# Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources

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**Primary Audience:** Nutritionists, Broiler Breeder and Hatchery Managers, Researchers

## SUMMARY

The objective of this study was to investigate the effects of maternal dietary Zn, Mn, and Cu sources on egg production, eggshell quality, hatchability, and hatched chick grading. Inorganic sources of Zn, Mn, and Cu (IZMC) as zinc sulfate monohydrate (35% Zn), manganese sulfate monohydrate (31% Mn), and copper sulfate pentahydrate (25% Zn) or organic sources of Zn, Mn, and Cu as amino acid-mineral complexes (OZMC) were used. The 3 experimental treatments consisted of diets supplemented with 1) 100, 100, and 10 mg/kg of Zn, Mn, and Cu, respectively, from IZMC (control); 2) 60, 60, and 3 mg/kg of Zn, Mn, and Cu, respectively, from IZMC plus 40, 40, and 7 mg/kg of Zn, Mn, and Cu, respectively, from OZMC (ISO); and 3) a diet with 100, 100, and 10 mg/kg of Zn, Mn, and Cu, respectively, from IZMC as in the control plus 40, 40, and 7 ppm of supplemental Zn, Mn, and Cu from OZMC (on top). Treatments were fed from 22 to 68 wk of age. Each treatment had 10 replications of 20 females and 2 males. Feeding the ISO diet compared with the control diet increased eggshell weight and thickness ($P < 0.05$) and decreased early embryo mortality ($P < 0.01$). Feeding the on top diet compared with the control diet resulted in thicker and heavier eggshells ($P < 0.05$). An improvement in eggshell quality was observed in breeder hens consuming the OZMC-supplemented ISO diet or the on top diet with IZMC.

**Key words:** amino acid complex, broiler breeder, fertile egg, trace mineral

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## DESCRIPTION OF PROBLEM

Trace minerals, such as Zn, Mn, and Cu, are components of eggs that are essential for broiler breeder performance as well as chicken embryo development [1, 2]. These trace minerals are constituents of several proteins involved in intermediary metabolism, hormone secretion, and the immune system [3] and are required in small amounts in the diet. The effects of Zn, Mn, and Cu deficiencies on breeder performance and embryo development have been well documented

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and can result in low egg production, reduced eggshell strength, poor hatchability, reduced fertility, increased embryo bone abnormalities, poor feathering, and dermatitis [4–6]. Mineral concentration in conventional feedstuffs can fluctuate widely because of the trace mineral composition in soils, geographic area, climate, and crop yield. [7]. Thus, in practical poultry formulations, nutritionists usually include a wide margin of safety for trace minerals to ensure appropriate bird growth and maximize performance [8].

In breeder hens, Zn is important as a component of carbonic anhydrase, which is involved in the supply of carbonate ions during eggshell formation [9]. Other important Zn metalloenzymes include carboxypeptidases and DNA polymerases, which are important in immune responses, skin and wound healing, and hormone production. Breeder diets that are deficient in Zn can lead to a decrease in egg production and eggshell quality, as well as in hatchability [4]. Essential for formation of the bone cartilage, Mn plays a significant role in the formation of chondroitin sulfate. Manganese-deficient avian embryos exhibit shortening of the long bones, parrot beak, and wiry down [5]. The involvement of Cu in the synthesis of hemoglobin, erythrocuprein, and other plasma proteins is well known [10]. Moreover, Cu is closely associated with iron metabolism because it is a part of ceruloplasmin. Copper also plays an important role in eggshell membrane formation, which in turn influences eggshell structure, texture, and shape [11].

The trace minerals are traditionally supplemented in broiler feeds by using inorganic sources (**ITM**), such as oxides or sulfates [12]; however, these sources have variable bioavailability [13, 14], which can impair bird performance and may contribute to environmental pollution. During transit through the gastrointestinal system, ions from dissolved ITM can potentially bind with other dietary components, forming insoluble complexes that are excreted, thus reducing their availability [14–16]. The organic trace minerals (**OTM**) have been increasingly used in avian nutrition because they seem to have greater bioavailability compared with inorganic sources [13, 16]. These types of minerals have been shown to improve bird performance, enhance immunity, and potentially reduce minerals in excreta [17–21]. However, data from studies conducted with broiler breeders evaluating the effects of OTM are still limited.

The NRC [22] does not provide specific recommendations for Zn, Mn, and Cu for broiler breeder hens; instead, fixed amounts are recommended for meat-type chickens until 8 wk; Leghorn-type laying hens; and growing, holding, and laying turkeys, with the exception of Cu requirements for Leghorn-type laying hens. National Research Council [22] recommendations do not take mineral availability into consideration, which can change broiler breeder and progeny performance. Currently, the main sources of information used by breeder nutritionists to set Zn, Mn, and Cu concentrations in breeder diets derive from primary broiler breeder manuals [23] because a few recent research reports exist in this area [17–20, 24]. The objective of this study was to evaluate the reproductive response of broiler breeder hens fed sulfate and amino acid-complexed sources of Zn, Mn, and Cu in breeder diets.

## MATERIALS AND METHODS

### *Bird Husbandry*

Birds in the study were managed according to the directives of the Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. Six hundred slow-feathering Cobb 500 broiler breeder hens and 60 Cobb 500 breeder males, 22 wk of age, were obtained from a commercial breeder farm [25]. Birds were individually weighed and placed in 30 floor pens in a tunnel-ventilated house, with 20 females and 2 males per pen. Pens were 2.0 × 2.5 m and had 5 nipple drinkers, separate male and female feeders, and 6 nests located at one side of the pen. Forty-eight males were maintained in separated floor pens to replace sexually inactive or dead males. Litter was of new rice hulls 15 cm deep at placement. Birds were monitored daily, and any abnormalities were recorded and treated as necessary.

Lighting and feeding throughout the study were provided according to primary breeder recommendations [26]. All birds were individually weighed every 4 wk for assessment of BW and

uniformity. According to the BW and development, males and females were feed restricted following the recommended daily allowances for each sex. All dietary treatment groups were provided the same amount of feed on a per-bird basis, and water was provided for ad libitum consumption.

### *Experimental Diets*

Two diet phases were used (22 to 32 wk and 33 to 68 wk) for hens, whereas males were fed the same diet throughout the entire study (Table 1). Inorganic sources of Zn, Mn, and Cu (**IZMC**) as zinc sulfate monohydrate (35% Zn), manganese sulfate monohydrate (31% Mn), and copper sulfate pentahydrate (25% Zn) or organic sources of Zn, Mn, and Cu as amino acid-mineral complexes (**OZMC**) were used. The 3 experimental treatments consisted of diets supplemented with 1) 100, 100, and 10 mg/kg of Zn, Mn, and Cu, respectively, from IZMC (control); 2) 60, 60, and 3 mg/kg of Zn, Mn, and Cu, respectively, from IZMC plus 40, 40, and 7 mg/kg of Zn, Mn, and Cu, respectively, from OZMC (**ISO**); and 3) a diet with 100, 100, and 10 mg/kg of Zn, Mn, and Cu, respectively, from IZMC as in the control diet plus 40, 40, and 7 ppm of supplemental Zn, Mn, and Cu from OZMC (on top). A commercially available OZMC was used [27]. Each dietary treatment was replicated 10 times (3 dietary treatments in 30 floor pens). Formulated and analyzed nutrient contents of the diets are shown in Table 1.

The diets were manufactured every 4 wk. Diets samples were collected and analyzed, in duplicate, for CP, Ca, and P [28] (Table 1). Diet subsamples and ingredients such as corn, soybean meal, and wheat bran meal were analyzed, in duplicate, for Zn, Mn, and Cu concentrations from each feed mix batch (4 wk). Drinking water samples were collected at the nipple drinker (breeder house) and analyzed, in duplicate, for Zn, Mn, and Cu. All trace mineral analyses were performed using inductive coupled plasma atomic emission spectroscopy (Table 2) [29].

### *Hen Performance Measurements*

Eggs were collected 4 times daily, recorded as nest or floor laid, and classified as settable, cracked, shell-less, double-yolked, or abnormally shaped, regardless the location of lay (nest or floor). Mortality was recorded daily and feed allowance was adjusted accordingly. Total and settable egg production on a hen-housed and hen-day basis and cumulative mortality (total number of dead birds/initial number of birds × 100%) were summarized weekly. Average egg weight and specific gravity were determined at 35, 45, 55, and 65 wk of age using all settable nest-laid eggs on a single day. Specific gravity was measured using saline solutions with concentrations ranging from 1.065 to 1.095 in intervals of 0.005 units [30]. Eggshell percentage and thickness were measured using 5 settable eggs from a single day, per replication, at 35, 45, 55, and 65 wk of age. Eggshell weight was measured after washing and drying overnight at 105°C, with the eggshell membrane on. Eggshell thickness was measured at 3 points at the egg equatorial zone after inner membrane removal.

A total of 90 eggs per replicate pen were set for incubation every 3 wk, between the breeder ages of 45 to 68 wk. Only nest-laid eggs that were not dirty, double-yolked, misshapen, broken, cracked, or excessively small, or eggs with poor shell quality were incubated [31]. The eggs were stored for 7 d in a controlled-environment room at 18°C and 75% RH before incubation. The settable eggs were placed into replicate trays (n = 30 trays), which were randomly placed in a single-stage incubator [32] with a 3,600-egg capacity. The incubator was set as 37.5°C and 60% RH. On d 18.5, eggs were transferred to the hatcher [32], which was set at 36.5°C and 65% RH. The number of eggs that hatched was recorded at 21.5 d of incubation.

All incubated eggs that were cracked before incubation or at transference to the hatcher were removed from the data set. All unhatched eggs were broken open to determine the approximate day of embryonic death. Embryonic mortality was grouped into 4 categories: early (0 to 7 d), middle (7 to 14 d), late (15 to 21 d), and at pipping. Hatchability of fertile eggs was expressed as the number hatching chicks per fertile eggs set, and percentage of cumulative hatchability was expressed as percentage of hatching chicks to the total eggs set. Chicks per hen housed were estimated by multiplying weekly settable eggs

**Table 1.** Composition of basal diets supplied to broiler breeders from 22 to 68 wk of age[1]

| | Breeder hens | | Males |
|---|---|---|---|
| Item | 22 to 32 wk | 33 to 68 wk | 22 to 68 |
| Ingredient, % (as-is basis) | | | |
| Corn | 63.41 | 64.15 | 58.93 |
| Soybean meal | 22.14 | 21.40 | 12.93 |
| Wheat bran | 2.00 | 2.00 | 23.40 |
| Soybean oil | 1.74 | 1.68 | 1.00 |
| Limestone | 3.84 | 4.31 | 1.16 |
| Dicalcium phosphate | 1.91 | 1.64 | 1.64 |
| Sodium chloride | 0.37 | 0.27 | 0.33 |
| Sodium bicarbonate | 0.13 | 0.24 | 0.18 |
| Oyster shells | 3.50 | 3.50 | — |
| Potassium carbonate (98%) | 0.27 | 0.20 | 0.01 |
| L-Lys HCl (78%) | 0.03 | — | — |
| DL-Met (99%) | 0.18 | 0.15 | 0.08 |
| L-Thr (98.5%) | 0.03 | 0.01 | 0.08 |
| Choline chloride | 0.10 | 0.10 | 0.11 |
| Vitamin premix[2] | 0.10 | 0.10 | 0.10 |
| Mineral premix[3] | 0.05 | 0.05 | — |
| Male mineral premix[4] | — | — | 0.05 |
| Kaolin[5] | 0.20 | 0.20 | — |
| Total | 100.00 | 100.00 | 100.00 |
| Calculated nutrient composition, % or as shown | | | |
| ME, kcal/kg | 2,840 | 2,840 | 2,800 |
| CP | 15.40 | 15.06 | 13.77 |
| | (15.42 ± 1)[6] | (15.23 ± 1) | (14.21 ± 1) |
| Digestible amino acids | | | |
| Lys | 0.73 | 0.69 | 0.55 |
| TSAA | 0.63 | 0.59 | 0.50 |
| Thr | 0.55 | 0.52 | 0.51 |
| Ile | 0.60 | 0.59 | 0.48 |
| Val | 0.66 | 0.64 | 0.56 |
| Ca | 3.20 | 3.30 | 0.90 |
| | (3.33 ± 0.4) | (3.46 ± 0.3) | (0.95 ± 0.1) |
| Nonphytate P | 0.45 | 0.40 | 0.45 |
| Total P | 0.64 | 0.59 | 0.74 |
| Na | 0.20 | 0.20 | 0.20 |
| | (0.66 ± 0.1) | (0.63 ± 0.1) | (0.70 ± 0.1) |
| Dietary electrolyte balance, mEq/kg | 200.00 | 200.00 | 180.00 |
| Choline, mg/kg | 1,500 | 1,500 | 1,500 |

[1]Experimental diets were prepared by adding Zn, Mn, and Cu from either $ZnSO_4$, $MnSO_4$, $CuSO_4$, Availa-Zn, Availa-Mn, or Availa-Cu (last 3 from Zinpro, Eden Prairie, MN).

[2]Vitamin premix provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin $D_3$, 3,000 IU; vitamin E, 100 IU; vitamin C, 50 mg; vitamin $K_3$, 6 mg; vitamin $B_{12}$, 35 µg; thiamine, 3 mg; riboflavin, 15 mg; vitamin $B_6$, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4 mg; biotin, 0.3 mg.

[3]Supplied the following per kilogram of diet: Se (from sodium selenite), 0.3 mg; Fe (from ferrous sulfate), 75 mg; I (source = calcium iodate), 1 mg; cobalt (from cobalt sulfate), 1 mg.

[4]Supplied the following per kilogram of diet: Zn (from zinc sulfate), 100 mg; Mn (from manganese sulfate), 100 mg; Cu (from copper sulfate), 10 mg; Se (from sodium selenite), 0.3 mg; Fe (from ferrous sulfate), 75 mg; I (source = calcium iodate), 1 mg; Co (from cobalt sulfate), 1 mg.

[5]The trace mineral sources replace kaolin (Sericita M-200, Mineração Violani, Colombo, Paraná, Brazil).

[6]Values within parentheses are analyzed means ± SD; n = 12.

per hen housed per period and hatchability of total eggs set per batch set.

All hatched chicks were counted and feather-sexed, and chick quality was visually assessed according to commercial hatchery standards [33]. This evaluation was conducted on all incubations and always by the same personnel, and chicks were classified as salable chicks, un-

**Table 2.** Calculated[1] and analyzed[2] Zn, Mn, and Cu concentrations in the experimental diets and drinking water (ppm)

| Item | Zn | Mn | Cu |
|---|---|---|---|
| Diet[3] | | | |
| Control | 157 (146 ± 6)[4] | 164 (158 ± 7) | 16 (13 ± 1) |
| ISO[5] | 157 (145 ± 5) | 164 (155 ± 7) | 16 (13 ± 1) |
| On top | 197 (185 ± 6) | 204 (202 ± 8) | 23 (21 ± 1) |
| Main ingredient[6] | | | |
| Drinking water[7] | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.01 ± 0.001 |
| Corn | 54 ± 2 | 15 ± 1 | 2 ± 0.5 |
| Soybean meal | 81 ± 3 | 48 ± 2 | 15 ± 1 |
| Wheat bran | 104 ± 4 | 163 ± 4 | 11 ± 1 |

[1]Calculated values were obtained using a linear feed formulation based on the analyzed Zn, Mn, and Cu in all feed ingredients.

[2]Data were obtained using inductive coupled plasma atomic emission spectroscopy [29].

[3]Control = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$; ISO = 60 ppm of $ZnSO_4$, 60 ppm of $MnSO_4$, and 3 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex; on top = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex.

[4]Values within parentheses are analyzed means ± SD (n = 12).

[5]ISO and On top diets: Availa-ZMC produced by Zinpro Corporation (Eden Prairie, MN). Guaranteed composition: 4.00% of Zn; 4.00% of Mn; 0.70% of Cu.

[6]Corn, soybean meal, and wheat bran meal samples were collected from each batch (every 4 wk). Values are the mean ± SD; n = 12.

[7]Drinking water samples were collected at the nipple drinker (breeder house). Values are the mean ± SD; n = 12.

healed navels, culled (weak or red hocks), and physical abnormalities. Additionally, 200 newly hatched chicks (21.5-d incubation) per treatment (n = 10 of each sex per replicate pen) were weighed (average) and individually measured from the tip of the beak to the end of the middle toe (third toe) to determine the chick length as described by Molenaar et al. [34].

### *Statistical Analysis*

Birds were randomly allocated to floor pens that had previously been randomly assigned to the different diet treatments. Each pen was a replicate. All reproductive performance data were taken on the same experimental units, repeated in time. All percentage data were subjected to angular transformation to stabilize variances (arcsine square root percentage transformation) before statistical analysis [35]. Repeated-measurement analyses were applied to examine and compare responses over time in a factorial experiment with diet treatment and time as the 2 factors.

Data were analyzed using the repeated statement PROC MIXED of SAS [36], adding the factor time as a fixed effect, and pen was considered a random effect [37]. The following statistical model was used:

$$y_{ijk} = \mu + \alpha_i + d_{ij} + t_k + (\alpha t)_{ik} + e_{ijk},$$

where $y_{ijk}$ is the response at time k on experimental unit j in treatment group i, $\mu$ is the overall mean, $\alpha_i$ is a fixed effect of treatment i, $d_{ij}$ is a random effect of experimental unit j in treatment group i, $t_k$ is a fixed effect of time k, $(\alpha t)_{ik}$ is a fixed interaction effect of treatment i with time k, and $e_{ijk}$ is random error at time k on experimental unit j in treatment i. The best covariance structure was based on the Akaike information criterion [38] and used the smallest Akaike information criterion value for each variable [39]. The autoregressive covariance structure [AR(1)] fit the data best for hen-day egg production, hen-housed egg production, total eggs per hen housed, settable eggs per hen housed, egg weight, specific gravity, eggshell weight, eggshell thickness, and hatchling chick weight and length. The heterogeneous autoregressive covariance structure [ARH(1)] fit the data best for fertility, hatchability of eggs set, hatchability of fertile, chicks per hen housed, salable chicks, culls, unhealthy navels, and physical abnormalities. The unstructured (UN) fit the data best for

embryo mortality measurements. Differences were determined using the PDIFF option of the LSMEANS statement of SAS and the Tukey-Kramer adjustment for multiple contrasts for all pairwise comparisons [36]. In all analyses, significance was declared at $P < 0.05$.

## RESULTS AND DISCUSSION

Formulated and analyzed diet nutrients were similar (Table 1) except in the basal diet of the males, which was formulated to contain 13.77% of CP but analyzed as 14.21%. Increases in Zn, Mn, and Cu from the control and from ISO to the on top supplementation occurred, as expected. Analyzed Zn, Mn, and Cu compositions of corn, soybean meal, and wheat bran are presented in Table 2.

At the start of the experiment at 22 wk of age, BW and uniformity were similar between replications (Figure 1). Mean BW and uniformity were not affected by treatments ($P > 0.05$; data not shown). Average livability from 24 to 68 wk was 5.3% higher (98.2 vs. 93.0%) compared with the breeder strain guide [23] and was not affected by treatments ($P < 0.05$; data not shown). Breeder production (hen-day egg production, hen-housed egg production, eggs per hen housed, and settable eggs per hen housed) was affected only by period ($P < 0.001$; Figure 2 and Table 3). No period × diet interaction was observed for any of the parameters evaluated in this study ($P > 0.05$). All production data were affected similarly by period, with an initial increase from period 1 to period 2, coinciding with peak production, and a subsequent decrease between periods 2 and 3 and a further decrease between periods 3 and 4. As compared with the expected data from the primary breeder manual, peak of production was 4% higher (84.9 vs. 81.5%) and postpeak egg production was superior [23]. In addition, the number of eggs per hen housed (from 25 to 68 wk) was 7.4% higher (192.9 vs. 178.7) compared with the breeder strain from 24 to 65 wk [23]. When compared with commercial data, hen-day egg production could be considered typical for a Brazilian broiler breeder flock.

All eggshell quality measures were affected by period ($P < 0.001$; Table 4). Relative eggshell

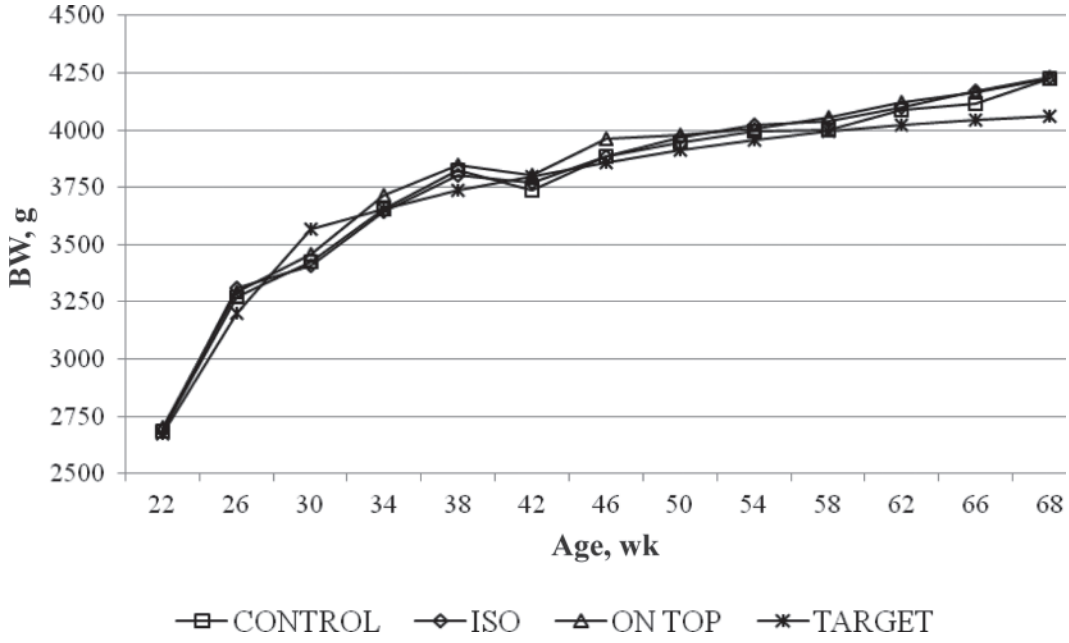


**Figure 1.** Effect of trace mineral treatment on mean BW of broiler breeder hens. Control = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$; ISO = 60 ppm of $ZnSO_4$, 60 ppm of $MnSO_4$, and 3 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex; on top = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex.

**Table 3.** Effect of trace mineral treatment and period on breeder performance measures

| Item | Hen-day egg production,[1] % | Hen-housed egg production,[2] % | Total eggs per hen-housed[3] | Settable eggs per hen-housed[4] |
|---|---|---|---|---|
| Diet[5] | | | | |
| Control | 65.97[6] | 62.26 | 191.79 | 185.81 |
| ISO | 66.41 | 62.73 | 193.21 | 188.46 |
| On top | 66.06 | 62.93 | 193.82 | 188.99 |
| SEM | 0.52 | 0.83 | 2.23 | 2.24 |
| Period, wk | | | | |
| 25 to 35 | 70.25[b,7] | 68.47[b] | 52.72[b] | 50.59[b] |
| 36 to 46 | 73.87[a] | 71.94[a] | 55.40[a] | 54.21[a] |
| 47 to 57 | 64.20[c] | 60.64[c] | 46.69[c] | 45.86[c] |
| 58 to 68 | 56.26[d] | 49.52[d] | 38.13[d] | 37.10[d] |
| SEM | 0.41 | 0.58 | 0.45 | 0.47 |
| *P*-value | | | | |
| Diet | 0.8267 | 0.8472 | 0.8105 | 0.5802 |
| Period | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Diet × period | 0.7603 | 0.6985 | 0.6985 | 0.5787 |

[a–d]Means within the same column with different superscripts differ ($P \leq 0.05$).

[1]Hen-day egg production, % = (number of egg produced on a daily basis/number of birds available in the flock on that day) × 100.

[2]Hen-housed egg production, % = (total number of eggs produced per pen/total number of hens housed) × 100.

[3]Number of total eggs produced from 25 to 68 wk of age divided by the number of hens housed.

[4]Number of total settable eggs produced from 25 to 68 wk of age divided by the number of hens housed.

[5]Control = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$; ISO = 60 ppm of $ZnSO_4$, 60 ppm of $MnSO_4$, and 3 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex; on top = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex.

[6]Means of 200 females per treatment at housing; n = 10 replications of 20 females and 2 males per replicate.

[7]Means of 600 females per period at housing; n = 30 replications of 20 females and 2 males per period.

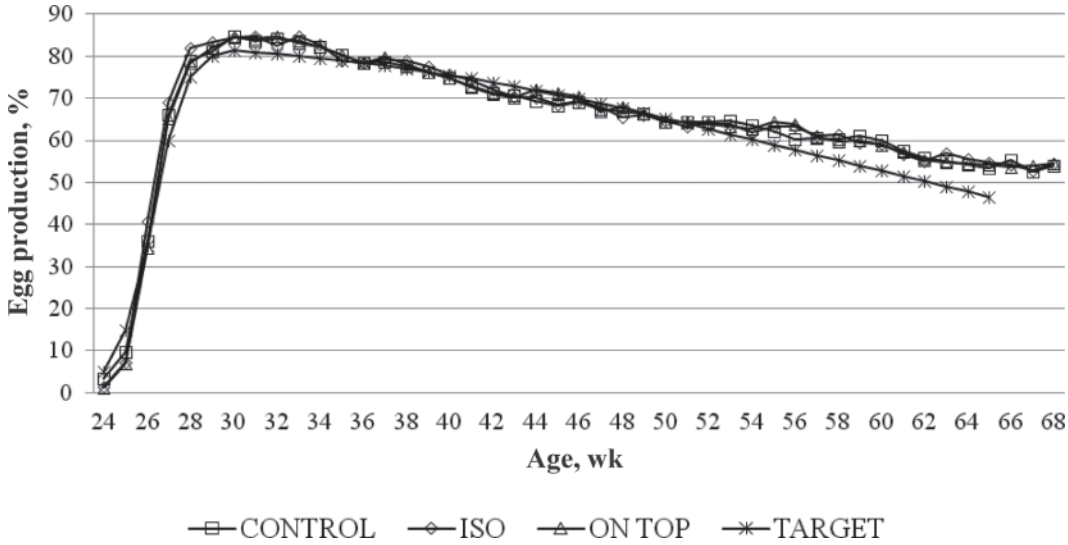


**Figure 2.** Effect of trace mineral treatment on hen-day egg production. Control = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$; ISO = 60 ppm of $ZnSO_4$, 60 ppm of $MnSO_4$, and 3 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex; on top = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex.

**Table 4.** Effect of trace mineral treatment and period on eggshell quality

| Item | Egg weight,[1] g | Egg specific gravity,[1] g/mL | Relative eggshell weight,[2] % | Eggshell thickness,[3] µm |
|---|---|---|---|---|
| Diet[4] | | | | |
| Control | 70.37[5] | 1.0852 | 8.96[b] | 382.09[b] |
| ISO | 70.68 | 1.0852 | 9.10[a] | 384.82[a] |
| On top | 70.20 | 1.0858 | 9.17[a] | 385.83[a] |
| SEM | 0.29 | 0.00025 | 0.05 | 0.97 |
| Period, wk | | | | |
| 35 | 65.03[d,6] | 1.0871[a] | 9.39[a] | 388.77[a] |
| 45 | 69.72[c] | 1.0861[b] | 9.33[a] | 383.47[b] |
| 55 | 72.74[b] | 1.0843[c] | 8.82[b] | 382.84[b] |
| 65 | 74.18[a] | 1.0841[c] | 8.76[b] | 381.23[b] |
| SEM | 0.24 | 0.00027 | 0.05 | 1.12 |
| *P*-value | | | | |
| Diet | 0.4829 | 0.1559 | 0.0164 | 0.0353 |
| Period | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| Diet × period | 0.0726 | 0.9376 | 0.9395 | 0.8125 |

[a–d]Means within the same column with different superscripts differ ($P \leq 0.05$).

[1]All settable nest-laid eggs on a single day were measured at 35, 45, 55, and 65 wk of age; n = 20 females per replication.

[2]Eggshell weight, % = (dry eggshell weight/egg weight) × 100; n = 5 eggs per replication per period (35, 45, 55, and 65 wk).

[3]Eggshell thickness was measured at 3 points on the egg equatorial zone (settable nest-laid eggs on a single day) after removal of the inner membrane; n = 5 eggs per replication per period (35, 45, 55, and 65 wk).

[4]Control = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$; ISO = 60 ppm of $ZnSO_4$, 60 ppm of $MnSO_4$, and 3 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm Cu-amino acid complex; on top = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex.

[5]Means of 200 females per treatment at housing; n = 10 replications of 20 females and 2 males per replicate.

[6]Means of 600 females per period at housing; n = 30 replications of 20 females and 2 males per period.

weight decreased after 45 wk ($P < 0.001$), and the specific gravity and eggshell thickness decreased after 35 wk of age ($P < 0.001$). Eggshell relative weight and eggshell thickness were affected by diet ($P < 0.05$); however, no differences were found in the egg specific gravity ($P > 0.05$). Eggs laid by hens fed the ISO or on top diet had an increased relative eggshell weight and thickness ($P < 0.05$) compared with those laid by hens fed the control diet. The specific gravity method is frequently used as an indicator of eggshell quality and is considered to be adequate when above 1.080 [8]. The relative eggshell weight and thickness were quite high in all treatments, which does not suggest that the control diet was negatively influencing shell formation in this study. Failure to show a consistent relationship between relative eggshell weight and thickness with the specific gravity may have been due to large differences between test sensitivities.

The role of trace minerals in poultry nutrition and embryo development is well documented [1, 2, 40]; however, less is known about the specific involvement in the actual process of shell formation. Both Zn and Mn act as cofactors in the enzymes needed for Ca metabolism (i.e., carbonic anhydrase). The shell and its associated membranes have varying amounts of Zn and Mn but contain an especially high concentration of Cu [1, 11]. Moreover, it has been shown that a Cu-deficient diet can influence eggshell membrane structure, which in turn influences eggshell structure, texture, shape, pigments, and egg weight by an increase in the thin albumen portion [11]. It is important to consider that mineral deficiency does not appear to affect the absolute amount of shell deposited by the hen, but that variation in egg size impairs the shell thickness [41]. This appears to be a plausible explanation in the present study for the increase in egg weight and eggshell quality measurements observed as the flock aged from period 1 to period 4.

Egg mineral composition is variable, and the total amount deposited in egg components may

**Table 5.** Effect of trace mineral treatment and period on fertility, hatchability, and embryo mortality of broiler breeder[1]

| Item | Fertility,[3] % | Hatchability of eggs set,[4] % | Hatchability of fertile,[5] % | Chicks per hen housed[6] | Embryo mortality[2] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Early dead, % | Middle dead, % | Late dead, % | Pips, % |
| Diet[7] | | | | | | | | |
| Control | 92.62[8] | 78.70 | 88.39[b] | 147.06[b] | 3.14[a] | 0.84 | 4.63 | 3.00 |
| ISO | 91.08 | 79.11 | 90.60[a] | 149.84[ab] | 2.08[b] | 0.51 | 4.31 | 2.48 |
| On top | 92.18 | 80.94 | 90.30[a] | 153.81[a] | 2.60[ab] | 0.71 | 3.99 | 2.39 |
| SEM | 1.02 | 1.17 | 0.92 | 1.90 | 0.30 | 0.15 | 0.56 | 0.43 |
| Period, wk | | | | | | | | |
| 45 to 50 | 93.12[a,9] | 84.69[a] | 92.50[a] | 42.82[b] | 1.41[c] | 0.56[b] | 3.43[c] | 2.09 |
| 51 to 56 | 92.04[a] | 81.15[b] | 90.72[b] | 43.98[a] | 2.04[bc] | 0.44[b] | 3.94[bc] | 2.86 |
| 57 to 62 | 93.57[a] | 80.21[b] | 89.04[c] | 36.75[c] | 2.69[b] | 0.68[b] | 4.98[a] | 2.59 |
| 63 to 68 | 89.09[b] | 72.16[c] | 86.80[d] | 26.69[d] | 4.30[a] | 1.07[a] | 4.88[ab] | 2.94 |
| SEM | 0.93 | 1.05 | 0.84 | 0.42 | 0.34 | 0.17 | 0.54 | 0.40 |
| Probability | | | | | | | | |
| Diet | 0.5379 | 0.1706 | 0.0659 | 0.0619 | 0.0058 | 0.1152 | 0.5473 | 0.3256 |
| Period | 0.0006 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0027 | 0.0206 | 0.1137 |
| Diet × period | 0.5742 | 0.3231 | 0.3169 | 0.6135 | 0.9120 | 0.2079 | 0.2887 | 0.7340 |

[a–d]Means within the same column with different superscripts differ ($P \leq 0.05$).

[1]Values are the means of 10 replications, each with 20 hens and 2 males at housing. Eggs were incubated and hatched every 3 wk from 45 to 68 wk; n = 8 incubations.

[2]All data were calculated as a percentage of fertile eggs.

[3]Fertility, % = (number of fertile eggs/number of total egg set) × 100.

[4]Hatchability of eggs set, % = (number of chicks hatched/number of eggs set) × 100.

[5]Hatchability of fertile, % = (number of chicks hatched/number of fertile eggs set) × 100.

[6]Estimate produced chicks by multiplication of weekly settable eggs per hen housed per period and hatchability of total eggs set per hatch per period.

[7]Control = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$; ISO = 60 ppm of $ZnSO_4$, 60 ppm of $MnSO_4$, and 3 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex; on top = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex.

[8]Means of 81 eggs per replicate per incubation; n = 10 replicates.

[9]Means of 2 incubations of 2,430 eggs per incubation per period; n = 30 replicates.

depend on the chemical form of the mineral and the amount fed to the hen [15, 42]. According to Richards [1], the transfer of trace minerals from the hen to the egg involves 2 possible routes: 1) via the ovary to the yolk, and 2) via the oviduct to the albumen, the eggshell, and its membranes. Feeding hen diets with high levels of Zn, Mn, and Cu does not mean that the egg mineral composition will increase significantly [4, 42], whereas it has been shown that OTM sources of Zn, Cu, and Se, as opposed to the respective ITM sources, result in higher total amounts of these minerals deposited in the egg [15, 20, 43, 44]. Significant improvements in the egg specific gravity and eggshell thickness were seen when using a racemic mixture of zinc sulfate and a Zn-amino acid complex compared with a control diet containing only zinc sulfate [20, 45]. Moreng et al. [46] found that feeding laying hens a zinc-methionine complex improved eggshell relative weight and thickness. Based on these results, it can be postulated that a synergistic effect occurs when using a mixture of amino acid-complexed and inorganic sources of trace minerals, and that this synergy may be due to enhanced mechanisms involved in the calcification of eggshells [20].

Fertility, hatchability, and chicks per hen housed were affected only by period ($P < 0.001$; Table 5). Embryo mortality (early, middle, and late dead) was affected by period ($P < 0.05$; Table 5); however, a reduction in early embryo mortality was observed in eggs laid by hens fed the ISO diet compared with the control diet ($P < 0.05$). Similarly, Hudson et al. [20] found a significant reduction in early embryo mortality

**Table 6.** Effect of trace mineral treatment and period on hatched chick grading[1]

| Item | Salable chicks,[2] % | Culls,[3] % | Unhealthy navels,[4] % | Physical abnormalities,[5] % | Hatchling weight, g | Hatchling length, cm |
|---|---|---|---|---|---|---|
| Diet[6] | | | | | | |
| Control | 84.69[7] | 3.44 | 10.69 | 1.12 | 51.28[8] | 19.45[b,9] |
| ISO | 85.39 | 3.56 | 10.13 | 0.92 | 51.35 | 19.50[ab] |
| On top | 87.00 | 3.19 | 8.90 | 0.83 | 51.18 | 19.58[a] |
| SEM | 1.36 | 0.57 | 0.95 | 0.19 | 0.21 | 0.05 |
| Period (wk) | | | | | | |
| 45 to 50 | 89.55[a,10] | 1.78[b] | 7.82[b] | 0.71 | 49.85[b,11] | 19.37[c,12] |
| 51 to 56 | 86.90[b] | 4.82[a] | 7.42[b] | 0.79 | 51.62[a] | 19.51[b] |
| 57 to 62 | 82.93[c] | 3.95[a] | 12.14[a] | 1.08 | 51.83[a] | 19.59[a] |
| 63 to 68 | 83.41[c] | 3.06[ab] | 12.19[a] | 1.34 | 51.78[a] | 19.58[a] |
| SEM | 1.06 | 0.55 | 0.94 | 0.20 | 0.17 | 0.03 |
| *P*-value | | | | | | |
| Diet | 0.2444 | 0.8907 | 0.1888 | 0.5520 | 0.8536 | 0.0772 |
| Period | 0.0001 | 0.0003 | 0.0001 | 0.1342 | 0.0001 | 0.0001 |
| Diet × period | 0.9699 | 0.9169 | 0.5911 | 0.4407 | 0.8193 | 0.7845 |

[a–c]Means within the same column with different superscripts differ ($P \leq 0.05$).

[1]All data were calculated as a percentage of hatched chicks. Values are the means of 10 replications, each with 20 hens and 2 males at housing. Eggs were incubated and hatched every 3 wk from 45 to 68 wk; n = 8 incubations.

[2]High-quality chicks.

[3]Weak and red-hocked chicks or chicks with some apparent abnormalities.

[4]Chicks at hatching with minor navel conditions: navel buttons (>2 mm) or leaky.

[5]Chicks with some physical problem and difficulty walking.

[6]Control = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$; ISO = 60 ppm of $ZnSO_4$, 60 ppm of $MnSO_4$, and 3 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex; on top = 100 ppm of $ZnSO_4$, 100 ppm of $MnSO_4$, and 10 ppm of $CuSO_4$ plus 40 ppm of Zn-amino acid complex, 40 ppm of Mn-amino acid complex, and 7 ppm of Cu-amino acid complex.

[7]Mean of 81 eggs per replicate per incubation; n = 10 replicates.

[8]Mean of 200 hatched chick weights; n = 10 replications per treatment.

[9]Mean of 20 chicks divided into 10 males and 10 females per replication per treatment; n = 10 replicates.

[10]Mean of 2 incubations of 2,430 eggs per incubation per period; n = 30 replicates.

[11]Mean of total hatched chick weights; n = 30 replications per period.

[12]Mean of 2 incubations of 600 males and 600 females per incubation per period; n = 30 replicates.

when breeder hens were fed a Zn-amino acid complex as the only source of Zn supplementation. Hudson et al. [20] also reported 3.6 more chicks per hen housed in hens consuming diets supplemented with a racemic mixture of zinc sulfate and a Zn-amino acid complex compared with hens consuming only the diet supplemented with the Zn-amino acid complex. Zinc, Mn, and Cu play important roles in embryo development as well as hatchability [2, 4, 5, 40], and a positive relationship has been shown between egg Zn content and hatchability [47]. Kidd et al. [17] found an increase in fertility in hens fed a diet supplemented with a Zn-amino acid complex compared with a control diet (without Zn supplementation); however, only a numerical response for hatchability was observed by them.

At hatch, chick grades (salable chicks, culls, and unhealthy navels), hatchling weight, and hatchling length were affected only by period ($P < 0.001$), except for physical abnormalities, for which no period effect was observed ($P > 0.05$; Table 6). No diet effect was observed in these parameters ($P > 0.05$). As expected, hatchling weight and length increased with flock age as a consequence of the increase in egg weight [48].

The improvements in relative eggshell weight and eggshell thickness, as well the reduction in early embryo mortality when broiler breeder hens were fed the ISO or on top diet may be due to an increase in the bioavailability of these minerals in the intestinal lumen. It has been hypothesized that OTM can resist dissociation in the relatively low pH of the proventriculus and giz-

zard, thus allowing the intact complex to be delivered to the absorptive epithelium of the small intestine [49]. This may result in decreases in antagonistic interactions between minerals and the binding with nutrients and nonnutritive components of the digesta [2]. Therefore, it is likely that Zn, Mn, and Cu bound to certain stable ligands could improve the reproductive performance of breeder hens. However, more detailed information about trace mineral metabolism is needed to clarify the difference between the OTM, ITM source, and their interaction when associated in breeder feed.

## CONCLUSIONS AND APPLICATIONS

1. Broiler breeders provided the ISO diet had improved eggshell weight and thickness and had less early embryo mortality than did hens consuming the control diet.
2. Hens consuming the on top supplementation had thicker and heavier eggshells compared with those consuming the control diet.
3. Total or partial replacement of sulfates with OTM could potentially reduce manure trace mineral levels without any negative effects on various aspects of broiler breeder production, fertility, or hatchability.

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