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Effect of Different Levels of Manganese and Zinc on Performance Traits and Breaking Eggs in Laying Hens

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Abstract: This experiment was conducted to evaluate the effects of supplementing the diet of laying hens with a combination of Zn and Mn on performance traits and broken eggs in a Completely Randomized Design with a 4×4 (16 treatment) factorial arrangement. Three hundred and twenty, 28 weeks old white leghorn laying hens, strain Hy-Line W_{36} were divided into 64 groups, five hens per group and each four groups were assigned to one of the 16 experimental diets. Sixteen experimental diets contained a corn-soybean basal diet containing 50 mg kg^{-1} Zn and 30 mg kg^{-1} Mn supplemented with 0-0, 0-30, 0-60, 0-90, 50-0, 50-30, 50-60, 50-90, 100-0, 100-30, 100-60, 100-90, 150-0, 150-30, 150-60 and 150-90 mg kg^{-1} of Zn and Mn, respectively. The results indicated that Mn and Zn in combination, Mn alone and age had not any effect on egg output, feed consumption and feed conversion ratio. Mn and Zn alone and in combination had a significant ($p<0.05$, $p<0.05$ and $p<0.01$, respectively) effect on broken eggs. Different levels of Zn had significant ($p<0.05$) effect on egg production, without any effect on egg output, feed consumption and feed conversion ratio. Age of birds had significant ($p<0.05$) effect on feed consumption but did not affect egg output, egg production and feed conversion ratio.

Key words: Mn, Zn, laying hen, egg

INTRODUCTION

The quality of the eggshell is of primary concern to the poultry industry. The successful development of chicken embryos is dependent upon a robust eggshell for mechanical protection, protection from infection, prevention of water loss and as a primary source of calcium for the embryonic skeleton. On the other hand, the commercial production and marketing of eggs exposes them to insults that cause a high rate of broken or cracked eggshells which are responsible for major economic losses to the egg producers. Additional losses occur in the highly mechanized washing, grading and packing procedures. The eggshell serves to protect the egg contents, but it is also the first barrier against bacterial penetration and must be free from defects in order to optimize the safety of the egg contents for human consumption. A great deal of effort has been applied to improve eggshell quality in the fields of genetics, environmental condition and nutrition, especially mineral nutrition[1]. Trace elements may affect eggshell quality either by their coenzymatic effect on key enzymes involved in the process of membrane and eggshell formation or by interacting directly with the calcite crystals in the forming eggshell. Mn, for example, activates the glycosyl transferases involved in the formation of mucopolysaccharides, which are components of proteoglycans[2]. In bone, proteoglycans from the epiphyseal zone contribute to its ability to resist compressive charges[2]. Likewise, keratan and dermatan proteoglycans are present in the eggshell matrix and may be involved in the control of its structure and texture[1,3], consequently, they may influence its mechanical properties[4]. Another trace element, Zn is a component of the carbonic anhydrase enzyme, which is crucial for supplying the carbonate ions during eggshell formation. Inhibition of this enzyme results in lowered bicarbonate ion secretion and, consequently, greatly reduces eggshell weight[4].

As previously mentioned, trace elements may also directly affect eggshell structure through a modifying effect on calcite crystal growth mechanisms. It has been shown that some trace elements can modify the morphology of the calcite crystal precipitated *in vitro*. For instance, Mn interferes with the face parallel to the 'c' axis and favors an elongation of the calcite crystal along its 'c' axis in contrast, Li inhibits the growth of this axis[5,6]. Thus, it is also possible that the presence of trace elements could alter the crystallographic texture of eggshells and consequently, the mechanical properties. Essary and Holmes[7] reported that even though adding Mn plus Zn to the diet increased shell strength, egg

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production decreased when Zn was added alone and also addition of Mn to diet decreased number of cracked eggs. Turk *et al.*[8] found no deficiency symptoms and normal offspring from laying hens fed a purified casein diet containing 15 mg kg^{-1} Zn. Kienhols *et al.*[9] found that laying hens fed an isolated soy-based diet containing 10 mg kg^{-1} Zn had lower egg production and hatchability than normal, unless the diet contained additional Zn, but the National Research Council[10] suggests 50 mg kg^{-1} Zn for laying hens. James *et al.*[11] found that hens receiving 20 mg kg^{-1} extra Zn (48 mg kg^{-1} total Zn) had significantly lower egg production than the 0 mg kg^{-1} (28 mg kg^{-1} total Zn) and also Zn-supplemented birds showed no significant differences in feed conversion or feed intake. In this relation others found no increase in egg production when extra Zn was added to a practical ration fed to layers maintained on the floor or cages[12,13]. Age of laying hens is also another factor which should be considered in these controversial results which is not available in these kinds of reports. As Balnave and Muheerazs[14] and Keshavars[15] found that age of hens produced significant effects on egg production, but Yahav *et al.*[16] reported that age of hens produced no significant effects on egg production.

Berry and Break[17] found increase in egg production when supplemental Zn was added to a practical ration fed to layers. Reviewing different reports about the trace elements in the laying hens diet makes these opposite results more complicated[18-20]. Holder and Huntley[12], Maurice and Whisenhut[21] and Sazzad *et al.*[22] reported no significant difference in egg production and feed conversion with increasing Mn level in the diet. Egg production and egg weight were higher with a dietary Mn level higher than the NRC[23] estimated requirements.

To decrease egg breaking it is necessary to identify the molecular constituents involved in the mineralization of the eggshell. Biological molecules guide mineralization processes through a series of specific and definable calcium-bio molecule interactions that lead to the deposition of specific and uniquely oriented crystalline structures[24]. Eggshell assembly and mineralization are guided by an array of bio molecules that follow a set of biological principles for the mineralization process. The purpose of this study was to evaluate the effect of adding Zn and Mn mineral supplements to a corn-soybean diet fed to laying hens on the performance traits and breaking eggs. The effects of relatively high and low levels of these minerals alone and in combination and the age of hens were also considered in this investigation.

MATERIALS AND METHODS

Experimental hens and diets: Three hundred and twenty laying hens (Hy-Line W_{36}) were divided into 64 Groups and four cages of 5 birds per cage were assigned to one of the 16 experimental diets. Birds kept under 16L: 8D lighting program. The birds had free access to food and water during experimental period. Initially the birds were fed with a conventional layer diet containing adequate levels of all nutrients as recommended by NRC[10]. The experimental period, which lasted 12 weeks, began when the birds were 28 weeks old. During the first 3 weeks of this experiment, all hens were fed a basal diet (Table 1). The experimental diets were introduced at the end of week-3 and were fed for a total of 9 weeks. These diets consisted of the basal diet supplemented with 0-0, 0-30, 0-60, 0-90, 50-0,50-30, 50-60,50-90, 100-0, 100-30, 100-60, 100-90, 150-0, 150-30, 150-60 and 150-90 mg kg^{-1} added Zn and Mn in combination. The supplementation minerals were ZnO and MnO.

Table 1: Composition of the basal diet

Ingredients	Composition(%)
Ground yellow corn	68.9000
Dehulled soybean meal	17.8000
Alfa Alfa meal dehydrated	2.0000
Fish meal	3.0000
Dicalcium phosphate	0.5500
Oyster shell	7.2000
Sodium chloride	0.2000
DL-Methionine	0.0200
Vitamin premix[1]	0.3000
Mineral premix[2]	0.0867
Mineral test[3]	0.0031
Vitamine D_3	0.0300
Nutrient composition	
ME (Kcal kg^{-1})	2900.0000
CP (%)	14.5000
Manganese (mg kg^{-1})	30.0000
Zinc (mg kg^{-1})	50.0000

[1] Provided per kilogram of diet: vitamin A, 5,000 IU; cholecalciferol, 750 IU; vitamin E, 7.5 mg; Menadione, 0.63 mg; Thiamine, 0.25 mg; Riboflavin, 1.6 mg; Pyridoxine, 0.500 mg; Vitamin B12, 4.0 μg; Niacin, 12.5 mg; Calcium pantothenate, 1.8 mg; Butylated hydroxyl toluene, 63 mg

[2] Provided per kilogram of diet: Iron, 44 mg; Iodine, 1.2 mg; Cobalt, 0.36 mg; Selenium, 0.24 mg; Mn, 30 mg; Zn, 24 mg and Cu, 2.4 mg

[3] Test mineral supplement contained variable amounts of $CaCo_3$, wheat straw and Zno

In order to examine breaking eggs, pieces of shell were cut from the equatorial region. The shells were fixed to aluminum stabs with silver paint and once dry were coated with gold/palladium prior to viewing in a Phillips 501 B scanning electron microscope at an accelerating voltage of 15 Kv. Transverse sections of each shell were mounted in grooved aluminum stubs.

Data collection: Breaking eggs and egg production was recorded every day and feed consumption was recorded every 1.5 weeks after introduction of the experimental diets with all groups of hens per treatment.

Statistical analysis: Statistical analyses were performed by using ANOVA in a factorial experiment with

Completely Randomized Design with the Statview software program[25]. Calculations were carried out with 4 eggs/cage and 16 eggs/treatment. When significant effects were detected by ANOVA, treatment means were compared using Duncan's Multiple Range Test.

RESULTS

The current study suggests that addition of combined Zn and Mn significantly ($p<0.05$) influence the amount of eggshell material deposited during shell formation and can improve some of the mechanical properties and decrease broken eggs. As the Table 2 shows except the feed consumption other performance traits were not affected by age. In the current study average egg production had no relation with the age of hens. Addition of Zn and Mn in combination to the basal diet did no significantly influence egg production and Zn and Mn in combination had not effect on the feed conversion (Table 3). Mn and Zn supplementation alone and in combination to the basal diet had no obvious effect on feed consumption (Table 3 and 4). Addition of Zn significantly influenced egg production. The average egg production was found to be significantly ($p<0.05$) higher in the birds that supplied with 150 mg kg^{-1} Zn than those receiving 0, 50 and 100 mg kg^{-1} Zn, but Mn did not influence egg production (Table 4). The results of the present study demonstrated that Zn and Mn in combination and alone and also periods of age in the birds did not significantly influence on egg output (Table 2-4).

The birds supplied with 30 or 60 mg kg^{-1} Mn and also the birds supplied with 150 mg kg^{-1} Zn had lower egg breaking (Table 4) which were statistically significant ($p<0.05$). Results about breaking eggs presented in Table 3 also confirm statistically significant ($p<0.01$) difference due to Zn and Mn in combination treatments, the average breaking eggs was found to be significantly lower in the birds that received mineral supplementation of 0-60, 100-30, 150-30 and 150-60 mg kg^{-1} of Zn and Mn, respectively. These findings are confirmed by electron microstructure photographs of the eggshell (Fig. 1 and 2).

Table 2: Effect of different periods of age on performance traits in laying hens

Age (weeks)	Egg production (%)	Feed consumption (g/hen/day)	Feed conversion (g/g)	Egg output (g/hen/day)
31-32.5	72.30	90.25^{b}	2.450	36.80
32.5-34	72.15	90.05^{b}	2.460	36.60
34-35.5	72.00	90.15^{b}	2.470	36.50
35.5-37	73.20	90.00^{b}	2.330	38.60
37-38.5	72.70	91.63^{ab}	2.400	37.70
38.5-40	72.20	94.26^{a}	2.460	37.00
SEM	0.089	00.62	0.021	00.68
Probability	NS	p<0.01	NS	NS

^{a}, ^{b}Means within a column with no common superscript differ significantly

Table 3: Effect of different levels of Mn and Zn in combination on performance traits and breaking eggs in laying hens

Supplement (mg/kg) Zn	Mn	Breaking eggs (%)	Feed consumption (g/hen/day)	Feed conversion (g/g)	Egg output (g/hen/day)	Egg production (%)
0	0	1.80^{a}	91.10	2.50	36.70	67.30
0	30	1.60^{a}	91.60	2.40	37.70	69.80
0	60	0.50^{b}	92.00	2.40	39.00	64.00
0	90	1.40^{a}	90.90	2.50	36.40	68.80
50	0	1.10^{a}	90.10	2.50	36.00	68.00
50	30	1.20^{a}	91.10	2.50	36.60	68.80
50	60	1.00^{a}	91.10	2.50	36.70	68.80
50	90	1.20^{a}	92.00	2.30	39.50	69.80
100	0	1.70^{a}	92.10	2.40	39.00	69.80
100	30	0.60^{b}	91.00	2.40	37.80	68.60
100	60	1.40^{a}	90.20	2.50	36.20	71.60
100	90	1.10^{a}	90.60	2.40	37.90	69.00
150	0	1.00^{a}	91.60	2.50	36.60	71.40
150	30	0.50^{b}	91.10	2.30	38.60	71.00
150	60	0.50^{b}	92.00	2.50	36.80	69.80
150	90	1.70^{a}	90.00	2.40	37.60	69.80
SEM		0.45	1.01	0.03	0.95	0.82
Probability		p<0.01	NS	NS	NS	NS

^{a}, ^{b}Means within a column with no common superscript differ significantly,
SEM: Standard Error Mean, NS: Non-Significant

Table 4: Effect of different levels of Mn and Zn alone on performance traits and breaking eggs in laying hens

Supplement (mg/kg)	Breaking eggs (%)	Feed consumption (g/hen/day)	Feed conversion (g/g)	Egg output (g/hen/day)	Egg production (%)
Main effect					
Zn					
50	1.30^{a}	91.40	2.40	37.40	67.50^{b}
100	1.10^{ab}	91.10	2.40	37.20	68.80^{b}
150	1.20^{ab}	91.00	2.40	37.70	69.70^{ab}
200	0.90^{b}	91.40	2.40	37.40	70.50^{a}
SEM	0.22	0.83	0.02	0.47	0.41
Probability	p<0.05	NS	NS	NS	p<0.05
Mn					
30	1.40^{a}	91.20	2.50	37.10	69.00^{ab}
60	1.00^{b}	91.20	2.40	37.70	69.50^{ab}
90	0.90^{b}	91.30	2.50	37.20	69.00^{ab}
120	1.40^{a}	91.10	2.40	37.80	69.30^{ab}
SEM	0.22	0.83	0.02	0.47	0.41
Probability	p<0.05	NS	NS	NS	p<0.05

^{a}, ^{b}Means within a column with no common superscript differ significantly
SEM: Standard Error Mean, NS: Non-significant

Fig. 1: Egg shell received diet with 200 mg kg^{-1} Zn in combination with 90 mg kg^{-1} Mn

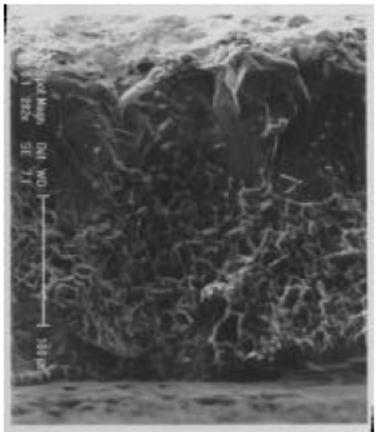

Fig. 2: Egg shell received diet with 50 mg kg^{-1} Zn in combination with 30 mg kg^{-1} Mn

As Fig. 1 and 2 demonstrate highest level of Zn (total 200 mg kg^{-1}) in combination with higher level of Mn (total 60 or 90 mg kg^{-1}) have more density than control group.

DISCUSSION

Trace element supplements are frequently fed to laying hens to reduce eggshell breakage[1,26,27]. In this study with using a wide range of Zn and Mn we obviously show these effects. These findings have been refuted by Balnave and Muheeraza[14] but are in agreement with Yahav *et al.*[16]. The difference between results is relatively related to the conditions and quantity of minerals used, which the present study shows higher level and combined form are more effective. Relation between production and mineral are also complicated, as some have reported decrease in the egg production with increase in Zn, another reports believe increase in level of Zn in diet have no effect on the egg production[17,18,28,29], but Watson[30] believes Zn increases egg production. This situation also exist about the Mn, as these findings are in agreement with Berry and Break[17] but have been refuted by Stahll *et al.*[31] who reported increasing of Mn in the diet increase egg production.

The average food consumption was found to be related to the age of hens (Table 2). These finding have already been reported which is in agreement with numerous studies[15,32-34] and development of the digestive system of the bird and requirement of more food is a dynamic response of the body. Feed conversion was found to be similar in the all periods of age (Table 2). This factor is also controversial and similar studies have found different results by Keshavars[16], Chen and Balnave[32], Hsu *et al.*[33], Xin *et al.*[34] and Balnave and Chen[35]. In this regard many factors would affect the feed conversion which is not obvious and interfere with the experiment. Despite all the efforts for controlling the circumstances there are many factors such as climate, antibiotics, kind of nutrients with different sources and more importantly micro-flora kind and population content of the digestive system, in different regions seriously affect the feed conversion. The addition of Mn supplement to the basal diet had no obvious effect on feed conversion (Table 4). These findings are partially in agreement with other studies in this relation[31,36]. As the final point this study very obviously represents the special effect of these minerals on crystallographic texture of egg shell and consequently decrease breaking eggs, that highest level of Zn (total 200 mg kg^{-1}) and higher levels of Mn (total 60 or 90 mg kg^{-1}) in the diet decrease egg breaking that is promising in this respect and further studies are necessary to be confident.

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