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Supplementation of a Corn-soybean Meal Diet with Manganese and Zinc Improves Eggshell Quality in Laying Hens

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Abstract: An experiment was conducted to evaluate the effects of supplementing the diet of laying hens with a combination of Zn and Mn on eggshell quality. Six hundred and forty white leghorn laying hens, strain Hy-Line W_{36} were divided into two groups according to age (28 and 44 week old). Each group then was divided into 64 and five birds per group (totally 128 groups). In both groups every four cages were assigned to one of the 16 experimental diets. Sixteen experimental diets included 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. Addition of Zn increased eggshell thickness, concentration of Ca in eggshell and eggshell index and decreased concentration of P in eggshell significantly ($p<0.05$) and did not affect the following measures of eggshell quality: percentage, stiffness, elastic modulus, breaking strength and fracture toughness of eggshell and egg weight. In contrast addition of Mn increased percentage, stiffness, elastic modulus, breaking strength and fracture toughness of eggshell significantly ($p<0.05$), but did not affect the following measures of eggshell quality: Concentration of P and Ca, index and thickness of eggshell and egg weight. Addition of Zn and Mn in combination increased percentage, index (shell weight per unit surface area), stiffness, elastic modulus, breaking strength, thickness, fracture toughness and concentration of Ca in eggshell and decreased concentration of P in eggshell and did not affect egg weight. In older hens there was a significantly increase in egg weight, breaking strength, elastic modulus, stiffness and fracture toughness of eggshell and significantly ($p<0.05$) decrease in thickness, percentage and index of eggshell without any effect on concentration of P and Ca in eggshell.

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

INTRODUCTION

Cracked or broken eggshells accounted for 80 to 90% of the eggs that are routinely downgraded. 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 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 coenzymatictic 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[3,4], consequently they may influence its mechanical properties[5]. In support of this hypothesis, Leach and Gross[6] observed that hens fed Mn-deficient diets produced eggs with thinner shells, with translucent areas and abnormalities in eggshell ultrastructure, particularly in the mammillary layer[6].

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[5]. As previously mentioned, trace elements may also directly affect eggshell structure through a modifying effect on calcite crystal growth mechanisms. During shell

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formation the first calcite crystals are deposited on the outermost surface of eggshell membranes at specific sites. Thereafter the spherulitic crystal growth continues to form a compact or palisade layer. The completed eggshell has a particular ultrastructure and crystallographic organization, such as preferential crystal orientation[7,8]. This organization may result from crystal growth competition between adjacent nucleation sites[7,8] and from the modifying effects on the size and orientation of the crystals caused by the presence of organic matrix components[3,5].

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[9,10]. Thus, it is also possible that the presence of trace elements could alter the crystallographic texture of eggshells and, consequently, the mechanical properties.

The purpose of this study was to evaluate the effect of adding a wide range of Zn and Mn mineral supplements in a corn soybean diet fed to laying hens on the mechanical properties and microstructure of the eggshells.

MATERIALS AND METHODS

Experimental hens and diets: Six hundred and forty laying hens (Hy-Line W_{36}) were divided into two groups according to age (28 and 44 weeks old). Each group then were divided into 64 groups (totally 128 groups), five birds per group and kept under 16L: 8D lighting program. The birds had free access to food and water during the experimental period. Initially the birds were fed with a conventional layer diet which contained adequate levels of all nutrients as recommended by NRC[11]. The experimental period which lasted 12 week, began when the birds were 28 and 44 week old for young and old groups, respectively. The first 3 week of the experimental period considered as adaptation period and all birds 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 week. These diets included 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 (ZnO and MnO), respectively. In both groups every 4 cages of 5 birds were assigned to 1 of the sixteen diets treatment.

In order to examine eggshell thickness with electron microscope, 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.

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
Vitamin 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 B_{12}, 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

Data collection: Feed consumption was recorded every 2 week after introduction of the experimental diets with all groups of hens per treatment. Eggshell quality was assessed four times: 3 week after the introduction of the basal corn-soybean diet without supplementation of Zn and Mn (pre-experimental diet phase); and then, 1, 5 and 9 week after the introduction of the experimental diets. Egg weight (g), egg length and breadth (mm), shell weight (g), stiffness (N mm^{-1}) and breaking strength (N) were measured on 3 eggs per cage during experimental period. Eggshell weight was measured after washing the interior egg membrane and after drying overnight at 80°C. The percentage shell [(eggshell weight/egg weight) × 100], shape index (SI = egg length/breadth), egg surface area ($S = 4.68 \times P^{2/3}$, where, P = egg weight), eggshell index [shell weight/unit surface, I = [P shell/S] × 100, where, P shell = shell weight (g)] were also calculated. Eggshell thickness (T; mm) was subsequently estimated using the formula T = P shell/(S × d), where, d = material density (d = 2.3 for calcium carbonate). Mechanical stiffness and breaking strength were measured by quasistatic compression using a machine fitted with a 50-N load cell. The deformation (mm) of the eggshell was measured using a nondestructive load of 10 N at a compression speed of 1 mm min^{-1}. The mean deformation of each egg was derived from 3 consecutive measurements that were carried out at different sites around the equator of each egg (spaced about 120° from each other). According to Voisey and Hamilton[12,13], the slope of the force versus deformation (F/d) curve provides a measure of the overall stiffness characteristics of an eggshell. In the current study the stiffness (Sd) of each egg was taken as the inverse function of the mean deformation (mm) at 10 N. Breaking strength (F) was measured as the maximum force (N) required to fracture each egg at a compression speed of 5 mm min^{-1}. Breaking strength, however, does not describe any physical property of the material from which the eggshell is constructed but describes the response of the eggshell as a composite complex structure[12]. The elastic modulus (E shell) and fracture toughness (KC) or resistance to fracture of each egg was therefore also computed, this time using formulas developed by Bain[14]. According to this author, these properties relate to the material strength of eggshells and allow more direct comparisons of the mechanical strength of eggs to be made as they take into account structural differences in shape, curvature and thickness in their calculation. The elastic modulus (N mm^{-2}) describes the contribution made by the eggshell material to the overall stiffness characteristics of the eggshell: Elastic modulus = C [(Sd.R)/T^2], where Sd = stiffness (N mm^{-1}), R = radius of egg (breadth/2 (mm)), T = thickness of the shell (which in this case was estimated from shell weight per unit surface area (mm)) and C = non dimensionalised constant. Bain[14] established that C is dependent on the Shape Index (SI), Radius of egg (R) and Thickness (T) of the eggshell and can be calculated as follows: C = A (0.408+(3.026T)/R) where, A = (-0.666 + (1.8666 × (SI))-(0.907 × (SI)2) + (0.153 × (SI)3))/0.444. Fracture toughness, or resistance to fracture (KC, N/mm3/2), is influenced by the nature and magnitude of inherent defects within a material. This value for eggshell was derived by the formula KC = Knd (F/T3/2). In this expression, Knd = 0.777 (2.388 + (2.9934 (6/R)), where R = radius of curvature (mm), F= breaking strength (N) and T= thickness of the shell (mm)[14].

Chemical analysis: A representative sample (n = 3) of each eggshell was ashed (550°C for 14 h) and then solubilized in 10 mL HCl (6 N) and 30 mL of demineralized water at a high temperature by using a sand heater (300°C for 15 min). After filtration (Whatman filter) the volume was increased to 100 mL with demineralized water. The Ca and P concentrations were subsequently measured by atomic absorption spectrophotometry.

Statistical analysis: Statistical analyses were performed by using ANOVA in a factorial experiment with Completely Randomized Design with the Statview software program[15]. Calculations were carried out in each group with hens of the same age (4 consecutive eggs/cage, 16 eggs/treatment). When significant effects were detected by ANOVA, treatment means were compared using Duncan Multiple Range test.

RESULTS

Egg weight: As these results indicate the average egg weight was found to be significantly ($p<0.05$) higher in the older group of birds (Table 2). The addition of Zn and Mn in combination and Mn alone to the basal diet did not influence egg weight (Table 3 and 4), but the birds supplied with 50 and 150 mg kg^{-1} Zn had higher average egg weight than those receiving 0 and 100 mg kg^{-1} Zn (Table 4).

Eggshell percentage, thickness and index values: As the results in Table 2 indicate the average percentage of eggshell was found to be significantly higher in the younger group of birds (28 vs 40 week). Similar nonspecific changes were also observed for the eggshell index values (Table 2) for this particular group of birds. The addition of Zn did not influence on eggshell percentage (Table 4). The average percentage of eggshell was found to be significantly ($p<0.05$) higher in the birds that received a mineral supplementation of 0-30, 50-60 and 50-90 mg kg^{-1} of Zn and Mn, respectively (Table 3). But birds supplied with 60 mg kg^{-1} Mn had higher average percentage of eggshell (Table 4). Addition of Zn and Mn in combination to the basal diet significantly ($p<0.05$) influenced eggshell index. The average eggshell Index was found to be significantly higher in the birds that received of 0-30, 50-0, 50-90, 100-30, 100-60, 100-90, 150-0, 150-60 and 150-90 of Zn and Mn, respectively (Table 3). But addition of Mn alone did not affect the eggshell index (Table 4). It is interesting that birds supplied with 100 mg kg^{-1} Zn had higher average eggshell index than those receiving 0, 50 and 150 mg kg^{-1} Zn (Table 4). The average eggshell thickness was found to be significantly ($p<0.05$) higher in the older group of birds (Table 2). The average eggshell thickness was found to be significantly higher in the birds that received mineral supplementation of 150-60 and 150-90 of Zn and Mn, respectively (Table 3). These results are also confirmed by microstructure figures of eggshell from different groups (Fig. 1 and 2). Zn also affected the eggshell thickness as the birds supplied with 50 and 150 mg kg^{-1} Zn had higher average eggshell thickness than those receiving 0 and 100 mg kg^{-1} Zn (Table 4).

Table 2: Egg weight, thickness, index, percentage and mechanical properties (stiffness, elastic modulus, Breaking strength, fracture toughness), Ca an of eggshell from hens of different ages

Experimental diets	Egg weight (g)	Thickness (mm)	Index (g/100 cm^2)	Stiffness (N mm^{-1})	Elastic modulus (N mm^{-1})	Breaking strength (N)	Fracture toughness	Eggshell (%)	Concentration of Ca (%)	Concentration of P (%)
Basal diet[1] (28-31 week)	48.7^{b}	0.34^{b}	8.1^{b}	154^{b}	15055^{b}	32.0^{b}	405^{b}	9.27^{a}	56.0	0.83
Week 1 (32 week)	49.0^{b}	0.34^{b}	8.4^{a}	163^{a}	15300^{a}	37.2^{a}	418^{a}	9.49^{a}	58.0	0.82
Week 5 (36 week)	49.1^{b}	0.34^{b}	8.6^{a}	163^{a}	15490^{a}	36.9^{a}	409^{a}	9.50^{a}	56.0	0.84
Week 9 (40 week)	49.5^{b}	0.34^{b}	8.5^{a}	162^{a}	15440^{a}	37.3^{a}	422^{a}	9.78^{a}	54.0	0.89
Means[2]	49.0^{b}	0.34^{b}	8.5^{a}	163^{a}	15410^{a}	37.1^{a}	416^{a}	9.59^{a}	56.0	0.85
Basal diet (44-47 week)	51.9^{ab}	0.35^{a}	8.3^{ab}	146^{b}	15350^{b}	30.6^{b}	374^{b}	8.47^{b}	52.0	0.91
Week 1 (48 week)	52.1^{a}	0.35^{a}	8.3^{ab}	152^{b}	14776^{b}	29.7^{b}	365^{b}	8.27^{b}	54.0	0.93
Week 5 (52 week)	52.3^{a}	0.35^{a}	8.1^{b}	149^{b}	15033^{b}	28.4^{b}	349^{b}	8.48^{b}	56.0	0.91
Week 9 (56 week)	52.7^{a}	0.35^{a}	8.0^{b}	140^{b}	14652^{b}	31.3^{b}	376^{b}	8.38^{b}	51.0	0.84
Means	52.3^{a}	0.35^{a}	8.1^{b}	146^{b}	14820^{b}	29.5^{b}	364^{b}	8.37^{b}	54.0	0.92
SEM	0.6	0.03	0.06	147	83	0.4	4.51	0.07	4.5	0.03
Probability	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	NS	NS

[1]Data obtained when hens were fed a standard diet including Zn and Mn. Basal diet contained by analysis 50 mg kg^{-1} Zn and 30 mg kg^{-1} Mn
[2]Average of means from 1, 5 and 9, [a, b]Means within a column with no common superscript differ significantly

Table 3: Effect of dietary supplementation of Zn and Mn in combination on egg weight, thickness, percentage and index of eggshell and also mechanical properties (stiffness, elastiodulus and fracture toughness), Ca and P in eggshell

Supplement (mg kg^{-1})		Egg weight (g)	Thickness (mm)	Index (g/100 cm^2)	Stiffness (N mm^{-1})	Elastic modulus (N mm^{-1})	Breaking strength (N)	Fracture toughness	Eggshell (%)	Concentration of Ca (%)	Concentration of P (%)
Zn	Mn										
0	0	50.30	0.34^{b}	8.2^{b}	150^{b}	14702^{b}	31.3^{b}	390^{b}	8.87^{b}	33^{b}	1.02^{a}
0	30	50.60	0.34^{b}	8.4^{a}	154^{b}	15030^{b}	33.0^{b}	390^{b}	9.48^{a}	48^{b}	0.99^{a}
0	60	50.90	0.34^{b}	8.1^{b}	160^{a}	15778^{a}	35.7^{a}	404^{a}	9.12^{ab}	40^{b}	1.60^{a}
0	90	50.00	0.34^{b}	8.2^{b}	155^{a}	15010^{b}	33.2^{b}	386^{b}	8.74^{b}	42^{b}	0.98^{a}
50	0	51.40	0.34^{b}	8.4^{a}	147^{b}	14130^{b}	31.0^{b}	373^{b}	8.94^{b}	41^{b}	0.97^{a}
50	30	50.90	0.34^{b}	8.3^{b}	150^{b}	15060^{b}	33.9^{a}	379^{b}	8.64^{b}	44^{b}	0.97^{a}
50	60	51.10	0.34^{b}	8.2^{b}	164^{a}	15700^{a}	35.1^{a}	412^{a}	9.19^{a}	57^{b}	0.71^{b}
50	90	51.20	0.34^{b}	8.4^{a}	155^{b}	15110^{b}	33.1^{b}	377^{b}	9.18^{a}	54^{b}	0.92^{a}
100	0	49.70	0.34^{b}	8.3^{b}	150^{b}	14260^{b}	31.2^{b}	376^{b}	9.05^{b}	54^{b}	0.88^{a}
100	30	50.30	0.34^{b}	8.4^{a}	151^{b}	15160^{b}	34.2^{a}	387^{b}	9.15^{ab}	73^{a}	0.63^{b}
100	60	50.00	0.34^{b}	8.5^{a}	162^{a}	15600^{a}	35.1^{a}	420^{a}	8.85^{b}	63^{a}	0.90^{a}
100	90	49.60	0.34^{b}	8.4^{a}	149^{b}	15200^{b}	32.1^{b}	389^{b}	9.07^{b}	60^{a}	0.76^{b}
150	0	50.50	0.34^{b}	8.4^{a}	146^{b}	14530^{b}	33.2^{b}	370^{b}	8.71^{b}	65^{a}	0.77^{b}
150	30	50.50	0.34^{b}	8.1^{b}	157^{a}	15120^{b}	34.3^{a}	381^{b}	8.91^{b}	60^{a}	0.81^{b}
150	60	51.60	0.35^{a}	8.4^{a}	163^{a}	15360^{a}	34.0^{a}	422^{a}	9.12^{ab}	68^{a}	0.64^{b}
150	90	51.70	0.35^{a}	8.4^{a}	155^{b}	15010^{b}	33.1^{b}	386^{b}	8.69^{b}	69^{a}	0.76^{b}
SEM		0.764	0.062	0.456	1.57	69	0.34	3.48	0.092	8.6	0.77
Probability		NS	p<0.05	p<0.05	p<0.05	p<0.01	p<0.05	p<0.01	p<0.05	p<0.05	p<0.05

[a, b]Means within a column with no common superscript differ significantly

Table 4: Effect of dietary supplementation of Zn and Mn alone on egg weight, thickness, percentage, index, mechanical properties (stiffness, elastiodulus and fracture toughness) and also Ca and P in eggshell

Supplement (mg kg^{-1})	Egg weight (g)	Thickness (mm)	Index (g/100 cm^2)	Stiffness (N mm^{-1})	Elastic modulus (N mm^{-1})	Breaking strength (N)	Fracture toughness	Eggshell (%)	Concentration of Ca (%)	Concentration of P (%)
Zn										
0	50.42^{b}	0.34^{b}	8.2^{b}	154	15130	33.3	390	9.060	41.0^{b}	1.15^{a}
50	51.41^{a}	0.35^{a}	8.3^{b}	152	15000	33.0	386	8.990	49.0^{b}	0.89^{a}
100	49.85^{b}	0.34^{b}	8.4^{a}	153	15020	33.1	393	9.120	62.5^{a}	0.79^{b}
150	51.81^{a}	0.35^{a}	8.3^{b}	155	14980	33.2	390	8.850	65.5^{a}	0.74^{b}
SEM	0.382	0.031	0.23	0.79	34.5	0.17	1.74	0.046	4.3	0.37
Probability	p<0.05	p<0.05	p<0.05	NS	NS	NS	NS	NS	p<0.01	p<0.05
Mn										
0	50.47	0.34	8.3	148^{b}	14652^{b}	31.4^{b}	377^{b}	8.93^{b}	48.1	0.91
30	50.59	0.34	8.3	153^{b}	15092^{b}	33.4^{a}	381^{b}	9.06^{ab}	56.0	0.85
60	50.89	0.34	8.3	162^{a}	15584^{a}	35.1^{a}	412^{a}	9.57^{a}	57.1	0.96
90	50.62	0.34	8.3	153^{b}	15082^{b}	32.8^{b}	384^{b}	8.95^{b}	56.1	0.83
SEM	0.382	0.031	0.23	0.79	34.5	0.17	1.74	0.046	4.3	0.37
Probability	NS	NS	NS	p<0.05	p<0.01	p<0.05	p<0.01	p<0.01	NS	NS

[a, b]Means within a column with no common superscript differ significantly

Eggshell mechanical properties and composition: The addition of mineral supplements to the basal diet had obvious effects on the stiffness characteristics of eggshells as derived directly from the nondestructive deformation measurements. In the current study addition of Zn did not significantly influence on eggshell stiffness (Table 4). The average eggshell stiffness was found to be significantly higher in the birds that received a mineral

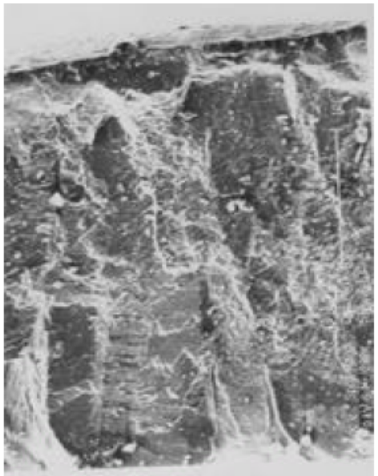

Fig. 1: Eggshell thickness and microstructure (Eggshell related to birds that received supplementation of 150-90 mg kg^{-1} of Zn and Mn, respectively)

Fig. 2: Eggshell thickness and microstructure (Eggshell related to birds that received basal diet contained 50-30 mg kg^{-1} of Zn and Mn, respectively)

supplementation of 0-60, 0-90, 50-60, 100-60, 150-30 and 150-60 mg kg^{-1} of Zn and Mn, respectively (Table 3). The birds supplied with 60 mg kg^{-1} Mn had higher average eggshell stiffness (Table 4). The fracture, breaking strength, stiffness and elastic modulus of eggshell values, according to Bain[14], however, permit a more direct comparison of the stiffness characteristics of eggs to be made and in this respect, there was a significant increase in the elastic modulus fracture, breaking strength and stiffness of eggshell in the eggs associated with younger group of hens (28 vs 40 week, Table 2).

The additions of Zn and Mn in combination to the basal diet significantly ($p<0.05$) influenced eggshell elastic modulus. The average eggshell elastic modulus was found to be significantly higher in the birds that received a mineral supplementation of 0-60, 50-60, 100-60 and 150-60 mg kg^{-1} of Zn and Mn respectively (Table 3). But addition of Zn alone to the basal diet did not influence eggshell elastic modulus (Table 4). The birds supplied with 60 mg kg^{-1} Mn had higher average eggshell elastic modulus than those receiving 0, 30 and 90 mg kg^{-1} Mn (Table 4). The addition of Zn did not affect the eggshell breaking strength (Table 4). The average breaking strength of eggshell was found to be significantly higher in the birds that received a mineral supplementation of 0-60, 50-30, 50-60, 100-30, 100-60, 150-30 and 150-60 mg kg^{-1} of Zn and Mn, respectively (Table 3). The birds supplied with 30 and 60 mg kg^{-1} Mn had higher average breaking strength of eggshell (Table 4). The addition of Zn and Mn in combination to the basal diet significantly influenced eggshell fracture toughness (eggshell resistance to fracture). The average fracture toughness of eggshell was found to be significantly higher in the birds that received a mineral supplementation of 0-60, 50-60, 100-60 and 150-60 mg kg^{-1} Zn and Mn respectively (Table 3). The birds supplied with 60 mg kg^{-1} Mn had higher average fracture toughness of eggshell (Table 4). The addition of Zn did not influence eggshell fracture toughness (Table 4).

The average concentration of Ca and P in eggshell was found to be similar in whole birds (young and old, Table 2). The birds supplied with 100-30, 100-60, 100-90, 150-0, 150-30, 150-60 and 150-90 mg kg^{-1} of Zn and Mn, respectively had higher average of Ca in eggshell (Table 3). The birds supplied with 50-60, 100-30, 100-90, 150-0, 150-60 and 150-90 mg kg^{-1} of Zn and Mn respectively had lower average of P in eggshell (Table 3). The addition of Mn supplement to the basal diet had no obvious effect on the concentration of Ca and P in eggshell (Table 4). The birds supplied with 100 and 150 mg kg^{-1} of Zn had higher average concentration of Ca and lower average concentration of P in eggshell (Table 4).

DISCUSSION

According to Abdallah *et al.*[16] who reported withdrawal of Zn, Fe and Cu supplementation for 10 week has no effect on egg production or egg weight[16]. Moreover removing Mn from the diet reduced eggshell weight in hens laying eggs with characteristically heavier shells. Recently, Inal *et al.*[17] showed that diets not supplemented with vitamins and trace minerals and containing 24 mg kg^{-1} Zn, 15 mg kg^{-1} Mn and 6 mg kg^{-1} Cu decreased egg weight in old hens (62 to 74 week) but not in young hens (30 to 40 week)[17]. Thus, according to the literature, the effect of mineral supplementation on egg weight is inconsistent and seems the age of the bird is an important factor which should be noticed. If this conclusion is true, then managing the trace elements in different ages needs a particular research to be confident for balancing the ration.

The addition of Mn did not significantly influence on eggshell thickness (Table 4). Supplementation of this basal diet had elicited any major changes in the amount of eggshell material being laid down during shell formation. These findings are in agreement with some of the data available from the existing literature. For example, Karunajeewa and Tham[18] found that Mn did not improve eggshell quality when it was increased from 74 to 141 mg kg^{-1}[18]. Ochrimento *et al.*[19] also reported no improvement in eggshell quality when greater than 50 mg kg^{-1} Mn was added to the layer ration[19]. The more recent study of de Faria *et al.*[20] also supports this contention[20]. Holder and Huntley[21] however found that Mn supplementation (65 mg kg^{-1}) in a basal diet (30 mg kg^{-1} Mn) significantly increased specific gravity[21], whereas the work of Sazzad *et al.*[22] reported that the Mn level higher than 105 mg kg^{-1} in the diet will improve eggshell quality[22]. Stevenson[23] found that elevated levels of Zn, (100 to 200 mg kg^{-1}) did not affect shell thickness. Dietary supplementation of 80 mg kg^{-1} Zn or 100 mg kg^{-1} Zn as Zn-methionine however, has been shown to improve eggshell weight and reduce shell defects in hens exposed to high temperatures or watered with saline water[24,25]. These findings, however, have since been refuted by Kita *et al.*[26] who did not find any positive effects of adding a Zn supplement on eggshell index in their study, which attempted to reverse a situation in which poor eggshell quality had been induced by elevated temperatures. The varieties of methods used to assess eggshell quality provide the key to these differing opinions. Thus, standardization of methods and adoption of a multidisciplinary approach in the current studies seems very useful.

Trace element supplements are frequently fed to laying hens to reduce eggshell breakage[1,16,17]. Studies of trace element supplementation on the eggshell mechanical properties are rare and the majority of this record only the eggshell mass. Whisenhunt and Maurice[27], however, did report an improvement in eggshell breaking strength when hens were supplied with Mn (200 mg kg^{-1}). Ochrimento *et al.*[19] likewise demonstrated an increase of 4 N in breaking strength when hens were supplied with 300 mg kg^{-1} Mn[19]. These observations suggest that Mn may be the mineral responsible for the improvements in the percentage, stiffness, elastic modulus, breaking strength and fracture toughness of eggshell. There is however, a lack of information in the literature on Zn dietary supplementation and its effects on eggshell strength and so no definitive conclusions can be made at this time. The dose effects observed on some of the mechanical properties of the eggshell, independent of eggshell mass, suggest that trace elements may directly interact with the calcium carbonate fabrication and the resulting texture of the eggshell. In this respect, one possibility is that the presence of trace elements alters the initial phase of eggshell formation. Bain[14] for example, looked at the relationship between fracture toughness and the ultra-structural organization of the eggshell and explained a decline in eggshell fracture toughness in terms of an increase in the incidence of late fusion of adjacent mammillary columns during shell formation. According to this author the presence of late fusion coincides with more rapid crack propagation through the shell wall when the egg is subjected to load. Thus, it could be hypothesized that trace element supplementation promotes early fusion during the initial stages of shell formation and hence improves the mechanical strength of the egg irrespective of its thickness. Alternatively, there is increasing evidence to suggest that the presence of trace elements influences the morphology of calcite in vitro by altering growth of the crystal in various directions. Lithium, for example, inhibits the growth along the c axis of calcite grown *in vitro*, but more interestingly Mn apparently favours elongation of the crystal[9,10]. Thus it is equally likely that similar effects occur *in vivo*, thereby altering the eggshell texture and hence mechanical properties. In support of this hypothesis, Rodriguez-Navarro *et al.*[8] established that there was a negative relationship between the degree of orientation of calcite in eggshells from young hens and their breaking strengths. However it has not yet been possible to relate the widely documented decrease in eggshell strength observed with advancing bird age to specific changes in the textural properties of eggshells.

Finally, it is highly likely that some or all of these properties will be altered positively or negatively in the presence of a controlled amount of specific ions. The current study suggests that addition of combined Zn and Mn significantly influence the amount of eggshell material deposited during shell formation and can improve some of the mechanical properties. In this study, the positive improvement in mechanical properties in birds that receiving the Mn and Zn supplemented diets is promising in this respect.

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