</th>
<th>Wks post-adding vit. E</th>
<th>Diets (added vitamin E, mg/kg)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>ND2 (log2)</td>
<td>31</td>
<td>8</td>
<td>11.8±0.9</td>
<td>11.8±0.4</td>
</tr>
<tr>
<td>IBD3 (×103)</td>
<td>31</td>
<td>8</td>
<td>14.7±2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.4±1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IB3 (×103)</td>
<td>31</td>
<td>8</td>
<td>7.4±3.2</td>
<td>7.8±2.9</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d</sup> Values in a row without the same subscript are significantly different (p<0.05).
<sup>1</sup> Composition of basal diets, experiment design, and vaccination schedule are described as Table 1 and 2.
<sup>2</sup> Hemagglutinin inhibition titers.
<sup>3</sup> ELISA titers.

Immune response

Supplemental vitamin E was added beginning at 17 weeks of age for breeder pullets. There was no significant difference among the treatments for antibody titer in responses to SRBC, NDV at 35 weeks of age, or for skin response to PHA-P and for PCV percentage at 36 and 31 weeks of age, respectively (data not shown).

Cockerels fed the diets supplemented with 20 mg/kg vitamin E had the highest antibody titers in response to SRBC at 27 and 31 weeks of age (p<0.05) (Table 4). Significantly lower titers were found in cockerels fed with 0 and 160 mg/kg supplemental vitamin E at 27 and 31 weeks of age, respectively. Pooled data indicated that cockerels fed the diets supplemented with 20 mg/kg vitamin E had significant higher anti-SRBC titers than those fed with 0, 80 and 160 mg/kg vitamin E (p>0.01). Diets supplemented with vitamin E had no significant difference on cockerels' PCV. Cockerels' PCV at 31 weeks of age was higher than that at 26 weeks of age.

For antibody titers in response to NDV, IBDV and IBV at 31 weeks of age, the results indicated that cockerels' diets supplemented with 20 mg/kg vitamin E had an IBD-titer comparable with the control birds and birds supplemented with more than 20 mg/kg had lower (p<0.01) antibody levels (Table 5). There was no significant difference in anti-ND and IB titers.

DISCUSSION

Results of this study showed that high levels (120 and 160 mg/kg) of supplemental vitamin E significantly increased body weight gain only when the pullets reached their peak egg-production period (26 to 35 weeks of age), suggesting that breeder pullets fed corn-soybean meal diets without supplemental vitamin E had received minimum nutrient requirement for maintenance of growth before peak egg-production period. In this study, cockerels fed with corn-soybean meal diets without supplemental vitamin E should have also received minimum requirement for growth maintenance. Similar report was found by Siegel et al. (2001) who observed greater BW gains from laying pullets fed on a 300 mg vitamin E/kg than those fed on 10 mg/kg, whereas BW gain was not significantly affected by the vitamin E treatments in broilers (Bartov and Frigg, 1992). These results led to the assumption that the vitamin E existing in the ingredients of corn-soybean meal diets was sufficient to meet the minimum requirement for growth performance of chickens, exclusive of laying pullets.

Humoral immune response was evaluated by antibody response to SRBC, NDV, IBDV and IBV. Cell-mediated immune response was evaluated by skin response to PHA-P (mitogen-driven proliferation). Compared with growth performance, immune response affected by supplemental vitamin E for laying pullets appeared to be less sensitive. In this study, female chickens fed corn-soy bean diets without supplemental E did not appear significantly lower in humoral and cell-mediated immune responses than those fed supplemental vitamin E. Richter et al. (1986) also reported no relation between the humoral immune response and vitamin E supplementation (0 and 20 mg/kg) for laying pullets.

From the results of cockerels, it was shown that moderate supplementation of vitamin E (20 mg/kg) could enhance immune response to SRBC. Previous research indicated that deficiency of vitamin E caused a number of reduced immune responses and deficiency of both vitamin E and selenium in chicks impaired bursal growth and reduced the number of lymphocytes in the bursa and the thymus gland (Marsh et al., 1986). From this point of view, vitamin E seemed to be beneficial for the ontogeny of immune response in terms of humoral immunity. Compared with cockerels fed supplemental vitamin E in the mature period, long-term (27 to 31 weeks) omission of supplemental vitamin E appeared to depress antibody titers in response to SRBC but not in response to ND, IBD or IB vaccine. We assume that long term omission of supplemental vitamin E might reach the margin of vitamin deficiency for specific immune responses.

Previous works were not consistent on relation between supplemental vitamin E and immune response. Some
research indicated that high levels of vitamin E (greater than 10 times the required level) were immunostimulatory in chicks (Latshaw, 1991). However, Qureshi et al. (1993) and Marsh et al. (1981) found no effect by supplementing 100 or 250 mg vitamin E/kg diet. The present study in cockerels showed that high levels of supplemental vitamin E (80 to 160 mg/kg) had no or adverse effects on antibody titers in response to SRBC or IBD vaccine. Our results are consistent with those reported by Leshchinsky and Klasing (2001) who reported that moderate levels of supplemental vitamin E (25 to 50 mg/kg) modulate the immune system more than high levels (100 to 200 mg/kg).

Significant differences in immune response to SRBC and IBDV were observed but not in NDV or IBV. It might be due to different types of those antigens. Antibody levels of IBDV and NDV were determined following vaccination with a live vaccine and booster with inactivated vaccines. It is known that SRBC and IBDV are antigens which require the presence of T helper cells (CD4) in order to induce humoral immune response (T-cell dependent), whereas NDV does not (T-cell independent). Research has shown that more T helper cells (CD4) are presented with increased dietary vitamin E (0 to 87 mg/kg) and thus improved responsiveness to immunologic stimuli (Erf et al., 1998). Further study is needed to clarify whether the different results of immune response to SRBC and IBDV but not to NDV and IBV by vitamin E supplementation correlate with the views described above.

Although higher antibody response to vaccination of chickens may result in lower mortality caused by infectious diseases (Yunis et al., 2000) and higher clinical protection against virulent challenge (Maas et al., 1999), the beneficial effect of supplemental vitamin E in terms of disease resistance needs to be further investigated.

For laying hens, vitamin E is one of the nutrients that can be increased in the egg by increasing the dietary level of this vitamin (Cherian and Sim, 1997; Grobas et al., 2001). Meydani and Hayek (1992) reported that low vitamin E levels led to unstable immune cell membranes, which led to enhanced production of immunosuppressors (eg, prostaglandins). In addition, laying hens showed higher serum concentration of prostaglandins than nonlaying hens or cocks (Takahashi et al., 1999). In the study of mice, Meydani et al. (1986) reported that the enhancement of immune response of aged mice by vitamin E appeared to be mediated by decreased prostaglandin synthesis. It remains an unsettled question whether egg-laying itself or depletion of supplemental vitamin E for laying influences the immune response.

It is well-known that vitamin E works as a part of cellular antioxidant systems in close cooperation with other cellular antioxidants, particularly with the ascorbate and glutathione systems (Di Mascio et al., 1991). Parts of this antioxidant system are able to regenerate each other (Beyer 1994; Packer et al., 1997). However, under various conditions, vitamin E has been found to have diverse roles; that is, neutral, anti-, or pro-oxidant activity. For example, Thomas and Stocker (2000) reported that vitamin E at high concentrations can serve as a prooxidant. In addition, Yang et al. (1976) reported that both excess and deficiency of vitamin E in rats significantly depress the activity of glutathione peroxidase and thus modulates the intracellular glutathione pool. Thus, we speculated that excess dose of vitamin E could modify the overall balance of antioxidants in a cell, resulting in different pools of antioxidant compound that carry different immunoregulatory properties.

Supplemental vitamin E had no effect on PCV of pullets or cockerels. Similar results were obtained in broiler chickens (Jakobsen et al., 1995; Tras et al., 2000). In addition, this study has shown that older cockerel’s PCV (41.5) was higher than younger PCV (39.5) and cockerel’s PCV (40.8 to 42.8) was higher than hen’s PCV (29.1 to 30.3). Sex and age effects on PCV in this study may partly account for previous reports that broilers selected for high body weight, rapid protein accretion, or superior feed efficiency have higher PCV (Lubritz and McPherson 1994; Silversides et al., 1997) and PCV increased with age (Price et al., 1998). Yahav (1997) reported that a difference of temperature of 10°C or more may affect PCV. In this study, the difference of mean temperature in the determined weeks (week 27 and 31) was only 1°C, thus excluding the temperature effect. There were no diet×age interaction on SRBC or PCV. However more data are needed to elucidate the age effect.

In conclusion, the results of the present study indicated that vitamin E existing in the ingredients of corn-soybean meal diets was sufficient to meet the minimum requirement for growth performance of breeder chickens, exclusive of peak-laying pullets. Supplemental vitamin E has no effect on immune response of laying pullets. For cockerels, moderate supplementation of vitamin E was shown to enhance immune responses to SRBC, whereas for antibody titers in response to IBD depression was observed when supplementation level was over 80 mg/kg. Although a vitamin E deficiency would not be expected to occur in practice from the results of this study, caution must be taken as excessive vitamin E may depress specific immune responses.

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