ABSTRACT This research, which was designed and carried out as two consecutive experiments, investigated the effects of four different levels (0, 4,000, 12,000, and 24,000 IU/kg) of vitamin A supplementation on egg yield, plasma vitamin A levels, and immune responses of laying hens. Transmission of maternal immunity to their descendants was also studied. In the first experiment, egg yield, blood vitamin A levels, and various parameters of the immune system such as T lymphocyte levels in the peripheral blood, plasma cell counts in the spleen, and antibody titers against Newcastle Disease Virus (NDV) in the sera were investigated for a 1-yr period. A total of 864 Hisex-brown laying hens were used in this experiment. The chicks were reared as commercial flocks until the 18th wk of age. No significant differences occurred among the parameters of the different diet groups.

In the second experiment, maternal immunity was assessed in the chickens, supplied by hatching the eggs from hens in the first experiment. Maternal immunity was assayed by using the parameters as in Experiment 1. For this purpose, both blood and tissue samples were taken on the 2nd, 7th, and 10th d posthatch. Vitamin A supplementation had no significant effects on maternally derived antibody titers or histologic structure of the lymphoid organs.

(Key words: vitamin A, immunity, egg yields, antibody titers, T lymphocytes)

INTRODUCTION

Essential functions of vitamin A in growth, visual development, and reproductive physiology are well established. In poultry, as well in other animals. Vitamin A deficiency is closely associated with increased susceptibility to infections. The exact manner in which vitamin A deficiency affects the host immune system is attributed to destruction of mucosal epithelium acting as the first defense barrier (Bains, 1988). However, results of many experiments (Sijtsma et al., 1989a,b) have revealed that increased morbidity is observed in chicks experimentally infected Newcastle Virus (NDV) that were fed a diet marginally deficient in vitamin A (Sijtsma et al., 1989a,b).

Davis and Sell (1983) have reported that vitamin A deficiency had detrimental effects on the avian immune system functions and vitamin A-deficient chickens had lower thymus and bursa weights than controls. In a study by Butera and Krakowka (1986), lymphoid cell depletions in both germinal centers of lymphoid follicles (B cell areas) and periarteriolar lymphoid sheets (PALS, T cell areas) of the spleen, striking declines in the cellularity of the thymic cortex, have been observed in vitamin A-deficient rats. Thymus and spleen weights, and their proportions to the body weight were also reduced in the vitamin A-deficient animals (Butera and Krakowka, 1986).

Although the association between vitamin A deficiency and impaired immune responses is clear, high level-intake of this vitamin possibly has detrimental effects on the immune system. Friedman et al. (1991) have observed increased susceptibility to Escherichia coli infections in the chickens fed 1,000 mg/kg retinol equivalent of the retinyl acetate supplemented diets, whereas in the animals provided low levels of vitamin A, the serum antibody titers were normal. In another study (Friedman and Sklan, 1989b), important declines were found in both T lymphocyte proliferation and serum antibody titers against BSA.

Abbreviation Key: ANAE = α-naphthyl acetate esterase; HI = microhemagglutination-inhibition; NDV = Newcastle Disease Virus; PALS = periarteriolar lymphoid sheets.
Different levels of vitamin A intake result in different levels of vitamin A in the plasma and variable storage in the liver. Friedman and Sklan (1989b) have observed different plasma and liver vitamin A levels in the chickens fed diets containing 0, 0.85, 35, and 1,000 mg/kg vitamin A. In this study, the effects of vitamin A supplemented at different levels to a commercial feed on the immune system of laying hens were investigated. For this purpose, the antibody response to mineral oil-adjuvant Newcastle vaccine, peripheral blood T lymphocyte percentages, splenic plasma cell counts, blood vitamin A levels, egg production performance, and maternal immunity of the chicks of these hens were determined.

**MATERIALS AND METHODS**

**Experimental Design**

This study was designed as two consecutive experiments. In the first experiment, a total of 864 commercial Hisex-brown laying hens were used. The hens were supplied as 1-d-old chicks. The chicks were fed with a diet containing 15,000 IU/kg vitamin A until 6 wk of age, followed by a diet containing 10,000 IU/kg vitamin A until 18 wk of age. Newcastle HB1 and LaSota vaccines were given on the 14th and 28th d. The animals were divided into four groups at the 18th wk and transferred to cages.

Each of the four groups were further divided into six subgroups, each containing six pullets that were housed in six different cages. The subgroups were distributed randomly among the different compartments of the cage system. This distribution resulted in 36 cages and 216 laying hens for each group. At the time of transfer to the cages, each hen was administered 0.5 mL of Newcastle Vaccine containing mineral oil as adjuvant s.c. in the back of the neck.

The groups consumed ad libitum the diets containing 0 (A1; Negative Control), 4,000 (A2; NRC recommendation), 12,000 (A3; Commercial level; Positive Control), and 24,000 (A4; twofold of the commercial level) IU/kg vitamin A. Different vitamin A levels in each diet were through addition of the vitamin A to 300 kg of the concentrated diet and mixing homogeneously. The composition of the diets is given in Table 1.

The hens were fed in groups and feed consumption was determined at monthly intervals. Egg yields were recorded daily. Mortality were calculated at the end of the experimental period. Egg weights were recorded monthly. The experiment continued through the 72nd wk.

In the second experiment, eight hens from each group were chosen randomly in the 53rd wk of age from the first experiment. One cockerel from the same strain was placed into each of four groups, each containing eight hens, and after 7 d, fertilized eggs were collected for 10 d. At least 25 of the fertilized eggs from each group were hatched. Chicks of each group were fed a commercial starter diet and reared in separate compartments. Serum antibody titers, blood T lymphocyte levels, and splenic plasma cell counts were determined for these chicks. The samples were taken on the 2nd, 7th, and 10th d posthatch.

**Determination of Serum Antibody Titers**

Serum antibody titers were determined by means of microhemagglutination-inhibition (HI) test (Erganis and Istanbulluoglu, 1993) at 23rd, 25th, 29th, 33rd, 37th, 42nd, 52nd, 57th, 65th, and 72nd wk of age in the first experiment, and on the 2nd, 7th, and 10th d of age in the second experiment.

**Histologic Investigations and Cell Counts**

Blood and lymphoid tissue samples were taken from three hens of each group at the 1st, 2nd, 4th, 5th, 10th, and 12th mo of the first experiment. In the second experiment, the samples were taken on the 2nd, 7th, and 10th d posthatching.

Peripheral blood T lymphocyte counts were determined by the histochemical demonstration of α-naphthyl acetate esterase (ANAE) on blood films according to Mueller et al. (1975) with minor modifications. The

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1. Intervet Netherland B.V., Posbus 50,5830 AB, Boxmeer, The Netherlands.
2. Roche Müstahzarlar A. Ş, İstanbul, Turkey.

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**TABLE 1. Ingredients and chemical composition of diet samples**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>51.90</td>
</tr>
<tr>
<td>Wheat</td>
<td>10.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>12.50</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>10.50</td>
</tr>
<tr>
<td>Molasses</td>
<td>3.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>1.50</td>
</tr>
<tr>
<td>Limestone</td>
<td>8.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.50</td>
</tr>
<tr>
<td>Salt</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamins1,2</td>
<td>0.25</td>
</tr>
<tr>
<td>Mineral1</td>
<td>0.10</td>
</tr>
</tbody>
</table>

1. Provided per kilogram of diet: cholecalciferol, 1,200 IU; vitamin E, 35 mg; vitamin K, 5 mg; vitamin B6, 7 mg; niacin, 20 mg; Ca-d-pantothenate, 10 mg; vitamin B, 5 mg; vitamin B12, 0.015 mg; folic acid, 1 mg; D-Biotin, 0.045 mg; choline chloride, 125 mg; vitamin C, 50 mg; charophyll red, 25 mg; charophyll yellow, 5 mg; Mn, 80 mg; Fe, 30 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.5 mg; I, 2 mg; CaCO3, 236 mg.
2. In the first experiment, vitamin A level in the mixture varied depending on the groups.
Feed conversion, kg feed:kg egg 2.17

Egg yield, % 81.2

Statistical Analysis

In this experiment, it was possible to maintain good egg production without any definite decline for 1 yr in the group without vitamin A supplements. This result does not indicate that laying hens do not require dietary vitamin A, but does provide some evidence that the vitamin A content of the ingredients itself, particularly that of corn, can meet the vitamin A requirements of laying hen so that extra supplementation might not be required. However, when the high vitamin A level (9,500 IU/kg) of corn, constituting 51.90% of the diet (Table 1) is taken into consideration, the NRC (1984) recommendation is met by vitamin A coming from corn alone.

McGinns (1988) has reported that the vitamin A content of the ingredients were rather variable; consequently, vitamin A supplementation was necessary in preventing animals from deficiency problems. In addition, McGinns (1988) has also suggested that additional vitamin A should be supplemented in order to compensate for fluctuations in the levels of vitamin A in feed ingredients, oxidation loses, and the demands due to the feed consumption, stress, genotype, and management. However, the hens have some mechanisms for tolerating the fluctuations in vitamin A intake. In the feeding experiments that were carried out with a purified diet (West et al., 1992), the chickens reached the lowest critical level at the 12th wk; that is, the vitamin A reserve in the liver possibly meets the animal’s need for at least 12 wk of age. Some of the evidence in the literature also shows that a high level of vitamin A has some toxicogenic effects and that the maximum tolerable dose for laying hen is 40,000 IU (NRC, 1987).

Plasma retinol levels increased due to the high vitamin A level of the diet (Table 4). Although plasma retinol levels have been suggested (West et al., 1992) as an indicator of dietary vitamin A level, only significant

### Statistical Analysis

One-way analyses of variance of egg yield, egg weight, feed intake, feed conversion, abnormal egg, and viability were conducted. Any significant differences were further analyzed using Duncan’s multiple range test. Antibody titers were evaluated after they were transformed into natural logarithms. Egg production data were converted by arc sine transformation before analyzing; however for interpretation, the data were presented as percentages. Mortality data was evaluated by chi-square (Düzgün et al., 1987).

### RESULTS AND DISCUSSION

Because the duration of the experiment was long and raw materials were purchased as six lots at different times, small differences occurred in the composition of the diets. The means of analyses of experimental diets are presented in Table 1.

There was no significant difference between Group A1 (vitamin A not supplemented) and Group A4 [supplemented with 24,000 IU/kg vitamin A, twofold the NRC recommendation (1984)] (Table 2). Significant differences were observed only between Group A3 (which contained 12,000 IU/kg feed vitamin A as in most of the commercial premixes) and the other groups. In Group A3, a prominent decline in egg production occurred between 5 and 6 mo of the experiment, although there were no significant differences for egg production during the other months.

In this experiment, it was possible to maintain good egg production without any definite decline for 1 yr in the group without vitamin A supplements. This result does not indicate that laying hens do not require dietary vitamin A, but does provide some evidence that the vitamin A content of the ingredients itself, particularly that of corn, can meet the vitamin A requirements of laying hen so that extra supplementation might not be required. However, when the high vitamin A level (9,500 IU/kg) of corn, constituting 51.90% of the diet (Table 1) is taken into consideration, the NRC (1984) recommendation is met by vitamin A coming from corn alone.

McGinns (1988) has reported that the vitamin A content of the ingredients were rather variable; consequently, vitamin A supplementation was necessary in preventing animals from deficiency problems. In addition, McGinns (1988) has also suggested that additional vitamin A should be supplemented in order to compensate for fluctuations in the levels of vitamin A in feed ingredients, oxidation loses, and the demands due to the feed consumption, stress, genotype, and management. However, the hens have some mechanisms for tolerating the fluctuations in vitamin A intake. In the feeding experiments that were carried out with a purified diet (West et al., 1992), the chickens reached the lowest critical level at the 12th wk; that is, the vitamin A reserve in the liver possibly meets the animal’s need for at least 12 wk of age. Some of the evidence in the literature also shows that a high level of vitamin A has some toxicogenic effects and that the maximum tolerable dose for laying hen is 40,000 IU (NRC, 1987).

Plasma retinol levels increased due to the high vitamin A level of the diet (Table 4). Although plasma retinol levels have been suggested (West et al., 1992) as an indicator of dietary vitamin A level, only significant
increases ($P < 0.01$) were observed in 4th and 10th mo of this experiment. Namely, plasma retinol levels may not be a practical indicator of the vitamin A status of diets.

The data of Table 4 demonstrate that serum antibody titers were not influenced by vitamin A level; however, in Group A, significantly ($P < 0.05$) higher titers were observed. It has been reported that vitamin A deficiency causes significant declines in peripheral lymphoid organ weights and low antibody response to human serum albumin in poultry (Zeutskaya and Fias, 1977). Rombout et al. (1992) did not observe significantly lower numbers of cytoplasmic IgA-, IgG-, and IgM-positive plasma cells in the spleens of the animals that were fed without vitamin A; however Pasatiempo et al. (1991) found that in rats, the numbers of IgM- and IgD-positive plasma cells or proportions of T lymphocytes (T helper:T suppressor) were not effected by the vitamin A level of the diet.

In the present study, there were no differences in splenic plasma cell concentrations or peripheral blood T lymphocyte proportions among the vitamin A groups, which confirms the results of others (Rombout et al., 1992; Zeutskaya and Fias, 1977). However, vitamin A has been reported to promote T cell function, but not to increase the number of T cells (Friedman and Sklan, 1989a).

Assessment of maternal immunity revealed that 2-d-old chicks hatched from 9-mo-old parent stocks fed with different vitamin A-supplemented diets, had similar antibody titers. Serum antibody titers over 7 protect chickens efficiently (Ergunis and Istanbuluoglu, 1993). On the 10th d, the titers were lower than 7 in all of the groups.

A close relationship between serum antibody levels of hens at the time of laying and of 2-d-old chicks was striking. However, the serum antibody titers of 9-mo-old parents were between 7.4 and 8.1; and in the sera of chicks hatched from those parents titers were between 7.0 and 9.6. It is evident that higher serum antibody titers result in higher maternal antibody transfer via the egg yolk, and, consequently, higher serum levels of maternal antibody in the chicks (Ergun, 1991). It has been suggested that protectivity of maternal immunity with high serum Ig levels may continue until the 29th d posthatch (FAO, 1987). Lymphoid organs were normal in histological structure in both vitamin A-supplemented and unsupplemented (negative control) groups. These findings demonstrate that the chick's reserve of vitamin A is sufficient for the early development of the immune system organs (Rombout et al., 1992).

Significant differences in splenic plasma cell concentrations and peripheral blood T cell proportions were not observed between control and experimental groups; however, a gradual increase was determined in all groups with aging.

### TABLE 4. Serum vitamin A levels of experimental groups

<table>
<thead>
<tr>
<th>Phase (mo)</th>
<th>$A_1$ Negative-control</th>
<th>$A_2$ NRC recommendation</th>
<th>$A_3$ Commercial level (positive control)</th>
<th>$A_4$ Twofold of commercial level</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>85.9 ± 15.06</td>
<td>69.4 ± 12.0</td>
<td>43.8 ± 11.2</td>
<td>87.8 ± 3.5</td>
</tr>
<tr>
<td>4</td>
<td>57.7 ± 8.8$^a$</td>
<td>56.8 ± 8.3$^b$</td>
<td>76.0 ± 10.0$^b$</td>
<td>97.3 ± 3.8$^a$</td>
</tr>
<tr>
<td>7</td>
<td>66.2 ± 13.1</td>
<td>65.0 ± 18.5</td>
<td>96.1 ± 12.0</td>
<td>74.2 ± 8.2</td>
</tr>
<tr>
<td>10</td>
<td>47.8 ± 8.5$^c$</td>
<td>66.5 ± 2.8$^b$</td>
<td>53.0 ± 4.3$^c$</td>
<td>85.9 ± 4.5$^a$</td>
</tr>
</tbody>
</table>

$^a$ $^c$ Means within rows with no common superscript differ significantly ($P < 0.05$).

### TABLE 4. Serum antibody titers at different phases of the first experiment

<table>
<thead>
<tr>
<th>Phases (wk)</th>
<th>$A_1$ Negative-Control</th>
<th>$A_2$ NRC recommendation</th>
<th>$A_3$ Commercial level (positive control)</th>
<th>$A_4$ Twofold of commercial level</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>11.7 ± 0.2</td>
<td>11.4 ± 0.3</td>
<td>11.4 ± 0.3</td>
<td>12.1 ± 1.5</td>
</tr>
<tr>
<td>5</td>
<td>14.1 ± 0.2$^a$</td>
<td>12.6 ± 0.2$^b$</td>
<td>13.9 ± 0.2$^a$</td>
<td>12.4 ± 2.0$^b$</td>
</tr>
<tr>
<td>9</td>
<td>10.8 ± 0.4</td>
<td>10.6 ± 0.3</td>
<td>10.3 ± 0.3</td>
<td>11.4 ± 1.3</td>
</tr>
<tr>
<td>13</td>
<td>10.5 ± 0.5</td>
<td>11.4 ± 0.3</td>
<td>10.4 ± 0.5</td>
<td>10.8 ± 1.7</td>
</tr>
<tr>
<td>17</td>
<td>8.8 ± 0.3</td>
<td>9.8 ± 0.3</td>
<td>9.9 ± 0.3</td>
<td>8.9 ± 2.2</td>
</tr>
<tr>
<td>22</td>
<td>10.2 ± 0.3</td>
<td>9.7 ± 0.2</td>
<td>10.1 ± 0.2</td>
<td>9.4 ± 1.3</td>
</tr>
<tr>
<td>27</td>
<td>9.2 ± 0.3$^a$</td>
<td>7.5 ± 0.2$^b$</td>
<td>8.8 ± 0.2$^a$</td>
<td>7.0 ± 1.2$^b$</td>
</tr>
<tr>
<td>32</td>
<td>8.2 ± 0.3$^a$</td>
<td>6.8 ± 0.2$^c$</td>
<td>7.8 ± 0.2$^a$</td>
<td>7.3 ± 1.2$^{abc}$</td>
</tr>
<tr>
<td>37</td>
<td>7.4 ± 0.3</td>
<td>8.0 ± 0.4</td>
<td>7.0 ± 0.2</td>
<td>8.1 ± 1.7</td>
</tr>
<tr>
<td>45</td>
<td>6.9 ± 0.2$^a$</td>
<td>7.5 ± 0.2$^a$</td>
<td>6.0 ± 0.2$^b$</td>
<td>6.9 ± 0.9$^a$</td>
</tr>
<tr>
<td>52</td>
<td>6.3 ± 0.3</td>
<td>6.2 ± 0.2</td>
<td>5.8 ± 0.2</td>
<td>6.0 ± 0.9$^a$</td>
</tr>
</tbody>
</table>

$^a$ $^c$ Means within rows with no common superscript differ significantly ($P < 0.05$).
The results of this study, covering a 1-yr experimental period, have revealed that feeding diets based on wheat, corn and soybean meal, or a sunflower oil meal, might meet the demand for vitamin A under normal conditions. Vitamin A supplementation was not needed in premixes to obtain high production performance and immune responsiveness. In the second experiment, extra vitamin A supplementation in the diets of parents did not improve maternal immunity of the chick transferred via egg yolk.

REFERENCES


