

## Effect of different levels of vitamin premix during finisher period on broiler on performance and immunocompetence in battery cage and floor systems

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Two trials were conducted to evaluate the withdrawal or reduction of vitamin premix from diets of finisher period (29 to 42 days of age) on broiler performance and immunocompetence. Trial 1 was conducted as a completely randomized design with 4 treatments of 6 replicates and 5 chicks in each battery cage system. Trial 2 was conducted with 4 treatments of 6 replicates and 18 chicks in pen floor system. The diets were formulated based on wheat and barley, and dietary treatments were: T1 = based diet without vitamin premix, T2 = based diet with 33.33% vitamin premix, T3 = based diet 66.66% vitamin premix, T4 = based diet 100% vitamin premix (Control Group). On day 34, two birds from each replicate were selected and antibody responses to inoculated sheep red blood cells were determined. The cell-mediated immunity was determined via phytohemagglutinin (PHA) and dinitrochlorobenzene (DNCB). Results of trial 1 showed that there were no significant differences in the bird's performance with reduction or withdrawal of vitamin premix from diets in 29-35 days among the experimental groups, but in 36-42 days of age, the performance of the group of the birds fed with diet without vitamin premix (T1) was significantly lower than other groups ( $P < 0.05$ ). Results of trial 2 showed that vitamin premix reduction and withdrawal from 29 d of age did not impair body weight, feed intake and feed conversion ratio during the final period of broilers (29-42d). The results of two trials demonstrated that immunocompetence response was not affected by treatments in finisher period. The results of the present study indicated that in the battery cage system it's possible to reduce dietary vitamin premix during finisher period but withdrawal can negatively affect performance of broilers. While in the floor system it is possible to withdraw vitamin supplements from finisher diets.

Key words: broiler; immunocompetence, rearing system, vitamin premix

Vitamins are micronutrients that participate in numerous organic metabolic processes and are therefore indispensable for excellent animal health and productive performance (Alahyari-Shahrasb *et al.* 2011). When compared to other nutrients, there are very few studies carried out in the last years to estimate the optimum levels of vitamins for broilers, and there is a huge variation in the levels used commercially (McDowall 2010).

Most levels recommended by the NRC (1994) were based on old studies, performed under controlled conditions and using the minimum levels to avoid signs of deficiency, not evaluating the best performance under the challenge conditions found in the field (Alahyari-Shahrasb *et al.* 2012a). Moreover, the modern breeds have higher growth and production rates, and have higher nutritional requirements to express their genetic potential. Besides production rates, other parameters are also presently evaluated to determine the vitamin requirements, as immunity, animal welfare, carcass characteristics and microbiological analysis.

It has been reported that minerals and vitamins are being added to the diet when they may not be needed (Christmas *et al.*, 1995). Khajali *et al.* (2006) suggested that the vitamin and trace mineral contents of the finisher diet were sufficiently high to maintain a humoral immune response. Alahyari-Shahrasb *et al.* (2012b) indicated that vitamin premix withdrawal at 29 d of age did not impair feed intake or weight gain, and feed conversion ratio.

Vitamin deficiencies have been shown to suppress immunocompetence (Myrvik 1988). Therefore, the response of the immune system needs to be considered when studying the effect of vitamin reduction or withdrawal. Deyhim and Teeter (1993) showed that removal of the vitamin and trace mineral premixes from broiler rations did not affect immunological competence as judged by antibody titer to sheep erythrocyte injection.

There are several reports about vitamin premix withdrawal in broilers diets based on corn and rearing floor system (Christmas *et al.* 1995; Maiorka *et al.* 2002; Khajali *et al.* 2006 and Alahyari-Shahrasb *et al.*, 2011, 2012a), but based on the following causes, it seems necessary to study the effect of withdrawal or reduction of vitamin supplements in finisher diets of broilers based on wheat and barley in the battery cage system. 1) There are differences in vitamin content of wheat, barley and corn; 2) Corn is the high energy grain favored by most poultry nutritionists and poultry producers. However, it is not always available at an economic price. Wheat and barley may be a more economic and readily available alternative and 3) Birds in cages require more dietary vitamins than those on floor housing because of their limited access to feces.

Therefore this study was carried out to evaluate the effects of reduction or withdrawal of the vitamin premix from broiler diets during finisher period on performance and immunocompetence in two rearing systems (battery cage and floor systems).

## Materials and Methods

*General procedure.* Two trials were conducted to evaluate the effect of reduction or withdrawal of vitamin premix during the final phase of broiler chicken growth (between 29 and 42 d of age) in battery cage (trial 1) and floor (trial 2) rearing systems. The average initial body weight of chicks in each pen was 42 g. Room temperature was kept at 34°C during the first 3 days of the trial and then reduced gradually according to age until reaching 22°C at 21 d. The light was continuous during the first 3 d, and then the lighting regimen was 23 h/d. The ingredient composition of the experimental diets and the nutrient composition are shown in table 1. The diets in all treatments were based on wheat and barley with different levels of vitamin premix as following: T1) based diet without vitamin premix, T2) based diet 33.33% vitamin premix, T3) based diet 66.66% vitamin premix and T4) based diet 100% vitamin premix during 29-42 days (table 2). Feed intake and weight gain were measured at the end of each week of finisher period and feed conversion ratio was calculated.

Mash feed and water were available for *ad libitum* consumption. Prior to formulation, all major dietary ingredients were analyzed for nitrogen-corrected apparent metabolizable energy (AMEn), amino acid (AA) profiles (according to prediction formula existing in NRC 1994), crude protein (CP), crude fiber (CF) and ether extract (EE) as described by AOAC (2000). The total nitrogen (N) content was measured by the Kjeldahl method (Kjeltec 1030 Autoanalyzer, Foss Tecator AB, Hogans, Sweden). CP was calculated as  $N \times 6.25$ . The crude fiber (CF) content was determined using an automated Fibertech Foss Tecator 1010 apparatus (AOAC 2000, ID 944.05). The ether extract (EE) content was determined by the Soxtec automated apparatus (Soxtec system, HT, Foss Tecator 1043) (AOAC 2000, ID 920.39)

*Trial 1:* One hundred-twenty male broiler chicks, Ross 308, were distributed with similar average body weight into battery cages at 23 d of age and fed similar grower diet up to 28 d of age and allocated into cages in a completely randomized design with four treatments

Table 1. Composition of the starter and grower diets used in the pre-experimental.

| Ingredient (%)                       | Starter diet (g/kg) | Grower diet (g/kg) |
|--------------------------------------|---------------------|--------------------|
| Wheat                                | 34.00               | 35.14              |
| Barley                               | 32.00               | 30.00              |
| Soya bean meal (440 g/kg CP)         | 23.98               | 26.93              |
| Corn gluten meal                     | 5.62                | 2.51               |
| Soya oil                             | 1.03                | 1.78               |
| Oyster shell                         | 1.30                | 1.29               |
| Dicalcium phosphate                  | 1.05                | 1.05               |
| Vitamin premix <sup>1</sup>          | 0.25                | 0.25               |
| Trace mineral premix <sup>2</sup>    | 0.25                | 0.25               |
| Sodium chloride                      | 0.28                | 0.28               |
| DL-Methionine                        | 0.06                | 0.21               |
| L-Lysine-HCl                         | 0.13                | 0.26               |
| Multi Enzyme (Rovabio®) <sup>3</sup> | 0.05                | 0.05               |
| Calculated compositions              |                     |                    |
| ME (MJ/kg)                           | 11.93               | 11.97              |
| CP (%)                               | 20.80               | 20.00              |
| Met (%)                              | 0.48                | 0.41               |
| Met + Cys (%)                        | 1.00                | 0.86               |
| Lys (%)                              | 1.35                | 1.12               |
| Na (%)                               | 0.15                | 0.14               |
| Ca (%)                               | 0.99                | 0.81               |
| Available Phosphorus (%)             | 0.47                | 0.41               |

<sup>1</sup>2.5 kg of vitamin premix contained: 2700mg retinal, 400mg calcidiol, 18g tocopheryl acetate, 2000mg menadione, 1800mg thiamine, 6600mg riboflavin, 10g niacin, 30g calcium pantothenate, 3g pyridoxine, 1g folic acid, 15mg cobalamin, 250g choline chloride, 100mg biotin.

<sup>2</sup>2.5 kg of trace mineral premix contained: 100g Mn, 50g Fe, 100g Zn, 10g Cu, 1g I, 200mg Se.

<sup>3</sup>These enzyme contained mainly  $\beta$ -glucanase and xylanase activities. The endo-1,3(4)- $\beta$ -glucanase 100 AGL/kg diet and endo-1,4- $\beta$ -xylanase 70 AXC units/ kg diet .

and six replicates per treatment and five birds per cage. Initial body weights were similar in all cages (1125±11.1g). The dimensions of the cages used for rearing broiler chicks in trial were 100 cm long × 60 cm wide × 46 cm high.

*Trial 2:* Four hundred and thirty-two, male chicks (Ross 308) were used in floor system. Chicks were raised until 29 d of age in floor system, as described in the general procedure, weighed (1130±13.6g), and distributed into pens (replicates) in a completely randomized design with four treatments of six replicates and 18 birds per floor pen replicate. The dimensions of the pens used for rearing broiler chicks in trial were 230 cm long × 150 cm wide × 90 cm high.

*Meat yield characteristics and lymphoid organs weight.* At 35 and 42 days of ages, one bird of each replicate (6/treatment) in trial 1 and two birds of each replicate (12/treatment) in trial 2, with similar average

weight of each replicate were selected, slaughtered and eviscerated in order to determine carcass weight and carcass yield as well as legs, breast (including skin and bone), thigh and the lymphoid organs (Bursa of Fabricius and Spleen) organ relative weights.

*Productive performance traits:* Mortality during 29 to 42 d was determined for each pen. Body weight gain (BWG) and average daily feed intake (ADFI) of chicks were determined at 35 and 42 d of age, then feed conversion ratio (FCR) was calculated.

*Immune system competence.* Humoral immune response to sheep red blood cell (SRBC). Sheep red blood cells (SRBC) were used as T-dependent antigens to quantify the antibody response. In trials, two birds were selected from each replicate and were inoculated intravenously with 0.1 mL of a 1% suspension of SRBC, at 34 d of age. Blood samples were collected from brachial vein 7 days after inoculation. The serum from each sample was collected; heat inactivated at 56°C for

Table 2. Compositions of the diets used during the experimental period (29-42 d of age) in trials 1 and 2

| Ingredient                        | Treatments <sup>1</sup> |        |        |        |
|-----------------------------------|-------------------------|--------|--------|--------|
|                                   | T1                      | T2     | T3     | T4     |
| Wheat                             | 36.38                   | 36.05  | 35.97  | 35.79  |
| Barley                            | 30.00                   | 30.00  | 30.00  | 30.00  |
| Soy-bean meal (44%)               | 27.93                   | 28.09  | 28.04  | 28.09  |
| Soya oil                          | 2.74                    | 2.80   | 2.86   | 2.90   |
| Oyster shell                      | 1.24                    | 1.25   | 1.24   | 1.24   |
| Dicalcium phosphate               | 0.89                    | 0.90   | 0.90   | 0.90   |
| Vitamin premix <sup>2</sup>       | 0.00                    | 0.08   | 0.16   | 0.25   |
| Trace mineral premix <sup>3</sup> | 0.25                    | 0.25   | 0.25   | 0.25   |
| Sodium chloride                   | 0.28                    | 0.28   | 0.28   | 0.28   |
| DL-Methionine                     | 0.17                    | 0.18   | 0.18   | 0.18   |
| L-Lysine-HCl                      | 0.07                    | 0.07   | 0.07   | 0.07   |
| Multi Enzyme (Rovabio®)           | 0.05                    | 0.05   | 0.05   | 0.05   |
| Total%                            | 100.00                  | 100.00 | 100.00 | 100.00 |
| Calculated compositions           |                         |        |        |        |
| ME (MJ/kg)                        | 12.14                   | 12.14  | 12.14  | 12.14  |
| CP (%)                            | 20.00                   | 20.00  | 20.00  | 20.00  |
| Met (%)                           | 0.37                    | 0.37   | 0.37   | 0.37   |
| Met + Cys (%)                     | 0.77                    | 0.77   | 0.77   | 0.77   |
| Lys (%)                           | 0.97                    | 0.97   | 0.97   | 0.97   |
| Na (%)                            | 0.16                    | 0.16   | 0.16   | 0.16   |
| Ca (%)                            | 0.76                    | 0.76   | 0.76   | 0.76   |
| Available Phosphorus (%)          | 0.37                    | 0.37   | 0.37   | 0.37   |

<sup>1</sup>T1) the basal diet without vitamin premix, T2, T3, T4) with 33%, 66%, 100% vitamin premix (VP) during 29-42 days respectively.

<sup>2</sup>2.5 kg of vitamin premix contained: 2700mg retinal, 400mg calcidiol, 18g tocopheryl acetate, 2000mg menadione, 1800mg thiamine, 6600mg riboflavin, 10g niacin, 30g calcium pantothenate, 3g pyridoxine, 1g folic acid, 15mg cobalamin, 250g choline chloride, 100mg biotin.

<sup>3</sup>2.5 kg of trace mineral premix contained: 100g Mn, 50g Fe, 100g Zn, 10g Cu, 1g I, 200mg Se.

30 min and then analyzed for total, mercaptoethanol-sensitive (MES) IgM and mercaptoethanol-resistant IgG anti-SRBC antibodies as previously described (Delhanty and Solomon 1966; Yamamoto and Glick 1982 and Qureshi and Havenstein 1994). Briefly, 50  $\mu$ L of serum was added in an equal amount of phosphate-buffered saline (PBS; pH 7.6) in the first column of a 96-well v-shaped bottom plate, and the solution was incubated for 30 min at 37°C. A serial dilution was then made (1:2), and 50  $\mu$ L of 2% SRBC suspension was added to each well. Total antibody titers were then read after 30 min of incubation at 37°C. The well immediately preceding a well with a distinct SRBC button was considered as the endpoint titer for agglutination. For MES (IgM) response, 50  $\mu$ L of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, followed by the aforementioned procedure. The difference between the total and the IgG response was considered to be equal to the IgM antibody level (Cheema *et al.* 2003).

**Cellular immune response.** DNCB challenge. On the 35<sup>st</sup> day 1-chloro-2, 4-dinitrobenzene (DNCB, Merck) solution (10 mg/mL) was spread and maintained over a 10 cm<sup>2</sup> area of featherless skin (0.25 mL) on the right side of the two birds per pen. Similar position on the left side of the bird treated by the solvent alone (acetone: olive oil, 4:1 v/v) to correct the solvent effect. The second treatment by DNCB solution (1 mg/mL) was applied on the 42 day (Karimi Torshizi *et al.*, 2010). The skin swelling was calculated as the difference between the thickness of the skin before and after DNCB treatment was measured using a digital caliper (Mitutoyo, Japan).

**PHA-M induced lymphoproliferation.** Phytohemagglutinin-M (Gibco, USA), T-cell mitogen was injected (100 mg dissolved in 100 mL of sterile PBS) to the right toe web of 2 birds per experimental group at 40 d. The increase in toe web thickness was measured 24 h after injection (Corrier 1990).

**Statistical Analysis.** The data were subjected to ANOVA as completely randomized design using the

GLM procedure of SAS software (SAS, Institute; 2002). Anti-SRBC titers and lymphoid organ weights data were transformed to log<sub>2</sub> and arc sin, respectively. Means were compared by Duncan's Multiple Range Test (1995) at significance level of  $P < 0.05$ .

## Results

**Performance. Trial 1.** The total mortality was 2 birds during all experiments. The results from trial 1 for performance are shown in table 3. Reduction or withdrawal of the dietary vitamin premix from the ration between 29-35 d had no significant impact on ADFI, BWG and FCR, whereas broilers fed a diet without vitamin premix (T1) at 36-42 and 29-42 days of age had poorer performance as compared to those receiving the vitamin premix ( $P < 0.05$ ). Reduction or withdrawal of the dietary vitamin premix from the ration between day 29 and 35 had no significant impact on carcass yield, breast and thigh weight, but vitamin premix withdrawn (T1) between 36-42 d has a significantly negative effect on carcass yield, breast and thigh weight (table 4).

**Trial 2.** The results from trial 2 for ADFI, BWG, and FCR are shown in table 5. Vitamin premix reduction or withdrawal at different ages did not significantly affect ADFI, BWG or FCR ( $P > 0.05$ ). Reduction or withdrawal of the dietary vitamin premix from the ration between day 29 and 42 had no significant impact on carcass yield, breast and thigh weight (table 6).

**Immune system competence.** The effect withdrawal or reduction of vitamin premix from diets of finisher on total anti-SRBC, IgG and IgM antibody titers is shown in table 7. In both experiments, humoral immunocompetence response (IgM, IgG and anti-SRBC titers) were not affected by different treatments. The bursa of fabricius and spleen weights were not significant different in chicks fed diets with various levels of vitamin premix in experiments 1 and 2 (tables 4 and 6). In both experiments, Cellular immune response (DNCB challenge and phytohemagglutinin-M) were not affected by different

Table 3. Vitamin premix reduction or withdrawal effects on performance; Trial 1

| Treatments       | 29-35 d           |                  |                  | 36-42 d             |                    |                   | 29-42 d             |                    |                   | BW42d <sup>4</sup>  |
|------------------|-------------------|------------------|------------------|---------------------|--------------------|-------------------|---------------------|--------------------|-------------------|---------------------|
|                  | ADFI <sup>1</sup> | BWG <sup>2</sup> | FCR <sup>3</sup> | ADFI                | BWG                | FCR               | ADFI                | BWG                | FCR               |                     |
| T1               | 138.38            | 80.75            | 1.72             | 153.83 <sup>b</sup> | 63.58 <sup>b</sup> | 2.42 <sup>b</sup> | 146.33 <sup>b</sup> | 72.17 <sup>b</sup> | 2.03 <sup>b</sup> | 2140.3 <sup>b</sup> |
| T2               | 139.51            | 79.66            | 1.75             | 174.77 <sup>a</sup> | 93.46 <sup>a</sup> | 1.87 <sup>a</sup> | 157.14 <sup>a</sup> | 86.57 <sup>a</sup> | 1.82 <sup>a</sup> | 2332.6 <sup>a</sup> |
| T3               | 139.59            | 81.27            | 1.72             | 172.04 <sup>a</sup> | 94.71 <sup>a</sup> | 1.82 <sup>a</sup> | 155.81 <sup>a</sup> | 87.99 <sup>a</sup> | 1.77 <sup>a</sup> | 2354.9 <sup>a</sup> |
| T4               | 139.23            | 83.24            | 1.67             | 179.65 <sup>a</sup> | 97.83 <sup>a</sup> | 1.84 <sup>a</sup> | 159.44 <sup>a</sup> | 90.53 <sup>a</sup> | 1.76 <sup>a</sup> | 2377.1 <sup>a</sup> |
| SEM <sup>5</sup> | 2.52              | 4.63             | 0.05             | 5.11                | 4.32               | 0.01              | 4.52                | 2.35               | 0.05              | 29.58               |

<sup>1</sup>ADFI= average daily feed intake (g).

<sup>2</sup>BWG= body weight gain (g).

<sup>3</sup>FCR= feed conversion ratio (g/g).

<sup>4</sup>BW42d= body weight 42 day (g).

<sup>5</sup>Standard error of the mean

Means in each column not bearing a common superscript differ significantly ( $P < 0.05$ ).

Table 4. Vitamin premix reduction or withdrawal effects on carcass composition (g) and lymphoid organs weight (g); Trial 1

| Treatments | 35 d          |              |             |       |        | 42 d                 |                     |                     |       |        |
|------------|---------------|--------------|-------------|-------|--------|----------------------|---------------------|---------------------|-------|--------|
|            | Carcass yield | Breast yield | Thigh yield | Bursa | Spleen | Carcass yield        | Breast yield        | Thigh yield         | Bursa | Spleen |
| T1         | 1176.00       | 371.40       | 321.00      | 2.22  | 2.25   | 1498.00 <sup>b</sup> | 460.90 <sup>b</sup> | 419.80 <sup>b</sup> | 2.39  | 2.06   |
| T2         | 1170.00       | 407.00       | 324.90      | 2.18  | 2.55   | 1633.00 <sup>a</sup> | 573.30 <sup>a</sup> | 493.10 <sup>a</sup> | 2.47  | 2.10   |
| T3         | 1180.00       | 372.20       | 318.50      | 2.19  | 2.30   | 1648.00 <sup>a</sup> | 557.70 <sup>a</sup> | 485.30 <sup>a</sup> | 2.40  | 1.86   |
| T4         | 1184.00       | 382.30       | 323.80      | 2.21  | 2.30   | 1664.00 <sup>a</sup> | 573.10 <sup>a</sup> | 497.20 <sup>a</sup> | 2.61  | 2.15   |
| SEM        | 17.02         | 21.00        | 6.30        | 0.04  | 0.17   | 16.23                | 27.78               | 17.40               | 0.13  | 0.16   |

Means in each column not bearing a common superscript differ significantly ( $P < 0.05$ ).

Table 5. Vitamin premix reduction or withdrawal effects on performance; Trial 2

| Treatments | 29-35 d |        |        | 36-42 d |      |                      | 29-42 d             |                     |      | BW42d |
|------------|---------|--------|--------|---------|------|----------------------|---------------------|---------------------|------|-------|
|            | ADFI    | BWG    | FCR    | ADFI    | BWG  | FCR                  | ADFI                | BWG                 | FCR  |       |
| T1         | 1176.00 | 371.40 | 321.00 | 2.22    | 2.25 | 1498.00 <sup>b</sup> | 460.90 <sup>b</sup> | 419.80 <sup>b</sup> | 2.39 | 2.06  |
| T2         | 1170.00 | 407.00 | 324.90 | 2.18    | 2.55 | 1633.00 <sup>a</sup> | 573.30 <sup>a</sup> | 493.10 <sup>a</sup> | 2.47 | 2.10  |
| T3         | 1180.00 | 372.20 | 318.50 | 2.19    | 2.30 | 1648 <sup>a</sup>    | 557.7 <sup>a</sup>  | 485.30 <sup>a</sup> | 2.40 | 1.86  |
| T4         | 1184.00 | 382.30 | 323.80 | 2.21    | 2.30 | 1664.00 <sup>a</sup> | 573.10 <sup>a</sup> | 497.20 <sup>a</sup> | 2.61 | 2.15  |
| SEM        | 17.02   | 21.00  | 6.30   | 0.04    | 0.17 | 16.23                | 27.78               | 17.40               | 0.13 | 0.16  |

Table 6. Vitamin reduction or withdrawal effects on carcass composition (g) and lymphoid organs weight (g); Trial 2

| Treatments | 35 d          |              |             |       |        | 42 d          |              |             |       |        |
|------------|---------------|--------------|-------------|-------|--------|---------------|--------------|-------------|-------|--------|
|            | Carcass yield | Breast yield | Thigh yield | Bursa | Spleen | Carcass yield | Breast yield | Thigh yield | Bursa | Spleen |
| T1         | 1205.00       | 353.49       | 306.83      | 2.24  | 1.96   | 1655.00       | 569.36       | 479.32      | 2.50  | 1.85   |
| T2         | 1214.00       | 385.95       | 330.20      | 2.23  | 2.03   | 1673.00       | 586.90       | 496.23      | 2.58  | 2.08   |
| T3         | 1218.00       | 375.60       | 305.62      | 2.15  | 2.16   | 1663.00       | 574.08       | 482.19      | 2.78  | 1.89   |
| T4         | 1211.00       | 372.90       | 315.15      | 2.22  | 2.00   | 1677.00       | 600.45       | 499.86      | 2.29  | 2.36   |
| SEM        | 8.03          | 10.15        | 8.47        | 0.03  | 0.11   | 19.06         | 11.34        | 9.87        | 0.09  | 0.12   |

Table 7. Effect of experimental both on IgM and IgG anti-SRBC titers\*; Trials 1 and 2

| Treatments | Trail 1 |      |      | Trail 2 |      |      |
|------------|---------|------|------|---------|------|------|
|            | SRBC    | IgG  | IgM  | SRBC    | IgG  | IgM  |
| T 1        | 6.00    | 2.50 | 3.50 | 7.75    | 2.50 | 5.25 |
| T 2        | 5.75    | 2.25 | 3.50 | 5.75    | 2.25 | 3.50 |
| T 3        | 6.50    | 2.50 | 4.00 | 7.00    | 2.75 | 4.25 |
| T 4        | 7.00    | 2.75 | 4.25 | 6.75    | 2.75 | 4.00 |
| SEM        | 0.70    | 0.30 | 0.40 | 1.07    | 0.25 | 0.80 |

\*Data expressed as log<sub>2</sub>

Table 8. Vitamin reduction or withdrawal effects on cell-mediated immunity by response of skin to DNCB and toe web swelling by PHA; Trials 1 and 2

| Treatments | Increase in skin thickness (%) to PHA and DNCB in trail 1 |              |               | Increase in skin thickness (%) to PHA and DNCB in trail 2 |               |               |
|------------|---|--------------|---------------|---|---------------|---------------|
|            | PHA   | NCB (35 day) | DNCB (42 day) | PHA   | DNCB (35 day) | DNCB (42 day) |
|            | T 1   | 0.55         | 1.45          | 0.86  | 0.51          | 1.68          |
| T 2        | 0.53  | 1.40         | 0.83          | 0.54  | 1.60          | 0.85          |
| T 3        | 0.54  | 1.44         | 0.76          | 0.49  | 1.58          | 0.79          |
| T 4        | 0.48  | 1.35         | 0.72          | 0.50  | 1.55          | 0.80          |
| SEM        | 0.071   | 0.067        | 0.048         | 0.024   | 0.084         | 0.032         |

treatments (table 8).

### Discussion

*Performance. Trial 1.* The findings of this study differ from those reported by Khajali *et al.* (2006) Alahyari-Shahrab *et al.* (2011) and Alahyari-Shahrab *et al.* (2012a,b) as they showed that vitamin premix withdrawal from the finisher diet of broiler chickens did not affect the performance. But the findings of this study were similar to the reports of Deyhim and Teeter (1993) and Alahyari-Shahrab *et al.* (2012c). Deyhim and Teeter (1993) demonstrated that broiler chickens reared in batteries under a cycling ambient temperature (24 to 35 °C, creating heat stress), and fed diets without vitamin and mineral premix had lower weight gains and poorer feed conversion as compared to the birds fed normally supplemented diets; Moreover results of carcass yield and breast and thigh weight confirm the results reported by Deyhim and Teeter (1993) and Alahyari-Shahrab *et al.* (2012a). It seems that birds in cages require more dietary vitamins than those on floor housing because of their limited access to feces (coprophagia) and they must endure more stress for storage in the cage (Alahyari-Shahrab *et al.* 2012c).

*Trial 2.* These findings were similar with those reported by Skinner *et al.* (1992) and Alahyari-Shahrab *et al.* (2012a,b), as they showed that vitamin premix withdrawal to the finisher diet of broiler chickens did not affect performance. Skinner *et al.* (1992) suggested that the lack of a withdrawal effect could be related to the availability in the body of vitamins and minerals for further growth, as the amount of these supplements usually exceeds two or three times the recommended broiler chicken requirements in poultry diets. In opposition, omitting vitamin from the finisher diet for the same period decreased weight gain in three different broiler strains (Patel *et al.* 1997; Maiorka *et al.* 2002 and Alahyari-Shahrab *et al.* 2012c). These differences may be due to the type of rearing system (floor litter or cages) or differences in diet composition. Maiorka *et al.* (2002) suggested that withdrawal of vitamin mix during the final period of broiler chicken growth is more deleterious than withdrawal of mineral mix, because its affect on FCR, but carcass yield was not affected by withdrawal of vitamin or mineral mix. Alahyari-Shahrab *et al.* (2012c) demonstrated that birds reared in cages were slightly more sensitive to vitamin premix reduction/withdrawal, probably due to the impracticality of performing coprophagy. Results of carcass yield and breast and thigh weight verify the results obtained by Maiorka *et al.* (2002) and Alahyari-Shahrab *et al.* (2011).

Based on the results of trial 1 and 2 it seems that, it is possible to reduce vitamin premix in the finisher broiler diets (up to 33%) in 29-42 (T2), without any adverse effect on the performance of broilers reared in cages, while in the floor system it can be possible to withdraw vitamin supplements in the finisher broiler diets.

*Immune system competence.* Research regarding the effect of water-soluble vitamins nutrition on the immunological response of avian species has been limited to a few number of water-soluble vitamins, mostly vitamins C and B6, but the results have been conflicting (Gay and Meydani, 2002 and Hesabi Nameghi *et al.*, 2007). More work has been done on the effect of vitamin E nutrition on immunity. The effect of vitamin E on immunocompetence of chickens is well known (Niu *et al.* 2009).

In practice, nutritionists do not take into account the vitamins and trace minerals supplied from the natural feedstuffs (Alahyari-Shahrab *et al.* 2011). Consequently, the bird's requirements for these nutrients are possibly met from natural feedstuffs as well as body reserves during a short-term vitamin premix withdrawal (Alahyari-Shahrab *et al.* 2012a). It has been demonstrated that the immune system has a higher priority for circulating nutrients and is able to compete favorably with other tissues when nutrient levels are low (Klasing, 1998). In this experiment, there was no effect on immune response as indicated by total anti-SRBC, IgG, IgM antibody titers, DNCB challenge and phytohemagglutinin-M. These results agree with those reported by Deyhim and Teeter (1993), Khajali *et al.* (2006) and Alahyari-Shahrab *et al.* (2011; 2012b). These findings suggest that the vitamin premix contents of the finishing diet were sufficiently high to maintain a humoral immune response. Results of bursa of Fabricius and spleen weights of this study differ slightly from those reported by Alahyari-Shahrab *et al.* (2011; 2012b), as they showed that vitamin premix withdrawal from the finisher diet of broiler chickens did not affect lymphoid organs weight.

It should be emphasized that removal of vitamin premix from broiler diets does not imply that such diets are void of these essential nutrients. Unfortified diets, especially those that contain some animal protein feedstuff, may contain quantities of vitamins sufficient to meet or exceed minimum recommended needs (Alahyari-Shahrab *et al.* 2011; 2012a). Vitamin premixes used in the commercial broiler industry typically provide vitamin premix at two to fourfold or more of the minimum recommended levels (Gwyther *et al.* 1992); thus, some storage within the carcass should be expected, especially for the fat-soluble vitamins. Under commercial growing conditions, using practical feedstuffs, it may be difficult to produce vitamin premix deficiencies in birds during the finishing period following adequate supplementation early in the growing period.

In conclusion, the results of the present study indicated that in the battery cage system it's possible to reduce dietary vitamin premix during finisher period but withdrawal can negatively affect performance of broiler chickens. While in the floor system it can be possible to withdraw vitamin supplements in finisher broilers' diets.

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