SUPPLEMENTATION OF MANGANESE AND ZINC IN LAYING HENS DIET IMPROVES EGGSHELL QUALITY

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Abstract

This experimental study evaluated the supplementation effects of Zn and Mn from laying hens diets on the physical parameters of egg quality. The experiment was conducted for 42 days, on 120 Lohmann Brown laying hens, aged 22 weeks. Birds, accommodated in cages (3 birds/cage), were divided in 4 batches (C, E1, E2, E3). The basal diet (corn, rice, rape meal and soybean meal), identical for all four batches, was characterized by 17.96% protein and 2724.31 kcal/kg metabolisable energy. Diets of the 4 batches differentiated among them by Mn (source MnO) and Zn (source ZnO) levels (mg/kg). Conventional diet of batch C had 71,9 mg Mn/kg NC and 60 mg Zn/kg CF(compound feed). In batch E1 dietary levels of Mn and Zn (85 mg Mn, respectively 145 mg Zn /kg CF) were 5 times higher than NRC requirements, 1994. Batch E2 diet had 100 mg Mn/kg CF and 80 mg Zn/kg CF (according to Lohmann Brown Guide Manual), and batch E3 used double levels in regard to Lohmann Brown Guide Manual (200 mg Mn, respectively 160 mg Zn/kg CF). The results showed that eggshell breaking strength was significantly ($P \leq 0.05$) higher for E3 batch (4,68±0.16 kgF) compared to all other batches. Similarly, E3 batch had a significantly ($P \leq 0,05$) higher Zn concentration in eggshell compared to C and E2 batches. Eggshell thickness was not different between batches

Key words: *laying hen, Mn, Zn, egg, eggshell quality*

INTRODUCTION

Eggshell must be flawless especially because it represents the first barrier against bacterial penetration, a determinant factor in ensuring the safety of this valuable food. High eggshell breaking strength and flawless eggshell are essential for protection against egg penetration by pathogens such as Salmonella sp.

In this context, eggshell quality is one of the major concerns for avian industry. Eggs with damaged shell represent 6-10% of the total egg production, leading to great economic losses [11], [14].

Further efforts are made to improve eggshell quality in areas such as genetics, environment and nutrition, especially mineral nutrition [9]. Trace elements are among the nutrients used for hens feeding that may affect eggshell quality. Trace elements may affect eggshell quality, by their catalytic properties as key enzymes involved in the formation of membrane and eggshell or by direct interaction with calcite crystals during shell formation.

Mabe et al. [5] state that trace elements such as Mn, Zn, and Cu influence the mechanical properties of eggshell.

Manganese (Mn) activates glycosyl transferase that is involved in the formation of mucopolysaccharides, components of proteoglycans [3]. Leach and Gross [4] observed that hens fed with diets deficient in Mn (less than 7 mg / kg) produced eggs with thinner skin, translucent areas due to changes in the ultrastructure of the eggshell mammillary layer and a decrease in polysaccharides concentration, precursors of eggshell matrix.

Zinc (Zn) is one of the components of the carbonic anhydrase enzyme, which is crucial for providing carbonate ions during eggshell formation. Inhibition of this enzyme leads to decreased bicarbonate ion secretion and consequently greatly reduces eggshell weight [8]. Manganese and zinc are essential trace elements, usually supplemented in laying hens diets. However, starting from the premise that the bioavailability of minerals in feed is low, nutritionists and hybrids manufacturers recommend diet supplementation with these elements, in excess, compare to animal requirements. According to NRC (1994) [7], Mn and Zn requirements for laying hens are 28 mg Mn / kg and 33 mg Zn / kg. Manufacturers of laying hens hybrids recommend Mn and Zn additional supplementation at levels 3-4 times higher.

The purpose of this study was to monitor if laying hens basic diet supplementation with established levels of Zn and Mn from inorganic sources improves eggshell quality.

MATERIAL AND METHOD

The experiment was conducted in an experimental room of IBNA Balotesti, on 120 Lohmann Brown hens, aged 22 weeks, divided into 4 batches of 10 repetitions per batch (repetition being a cage with 3 hens). Experimental room is equipped with cages (59.5 cm length × 55.5 cm depth × 41.5 cm height), type Zucami (Zucami Poultry Equipment, Spain). During the experiment (22 to 28 weeks) the light regimen was 16 hours/day, between 04:30 a.m and 8:30 p.m. Food and water were provided *ad libitum*. Diet formulation considered both the nutritional requirements for laying hens [7] and Lohmann Brown hybrid producer compliance requirements. The basic structure of experimental diets (Table 1) was the same for all four groups.

Table 1 Basic diet structure

Nr. crt.	Ingredient	Structure (%)
1.	Corn	40.09
2.	Rice	20
3.	Rapeseed meal	15
4.	Soybean meal	10
5.	Gluten	2
6.	Sunflower oil	2
7.	Phosphate	1.26
8.	Calcium Carbonate	8.3
9.	Salt	0.2
10.	Methionine	0.1
11.	Choline	0.05
12.	Premix*	1*
Total		100
Determined crude chemical composition		
Metabolizable energy kcal/kg		2724.31
Dry substance %		96.47
Crude protein %		17.96
Crude fat %		5.86
Crude fiber %		5.95
Ash %		14.48
Calcium %		3.79
Total phosphorus %		0.68
Available phosphorus %		0.34

* Premix structure was different between batches by Mn oxide and Zn oxide concentration

The vitamin mineral premix of the C batch was a conventional one (ZOOFORT produced by IBNA Balotesti). This premix provides 72 mg of Mn / kg and 60 mg of Zn / kg feed (Table 2). These values exceed the requirements of minerals published in NRC (1994) [9]. Mn and Zn levels of E1 exceed five times NRC (1994) requirements [7]. E2 premix provides Mn and Zn in feed according to Lohmann Brown recommendations (100 mg Mn / kg and 80 mg Zn / kg). P3 premix provides Mn and Zn in excess both to the requirements and to guideline recommendations (two times higher than the guide concentration - 200 mg Mn / kg and 160 mg Zn / kg). Table 2 shows C, E2 and E3, the Mn / Zn ratio in the premix is higher than one, whereas in E1 it 0.85, similar with NRC (1994) [7].

Table 2 Mn and Zn levels supplemented into diets through vitamin-mineral premix

Dietary sources of Mn and Zn	C mg/kg feed	E1 mg/kg feed	E2 mg/kg feed	E3 mg/kg feed
Mn (MnO) from premix	71.90	85.00	100.00	200.00
Zn (ZnO) from premix	60.00	145.00	80.00	160.00
Mn/Zn ratio in premix	1.20	0.850	1.25	1.25

Parameters of bioproductivity monitored in the experiment were: chicken weight, average daily feed intake, laying percentage, feed conversion ratio (kg feed / kg egg), average egg weight. Feed ingredients and compound feed samples were sampled and analyzed for: dry matter, protein, fat, cellulose, ash, Ca, P, Mn and Zn according to Regulation (EC) no. 152/2009 [12]. At the beginning of the experiment and on weeks 24, 25, 26 (hen age) and in the final week (week 28) 18 eggs / batch were collected and analyzed for: physical parameters of eggshell quality: eggshell weight (Kern maximum precision balance (0.01 g), thickness (Egg Shell Thickness Gauge, Sanovo engineering A/ S, Denmark), breaking strength (Egg Force Reader, Sanovo engineering A/S, Denmark). Of the 18 eggs harvested / batch, 6 samples of shell (3 eggs / batch) were formed and analyzed for: ash (gravimetric, SR EN ISO 2171: 2010), Ca (Titrimetric, ISO 64901: 2006), Mn (atomic absorption spectrophotometry, SR EN ISO 6869: 2002) and Zn (atomic absorption spectrophotometry, SR EN ISO 6869: 2002). Blood samples were collected (10 hens / group) to be assessed by a third party laboratory in order to determine Ca, P, Mn and Zn concentration in serum.

RESULTS AND DISCUSSIONS

Productive performance data (Table 3) show that E3 recorded an average daily feed intake significantly ($P \leq 0.05$) lower than the other batches. However, feed conversion ratio at E3 was significantly ($P \leq 0.05$) higher than E1 and E2. The average weight of egg on E1 was significantly ($P \leq 0.05$) lower than C, E2 and E3.

During the experiment, significant differences appeared between some of eggshell quality parameters collected from the 4 batches (Table 4).

Table 3- Bioproductive performances

Specification	C	E1	E2	E3
Initial weight, kg	1.743±0.152	1.747±0.066	1.740±0.099	1.736±0.077
Final weight, kg	1.865±0.152	1.822±0.167	1.857±0.137	1.863±0.126
ADFI, g /head/day	115.672±14.584 d	115.315±9.488 d	114.229±11.874 d	111.012±14.282 a,b,c
FCR, kg /kg egg	2.316±0.815 b,c	2.137±0.414 a,d	2.075±0.397 a,d	2.33±0.845 b,c
Egg intensity,%	96.906±20.637	96.072±13.984	96.979±13.245	95.182±22.21
Average egg weight, g	59.639±4.84 b	57.482±3.82 a,c,d	58.113±5.22 b	58.409±4,584 b

Note 1: Where: a = significantly different (P≤0,05) compared to C; b = significantly different (P≤0.05) compared to E1; c= significantly different (P≤0,05) compared to E2; d= significantly different (P≤0,05) compared to E3; ADFI= Average daily feed intake; FCR= Feed conversion ratio

The concentration of Mn in eggshell samples could not be determined being below the limit of detection of the method used but there are references in the literature according to which the level of Mn in diet influences shell quality. Mabe et al. [5] show that manganese supplementation (50-80 mg / kg) increased eggshell weight and, also, improves the mechanical properties of eggshell independently of the quantity effect on the composition of eggshell. Data from Table 4 are according with these observations of Mabe et al. [5]. Right from the beginning of the experiment (Figure 1), eggshell breaking strength values were significantly($P \leq 0.05$) higher (Table 4) for the batch with the highest Mn supplementation (E3) compare to the other batches. Compared to C and E1 and also E2, where large supplements of Mn and Zn were added, egg shell breaking strength was significantly ($P \leq 0.05$) higher.

Table 4 Eggshell quality parameters

Batch	C	E1	E2	E3
Mn supplement (mg /kg feed)	72.00	140.00	100.00	200.00
Zn supplement (mg /kg feed)	60.00	165.00	80.00	160.00
Eggshell weight . g	6.782 ±0.36d	6.900 ±0.50d	7.083 ±0.53	7.247 ±0.51 a.b
Eggshell thickness. mm	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01
Eggshell breaking strength. kgF	4.09 ± 0.07bcd	4.46 ± 0.07ad	4.41 ± 0.09ad	4.68 ± 0.16abc
Ash. g %	52.50 ± 0.52	52.65 ± 0.40	52.49 ± 0.33	52.67 ± 0.62
Ca. g%	35.38 ± 0.12d	35.37 ± 0.19d	35.58 ± 0.16d	35.93 ± 0.33abc
Zn. mg/kg	2.92 ± 0.08d	2.98 ± 0.07	2.88 ± 0.25d	3.19 ± 0.29ac

Note 2: Where: a = significantly different ($P \leq 0{,}05$) compared to C; b = significantly different ($P \leq 0{,}05$) compared to E1; c= significantly different ($P \leq 0{,}05$) compared to E2; d= significantly different ($P \leq 0{,}05$) compared to E3

From Table 4 data, taking into consideration the fact that the ratio of dietary calcium was similar for all batches, it is acceptable that a supplementation of Zn and Mn trace elements in diet has a beneficial effect on eggshell quality.

Eggshell thickness (Table 4) was not affected by Mn and Zn supplements, neither was the relationship between the levels of these trace elements in the diet (Table 2). Data of the Table 5 show however that it was a good correlation between eggshell Zn concentration and eggshell thickness (0.822). The literature too displays quite a range of different reviews concerning the optimal levels of Mn and Zn in order to ensure eggshell mechanical qualities. Stahl et al. [13] showed that the level of 30 mg Zn / kg diet is sufficient to ensure a high quality of the eggshell, while Zamani et al. [15] reported that only an additional 50 mg Zn / kg diet had a positive effect on eggshell thickness. Inal et al. [2] consider that a quantity of 25 mg Mn / kg diet is sufficient for a maximum bioproductive parameters but the requirement for an optimum eggshell quality in laying hens is much higher. Some authors believe, however, that supplements more than 70-100 mg Mn / kg do not have further beneficial effects in terms of eggshell quality [1]

It is interesting that eggshell calcium concentration (Table 4) was significantly higher ($P \leq 0.05$) in E3, although Milos and Dranceanu [6] show that Zn and Mn have antagonistic effects on the absorption of calcium. As it can be seen in Table 5, there is a strong correlation between eggshell concentration of Ca and Zn and eggshell breaking strength. Eggshell Zn concentration was in good correlation with shell thickness (0.822) and eggshell ash (0.829). Also, there was a good correlation (0.822) between eggshell Zn concentration and eggshell Ca concentration.

A good correlation was registered between eggshell breaking strength and Mn concentration from diet (0,971), respectively, Zn concentration from diet (0,953).

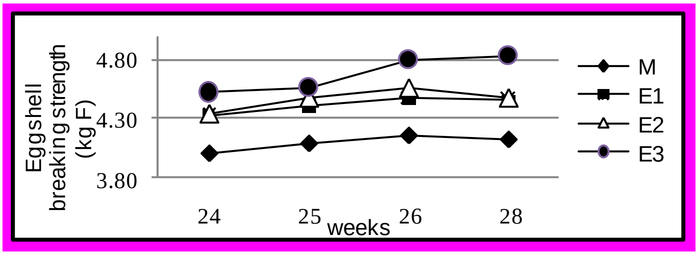

Figure 1- Eggshell breaking strength time evolution

Table 5 Eggshell quality parameters correlation matrix

	Eggshell breaking strength, kg F	Eggshell thickness, mm	Eggshell ash %	Eggshell calcium %	Eggshell zinc mg/kg
Eggshell breaking strength, kg F	1.000	0.433	0.650	0.829	0.704
Eggshell thickness, mm	0.433	1.000	0.368	0.829	0.822
Eggshell ash, %	0.650	0.368	1.000	0.434	0.829
Eggshell calcium, %	0.829	0.829	0.434	1.000	0.822
Eggshell zinc, mg/kg	0.704	0.822	0.829	0.796	1.000

From the data obtained above, taking into consideration the fact that the ratio of dietary calcium was similar for all batches, it is acceptable that a supplementation of Zn and Mn trace elements in diet has a beneficial effect on eggshell quality.

The highest serum Mn and Zn values (Table 6) were obtained in E3. It is noticed, that between Mn concentration in serum and eggshell breaking strength there was a good correlation (Figure 2). Ochrimento et al.[10], showed an increase of 4 N force of eggshell breaking strength when supplemented by 300 mg / kg Mn diet. This observation may suggest that Mn may be the mineral responsible for the improvements concerning eggshell breaking strength.

Table 6 Biochemical parameters determined in laying hens serum (28 weeks)

Parameter	C	E1	E2	E3
Ca (mg/dL)	20.93 ± 2.40	21.47 ± 3.14	20.34 ± 4.78	24.05 ± 2.94
P (mg/dL)	4.22 ± 0.88	5.02 ± 1.30	5.04 ± 1.48	4.97 ± 0.59
Mn (µg/dL)	106.82 ± 24.45d	89.37 ± 6.02d	109.01 ± 28.22d	130.23 ±30.60abc
Zn (µg/dL)	418.33 ± 50.34	395.70 ± 86.08	389.62 ± 63.29	426.12 ± 115.90

Note 3: Where: a = significantly different ($P \leq 0,05$) compared to C ; b = significantly different ($P \leq 0,05$) compared to E1; c= significantly different ($P \leq 0,05$) compared to E2; d= significantly different ($P \leq 0,05$) compared to E3

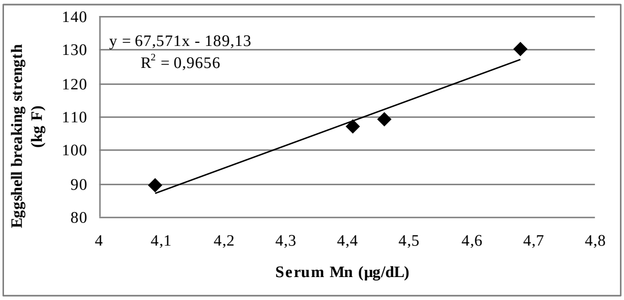

Figure 2 Correlation between serum Mn concentration and eggshell breaking strength

CONCLUSIONS

The batch with the highest Mn supplementation (200 mg / kg feed) and Zn supplementation (160 mg / kg feed) recorded the highest eggshell weight and the highest eggshell breaking strength. A differentiation ($P \leq 0.05$) between batches was noticed, after only 2 weeks of trial, in favour of E3 in terms of eggshell breaking strength. According to this experiment, a supplementation over 100 mg Mn / kg feed influences beneficially the mechanical properties of the eggshell.

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