The effect of zinc and manganese source in the diet for laying hens on eggshell and bones quality

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ABSTRACT: The study was conducted to evaluate the effect of zinc and manganese source (inorganic vs. organic) in the diet for hens on laying performance, eggshell quality and chosen parameters of bones. Experiment was carried out on 84 Hy Line Brown hens, from 25 to 70 weeks of age, allocated to seven experimental groups, each containing 12 hens individually caged on wire-mesh floor. All layers were fed the same basal diet contained in 1 kg 52 mg Zn and 30 mg Mn. In experimental treatments basal diet was supplemented with 30 mg Zn/kg and 50 mg Mn/kg and inorganic forms of Zn (ZnO) and Mn (MnO) were gradually replaced (in 0, 50 or 100%) with their organic sources (amino acid complexes). Egg production, egg weight, feed intake and feed efficiency were not affected by dietary treatments. Substitution of Zn and Mn oxides with amino acid complexes of microelements had also no effect on physical and geometrical parameters of tibia, ash content in tibia and in toes, eggshell percent, eggshell thickness and eggshell density, but improved ($P < 0.05$) eggshell breaking strength in late phase of laying cycle (at 62 and 70 weeks of age). Obtained results indicate that use of organic complexes of Zn and Mn could alleviate the negative effect of hen age on eggshell breaking strength.

Keywords: laying hens; zinc; manganese; organic complexes; eggshell quality; tibia bones; laying performance

Eggshell quality is one of the most important problems in poultry industry, influencing economic profitability of eggs production and eggs hatchability. High eggshell breaking strength and lack of shells defects are essential for protection against penetrating of pathogenic bacteria such as *Salmonella* sp. into the eggs. It has been estimated that eggs with damaged shells account for 6–10% of all produced eggs, what leads to great economic losses (Washburn, 1982; Roland, 1988). One of the main concerns is the decrease of eggshell quality with hen age, because the incidence of cracked eggs could be even more than 20% at the end of laying period (Nys, 2001). The other problem observed in highly productive caged layers in the modern poultry industry is poor bone quality related mainly to osteoporosis. Osteoporosis could be defined as a decrease of fully mineralised structural bone leading to increased bones fragility and susceptibility to fracture (Whitehead and Fleming, 2000). The consequence of this syndrome can be severe – bone weakness, deformities and breakage, so the osteoporosis is an important welfare problem causing pain and distress to birds (Webster, 2004). It was found, that almost 30% of caged hens in ending phase of lay had one or more broken bones during their lifetimes (Gregory and Wilkins, 1989).

Most of studies on nutrition effects on eggshell and bone quality in laying hens have been focused on macrominerals (Ca, P) and vitamin D_3. Although it is known, that enzymes related with some microelements are important in mineralization process, the number of research on relationship between trace elements and eggshell and bones quality is limited. Zinc and manganese, as cofactors of metaloenzymes responsible for carbonate and mucopolysaccharides synthesis, play an important role in eggshell formation. Mabe et al. (2003) suggested that trace elements as Zn, Mn and Cu could affect mechanical properties of eggshell by effect on calcite crystal formation and modifying crystallographic structure of eggshell. In previous study of Stahl et al. (1986) the level of 30 mg Zn/kg diet was sufficient for high eggshell quality, whereas

Zamani et al. (2005) reported, that supplementation of basal diet contained 50 mg Zn/kg with additional amounts of Zn had positive effect on eggshell thickness. Guo et al. (2002) found, that 80 mg dietary Zn/kg is needed to obtain improved eggshell strength in older hens (at 55 and 59 weeks of age). In the study of Stevenson (1985) high dietary levels of Zn (100 or 200 mg/kg) had no beneficial influence on egg quality measured as eggshell thickness. Positive effect of manganese addition to the diet on some eggshell parameters was observed by Abdallah et al. (1994), Hossain et al. (1994) and Sazzad et al. (1994). Inal et al. (2001) found, that 25 mg Mn/kg in the diet is sufficient for maximum egg production, egg weight and feed conversion, but for optimal shell quality the requirement of layers is much higher. In the study of Fassani et al. (2000), Mn addition (40–200 mg/kg) to the diet for leghorn hens in the second cycle of production, improved shell thickness and egg loss index. The highest shell thickness was observed when the diet was supplemented with 200 mg Mn/kg. Leach and Gross (1983) reported, that eggshells from hens fed Mn deficient diet are thinner and show alterations in eggshell ultra structure in mammillary layer and decreased content of hexozamine and hexuronic acid in the organic matrix. There is a lack of literature data on the effect of dietary Zn and Mn on the bone quality in laying hens, but Zn deficiency negatively affected skeletal metabolism in young chickens (Wang et al., 2002) and growing rats (Ovesen et al., 2001; Rossi et al., 2001).

Due to some reports indicated that organic mineral sources such amino acid complexes or proteinates have higher bioavailability than traditionally used inorganic forms (Henry et al., 1989; Wedekind et al., 1992; Smith et al., 1995; Li et al., 2005; Ao et al., 2006; Yan and Waldroup, 2006) there is an increasing interest in their using as a source of microelements for poultry. The higher bioavailability of organic minerals is probably related to different mechanism of absorption (by peptide or amino acid uptake mechanisms in the intestine) and to better protection from binding to dietary constituents such phytates and forming of indigestible complexes (Wedekind et al., 1992; Power and Horgan, 2000; Swiatkiewicz et al., 2001). The results of studies on the effect of mineral complexes in laying hens are inconsistent. Some reports indicated that use of organic sources of Zn and Mn beneficially affects laying performance and eggshell quality measured as eggshell weight and eggshell thickness (Bunesova, 1999; Klecker et al., 2002), whereas others authors found no differences between inorganic and organic sources of Zn and Mn (Lim and Paik, 2003; Mabe et al., 2003).

The aim of the present experiment was to study the effect of zinc and manganese source (inorganic vs organic) in the diet for hens on laying performance, eggshell quality and chosen parameters of bones.

MATERIAL AND METHODS

Experiment was carried out on 84 Hy Line Brown laying hens from 25 to 70 weeks, randomly assigned to seven experimental treatments. Each treatment contained 12 laying hens, individually caged on

Table 1. Composition of basal diet (%)

Components	Contents
Maize	34.0
Wheat	25.5
Soybean oil meal	19.2
Rapeseed oil meal	4.00
Grass meal	3.00
Rapeseed oil	3.20
Limestone	8.50
Dicalcium phosphate	1.70
NaCl	0.30
DL-Methionine	0.10
Vitamin-mineral premix (without Zn and Mn)[1]	0.50
Content of nutrients (g/kg)	
Crude protein[2]	168
Metabolizable energy[3] (MJ/kg)	11.6
Lys[2]	8.00
Met[2]	3.70
Ca[2]	3.60
Total P[2]	6.90
Zn[2] (mg/kg)	52
Mn[2] (mg/kg)	30

[1]used premix contained no sources of Zn and Mn and provided per 1 kg of diet: 10 000 IU vitamin A, 2000 IU vitamin D_3, 15 mg vitamin E, 2 mg vitamin K_3, 1 mg vitamin B_1, 4 mg vitamin B_2, 1.5 mg vitamin B_6, 10 μg vitamin B_{12}, 8 mg Ca-pantothenate, 25 mg niacin, 0.5 mg folic acid, 250 mg choline-Cl, 50 mg Fe, 8 mg Cu, 0.8 mg I, 0.2 mg Se and 0.2 mg Co

[2]analysed

[3]calculated according to European Table (1989) as a sum of ME content of components

wire-mesh floor. Cage dimensions were 40 cm × 40 cm equaling 1 600 cm^2 total floor space. During experiment hens had constant access to feed and water and were maintained on a 14 L:10 D lighting schedule, with a dark period in the night.

All layers were fed the same basal maize-wheat-soybean, mash diet formulated to meet or exceed nutrient requirements (NRC, 1994), except for zinc and manganese (vitamin-mineral premix without these microelements was used) (Table 1). Basal diet contained in 1 kg 52 mg Zn and 30 mg Mn. All experimental diets were supplemented with 30 mg Zn/kg and 50 mg Mn/kg and inorganic forms of Zn (ZnO) and Mn (MnO) were substituted in 0, 50 or 100% by their organic sources (amino acid complexes – ZnAA and MnAA).

The proximate composition of feed components and basal diet (dry matter, crude protein, crude fiber, crude ash, ether extract) was analyzed using standard procedures (AOAC, 1990), total phosphorus – calorimetrically with molibdeno-vanadate method (AOAC, 1990), Ca, Zn and Mn – by flame atomic absorption spectrophotometry (AOAC, 1990). Amino acids in acid hydrolysates (methionine after prooxidation to methionine sulphone) were estimated in a colour reaction with ninhydrin reagent using a Beckman-System Gold 126 AA automatic analyser. Metabolizable energy content of basal diet was calculated from content of basic nutrients and calculation factors from European Table (1989).

During the experiment, feed intake, number and mass of laid eggs were registered. Using collected data, basic production parameters (laying performance, daily egg mass per hen, daily feed intake, and feed conversion per 1 g of eggs) were calculated.

At 35, 48, 62 and 70^{th} weeks of age two eggs from each hen (24 eggs per treatment) were collected to determine the eggshell quality parameters (shell thickness, shell density, shell percent in egg) using Egg Quality Measurement apparatus (Micro version 3.2, 1990). Another two eggs from each bird were collected for measurement of eggshell breaking strength (using Instron 5542 with constant speed of head – 10 mm/min). Average value for two eggs laid by the same hen was subjected to statistical analysis.

At the end of the experiment six hens from each group were sacrificed by cervical dislocation. The tibias from both legs were prepared, cleaned of soft tissues, weighed and stored frozen (–20°C) until analysis. For determination of ash – left tibias and toes were dried for 24 h at 105°C, weighed and dry-ashed in a muffle furnace at 600°C.

For measurements of biomechanical and geometrical properties of bones the right tibias were used. Mechanical properties in three point bending test were determined, using Instron 5542 testing machine (a constant speed of crosshead – 10 mm/min and distance between supports – 50 mm). Ultimate load, yielding load, maximum deformation were measured as a graphical record from post deformation curves. Stiffness in elastic conditions was calculated as a yielding load/elastic deformation ratio.

Tibia length, cortex thickness, external and internal diameters (for cross section area calculations) were measured in the breaking place using electronic slide caliper. Cross section area was calculated from equation: 3.14 (HB – hb)/4, where H was external, vertical diameter; B – external, horizontal diameter, h – internal, vertical diameter and b – internal, horizontal diameter.

All data were subjected to statistical analysis using one-way ANOVA. When significant differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. Differences were considered significant at $P < 0.05$. All statistical analyses were performed with Statistica 5.0 PL software (Statsoft Inc.).

RESULTS AND DISCUSSION

Laying performance

The average egg production for whole laying period (25–70 weeks of hens age) was 89.3%; egg weight – 62.2 g; daily egg mass per hen – 55.6 g, daily feed intake – 117 g, feed conversion per 1 g of eggs – 2.10 g (Table 2). Partial or complete substitution of inorganic Zn and Mn oxides with amino acid complexes had no effect on laying performance parameters ($P > 0.05$). Similar results were observed by Lim and Paik (2003) who found no positive effect of zinc and manganese organic forms on laying performance. However Klecker et al. (2002) reported, that substitution of 20 or 40% Mn and Zn from inorganic sources by their organic chelates significantly increased laying performance of hens from 20 to 60 weeks of age. In broiler breeders partial (50%) or complete substitution of zinc sulphate with Zn amino acid complexes increased hen-day production, but only in early phase of laying cycle (from 24 to 37 weeks of age) (Hudson et al., 2004).

Eggshell quality

The average eggshell percent in egg mass for all experimental treatments was – 9.57% (overall mean); eggshell thickness – 373 µm and eggshell density – 81.2 mg/cm (Tables 3, 4 and 5). At any of experimental period (35, 48, 62 and 70 weeks of hens age) there were no differences between treatments (P > 0.05). Similar results were found by Mabe et al. (2003) who reported no differences in eggshell percent and eggshell density between hens fed diet supplemented with inorganic and organic sources of Zn, Mn and Cu. Dale and Strong (1998) and Lim and Paik (2003) observed no beneficial influence of Zn and Mn organic sources on eggshell quality measured as specific gravity of eggs. In contrary Bunesova (1999) and Klecker et al. (2002) found positive effect of partial substitution of inorganic Zn and Mn sources with their organic forms on eggshell weight and eggshell thickness. In broiler breeder hens the use of amino acid complexes of Zn increased specific gravity and reduced amount of cracked eggs (Hudson et al., 2004).

In our experiment the tendency for decline of eggshell breaking strength with hens age was observed (Table 6). Average breaking strength at 35 weeks of age was 39.6 N and diminished to 35.0 N at 70 weeks (8.8%). Similar tendency was

Table 2. Effect of Zn and Mn source on laying performance from 25 to 70 weeks of age

Groups	Organic source of Zn in total Zn addition (%)	Organic source of Mn in total Mn addition (%)	Egg production (%)	Egg weight (g)	Egg mass (g of eggs/hen per day)	Feed intake (g/hen per day)	Feed conversion (g of feed/g of egg)
1	0	0	89.0	62.4	55.5	117	2.11
2	50	0	89.1	62.4	55.6	116	2.09
3	0	50	89.3	62.1	55.5	117	2.10
4	50	50	89.4	62.5	55.9	117	2.09
5	100	0	89.0	61.9	55.1	117	2.12
6	0	100	89.4	62.3	55.7	118	2.11
7	100	100	90.0	61.9	55.7	117	2.10
SEM			0.288	0.143	0.235	0.234	0.009
P-value			NS*	NS	NS	NS	NS

*P > 0.05

Table 3. Effect of Zn and Mn source on eggshell (%)

Groups	Organic source of Zn in total Zn addition (%)	Organic source of Mn in total Mn addition (%)	Weeks of hens age				Overall mean
			35	48	62	70	
1	0	0	9.77	9.76	9.56	9.24	9.58
2	50	0	9.76	10.08	9.69	9.31	9.71
3	0	50	9.71	9.68	9.97	9.25	9.65
4	50	50	10.02	9.86	9.62	9.24	9.68
5	100	0	9.59	9.75	9.21	9.20	9.44
6	0	100	9.46	9.39	9.53	9.26	9.41
7	100	100	9.78	9.45	9.50	9.28	9.50
SEM			0.080	0.073	0.084	0.074	0.041
P-value			NS*	NS	NS	NS	NS

*P > 0.05

Table 4. Effect of Zn and Mn source on eggshell thickness (μm)

Groups	Organic source of Zn in total Zn addition (%)	Organic source of Mn in total Mn addition (%)	Weeks of hens age				Overall mean
			35	48	62	70	
1	0	0	379	378	366	375	375
2	50	0	371	388	377	367	376
3	0	50	376	376	374	363	372
4	50	50	388	384	377	365	378
5	100	0	376	383	360	364	371
6	0	100	370	370	369	369	369
7	100	100	380	371	372	370	373
SEM			2.53	2.86	3.02	3.21	1.63
P-value			NS*	NS	NS	NS	NS

*$P > 0.05$

Table 5. Effect of Zn and Mn source on eggshell density (mg/cm^2)

Groups	Organic source of Zn in total Zn addition (%)	Organic source of Mn in total Mn addition (%)	Weeks of hens age				Overall mean
			35	48	62	70	
1	0	0	82.6	82.7	79.7	79.7	81.2
2	50	0	83.0	83.8	82.7	80.3	82.5
3	0	50	81.6	81.1	82.9	78.9	81.1
4	50	50	83.8	83.1	81.1	78.4	81.6
5	100	0	81.1	82.0	79.7	79.3	80.5
6	0	100	80.6	80.9	81.1	79.1	80.4
7	100	100	82.1	80.7	80.9	80.6	81.1
SEM			0.580	0.626	0.730	0.721	0.342
P-value			NS*	NS	NS	NS	NS

*$P > 0.05$

observed among others by Al-Batshan et al. (1994) and De Ketelaere et. al. (2002).

From 62 weeks of hens age the positive effect of Zn and Mn organic complexes on eggshell quality was observed (Table 6), but differences as compared to control group were statistically confirmed ($P < 0.05$) only when Zn and Mn oxides were completely replaced with their amino acid complexes (Group 7). Klecker et al. (2002) found, that replacement of 20 or 40% of inorganic Zn and Mn with chelated forms of these elements significantly increased eggshell breaking strength. Guo et al. (2002) reported, that at 59 weeks of age the eggshell strength was 9% higher when inorganic Zn source ($ZnSO_4$) was substituted with Zn amino acid complex. However Bunesova (1999) found no statistically confirmed effect of partial replacement of inorganic Zn and Mn with their proteinates on eggshell strength. Also Mabe et al. (2003) reported no differences in eggshell mechanical properties (breaking strength, stiffness and elastic modulus) between aged layers fed diet supplemented with inorganic sources of Zn, Mn and Cu or organic amino acid complexes of these elements.

Bone parameters

Gradual substitution of Zn and Mn oxides with organic sources of these microelements (amino acids complexes) had no significant effect on biomechanical and geometrical parameters of tibia bones (ultimate load, yielding load, stiffness, maximum deformation, tibia length and tibia cross section area), and on ash content in tibia bones and toes (Table 7 and 8). These results indicate, that bone properties in older hens are less sensitive than eggshell breaking strength to the availability of zinc and manganese in the diet. There is a lack of literature data on the effect of dietary Zn and Mn on the bone quality in laying hens, but results of study on newly hatched chickens indicate that use of low Zn diet (10 mg/kg) could negatively affect bone formation (Wang et al., 2002). Among nutritional factors there are some results in literature data on beneficial effect of particulate limestone and active forms of vitamin D_3 on bone quality in laying hens. The positive influence of large particle limestone on bone quality was noted by Guinotte and Nys (1991) and Fleming et al. (1998). It could be explained as the effect of particulate limestone on constant metering of Ca in gastrointestinal tract

Table 6. Effect of organic sources of Zn and Mn on eggshell breaking strength (N)

Groups	Organic source of Zn in total Zn addition (%)	Organic source of Mn in total Mn addition (%)	Weeks of hens age				Overall mean
			35	48	62	70	
1	0	0	39.3	35.8	34.6^{a}	33.5^{a}	35.8^{a}
2	50	0	39.8	36.2	36.5^{ab}	34.1^{ab}	36.6^{a}
3	0	50	39.4	37.6	36.9^{b}	34.8^{abc}	37.2^{ab}
4	50	50	39.0	37.1	36.8^{b}	35.3^{abc}	37.1^{ab}
5	100	0	39.7	37.1	36.2^{ab}	35.5^{abc}	37.1^{ab}
6	0	100	39.6	37.6	36.3^{ab}	35.1^{abc}	37.2^{ab}
7	100	100	40.0	38.7	37.8^{b}	36.7^{c}	38.3^{b}
SEM			0.174	0.388	0.256	0.224	0.187
P-value			NS*	NS	0.0410	0.0252	0.0248

*$P > 0.05$; a,b,c = means in the same column with different superscrips are significantly different ($P < 0.05$)

Table 7. Effect of Zn and Mn source on physical parameters of tibia bones

Groups	Organic source of Zn in total Zn addition (%)	Organic source of Mn in total Mn addition (%)	Ultimate load (N)	Ultimate load/ cross section area ratio (N/mm^2)	Yielding load (N)	Stiffness (N/mm)	Maximum deformation (mm)
1	0	0	161	10.5	111	169	1.57
2	50	0	175	11.5	120	175	1.58
3	0	50	166	11.9	115	173	1.57
4	50	50	164	12.2	117	169	1.58
5	100	0	162	11.1	108	169	1.62
6	0	100	181	11.6	113	171	1.64
7	100	100	173	12.5	117	176	1.68
SEM			4.09	0.282	2.39	2.64	0.0146
P-value			NS*	NS	NS	NS	NS

*$P > 0.05$

Table 8. Effect of organic sources of Zn and Mn on geometrical parameters of tibia bones and ash content in tibia bones and in toes

Groups	Organic source of Zn in total Zn addition (%)	Organic source of Mn in total Mn addition (%)	Cortex thickness (mm)	Cortex cross section area (mm^2)	Tibia length (mm)	Relative weight of tibia (g/100 g of body weight)	Ash content in tibia (%)	Ash content in toe (%)
1	0	0	0.707	15.3	121	0.578	29.7	8.39
2	50	0	0.717	15.2	123	0.590	31.5	8.14
3	0	50	0.686	14.2	123	0.592	29.5	8.10
4	50	50	0.688	14.1	123	0.591	29.3	8.07
5	100	0	0.724	14.6	122	0.584	31.9	8.61
6	0	100	0.726	15.8	123	0.592	30.3	8.13
7	100	100	0.692	14.3	123	0.595	29.8	8.23
SEM			0.0093	0.205	0.478	0.0046	0.376	0.0928
P-value			NS*	NS	NS	NS	NS	NS

*$P > 0.05$

during eggshell formation and in consequence on lower bone Ca resorption (Guinotte and Nys, 1991). Newman and Leeson (1999) reported beneficial effect of 1,25-dihydroxy-cholecalciferol on bone breaking strength in older laying hens. Similarly Swiatkiewicz and Koreleski (2005) found that simultaneous addition of grind particle limestone and active form of vitamin D_3 (25-OH-D_3) to the diet significantly improved biomechanical, geometrical and chemical parameters of tibia bones in laying hens at 70 weeks of age.

In conclusions, it could be stated, that substitution of inorganic Zn and Mn with amino acid complexes in the diet for laying hens had no effect on the laying performance and bone quality, but could alleviate the negative effect of hen age on eggshell breaking strength.

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