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Effects of Supplementation of Ionized or Chelated Water-Soluble Mineral Mixture on the Live Performance, Nutrient Digestibility, Blood Profile, Egg Quality, and Excreta Microbiota of Laying Hens

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■Keywords

Ionized mineral mixture, laying hen, chelated mineral mixture.

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ABSTRACT

In total, 216 Hy-Line brown laying hens (40-week-old), were used in a 5-week experiment to evaluate the effects of ionized or chelated water-soluble mineral mixture supplementation on live performance, nutrient digestibility, blood characteristics, egg quality, and excreta microbiota. Layers were randomly assigned to one of three dietary treatments with 12 replicates of six adjacent cages each. The dietary treatments consisted of: 1) CON (basal diet + normal tap water), 2) T1 (CON+0.5% ionized mineral mixture in tap water, pH 3.0); and 3) T2 (CON+ 0.5% chelated mineral mixture in tap water, pH 3.0). Egg production tended to increase in week 1, week 3 and week 4 in the birds supplemented with T1 and T2 diet compared with CON. Moreover, the dietary supplementation of water-soluble mineral mixture improved ($p=0.02$) eggshell thickness in week 4 and tended to improve in week 5 of the experimental period in T2 hens compared with CON. The layers fed the T1 diet presented higher ($p<0.05$) Ca digestibility than CON hens, while the T2 diet promoted a numerical increase in Ca digestibility. Blood calcium concentration increased ($p<0.05$) with T1 and T2 treatments compared with CON. The laying hens fed the T1 and T2 diets tended to present lower *Salmonella* and *E. coli* counts isolated from excreta compared with CON. In conclusion, ionized and chelated mineral mixtures had a positive impact on production performance and eggshell quality, improved Ca digestibility and blood Ca level.

INTRODUCTION

The knowledge of the factors that affect the performance and quality of the eggshells produced by commercial layers is crucial for the production of high quality eggs. Eggshell influences the economic profitability of egg production and hatchability. Eggs with damaged eggshells have been estimated to account for 6-10% of all eggs produced, which causes significant economic losses (Roland, 1988). Therefore, mineral nutrition and supplementation have been considered for the improvement of egg quality. However, the effectiveness of minerals to improve animal performance depends on their bioavailability.

For instance, ionized minerals are considered to be readily available to the body for absorption. The ionization of minerals means that the mineral has either negative or positive charge. Minerals in ionic state carry an electric charge, which allows them to readily bind with water, resulting in their easy absorption in the body.

Also, chelated minerals are said to be more bioavailable than inorganic salts because inorganic salts are reported to rapidly dissociate and freely interact with antagonists, which leads to their loss prior to absorption (Henry *et al*., 1992; Ward *et al*., 1996). However, organic mineral complexes, formed by the chelation of mineral with organic acids, amino acids or peptides, prevent minerals from interacting with

antagonists because the minerals are bound to organic ligands through covalent bonds, which improves their bioavailability (Ward *et al*., 1996). The acidic status of the small intestine is necessary for the ionization of dissolved minerals (Tim O'Shea, 1998). Chelation involves the binding of a mineral ion to another component, which is usually an organic compound such as amino acids, protein, organic acids, etc.

Broilers fed chelated zinc (Zn), copper (Cu), and manganese (Mn) showed better bone strength, skin integrity, and immune response than inorganic trace minerals (Decoux *et al*., 2013). Minerals have a strong influence on egg quality of laying hens. The study performed by Ceylan & Scheideler (1999) showed that organic Zn is associated with better activity of the enzyme carbonic anhydrase, increasing eggshell quality, and Mn promotes eggshell calcification and strength.

Organic trace mineral supplementation to breeder and layer diets was shown to improve hatchability, albumen quality, and eggshell thickness (Rutz *et al*., 2004); however, there is less information in literature on eggshell formation. We hypothesized that ionized or chelated water-soluble mineral complex, a novel product obtained by mixing ionized minerals with organic acids and a natural extract produced from some plants, such as wild grapes and prickly pear, through *Bacillus* fermentation could improve the bioavailability of minerals to poultry and hence improve the production performance of layers.

Therefore, the objective of the present experiment was to determine the effects of ionized or chelated water-soluble mineral mixture on the performance, nutrient digestibility, blood profile, and isolation and enumeration of *Salmonella* and *E. coli.* in the excreta of layers.

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University. All birds used in this trial were handled in accordance with guidelines set forth by the Dankook University Committee on Laboratory Animal Care.

Experimental Design, Birds, Diets and Housing

In total, 216 40-week-old layers (Hy-LineBrown) were housed in a windowless laying house under a photoperiod of 17 h light: 7 h dark at approximately 23°C. Layers were individually housed in cages (20-cm-width × 50-cm length × 40-cm height).

Layers were randomly assigned to one of three dietary treatments with 12 replicates of six adjacent cages each. The dietary treatments consisted of: 1) CON (basal diet + normal tap water, pH 6.1), 2) T1 (CON+0.5% ionized mineral mixture in tap water, pH 3.0); and 3) T2 (CON+ 0.5% chelated mineral mixture in tap water, pH 3.0). All cages were equipped with nipple drinkers and common trough feeders. Water was provided *ad libitum* during the entire 5-week experimental period. The birds were fed experimental diets during the peak of lay (40 to 45 wk of age). All diets used in the present study were formulated to meet or exceed the nutrient recommendations of the NRC (1994) and provided in mash form (Table 1).Birds were housed in the cages for seven days prior to the start of the experiment for habituation, during which the control (CON) diet was supplied.

Table 1 – Basal diet composition (as-fed basis)

Item	
Ingredients (%)	
Corn	50.4
Soybean meal (CP, 46%)	18.7
Wheat grain	10.0
Corn gluten meal	2.0
Wheat bran	5.0
Animal fat	4.4
Limestone	7.5
Dicalcium phosphate	1.4
Salt	0.3
DL-Met (50%)	0.1
Vitamin premix[1]	0.1
Trace mineral premix[2]	0.1
Calculated values	
ME (kcal/kg)	2,904
CP (%)	15.02
Lys (%)	0.78
Met + Cys (%)	0.65
Ca (%)	3.25

[1]Provided per kg of diet: vitamin A, 125,000 IU; vitamin D3, 2,500 IU; vitamin E, 10 mg; vitamin K3, 2 mg; vitamin B1, 1 mg; vitamin B2, 5 mg; vitamin B6, 1 mg; vitamin B12, 15 mg; folic acid, 500 mg; niacin, 35,000 mg; Ca-pantothenate acid, 10,000 mg and biotin, 50 mg.

[2]Provided per kg of diet: 8 mg Mn (as MnO2); 60 mg Zn (as ZnSO4); 5 mg Cu (as CuSO4·5H2O); 40 mg Fe (as FeSO4·7H2O); 0.3 mg Co (as CoSO4·5H2O); 1.5 mg I (as KI), and 0.15 mg Se (as Na2SeO3·5H2O).

Tested product

The ionized or chelated mineral mixture was obtained from a commercial company (Jino Biotech Inc., South Korea). According to the supplier's information, inorganic mineral ions were extracted effectively in ceramics (natural mineral) and the extractions of inorganic ions were maximized using hydrodynamic cavitation as mentioned by Zhou *et al.*

(1997). The chelated mineral mixture was produced by mixing the ionized minerals with a natural extract. This natural extract was obtained by *Bacillus* fermentation of plants, such as wild grapes and prickly pear. This impure mineral mixture was purified using ion exchange membrane filters with micro pores. The water soluble ionized and chelated mineral mixture consisted of Na (1458.0mg), Mg (400mg), K (95.0 mg), Ca (1430.0mg), Cu (200mg), Zn (8.0mg), Fe (389.0mg) , Si (180mg) P (18mg), Mn (28mg) Co (1.2 mg), Al (125), S (44.6mg), V (13mg), and Se (0.3 mg). These minerals were mixed in 1 L water and 0.5% of this water soluble mineral mixture was administered to the laying birds during the experimental period.

Chemical Analysis

Feed samples were ground to pass through a 1-mm screen, after which they were analyzed for N (method 968.06; AOAC, 2000), Ca (method984.01; AOAC, 1995), and P (method 965.17; AOAC, 1995). Lysine was measured using an AA analyzer (Beckman 6300; Beckman Coulter Inc., Fullerton, CA) after a 24-h hydrolysis in HCl (Spackman *et al*., 1958). For the determination of Cys and Met, the samples were oxidized with performic acid overnight at 0°C. Performic acid is an oxidizing reagent that convertsCys quantitatively to cysteic acid and Met to Met sulfone (Moore, 1963). Nitrogen was determined (Kjectec2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as N × 6.25.

Egg production and quality

Hens were allowed *ad libitum* access to feed and water throughout the experimental period. Eggs were collected on a daily basis, and egg production was calculated as total number of eggs producted divided by the total number of days and hens. The rate of cracked eggs was also assessed.

In addition, egg quality was checked in weeks 1, 2, 3, 4 and 5of the experimental period. A total of 36 saleable eggs (no eggshell defects, cracks, or double-yolks) were randomly collected from each treatment (three eggs per replicate) at 17:00 h and were used to determine egg quality at 20:00 h the same day. Eggshell breaking strength (kg/cm^2) was evaluated using an Eggshell Force Gauge Model II (Robotmation Co., Tokyo, Japan). Egg weight, yolk color, yolk height, and Haugh units were evaluated using an Egg Multi Tester (Touhoku Rhythm Co., Tokyo, Japan). Finally, eggshell thickness was measured on the large end, equatorial region, and small end respectively using a Dial Pipe Gauge (Ozaki MFG. Co., Tokyo. Japan).

Nutrient digestibility

Laying hens were fed their respective diets containing chromic oxide (Cr_2O_3 at 0.20% level) for 4 days prior to the collection period to determine nutrient digestibility. Whole excreta collection was performed daily for three days in week 5 and stored at -20°C until further analysis. All excreta and feed samples were analyzed according to the AOAC procedures (AOAC, 2000).

Blood profiles

Blood samples were randomly collected from two layers per replicate (24 layers per treatment) from their wing veins using a sterilized needle at the end of the experiment. Blood samples were then transferred into a K_3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The samples for serum analyses were then centrifuged at 3,000 × g for 15 min, and an aliquot of 4 mL was stored at -4°C until it was analyzed for Ca using colorimetry, and for P, using phosphomolydate, in an automatic biochemistry modular analytic system (PE, Roche, Germany). Red blood cell (RBC), white blood cell (WBC), and lymphocyte counts in whole blood samples were determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

E. coli and *Salmonella* isolation and enumeration

At the end of the experiment, fecal samples were collected from six layers randomly selected from each replicate, pooled, and placed on ice for transportation to the lab. Excreta samples were processed at arrival according to method mentioned by Wang & Kim (2011). One gram of the excreta sample pooled per replication was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Rutherford, NJ) and homogenized. Viable counts of *Salmonella* and *E.coli* in the excreta samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Salmonella-Shigella* agar plates to isolate *E. coli* and *Salmonella*, respectively. The MacConkey agar and *Salmonella Shigella* agar plates were incubated for 24 hat 37 °C under anaerobic conditions. Bacterial colonies were counted immediately after removal from the incubator.

Statistical Analysis

Data were analyzed according to a randomized block design using GLM procedures (SAS Inst. Inc.,

Cary, NC) and significant differences were examined by least significant different (LSD) test. The cage was used as the blocking criterion. Data variability is expressed as standard error of mean (SEM), and a probability level of $p<0.05$ was considered as statistically significant and a probability level of 0.1 or less was considered as tendency.

RESULT AND DISCUSSION

Egg production and quality

As shown in Table 2, egg production tended be higher in the birds supplemented with water soluble ionized or chelated mineral mixture compared with CON in week 1 ($p=0.08$) and week 4 ($p=0.09$). Harbaugh & Sanford (1970) reported egg production per hen housed increased when optimal levels of zinc methionine were supplemented to layer diets. Also, Zn supplementation of 3,000-5,000 ppm from ZnO increased weight and feed intake in weanling pigs (Hahn & Baker, 1993; Carlson *et al*., 1995; Lemieux *et al*., 1995). Pesti & Bakalli (1998) reported the results of two layers' experiments in which egg production linearly increased as supplemental Cu from Cu sulfate pentahydrate increased from 0 to 125 and 250 mg/kg diet. Xavier *et al*. (2004) also reported that that the use of organic Se, Zn. and Mg combinations improved the performance and egg quality of brown layers during second laying cycle. Conversely, Sechinato *et al*. (2006) reported that there was no effect of organic minerals on the performance and egg production compared with inorganic minerals in 48- to 60-week-old layers. Likewise, Fernandes *et al.* (2008) noted that supplementation of organic trace minerals did not affect egg production or quality. The response to mineral supplementation depends on mineral concentration in the basal diet (Payane *et al*. 2005).

Cracked egg rate was not affected by the dietary supplementation with ionized or chelated water-soluble mineral mixture (Table 2). However, Hudson (2004) reported that feeding layers with Zn-amino acid complexes increased egg specific gravity and reduced amount of cracked eggs compared with inorganic Zn sources. Ceylan & Scheideler (1999) also reported that the percentage of cracked eggs during processing was also reduced by the supplementation of zinc and manganese to the layer diets. Eggshell strength is influenced by copper-containing enzymes that provide collagen stability and strength (Underwood, 1977), but no effect on eggshell strength was observed in our study. More research is needed to clarify the effect of ionized or chelated minerals on eggshell strength and rate of cracked eggs.

The effects of ionized or chelated water-soluble mineral mixture on egg quality are shown in Table 3. Layers fed the T2 diet produced ($p<0.05$) thicker eggshells than that compared with CON in week 4. However, Fernandes *et al*. (2008) did not find any positive effect of organic trace minerals on eggshell thickness, specific gravity, or cracked egg percentage. The improvement in eggshell thickness observed in the present experiment may be due to the interaction of trace minerals with calcium crystals during eggshell formation or to the catalytic properties of minerals that stimulate some key enzymes involved in the process of membrane or eggshell synthesis. In the current study, no effects ($p>0.05$) of the treatments were observed on eggshell color, egg weight, yolk color, or eggshell strength during the experimental period.

Table 2 – The effects of ionized or chelated water-soluble mineral mixture on egg production and cracked egg rate in laying hens

Items,%	CON[1]	T1[1]	T2[1]	P-value
Egg production				
41 wk	94.35±0.29	95.43±1.30	95.79±0.60	0.08
42 wk	95.24±0.0	96.03±0.90	96.12±0.90	0.19
43 wk	94.55±0.61	96.63±1.20	96.45±0.59	0.10
44 wk	94.94±0.30	96.03±0.34	95.86±0.59	0.09
45 wk	94.74±0.61	95.64±1.40	96.85±0.91	0.20
Cracked egg rate				
41 wk	1.58±0.95	1.25±0.02	1.24±0.01	0.70
42 wk	1.25±0.0	1.04±1.30	0.95±0.94	0.91
43 wk	0.94±0.31	0.83±0.96	0.92±0.32	0.95
44 wk	1.57±0.32	1.24±0.62	1.24±1.23	0.86
45 wk	1.26±0.01	1.05±1.32	1.2±0.61	0.93

[1]Abbreviation: CON (basal diet + plain tap water), T1 (CON+0.5% ionized mineral mixture in water, pH 3.0) and T2 (CON+ 0.5% cheated mineral mixture in water, pH 3.0).

Table 3 – Effects of ionized or chelated water-soluble mineral mixture on egg quality

Items	CON[1]	T1[1]	T2[1]	P-value
41 wk				
Eggshell color	12.2±1.2	12.7±0.98	12.6±1.23	0.43
Egg weight, g	59.48±3.6	60.18±4.87	59.07±4.03	0.71
Yolk height, mm	8.59±0.75	8.81±0.83	8.66±0.43	0.62
Yolk color	7.65±0.98	8.15±0.93	7.95±0.75	0.22
Haugh Unit	94.85±4.3	94.9±4.3	93.89±3.0	0.67
Eggshell strength, kg/cm^2	3.61±0.55	3.85±0.50	3.79±0.78	0.48
Eggshell thickness, mm^{-2}	39.77±1.6	40.35±2.06	39.87±1.5	0.55
42 wk				
Eggshell color	12.1±1.02	12.7±1.12	12.8±1.32	0.13
Egg weight, g	59.68±2.69	60.61±2.35	59.91±2.21	0.44
Yolk height, mm	8.74±0.48	8.72±0.84	8.58± 0.54	0.67
Yolk color	8.0±0.79	8.25±0.79	8.2±0.83	0.63
Haugh Unit	94.55±1.60	95.37± 1.72	95.07±1.02	0.16
Eggshell strength, kg/cm^2	3.68±0.34	3.676±0.59	3.77±0.55	0.80
Eggshell thickness, mm^{-2}	39.99±1.14	40.48±1.03	40.22±0.88	0.39
43 wk				
Eggshell color	12±0.94	12.6±1.1	12.4±1.31	0.19
Egg weight, g	58.95±2.5	59.83±2.9	60.28±2.81	0.26
Yolk height, mm	8.74±0.39	8.54±0.68	8.59±0.39	0.45
Yolk color	8.6±0.50	8.35±0.59	8.3±0.47	0.14
Haugh Unit	94.26±4.06	95.71±3.91	94.85±2.9	0.51
Eggshell strength, kg/cm^2	3.53±0.36	3.50±0.75	3.65±0.71	0.75
Eggshell thickness, mm^{-2}	39.94±1.32	40.28±1.15	40.46±0.81	0.35
44 wk				
Eggshell color	12.4±0.74	12.7±0.75	12.5±1.23	0.60
Egg weight, g	59.67±3.47	60.54±3.63	60.99±3.68	0.26
Yolk height, mm	8.72±0.28	8.66±0.67	8.64±0.39	0.86
Yolk color	8.5±0.51	8.65±0.58	8.65±0.49	0.62
Haugh Unit	94.3±3.43	94.9±3.78	95.04±4.6	0.74
Eggshell strength, kg/cm^2	3.64±0.42	3.62±0.72	3.76±0.79	0.75
Eggshell thickness, mm^{-2}	39.72^{b}±0.83	40.29^{ab} ±0.75	40.44^{a}±0.68	0.02
45 wk				
Eggshell color	12.4±1.23	12.4±1.35	12.5±1.32	0.97
Egg weight, g	59.82±2.47	60.65±2.99	61.0±3.9	0.37
Yolk height, mm	8.64±0.43	8.78±0.39	8.59±0.40	0.42
Yolk color	8.5±0.76	8.55±0.60	8.65±0.49	0.77
Haugh Unit	95.11±2.97	95.55±2.95	95.33±3.12	0.86
Eggshell strength, kg/cm^2	3.63±0.37	3.71±0.54	3.75±0.84	0.82
Eggshell thickness, mm^{-2}	39.68±2.19	40.33±0.79	40.59±0.55	0.10

[1]Abbreviation: CON (basal diet + normal tap water), T1 (CON+0.5% ionized mineral mixture in water, pH 3.0) and T2 (CON+ 0.5% cheated mineral mixture in water, pH 3.0).

[a,b]Means in the same row with different superscripts differ ($p<0.05$).

In contrast, Paik (2001) observed that layers supplied feed containing only chelated zinc produced heavier eggs compared with those fed an organic trace-mineral mixture of an association of organic zinc and manganese. Zinc plays an important role in poultry, particularly in layers, as a component of a number of metalloenzymes, such as carbonic anhydrase which is essential for eggshell formation in the shell gland (Scheideler, 2008). Other important zinc metalloenzymes in the hen include carboxypeptidases and DNA polymerases. These enzymes play important roles in the hens' immune response, in skin and wound healing, and in hormone production (testosterone and corticosteroids). Ceylan & Scheideler (1999) reported significant positive effects of zinc and manganese supplementation from organic Zn and Mn sources on carbonic anhydrase activity levels in the shell gland of laying hens and a correlated improvement in eggshell percentage relative to egg weight. Increasing dietary calcium from 2.5% to 4.5 % improved eggshell weight (Clunies *et al*. 2002).There are also evidences that feed additives that increase the availability of Ca

and other minerals may also improve eggshell quality (Sątkiewicz, 2010). Klecker *et al*. (2002) found positive effects of the partial substitution of inorganic Zn and Mn sources by their organic forms on eggshell weight and eggshell thickness. Therefore, in our study, the improvement in eggshell thickness was possibly due to the higher mineral bioavailability, particularly of Ca, or to their influence on enzymes that is responsible for eggshell synthesis.

Nutrient digestibility

Table 4 shows the effects of the tested mineral complex on nutrient digestibility. At the end of experiment, higher ($p<0.05$) Ca digestibility was obtained in layers fed the T1 diet compared with CON. No effects of the treatments on the digestibility of the dry matter, nitrogen, energy, or phosphorus were observed ($p>0.05$). Webb *et al*. (2005) reported that organic minerals chelated to small peptides have much greater bioavailability than their inorganic forms through increased selective transport of peptides at gut level. Calcium is absorbed across the gut wall in the ionized form and the solubility of the Ca source plays an important role in its absorption (Pak *et al*., 1989). The increased solubility of Ca in ionized form may be the reason of the increase in its digestibility in the present study.

Table 4 – Effects of ionized or chelated water-soluble mineral mixture on nutrient digestibility in laying hens

Items,%	CON[1]	T1[1]	T2[1]	P-value
Dry matter	73.43±2.4	74.66±2.9	74.08±2.15	0.68
Nitrogen	60.65±4.4	62.37±5.01	62.41±2.33	0.67
Gross Energy	77.16±2.15	78.26±2.43	77.7±2.73	0.68
Ca	51.15^{b}±3.3	57.58^{a} ±3.98	55.27^{ab}±1.61	0.004
P	44.56±8.1	48.48±6.61	47.31±2.98	0.48

[1]Abbreviation: CON (basal diet + normal tap water), T1 (CON+0.5% ionized mineral mixture in water, pH 3.0) and T2 (CON+ 0.5% cheated mineral mixture in water, pH 3.0).
[2]Standard error of mean.
[a,b]Means in the same row with different superscripts differ ($p<0.05$).

Blood profiles

The effect of the water-soluble mineral mixture on the layers' blood profiles is shown in Table 5. Birds fed the T1 and the T2 diet presented higher ($p<0.05$) blood Ca levels compared with those fed water with no mineral supplementation. On the other hand, WBC, RBC, and lymphocytes were not influenced by the water supplementation with the ionized or chelated mineral complex. It was suggested that the absorption and balance of plasma calcium, phosphorus, and magnesium levels improved with the dietary supplementation of 2.72 ppm boron in Vitamin D-deficient rats (Hegsted *et al*., 1991). The potential reason for the increase in Ca absorption in the present study may be the bioavailability of minerals supplemented in the diet.

Table 5 – Effects of ionized or chelated water-soluble mineral mixture on blood profiles in laying hens

Items	CON[1]	T1[1]	T2[1]	P-value
WBC, 10^3/µL	165.7±8.96	165.5±29.1	162.8±22.6	0.96
RBC, 10^6/µL	1.65±0.05	1.69±0.18	1.64±0.13	0.85
Lymphocyte,%	49.0±7.02	50.3±4.64	49.5±3.10	0.89
Ca, mg/dL	22.45^{b}±2.08	27.23^{a}±0.95	27.7^{a}±2.08	0.01
P, mg/dL	4.58±0.50	5.28±0.50	5.20±0.50	0.42

[1]Abbreviation: CON (basal diet + normal tap water), T1 (CON+0.5% ionized mineral mixture in water, pH 3.0) and T2 (CON+ 0.5% cheated mineral mixture in water, pH 3.0). WBC: white blood cell, RBC: red blood cells
[2]Standard error of means.
[a,b]Means in the same row with different superscripts differ ($p<0.05$).

Excreta *Salmonella* and *E. coli* isolation and enumeration

The effects of water-soluble mineral complex water supplementation on excreta *Salmonella* and *E. coli* isolation and enumeration are shown in Table 6. *Salmonella* and *E. coli* counts in the excreta tended to be reduced ($p=0.07$, $p=0.05$ respectively) in birds fed the T1 and T2 treatments compared with CON. Copper supplementation may also affect intestinal microflora and has been shown to affect the presence of bacteria in the litter (Johnson *et al*., 1985). Dietary calcium phosphate has a trophic effect on the intestinal microflora and strongly protects against *Salmonella* infection in rats (Bovee-Oudenhoden *et al*., 1997). The effect of Zn on the intestinal microflora resembles the mode of action suggested for antibiotic growth promoters (Hojberg *et al*., 2005). Hedemann *et al*. (2006) reported that feeding pigs the high dietary Zn concentrations mucin secretion in the cecum and colon, perhaps as a consequence of altered microbial activity in the large intestine. In the present study, our results indicate that mineral mixture supplementation led to reduction of *E. coli* and *Salmonella* counts compared with the control treatment. The observed trends of lower counts of these microorganisms may be due to the antibacterial activity of the supplemented mineral complex. Newman & Cragg (2007) also reported that mineral clays could function as an inexpensive bactericidal compound against resistant bacterial pathogens.

Table 6 – Effects of ionized or chelated water-soluble mineral mixture fecal*Salmonella* and *E. coli* counts

Items, log10cfu/g	CON[1]	T1[1]	T2[1]	P-value
Salmonella	2.4±0.14	2.24±0.04	2.19±0.09	0.07
E. coli	6.5±0.12	6.24±0.06	6.2±0.19	0.05

[1]Abbreviation: CON (basal diet + normal tap water), T1 (CON+0.5% ionized mineral mixture in water, pH 3.0) and T2 (CON+ 0.5% cheated mineral mixture in water, pH 3.0).
[2]Standard error of means.

CONCLUSION

In conclusion, our results indicated that chelated or ionized mineral water supplementation significantly improved blood Ca level and improved Ca digestibility compared with the control treatment. Trends of higher egg production, and reduced excreta *E. coli and Salmonella* counts were also observed. Therefore, the water supplementation with a water-soluble mineral mixture showed partial benefits in laying hens, and suggests that the tested mineral mixture could be used as a substitute for antibiotics.

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