Effects of different levels of trace minerals premix in finisher diets on performance, immune system and meat lipid oxidation of chicken fed barley- or wheat-based diet

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ABSTRACT

The present study was carried out to examine the effects of a trace mineral premix (MP) reduction or withdrawal from finisher diet (29–42 d) on performance, meat lipid oxidation, and immune system of chicks fed wheat- or barley-based diet. The diets were formulated based on wheat and barley for seven treatments and five replicates of each treatment. At 29 and 36 d, 4 birds from each replicate were injected with sheep red blood cells. The cell-mediated immunity was determined via phytohemagglutinin and dinitrochlorobenzene at 34 and 42 d of age. At 35 and 42 d of age, the oxidative stability was evaluated by thiobarbituric acid reactive substances on the thigh samples that were stored for 180 days at −20°C. The reduction or withdrawal of MP from diets did not affect the performance or the immune system. Results of TBARS showed that lipid peroxidation of the treatment without MP was significantly higher than of the other treatments when slaughtered at 42 days of age. Finally, the results of this study demonstrated that it is not possible to remove the MP in finisher broilers’ diets without negative effects on meat quality during the time of freezing.

1. Introduction

Minerals have important biological functions, and the requirements of broiler chickens have to be met for optimum growth and performance. NRC (1994) gives the minimum levels that are necessary for optimum productivity (Maiorka et al. 2002). In practice, food manufacturers use much higher concentrations than those specified by NRC (1994) (Jafari Sayadi et al. 2005). For this reason, mineral deficiencies are not commonly observed. The mineral requirements are determined in specially selected, appropriately managed and sufficiently fed animals. These are minimal requirement levels for maximal weight gain and performance; however, factors such as environmental variation and nutrients–mineral interactions are not considered in the determination of requirements. Thus, in practice, food manufacturers, nutritionists and producers use twice to 10 times more of these nutrients than stated requirements (Inal et al. 2001).

Trace mineral deficiencies have been shown to suppress immunocompetence (Khajali et al. 2006). Therefore, the response of the immune system needs to be considered when studying the effect of mineral premix (MP) withdrawal. Duration of removal period, different levels of MP, meat quality, and immunocompetence can be important factors in these kinds of studies. Moreover, the effect of selenium (Se) on meat quality of chickens is well known (Scholik et al. 2004).

There are several reports about MP withdrawal in broilers’ diets based on corn diets (Christmas et al. 1995; Maiorka et al. 2002; Khajali et al. 2006), but there is a lack of reports on the effect of withdrawal or reduction of MP in finisher diets of broilers based on wheat and barley. Indeed, several reasons should be taken into account: (1) the instability of world corn prices increases the propensity of producers to use wheat and barley instead of corn in the diet; (2) differences between mineral content in wheat, barley and corn and (3) lack of reports about effect of withdrawal or reduction of MP on the meat lipid oxidation.

Therefore, this study was carried out to evaluate the effects of reduction or withdrawal of the MP from broiler diets during finisher period on performance, immune system and meat lipid oxidation.

2. Materials and methods

2.1. General procedure

Birds and housing: the average initial body weight of day-old chicks in each pen was 42 ± 2 g. Room temperature was kept at 34°C during the first 3 d 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. Chicks were raised until 29 d of age and fed on commercial starter and grower diets that met their nutrient requirements (Ross 308, 2007; Table 1), as described in the general procedure, weighed (1125 ± 9.6 g) and distributed at random into pens with 7 treatments with 5 repetitions per treatment and 20 birds per floor pen replicate. The dietary treatments were: (T1) without MP, during 29–42 d of age; (T2) 33% MP, during 29–42 d of age; (T3) 33% MP, during 29–35 d of age and without MP during 36–42 d of age; (T4) 66% MP, during 29–42 d of age; (T5) 66% MP, during 29–35 d of age and without MP during 36–42 d of age; (T6) 100% MP, during...
Table 1. Composition of the starter and grower diets used in the pre-experimental.

| Ingredients               | Composition (g/kg) |
|---------------------------|--------------------|
|                          | Starter diet (1–10 d) | Grower diet (11–28 d) |
| Wheat                     | 332.0               | 345.2               |
| Barley                    | 325.0               | 306.2               |
| Soya bean meal (440 g/kg CP) | 222.5             | 254.0               |
| Corn gluten meal          | 72.0                | 40.4                |
| Soya oil                  | 14.8                | 16.9                |
| Oyster shell              | 13.0                | 13.7                |
| Dicalcium phosphate       | 10.5                | 10.6                |
| Vitamin premix           | 2.5                 | 2.5                 |
| Trace mineral premix      | 2.5                 | 2.5                 |
| Sodium chloride           | 2.8                 | 2.8                 |
| DL-methionine             | 0.6                 | 2.1                 |
| L-lysine-HCl              | 1.3                 | 2.6                 |
| Multi enzyme (Rovabio®)   | 0.5                 | 0.5                 |

Calculated composition

| ME (Kcal/kg) | 2900 | 2980 |
| Analyses CP (%) | 20.65 | 20.15 |
| Met (%) | 0.50 | 0.45 |
| Met + Cys (%) | 1.02 | 0.90 |
| Lys (%) | 1.37 | 1.17 |
| Na (%) | 0.15 | 0.15 |
| Ca (%) | 1.01 | 0.85 |
| Available P (%) | 0.47 | 0.42 |

**Note:**
- a2.5 kg of vitamin premix contained: 2700 mg retinal, 400 mg calcidiol, 18 g tocopheryl acetate, 2000 mg menadione, 1800 mg thiamine, 6600 mg riboflavin, 10 g niacin, 30 g calcium pantothenate, 3 g pyridoxine, 1 g folic acid, 15 mg cobalamin, 250 g choline chloride, 100 mg biotin.
- b2.5 kg of trace mineral premix contained: 100 g Mn, 50 g Fe, 100 g Zn, 10 g Cu, 1 g I, 200 mg Se.
- cThese enzyme contained mainly β-glucanase and xylanase activities. The endo-1,3-β-glucanase 100 AGL/kg diet and endo-1,4-β-xylanase 70 AXC units/kg diet.

29–42 d of age and (T7) 100% MP, during 29–35 d of age and without MP during 36–42 d of age. Mash feed and water were available for ad libitum consumption. Prior to formulation, all major dietary ingredients were analysed for AMEn, amino acid profiles (according to prediction formula existing in NRC), crude protein, crude fibre and ether extract contents as described by (AOAC 2005). The components and composition of diet presented during the experimental period is presented in Table 2.

2.2. Production performance

Mortality during 29–42 d was determined for each pen. Body weight gain (BWG) and feed intake (FI) of chickens were determined at 35 and 42 d of age, and then feed conversion ratio (FCR) was calculated from these data.

Lymphoid organs weight: at 35 and 42 d of age, 2 birds of each replicate in the experiment, with similar average weight, were selected and slaughtered. The relative weight of the lymphoid organs (bursa of fabricius and spleen) was measured to the nearest 0.01 g.

2.3. Humoral immune response

The sheep red blood cells (SRBC) were used as B-dependent antigens to quantify the antibody response. In the trial, 4 birds were selected from each of the replicated groups (20/treatment) and were injected intravenously with SRBC (1% suspension in PBS, 0.1 mL/chick) at 29 d of age followed by a booster injection of SRBC suspension 7 d after the first injection. Blood samples were collected at 6 d after the first injection and again at 7 d post booster. The serum from each sample was collected; heat inactivated at 56°C for 30 min and then analysed for total anti-SRBC antibodies as previously described (Qureshi & Havenstein 1994). Briefly, 50 μL 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 serum dilution was made (1:2), and 50 μL of 2% SRBC suspension was added to each well. Total antibody titres 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 titre for agglutination. For MER-IgG response, 25 μL of 0.02 M mercaptoethanol in PBS was used instead of PBS alone, followed by the previous mentioned procedure. The difference between the total and IgG response was considered to be equal to the IgM antibody level (Cheema et al. 2003).

Dinitrochlorobenzene (DNCB) challenge: at 34 and 42 d of age, 1-chloro-2, 4-dinitrobenzene (DNCB, Merck) solution (10 mg/mL) was spread and maintained over a 10 cm² area of featherless skin (0.25 mL) on the right side of the 2 birds per pen. A similar position on the left side of the bird was treated with a distinct SRBC button was considered as the endpoint titre for agglutination. For MER-IgG response, 25 μL of 0.02 M mercaptoethanol in PBS was used instead of PBS alone, followed by the previous mentioned procedure. The difference between the total and IgG response was considered to be equal to the IgM antibody level (Cheema et al. 2003).

Table 2. Compositions of the diets used during the experimental period (29–42 d of age) in experiment.

| Ingredients               | Treatment 1* | Treatment 2/3 | Treatment 4/5 | Treatment 6/7 |
|---------------------------|--------------|---------------|---------------|---------------|
| Wheat                     | 385.5        | 383.9         | 382.6         | 381.2         |
| Barley                    | 280.0        | 280.0         | 280.0         | 280.0         |
| Soybean meal (44%)        | 276.1        | 275.5         | 276.2         | 276.7         |
| Soybean oil               | 30.0         | 30.0          | 30.0          | 30.0          |
| Dicalcium phosphate       | 12.5         | 12.4          | 12.4          | 12.4          |
| Vitamin premix           | 2.5          | 2.5           | 2.5           | 2.5           |
| Mineral premix            | 0.0          | 0.8           | 1.6           | 2.5           |
| Sodium chloride           | 2.8          | 2.8           | 2.8           | 2.8           |
| DL-methionine             | 1.7          | 1.8           | 1.7           | 1.7           |
| L-lysine-HCl              | 0.7          | 0.7           | 0.7           | 0.7           |
| Multi enzyme (Rovabio®)   | 0.5          | 0.5           | 0.5           | 0.5           |

Calculated composition

| ME (Kcal/kg) | 3050 | 3050 | 3050 | 3050 |
| CP (%)       | 20.00 | 20.00 | 20.00 | 20.00 |
| Met (%)      | 0.39  | 0.39  | 0.39  | 0.39  |
| Met + Cys (%)| 0.82  | 0.82  | 0.82  | 0.82  |
| Lys (%)      | 1.04  | 1.04  | 1.04  | 1.04  |
| Na (%)       | 0.14  | 0.14  | 0.14  | 0.14  |
| Ca (%)       | 0.81  | 0.81  | 0.81  | 0.81  |
| Available P (%) | 0.40 | 0.40 | 0.40 | 0.40 |

**Note:**
- a(T1) Without MP, during 29–42 d of age; (T2) 33% MP, during 29–42 d of age; (T3) 33% MP, during 29–35 d of age and without MP during 36–42 d of age; (T4) 66% MP, during 29–42 d of age; (T5) 66% MP, during 29–35 d of age and without MP during 36–42 d of age; (T6) 100% MP, during 29–42 d of age and (T7) 100% MP, during 29–35 d of age and without MP during 36–42 d of age.
- b2.5 kg of vitamin premix contained: 2700 mg retinal, 400 mg calcidiol, 18 g tocopheryl acetate, 2000 mg menadione, 1800 mg thiamine, 6600 mg riboflavin, 10 g niacin, 30 g calcium pantothenate, 3 g pyridoxine, 1 g folic acid, 15 mg cobalamin, 250 g choline chloride, 100 mg biotin.
- c2.5 kg of trace mineral premix contained: 100 g Mn, 50 g Fe, 100 g Zn, 10 g Cu, 1 g I, 200 mg Se.
- dThese enzyme contained mainly β-glucanase and xylanase activities. The endo-1,3(4)-β-glucanase 100 AGL/kg diet and endo-1,4-β-xylanase 70 AXC units/kg diet.
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 at 34 and 42 d of age (Karimi Torshiz et al. 2010). The skin swelling was calculated as the difference between the thickness of the skin before and after DNCB treatment as 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 two birds’ experiment group at 34 and 42 d of age. The increase in toe web thickness was measured 24 h after injection (Corrier & Deloach 1990).

Thiobarbituric acid reactive substances (TBARS) value: the two thighs from each replicate in 2 slaughtered (35 and 42 d) were stored in separate oxygen permeable plastic bags at –20°C until required for chemical analysis from 180 d. The extent of lipid oxidation in thighs was assessed by measuring TBARS according to the method described by Cortinas et al. (2005), using third derivative spectrophotometry. The height of the third-order derivative peak that appeared at approximately 521.5 nm was used for calculation of the MDA concentration in the samples. Tetraethoxypropane was used as an MDA precursor in the standard curve. TBARS was expressed as micrograms of MDA per kilogram of sample.

### 2.4. Statistical analysis

The data were subjected to analysis of variance as a completely randomized design using the general linear model procedure of SAS software (SAS Institute 2002). Anti-SRBC titres and lymphoid organ weights data were transformed to log2 and arc sin, respectively. Means were separated by Duncan’s multiple range test at the significance level of P < .05.

### 3. Results and discussion

Performance: mortality for all groups was within the expected range and there was no significant difference in mortality of all treatments. The results from FI, BWG and FCR are shown in Table 3. Reduction or withdrawal of the MP at different ages did not significantly affect FI, BWG or FCR (P > .05). The findings of this study were similar to those reported by Skinner et al. (1992) and Maiorka et al. (2002), as they showed that vitamin and MP withdrawal from 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 requirement in poultry diets. In opposition, omitting MP from the finisher diet for the same removal period decreased weight gain in three different broiler strains (Deyhim & Teeter, 1993; Patel et al. 1997; Jafari Sayadi et al. 2005). These differences may be due to the type of rearing system (floor litter or cages) or differences in the diet composition.

It should be emphasized that the removal of MP 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 the minimum recommended needs. MP used in the commercial broiler industry typically provides MP at two to fourfold or more of the minimum recommended levels (Gwyther et al. 1992); thus, some storage within the carcass should be expected. Under commercial growing conditions, using practical feedstuffs, it may be difficult to produce MP deficiencies in birds during the finishing period following adequate supplementation early in the growing period. Reduction of these supplements in diets fed from 29 to 42 d of age could significantly reduce growing costs with no adverse effects on performance.

Immune competence: the effect of MP reduction or withdrawal on lymphoid organs weights and humoral immune system response (total anti-SRBC antibody, IgG, IgM titres) are given in Tables 4 and 5. In the experiment, the humoral immune response (total anti-SRBC antibody, IgG, IgM titres) was not affected by the different treatments (P > .05). The bursa of fabricius and spleen weights were not significantly different in chicks fed diets with various levels of MP in the experiment (P > .05). In the experiment, cellular immune response (DNBC challenge and phytohemagglutinin-M) were not affected by different treatments at 34 and 42 d of age (P > .05; Table 6).

These results agree with those reported by Deyhim and Teeter (1993) and Khajali et al. (2006). Our findings suggest that the mineral contents of the finisher diet were sufficiently high to maintain an adequate humoral immune response or that the mineral contents of corn and soybean meal diet were sufficiently high to compensate the lack of a MP during 29–42 d post-hatching. Research regarding the effect of trace mineral nutrition on the immunological response of avian species has been limited to a small number of trace elements, mostly selenium and zinc, but the results have been conflicting.

| Treatments | Feed intake (g) | Body weight (g) | FCR (g/g) | Feed intake (g) | Body weight (g) | FCR (g/g) |
|------------|----------------|----------------|----------|----------------|----------------|----------|
| T 1        | 144.61         | 86.78          | 1.66     | 168.76         | 86.47          | 1.95     |
| T 2        | 145.54         | 85.82          | 1.70     | 169.40         | 96.04          | 1.80     |
| T 3        | 143.22         | 82.87          | 1.73     | 169.07         | 88.66          | 1.91     |
| T 4        | 145.86         | 86.93          | 1.68     | 171.28         | 92.69          | 1.85     |
| T 5        | 146.56         | 86.09          | 1.70     | 169.05         | 93.59          | 1.81     |
| T 6        | 145.14         | 87.30          | 1.66     | 171.89         | 93.53          | 1.84     |
| T 7        | 145.36         | 85.11          | 1.71     | 172.02         | 91.43          | 1.88     |
| SEM        | 2.23           | 1.96           | 0.04     | 3.12           | 4.92           | 0.07     |

*feed conversion efficiency.

| Treatments | Total anti-SRBC (log HA) | IgG (log HA) | IgM (log HA) | IgG/IgM |
|------------|--------------------------|-------------|-------------|---------|
| T 1        | 3.81                     | 4.75        | 3.25        | 1.16    |
| T 2        | 3.95                     | 4.84        | 3.20        | 1.28    |
| T 3        | 4.00                     | 4.96        | 3.71        | 1.32    |
| T 4        | 3.96                     | 4.92        | 3.41        | 1.26    |
| T 5        | 3.93                     | 4.87        | 3.37        | 1.2    |
| T 6        | 4.01                     | 4.98        | 3.43        | 1.26    |
| T 7        | 3.95                     | 4.91        | 3.27        | 1.28    |
| SEM        | 0.11                     | 0.12        | 0.09        | 0.08    |

Table 3. Mineral premix reduction or withdrawal effects on feed intake, body weight and FCR* at 29–42 d of age.

Table 4. Total anti-SRBC antibody, IgG, IgM titres (log HA) and IgG/IgM ratio by HA method on 34 and 42 d of age of broilers.
Lipid oxidation of poultry meat (Ryu et al. 2005; Rife et al. 2006) and discolouration are believed to be major causes of the deterioration in meat quality during refrigeration (Ryu et al. 2006). Results of TBARS values showed that there were no significant differences between TBARS values of thigh samples for birds slaughtered at 35 d of age. However, TBARS values of treatment without MP were significantly higher than that of the other treatments in the birds slaughtered at 42 d of age ($P < .05$; Table 7). Selenium is recognized as an essential trace element that plays an important role in antioxidative system efficiency or as a component of Se-dependent glutathione peroxidase (Yoon et al. 2007). Adding Se to poultry diets can provide as a component of Se-dependent glutathione peroxidase plays an important role in antioxidative system efficiency or maintain a humoral immune response when the grower diet was not fortified and is able to compete favourably with other tissues when nutrient levels are low (Klasing 1998).

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4. Conclusions
The results of this study demonstrated that it is not possible to entirely withdraw, but that it is possible to reduce the mineral supplements in finisher broilers’ diets without negative effects on performance, immune system and meat quality during the time of freezing.

Table 5. Mineral premix reduction or withdrawal effects on lymphoid organs’ weight.

| Treatments | Bursa (g/kg) | Spleen (g/kg) | Bursa (g/kg) | Spleen (g/kg) |
|------------|-------------|---------------|-------------|---------------|
| T 1        | 1.33        | 1.10          | 0.98        | 1.47          |
| T 2        | 1.28        | 1.12          | 1.00        | 1.50          |
| T 3        | 1.29        | 1.09          | 1.03        | 1.49          |
| T 4        | 1.39        | 1.10          | 1.00        | 1.51          |
| T 5        | 1.30        | 1.13          | 0.99        | 1.55          |
| T 6        | 1.31        | 1.07          | 1.05        | 1.53          |
| T 7        | 1.29        | 1.05          | 1.01        | 1.53          |
| SEM        | 0.06        | 0.05          | 0.04        | 0.05          |

Table 6. Mineral premix reduction or withdrawal effects on cell-mediated immunity by response of skin to DNCB and toe web swelling by PHA.

| Treatments | Increase in skin thickness (%) to PHA and DNCB at 34 d | Increase in skin thickness (%) to PHA and DNCB at 42 d |
|------------|--------------------------------------------------------|--------------------------------------------------------|
|             | PHA | DNCB | PHA | DNCB |
| T 1        | 0.52 | 1.66 | 0.51 | 0.86  |
| T 2        | 0.55 | 1.62 | 0.54 | 0.83  |
| T 3        | 0.54 | 1.58 | 0.49 | 0.76  |
| T 4        | 0.48 | 1.55 | 0.50 | 0.72  |
| T 5        | 0.30 | 1.57 | 0.49 | 0.79  |
| T 6        | 0.53 | 1.54 | 0.52 | 0.80  |
| T 7        | 0.49 | 1.55 | 0.51 | 0.82  |
| SEM        | 0.045 | 0.062 | 0.031 | 0.084 |

Table 7. Mineral premix reduction or withdrawal effects on the oxidative stability of broiler thighs from birds, slaughtered at 35 and 42 d of age.

| Treatments | TBA * 35 d | TBA 42 d |
|------------|------------|----------|
| T 1        | 1.543      | 4.251    |
| T 2        | 1.353      | 1.500    |
| T 3        | 1.368      | 1.250    |
| T 4        | 1.365      | 1.083    |
| T 5        | 1.443      | 1.300    |
| T 6        | 1.394      | 1.267    |
| T 7        | 1.346      | 1.425    |
| SEM        | 0.176      | 0.386    |

$^a$μg of malondialdehyde (MDA)/kg on a dry matter basis.
$^b$Values in the same column within each experiment with different superscripts differ significantly ($P < .05$).

Notes
1. Coming, Corning, NY.
2. Sigma, St. Louis, MO.

Disclosure statement
No potential conflict of interest was reported by the authors.
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