

## **Eggshell Quality is Improved by Excessive Dietary Zinc and Manganese**

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### **Abstract**

640 Hy-Line W36 white Leghorn laying hens regarding their ages (28 and 44 weeks) were divided into two 64 groups of 5 bird per cage (16×4 factorial test). 16 experimental diets included a corn soybean meal basal diet containing 50mg/kg Zn and 30mg/kg 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 of Zn and Mn, respectively. Statistical analysis was done by ANOVA in a factorial experiment with completely randomized design and significant means compared by Duncan's multiple range test. Results showed addition of Zn significantly ( $P<0.05$ ) increases eggshell thickness, index and Ca concentration but, Mn supplementation significantly ( $P<0.05$ ) increases eggshell percentage, stiffness, elastic modulus and breaking strength.

### **Introduction**

Egg is protected by eggshell, because of its barrier against bacterial penetration. 80 to 90% of the routinely downgraded eggs are cracked or broken eggshell ones. Different strategies have been considered to improve eggshell quality including; genetics, environmental conditions, nutrition, particularly mineral nutrition (Nys, 2001). Trace elements may affect either by their co-enzymatic effect on key enzymes involved in eggshell formation or by interacting directly with the calcite crystals in this process. Mn activates glycosyl transferase in the mucopolysaccharides formation as the proteolytic components (Leach, 1976). Existence of keratin and dermatan proteolytic components in the eggshell matrix may be involved in the control of its structure and texture (Arias et al., 1993; Nys, 2001), or mechanical properties (NYS et al., 1999), as Leach and Gross (1983) reported thinner shells in hens fed Mn-deficient diets. Zn as another trace element is the cofactor of carbonic anhydrase enzyme, which is crucial for supplying the carbonic ions during eggshell formation. As Nys et al., (1999) reported inhibition of this enzyme results in lowered bicarbonate ion secretion and, consequently, greatly reduces eggshell weight. On the other hand trace elements may also affect eggshell structure and crystallographic texture through a modifying effect on calcite crystal growth mechanisms (Garcia-Ruiz and Rodrigues-Navarro, 1994; Nys et al., 1999).

### **Materials and Methods**

Six hundred and forty laying hens (HY-Line W36) regarding their ages were divided into two groups (28 and 44 weeks old), and each group of these were divided into 64 groups and 5 bird per cage (totally 128 groups) in a factorial design test (16×4). The birds were kept under 16:8 light, dark program and had free access to food and water. Initially they were fed with a conventional layer diet recommended by NRC, which contained 0.55% Dicalcium phosphate, 7.2% oyster shell, 44 mg iron, 1.2 mg iodine, 0.36 mg cobalt, 0.24 mg selenium, 30 mg Mn, 24 mg Zn, 2.4 mg Cu, and at ages 28 and 44 weeks they were fed experimental diets for 12 weeks, and the first 3 weeks considered as adaptation period. The 16 experimental diets were 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 of ZnO and MnO, respectively. Feed consumption, eggshell quality, egg weight, egg length and breadth, shell weight, stiffness and breaking strength were measured on 3 eggs per cage every week by standard methods. Statistical analyses were performed by using ANOVA in a factorial experiment with completely randomized design with the Statview program. Calculations were carried out in

each group with the same age, and significant effects were detected by ANOVA and treatment means  $\pm$  SE were compared by using Duncan's multiple range test.

## Results and Discussion

The results are shown in Table 1. Analyzed data in this table indicates that Zn and Mn alone or in combination significantly affect eggshell thickness, index, stiffness, elastic modulus, breaking strength and fracture toughness. The eggshell percentage, and Ca and P concentration have also been influenced, and the egg weight was not affected at all. These results are consistent with Abdallah et al., (1994) report which showed Zn, Fe and Cu do not affect egg production and egg weight but removing Mn reduces egg shell weight. But, reports about older (62 to 74 weeks) and younger (30 to 40 weeks) hens indicate that older birds are more sensitive to trace minerals (Inal et al., 2001) and our birds were much younger. Parts of the results are in agreement with some of the available data (Karunajeewa, and Tham, 1987; Ochrimento et al., 1992), but different from Stevenson., (1985) and Kita et al., (1997) findings. One important point which should be mentioned about the controversial reports about trace elements is the varieties of methods used to assess eggshell quality and standardization of methods may solve this problem. On the other hand different environmental circumstances such as temperature, humidity, health and immunity situation, stresses and microbial population of the environment all would affect the eggshell characteristics.

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TABLE 1-. Effect of dietary supplementation of Zn and Mn in combination on egg weight, thickness, index of eggshell and also mechanical properties (stiffness, elastic modulus, and fracture toughness), Ca and P in eggshell.

Zn Supplement(mg/kg)	Mn Supplement(mg/kg)	Egg weight (g)	Thickness (mm)	index (g/100cm <sup>2</sup> )	stiffness (N/mm)	elastic modulus (N/mm <sup>2</sup> )	breaking Strength (N)	fracture Toughness (N/mm <sup>3/2</sup> )	Eggshell percentage of Ca (%)	Concentration of Ca (%)	Concentration of P (%)
0	0	50.3	0.34 <sup>b</sup>	8.2 <sup>b</sup>	150 <sup>b</sup>	14702 <sup>b</sup>	31.3 <sup>b</sup>	390 <sup>b</sup>	8.87 <sup>b</sup>	33 <sup>b</sup>	1.02 <sup>a</sup>
0	30	50.6	0.34 <sup>b</sup>	8.4 <sup>a</sup>	154 <sup>b</sup>	15030 <sup>b</sup>	33.0 <sup>b</sup>	390 <sup>b</sup>	9.48 <sup>a</sup>	48 <sup>b</sup>	0.99 <sup>a</sup>
0	60	50.9	0.34 <sup>b</sup>	8.1 <sup>b</sup>	160 <sup>a</sup>	15778 <sup>a</sup>	35.7 <sup>a</sup>	404 <sup>a</sup>	9.12 <sup>ab</sup>	40 <sup>b</sup>	1.60 <sup>a</sup>
0	90	50.0	0.34 <sup>b</sup>	8.2 <sup>b</sup>	155 <sup>a</sup>	15010 <sup>b</sup>	33.2 <sup>b</sup>	386 <sup>b</sup>	8.74 <sup>b</sup>	42 <sup>b</sup>	0.98 <sup>a</sup>
50	0	51.4	0.34 <sup>b</sup>	8.4 <sup>a</sup>	147 <sup>b</sup>	14130 <sup>b</sup>	31.0 <sup>b</sup>	373 <sup>b</sup>	8.94 <sup>b</sup>	41 <sup>b</sup>	0.97 <sup>a</sup>
50	30	50.9	0.34 <sup>b</sup>	8.3 <sup>b</sup>	150 <sup>b</sup>	15060 <sup>b</sup>	33.9 <sup>a</sup>	379 <sup>b</sup>	8.64 <sup>b</sup>	44 <sup>b</sup>	0.97 <sup>a</sup>
50	60	51.1	0.34 <sup>b</sup>	8.2 <sup>b</sup>	164 <sup>a</sup>	15700 <sup>a</sup>	35.1 <sup>a</sup>	412 <sup>a</sup>	9.19 <sup>a</sup>	57 <sup>b</sup>	0.71 <sup>b</sup>
50	90	51.2	0.34 <sup>b</sup>	8.4 <sup>a</sup>	155 <sup>b</sup>	15110 <sup>b</sup>	33.1 <sup>b</sup>	377 <sup>b</sup>	9.18 <sup>a</sup>	54 <sup>b</sup>	0.92 <sup>a</sup>
100	0	49.7	0.34 <sup>b</sup>	8.3 <sup>b</sup>	150 <sup>b</sup>	14260 <sup>b</sup>	31.2 <sup>b</sup>	376 <sup>b</sup>	9.05 <sup>b</sup>	54 <sup>b</sup>	0.88 <sup>a</sup>
100	30	50.3	0.34 <sup>b</sup>	8.4 <sup>a</sup>	151 <sup>b</sup>	15160 <sup>b</sup>	34.2 <sup>a</sup>	387 <sup>b</sup>	9.15 <sup>ab</sup>	73 <sup>a</sup>	0.63 <sup>b</sup>
100	60	50.0	0.34 <sup>b</sup>	8.5 <sup>a</sup>	162 <sup>a</sup>	15600 <sup>a</sup>	35.1 <sup>a</sup>	420 <sup>a</sup>	8.85 <sup>b</sup>	63 <sup>a</sup>	0.90 <sup>a</sup>
100	90	49.6	0.34 <sup>b</sup>	8.4 <sup>a</sup>	149 <sup>b</sup>	15200 <sup>b</sup>	32.1 <sup>b</sup>	389 <sup>b</sup>	9.07 <sup>b</sup>	60 <sup>a</sup>	0.76 <sup>b</sup>
150	0	50.5	0.34 <sup>b</sup>	8.4 <sup>a</sup>	146 <sup>b</sup>	14530 <sup>b</sup>	33.2 <sup>b</sup>	370 <sup>b</sup>	8.71 <sup>b</sup>	65 <sup>a</sup>	0.77 <sup>b</sup>
150	30	50.5	0.34 <sup>b</sup>	8.1 <sup>b</sup>	157 <sup>a</sup>	15120 <sup>b</sup>	34.3 <sup>a</sup>	381 <sup>b</sup>	8.91 <sup>b</sup>	60 <sup>a</sup>	0.81 <sup>b</sup>
150	60	51.6	0.35 <sup>a</sup>	8.4 <sup>a</sup>	163 <sup>a</sup>	15360 <sup>a</sup>	34.0 <sup>a</sup>	422 <sup>a</sup>	9.12 <sup>ab</sup>	68 <sup>a</sup>	0.64 <sup>b</sup>
150	90	51.7	0.35 <sup>a</sup>	8.4 <sup>a</sup>	155 <sup>b</sup>	15010 <sup>b</sup>	33.1 <sup>b</sup>	386 <sup>b</sup>	8.69 <sup>b</sup>	69 <sup>a</sup>	0.76 <sup>b</sup>
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

<sup>a, b</sup>Means within a column with no common superscript differ significantly