

## Effects of feed supplementation with manganese from its different sources on performance and egg parameters of laying hens

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**ABSTRACT:** The objective of this study was to compare the effects of feed supplementation of laying hens with manganese from its inorganic and organic sources on performance and some parameters of egg quality. Ninety-six hens at 20 weeks of age were randomly allocated to 4 dietary treatments, each consisting of 6 replicates (4 birds per replicate). The control group was fed unsupplemented basal diet (BD) with only natural background Mn level of 46.4 mg/kg feed. For the three experimental treatments, the BD was supplemented with 120 mg Mn/kg either from Mn-sulphate or Mn-chelate of protein hydrolysate (Mn-Pro) or Mn-chelate of glycine hydrate (Mn-Gly). After 8 weeks of dietary treatments the egg production, egg weight, feed intake, and feed efficiency were not affected by dietary treatments. Regardless of the sources, Mn supplementation to feed resulted in significantly decreased percentages of cracked eggs compared to the unsupplemented control group. The thickness, weight, proportion, and index of eggshell were significantly elevated in all groups supplemented with Mn. The intake of Mn-Gly resulted in considerably increased Mn deposition in egg yolk compared to the control eggs. In the control and Mn-sulphate groups yolk malondialdehyde (MDA) started to increase after 20 and 30 days of egg storage respectively, whereas in eggs from hens given organic Mn-sources this parameter was not affected up to 40 days. Although there were no significant differences in MDA values between the treatments until 20 days of storage, the Mn-sulphate group showed significantly higher MDA concentration in yolks compared to the control group after 30 days of storage. These results demonstrate that supplementation of hens' diet with Mn has positive effects on eggshell quality. Feed supplementation with Mn from organic sources appears to be more effective in preventing yolk lipid oxidation during cold storage of eggs than that from Mn-sulphate.

**Keywords:** manganese chelates; layers; egg quality; laying performance; egg storage; lipid oxidation

Improving the production and quality of eggs is the topic of many scientific papers. It has been suggested that approximately 8% of all losses in egg production are directly connected with low eggshell quality (Klecker et al., 2002). From the consumers' point of view, the internal quality of eggs is very important (Tůmová and Gous, 2012). High eggshell breaking strength and shell defects incidence minimization are essential for protec-

tion against bacterial penetration into the eggs in order to ensure the safety of this food of animal origin for human consumption (Mabe et al., 2003). It has been documented that eggshell quality is related to macrominerals (Ca, P) and vitamin D<sub>3</sub>, but nowadays it is well known that trace elements are also very important in the mineralization process. Zinc, copper, and manganese from organic or inorganic sources could affect the mechanical

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properties of eggshell (Mabe et al., 2003; Swiatkiewicz and Koreleski, 2008).

Manganese as an essential trace element plays an important biological role in animals, in particular for normal bone formation, reproductive function, brain function, carbohydrate and lipid metabolism (Underwood, 1977). Mn also plays a crucial role in antioxidant protection as an integral part of Mn-superoxide dismutase (Robinson, 1998). In poultry, Mn is essential for eggshell formation and can positively affect eggshell quality. It has been shown that hens fed Mn-deficient diets produced eggs with thinner shells, with translucent areas and abnormalities in eggshell ultrastructure, particularly in its mammillary layer (Leach Jr. and Gross, 1983).

In poultry nutrition, either the inorganic and organic forms of the trace minerals as feed additives are commonly added to diets to improve hens' performance, production, and quality of eggs. Compared to inorganic sources, the organic mineral sources are reported to have several advantages, including protection from undesired chemical reactions in the gastrointestinal tract, easy passage intact through the intestine wall, and possibly different absorption, metabolic pathway, and mechanisms (Mateos et al., 2005). Several studies indicate that organic sources of trace minerals such as amino acid complexes, chelates, and proteinates have higher bioavailability than traditionally used inorganic forms (Henry et al., 1989; Smith et al., 1995; Li et al., 2005; Yan and Waldroup, 2006; Skřivan et al., 2010; Yuan et al., 2011). However, some results remain controversial. Some researchers have suggested that the use of organic sources of Mn substantially affects laying performance and eggshell quality (Klecker et al., 2002; Yildiz et al., 2011; Sun et al., 2012), whereas other authors have found no difference between inorganic and organic Mn sources (Lim and Paik, 2003; Mabe et al., 2003; Swiatkiewicz and Koreleski, 2008).

The present study was designed to evaluate the laying performance, some parameters of egg quality, susceptibility of yolk lipids to oxidation, and deposition of Mn in egg yolk of laying hens fed diets supplemented with Mn from its various sources (organic vs. inorganic).

## MATERIAL AND METHODS

**Animals and husbandry.** The experiment was carried out on 96 hens of Lohmann Brown laying

strain with the initial age of 20 weeks. On the basis of their body weight the birds were assigned evenly into four dietary treatments, each replicated six times with four hens (two cages) per replicate. During the whole experiment all birds were housed in battery cages for laying hens with randomized allocation of two birds per cage. The enriched cages sizing 43 × 42 × 68.5 cm provided 903 cm<sup>2</sup> of floor area per hen. Each cage was equipped with a two-nipple drinker, nest box, perch, dust bath, and equipment for sharpening claws. All birds were fed restricted amount of feed (120 g/day) during whole experiment while water was offered *ad libitum*. A constant lighting regimen of 15 h light (L) : 9 h darkness (D) was maintained throughout the adaptation and experimental periods. Environmental temperature was kept at 19–24°C and relative air moisture at 60–70%.

All experimental procedures were in accordance with established standards for the care and use of animals for research purposes. The experimental protocol was approved by the Ethical Committee of the Institute of Animal Physiology SASci and the State Veterinary and Food Office (Ro-1479/11-221/3).

**Experimental design and diets.** A period of 3 weeks was used for the adaptation of birds to feeding only the basal diet (BD) without manganese supplementation. All layers were fed the same wheat-maize-soybean meal basal diet formulated to contain adequate levels of all nutrients as recommended by the National Research Council (1994). The analyzed natural background content of the unsupplemented diet was  $46.4 \pm 2.9$  mg Mn/kg. The composition and nutrient content of the diet fed to the hens since week 20 of age are given in Table 1. The following 8-week experimental period (from week 23) was subdivided into two 4-week periods. The control group continued in feeding the same unsupplemented BD during the whole experiment. Experimental diets for groups 2–4 were supplemented with identical Mn doses of 120 mg/kg, either from Mn sulphate (laboratory grade) or Mn-chelate of protein hydrolysate (Mn-Pro) (Bioplex<sup>®</sup> Mn 15%; Alltech Inc., Nicholasville, USA) or from Mn-chelate of glycine hydrate (Mn-Gly) (Glycinoplex-Mn 22%; Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany). The mean analyzed values of Mn concentrations in supplemented layer diets (five replicates of each) for groups 2–4 fed to hens from 24 weeks of age were  $165.4 \pm 3.7$ ,  $171.1 \pm 7.7$ , and  $171.2 \pm 4.0$  mg/kg complete feed, respectively.

Table 1. Ingredients and chemical composition of the basal diet<sup>a</sup>

Ingredients	g/kg	Analyzed composition	
Wheat, ground (110 g CP/kg)	335	dry matter (g/kg)	892
Maize, ground (83 g CP/kg)	310	crude protein (g/kg)	186
Soybean meal, extracted (460 g CP/kg)	245	crude fat (g/kg)	18.3
Limestone	90	crude fibre (g/kg)	33.1
Premix HYD-10 <sup>b</sup>	20	ash (g/kg)	111
		calcium (g/kg)	36.6
<b>Calculated nutrients</b>		phosphorus (g/kg)	6.0
Methionine	3.8	zinc (mg/kg)	107.3
Methionine + cystine	6.9	copper (mg/kg)	17.4
Lysine	8.7	manganese (mg/kg)	46.4
ME (MJ/kg)	11.5	selenium (mg/kg)	0.3

CP = crude protein, ME = metabolizable energy

<sup>a</sup>diets for three experimental groups were supplemented with manganese, each from its different source, at the level of 120 mg Mn/kg feed

<sup>b</sup>vitamin-mineral premix provided per kg of complete diet: vitamin A 11 000 IU, vitamin D<sub>3</sub> 2 750 IU, vitamin K 2.2 mg, vitamin E 12.0 mg, vitamin B<sub>1</sub> 2.2 mg, vitamin B<sub>2</sub> 5.0 mg, vitamin B<sub>6</sub> 3.1 mg, vitamin B<sub>12</sub> 0.02 mg, niacin 24.6 mg, pantothenic acid 6.6 mg, biotin 0.1 mg, folic acid 0.6 mg, methionine 1.2 g, Ca 3.4 g, P 2.27 g, Cl 2.1 g, Na 1.4 g, K 5.2 mg, Zn 37.6 mg, I 0.4 mg, Co 0.2 mg, Cu 7.6 mg, Fe 48.1 mg, Se 0.1 mg, Mg 11.4 mg

**Data collection and measurements.** The laying hens were weighed individually at the beginning and at the end of the experiment. Feed intake (per cage) was recorded on weekly basis. Egg production, egg weight, number of cracked and soft-shelled eggs were monitored daily from 24 to 31 weeks of age. Eggs from each pen were collected 3 times a day (at 9:00, 12:00, and 15:00 h.). The feed consumption and feed to egg mass ratio were determined for each replicate weekly. Based on the collected data, the basic production parameters (laying rate, feed to egg mass ratio, daily feed intake) were calculated.

Egg quality was assessed twice during the experiment, after weeks 4 and 8 of feeding the experimental diets. Three eggs from each replicate (18 eggs per treatment) were collected on the two consecutive last days (totally 36 eggs per treatment) before the end of each 4-week feeding period for measurement of the weights of albumen, yolk, shell, and eggshell thickness. Length and width of each egg were measured for egg shape index (%) calculation (width/length × 100). Subsequently the eggs were first weighed and broken, and the yolk was then carefully separated from the albumen. The shell weight was measured after washing the interior egg membrane and after its drying at 60°C for 48 h. Albumen weight was calculated by subtracting the shell and yolk weights from the egg

weight. After manual removal of shell membranes, eggshell thickness was measured at three different egg points (air cell, sharp end, and any side of the equator) using a micrometer (Model 7313; Mitutoyo Corp., Kawasaki, Japan). An average of three different thickness measurements from each egg was used to estimate the eggshell thickness. The proportion of eggshell (ES), albumen (A), and yolk (Y) were calculated as ((ES or A or Y weight/egg weight) × 100). Eggshell index (g/100 cm<sup>2</sup>) was calculated as (shell weight (g)/shell surface (cm<sup>2</sup>)) × 100, where the shell surface area (cm<sup>2</sup>) was determined using the equation 4.68 × egg weight<sup>2/3</sup> (g) (Ahmed et al., 2005).

To investigate the effect of the diet on lipid oxidation of egg yolks during storage, all fresh eggs from each replicate for each treatment were collected at the end of the trial and placed in a refrigerator (4°C). For analysis of yolk malondialdehyde, at day 0 and then after 10, 20, 30, and 40 days of storage, 3 eggs were selected randomly for each replicate (in total 18 eggs/treatment).

**Chemical analysis.** The basal diet was analyzed for crude protein, crude fat, crude fibre, and ash using standard procedures (AOAC, 2005; methods 976.05, 2003.06, 973.18, and 942.05). Dry matter (DM) of feed was obtained by the standard method of drying the samples at 105°C. Total phosphorus

was determined colourimetrically with molybdeno-vanadate (AOAC, 1990; method 965.17), and selenium concentration using the fluorometric method of Rodriguez et al. (1994). Metabolizable energy (ME) content of basal diet components was calculated according to European Table (1989).

Eight weeks after the introduction of experimental diets, 6 pools of egg yolks per treatment (3 eggs/replicate/pool) were freeze dried. For quantification of manganese, zinc, and copper the samples of freeze-dried egg yolks were digested in 6 ml of concentrated HNO<sub>3</sub> (65%) and 2 ml H<sub>2</sub>O<sub>2</sub> (30%) (Suprapur®; Merck, Darmstadt, Germany) in 100-ml durable TFM-PTFE pressure vessels for microwave digestion using a Speedwave MWS-4 microwave (Berghof Company, Eningen, Germany). An atomic absorption spectrometer (Model AA-700) (Shimadzu, Kyoto, Japan) equipped with a GFA graphite furnace atomizer and deuterium lamp background correction was used. The content of Mn and Cu in samples of egg yolks was determined using an AAS graphite furnace with argon as inert gas, and the concentration of Zn using flame atomic absorption spectrophotometry (FAAS). Representative feed samples ( $n = 5$ ) were prepared for chemical analysis of four elements (Mn, Zn, Cu, and Ca) using FAAS as given above.

The secondary oxidation product, malondialdehyde (MDA), in the yolks of fresh and stored eggs was measured by the fluorometric method described by Jo and Ahn (1998) using 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, St. Louis, USA) as MDA precursor in the calibration curve. The values were expressed in mg MDA/kg egg yolk.

**Statistical analysis.** The differences between the groups were processed using One-Way Analysis of Variance followed by the *post hoc* Tukey's multiple comparison test using GraphPad Prism (Version 5.02, 2008). Differences between the mean values

of the different treatment groups were considered statistically significant at  $P < 0.05$ . Values in tables are means and pooled standard errors of the mean (SEM).

## RESULTS

No clinical symptoms of health disorders were observed, no mortality occurred during experiment. The effects of dietary supplementation with various sources of manganese on laying performance are presented in Table 2. No significant differences were observed for feed intake, laying rate, egg mass produced, and feed to egg mass ratio. Addition of Mn into the diet significantly reduced the percentage of cracked eggs compared to control birds ( $P < 0.001$ ). The hens supplemented with Mn-Gly produced fewer soft-shelled eggs than those receiving solely BD ( $P < 0.01$ ). The average weight gain per hen was not significantly different between the treatments.

Egg quality parameters are summarized in Table 3. During the entire experiment the egg weight, weight and proportion of yolk, and weight of albumen were not significantly affected by dietary treatments. After 8 weeks of feeding a diet enriched with manganese from Mn-sulphate and Mn-Gly, significantly lower albumen proportion in eggs was observed in those groups than in control hens fed BD only ( $P < 0.05$ ). The Mn supplementation from Mn-sulphate and Mn-Pro to feed significantly increased shell thickness ( $P < 0.01$  and  $P < 0.05$ , respectively) compared to control hens after 4 weeks of feeding. All treated groups had significantly thicker eggshell after 8 weeks of intake of diets enriched with Mn ( $P < 0.01$ ) compared with unsupplemented control. Regardless of the source, Mn supplementation to feed significantly increased the weight, mass proportion, and index of eggshell. The percentage of egg shape index was

Table 2. Effect of Mn source on laying performance during the 8-week feeding period (weeks 24–31 of age)

	Basal diet	Mn-sulphate	Mn-proteinat	Mn-glycine chelate	SEM	P-value
Feed intake (g/hen/day)	114.2	114.7	114.9	114.7	0.37	0.91
Egg mass produced (g/hen/day)	59.20	59.44	58.71	58.69	0.15	0.20
Feed to egg mass ratio (g/g)	2.00	1.99	1.95	2.00	0.01	0.11
Laying rate (egg/hen/day)	0.97	0.97	0.97	0.98	0.003	0.54
Cracked eggs (%)	4.73 <sup>a</sup>	0.92 <sup>b</sup>	0.56 <sup>b</sup>	1.52 <sup>b</sup>	0.31	< 0.001
Soft shelled eggs (%)	0.91 <sup>a</sup>	0.39 <sup>ab</sup>	0.38 <sup>ab</sup>	0.0 <sup>b</sup>	0.10	0.01
Body weight gain (g)	186.5	152.8	210.0	192.0	9.52	0.19

<sup>a,b</sup> means within a row not sharing common superscripts differ significantly ( $P < 0.05$ )

Table 3. Parameters of eggs from hens after 4 and 8 weeks of feeding experimental diets supplemented with manganese from various sources

	Basal diet	Mn-sulphate	Mn-proteinat	Mn-glycine chelate	SEM	P-value
<b>Egg weight (g)</b>						
week 4	59.65	60.56	60.61	59.25	0.24	0.12
week 8	61.27	61.59	61.03	60.90	0.22	0.73
<b>Yolk weight (g)</b>						
week 4	14.02	14.19	13.92	14.07	0.08	0.69
week 8	14.82	15.22	14.69	15.07	0.08	0.07
<b>Yolk proportion (%)</b>						
week 4	23.51	23.45	22.96	23.73	0.12	0.12
week 8	24.21	24.73	24.08	24.74	0.13	0.14
<b>Albumen weight (g)</b>						
week 4	40.07	40.14	40.56	39.21	0.20	0.10
week 8	40.95	40.33	40.26	39.76	0.20	0.21
<b>Albumen proportion (%)</b>						
week 4	67.17 <sup>a</sup>	66.26 <sup>ab</sup>	66.93 <sup>ab</sup>	66.18 <sup>b</sup>	0.14	< 0.05
week 8	66.82 <sup>a</sup>	65.51 <sup>b</sup>	65.97 <sup>ab</sup>	65.29 <sup>b</sup>	0.17	< 0.01
<b>Eggshell weight (g)</b>						
week 4	5.55 <sup>a</sup>	6.23 <sup>b</sup>	6.13 <sup>b</sup>	5.98 <sup>b</sup>	0.05	< 0.001
week 8	5.50 <sup>a</sup>	6.04 <sup>b</sup>	6.08 <sup>b</sup>	6.07 <sup>b</sup>	0.06	< 0.001
<b>Eggshell proportion (%)</b>						
week 4	9.31 <sup>a</sup>	10.29 <sup>b</sup>	10.11 <sup>b</sup>	10.09 <sup>b</sup>	0.08	< 0.001
week 8	8.98 <sup>a</sup>	9.77 <sup>b</sup>	9.95 <sup>b</sup>	9.96 <sup>b</sup>	0.09	< 0.001
<b>Eggshell thickness (mm)</b>						
week 4	0.38 <sup>a</sup>	0.41 <sup>b</sup>	0.41 <sup>b</sup>	0.39 <sup>ab</sup>	0.003	< 0.01
week 8	0.36 <sup>a</sup>	0.39 <sup>b</sup>	0.39 <sup>b</sup>	0.39 <sup>b</sup>	0.003	< 0.001
<b>Eggshell index (g/100 cm<sup>2</sup>)</b>						
week 4	7.85 <sup>a</sup>	8.45 <sup>b</sup>	8.51 <sup>b</sup>	8.37 <sup>b</sup>	0.06	< 0.001
week 8	7.53 <sup>a</sup>	8.38 <sup>b</sup>	8.41 <sup>b</sup>	8.34 <sup>b</sup>	0.08	< 0.001
<b>Egg shape index (%)</b>						
week 4	78.78 <sup>ab</sup>	78.33 <sup>a</sup>	78.10 <sup>a</sup>	79.93 <sup>b</sup>	0.22	< 0.05
week 8	78.40 <sup>ab</sup>	78.78 <sup>b</sup>	77.68 <sup>a</sup>	78.79 <sup>b</sup>	0.15	< 0.05

<sup>a,b</sup>means within a row not sharing common superscripts differ significantly ( $P < 0.05$ )

significantly elevated ( $P < 0.05$ ) due to addition of Mn-sulphate and Mn-Gly compared to the eggs from hens fed the diet with Mn-Pro for 8 weeks.

Only diet supplemented with manganese from Mn-Gly resulted in significantly increased ( $P < 0.05$ ) Mn content in egg yolk. No differences in

Table 4. Effects of feed supplementation with manganese from various sources on concentration of manganese, zinc, and copper in egg yolk (mg/kg yolk DM)

	Basal diet	Mn-sulphate	Mn-proteinat	Mn-glycine chelate	SEM	P-value
Manganese	1.65 <sup>a</sup>	2.07 <sup>ab</sup>	1.96 <sup>ab</sup>	2.41 <sup>b</sup>	0.10	0.05
Zinc	82.92	71.34	71.45	78.73	1.99	0.09
Copper	2.57	2.78	2.62	2.72	0.03	0.09

DM = dry matter

<sup>a,b</sup>means within a row not sharing common superscripts differ significantly ( $P < 0.05$ )

Table 5. Effects of feed supplementation with manganese from various sources on concentration of malondialdehyde (MDA) in fresh yolks (day 0) and yolks stored at 4°C for 10–40 days (mg MDA/kg yolk)

	Basal diet	Mn-sulphate	Mn-proteinat	Mn-glycine chelate	SEM	<i>P</i> -value
Day 0	0.70 <sup>A</sup>	0.85 <sup>A</sup>	0.80	0.82	0.04	0.59
Day 10	0.87 <sup>AB</sup>	1.09 <sup>AB</sup>	1.12	1.08	0.05	0.29
Day 20	1.07 <sup>B</sup>	1.03 <sup>AB</sup>	1.13	1.12	0.04	0.87
Day 30	0.95 <sup>Ba</sup>	1.17 <sup>Bb</sup>	1.07 <sup>ab</sup>	1.04 <sup>ab</sup>	0.03	0.07
Day 40	1.03 <sup>B</sup>	1.15 <sup>B</sup>	1.12	1.05	0.02	0.09
SEM	0.03	0.03	0.05	0.04		
<i>P</i> -value	<i>P</i> < 0.001	<i>P</i> < 0.05	0.11	0.10		

<sup>a,b</sup>means within a row not sharing common superscripts differ significantly (*P* < 0.05)

<sup>A,B</sup>means within a column not sharing common superscripts differ significantly (*P* < 0.05)

concentration of Zn and Cu in yolk were observed between treatments (Table 4).

The effect of dietary treatments on MDA concentration in yolks during eggs storage at 4°C is shown in Table 5. The MDA values of egg yolk increased during storage in the control (from day 20 of storage) and Mn-sulphate group (from day 30 of storage). During the whole storage time, the feed supplementation with Mn-Pro and Mn-Gly did not affect MDA concentration in egg yolk. No significant difference in MDA concentration between treatments was observed until 20 days of egg storage. However, the Mn-sulphate group showed significantly higher yolk MDA values (*P* < 0.05) than the control group after 30 days of storage.

## DISCUSSION

The supplementation of diets for laying hens with manganese from Mn-sulphate or from two organic sources did not affect the laying performance in our experiment (Table 2). The average daily feed intake and egg weight for all the treatments were within the range of values given by the management guide for commercial Lohmann Brown layers between 24–31 weeks of age (Lohmann Tierzucht, 2008). It has been reported that diet supplementation with 30–120 mg Mn/kg (50–140 mg total Mn/kg feed) did not influence the egg production in brown layers (Luo et al., 2003). Yildiz et al. (2011) showed that egg production, egg mass, and feed conversion ratio were not affected either by the dietary Mn amounts or by the Mn sources, whereas the feed intake was significantly increased in laying hens supplemented with Mn dose of 75 mg/kg for 12 weeks. Swiatkiewicz and Koreleski (2008) found no differences in the laying performance between

hens fed diets with organic and inorganic sources of Mn. On the other hand, it has been demonstrated that organic Mn supplementation of feed for laying hens has the potential to improve the production, weight, and quality of eggs (Gheisari et al., 2011). In general, the literature dealing with effects of Mn supplementation on performance parameters of laying hens gives inconsistent outcomes. In commercial poultry farms, peak production usually occurs when hens reach 24–26 weeks of age, and production steadily declines until the flock is taken out of production at approximately 76 weeks of age (Bell, 2002). Our study was conducted in the early phase of the laying cycle (24–31 weeks of age), and therefore it would be interesting to repeat a similar study to determine whether or not the manganese supplementation to feed has any impact on the productivity of laying hens during the later stages of the production cycle. Hossain and Bertechini (1998) reported that feed supplementation with Mn from Mn-sulphate (50 and 75 mg/kg feed) significantly increased the egg production and egg weight in 42–52 weeks old laying hens. Yildiz et al. (2011) observed that Mn supplementation from an organic source significantly increased the egg weight and the body weight gains of layers (49–61 weeks of age) and reduced the percentage of damaged eggs compared to the inorganic source of the element.

In the study by Inal et al. (2001), diet supplementation with 25 mg Mn/kg was shown as sufficient for maximum egg production, egg weight, and feed conversion, but for the optimal eggshell quality the requirement of laying hens was suggested to be much higher. The minimum requirement for the maximal eggshell quality was found to be 50–100 mg/kg. Our overall results show that sup-

plementation of the basal diet with Mn at a dose of 120 mg/kg from inorganic and organic sources positively affects the eggshell quality. Regardless of the different Mn sources in dietary treatments, the percentage of cracked eggs was significantly lower and also the numbers of soft-shelled eggs tended to be reduced by the dietary Mn supplementation. Eggshell breakage is known to be directly related to the quality of the shell, which can be affected by nutrition and breeding conditions (Emery et al., 1984; Tůmová et al., 2009). It is well established that trace elements may affect eggshell quality by their catalytic properties as key enzymes involved in the process of membrane and eggshell formation, or by interaction directly with the calcite crystals in the formation of eggshells (Zamani et al., 2005). Our results show significantly higher eggshell weight, proportion, and index after 4 and 8 weeks of dietary Mn intake. In addition, the eggshell was significantly thicker in eggs from all groups fed diets enriched with additional manganese but not influenced by the source of manganese. The explanation could be based on the fact that manganese activates the glycosyl transferases involved in the formation of mucopolysaccharides, which are components of proteoglycans (Leach Jr., 1976). Proteoglycans are present in the organic matrix that controls eggshell structure and texture and thus can influence its mechanical properties (Nys et al., 2001). Our findings are in agreement with the similar results observed by Sazzad et al. (1994), who reported that dietary Mn levels above 105 mg/kg improved eggshell quality. Fassani et al. (2000) also recorded that the greatest shell thickness was obtained in leghorn hens in the second cycle when their diet was supplemented with 200 mg Mn/kg compared to lower doses of Mn. In contrast, Zamani et al. (2005) and Hossain and Bertechini (1998) demonstrated no significant difference in eggshell thickness with increasing dietary Mn content. It has been shown that the use of organic sources of Mn beneficially affects the eggshell quality of laying hens, whereas other authors have found no difference between its inorganic and organic sources. Mabe et al. (2003) reported no differences in the eggshell proportion and eggshell density between hens fed diets supplemented with inorganic or organic sources of Mn. In contrast, Bunesova (1999) and Klecker et al. (2002) found positive effect of partial substitution of inorganic Mn sources with their organic forms on the eggshell weight and thickness.

The significant increase of manganese in egg yolk was observed only for hens fed diet enriched with Mn-chelate of glycine hydrate, the other two groups given feeds supplemented with equivalent amounts of this trace element from Mn-sulphate or Mn-Pro showed only the tendency to its increase (Table 4). The discrepancies in relative bioavailability between various organic Mn sources could be explained by different chemical characteristics and chelation strength of commercial organic Mn feed additives (Li et al., 2004). Gravena et al. (2011) observed an increase in the Mn content of egg yolk from quails supplemented with 60, 120, and 180 mg Mn/kg feed from an organic source. According to Mabe et al. (2003), feed supplementation with Mn levels at 30 or 60 mg/kg from its inorganic (MnO) and organic (Mn amino acid complex) sources resulted in higher deposition of this trace element in the yolk of eggs from hens receiving greater amounts of Mn in their diet, regardless of its source.

The level of MDA as a marker of lipid peroxidation was monitored in egg yolk during 40 days of cold storage. Significant increase in MDA levels was detected only in eggs from the control group (from day 20 of storage) and from the group supplemented with Mn-sulphate (from day 30 of storage). The oxidative stability of yolk lipids was not significantly different during whole time of storage (40 days) in both groups of birds fed diets enriched with manganese from organic sources. One of the most important functions of Mn is related to its antioxidative effect mediated by Mn-superoxide dismutase, or by increasing the synthesis of metallothionein (Kobayashi et al., 2007), which can reduce oxidative damage caused by free radicals. The suppression of lipid oxidation in egg yolk from hens supplemented with organic Mn sources may be explained by this hypothesis. A further explanation could be that Mn from organic sources may improve the stability of yolk lipids through superoxide dismutase (SOD), whose optimal activity could prevent the initiation of lipid peroxidation. However, additional studies are required to confirm the suggested mode of action. Wawrzykowski and Kankofer (2011) confirmed the presence of SOD and its activity in hen egg yolk. Their study also showed significant decrease in SOD activity after a 9-day storage of egg yolk samples. It appears that variation in SOD activity of egg yolk during storage may indicate such a role of manganese in the stability of egg yolk lipids.

## CONCLUSION

Neither the laying rate nor feed to egg mass ratio were affected by the source of manganese supplementation to diets for laying hens. The positive effect of manganese supplementation on eggshell quality was observed regardless of its source. However, manganese from its organic sources proved to be more effective in prevention of lipid oxidation in egg yolk during storage than that from manganous sulphate. A significant increase of manganese in egg yolk due to its feed supplementation was found only for hens fed diet enriched with manganese from glycine chelate.

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