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EFFECT OF INORGANIC AND ORGANIC MANGANESE SUPPLEMENTATION ON THE PERFORMANCE AND TISSUE MANGANESE CONTENT OF BROILER CHICKS

Erzsébet BERTA[1], Emese ANDRÁSOFSZKY[1], A. BERSÉNYI[1], R. GLÁVITS[2], A. GÁSPÁRDY[1] and S. Gy. FEKETE[1*]

[1]Institute of Animal Breeding, Nutrition and Laboratory Animal Science, Faculty of Veterinary Science Budapest, Szent István University, H-1400 Budapest, P.O. Box 2, Hungary; [2]Central Veterinary Institute, Budapest, Hungary

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The effects of dietary levels of manganese (Mn) in inorganic (MnO) and organic (Mn fumarate) forms were evaluated on cockerel chicks. A basal corn-soybean diet with 23 mg/kg Mn was supplemented with levels of 0, 30, 60 and 240 ppm Mn from both Mn sources. Each treatment was replicated in five pens of 10 chicks. The chicks were fed diets *ad libitum* from 14 to 49 days of age, after which five birds per treatment were sacrificed for pathomorphological examinations and analysis. The treatments did not exert significant effects on the body weight (BW), the feed/gain (F/G) ratio or the mortality rate. According to the necropsy findings, no growth retardation or emaciation occurred in either of the groups and the differences in the average absolute and relative organ weights were not significant ($P > 0.05$). Tissue analysis indicated that the tibia showed the greatest response to Mn, followed by the liver and kidney. Accumulation in the tibia was higher ($P < 0.05$) with supplements of 30, 60 and 240 mg/kg from both Mn sources (3.71, 3.78, 4.44, and 3.68, 4.00, 4.36 mg/kg DM, MnO and Mn fumarate, respectively) compared to the control group (3.21 mg/kg). Accumulation in the liver increased significantly ($P < 0.05$) only with supplements of 60 and 240 ppm independently of the Mn source (12.7, 14.2, and 14.0, 14.9 mg/kg, respectively) compared to the control (9.8 mg/kg). Similarly, kidney tissue Mn was higher ($P < 0.05$) only with supplements of 60 and 240 ppm (12.8, 12.8, and 13.1, 12.5 mg/kg, respectively) compared to the control (10.2 mg/kg). At the same level of supplementation of the two Mn sources there were no significant differences ($P > 0.05$) between the Mn concentrations of organs and tissues. Droppings sensitively reflected the intake, whereas blood plasma and feathers showed only the extreme Mn loading.

Key words: Manganese supplementation, inorganic and organic, chick, tissue manganese, indicator organs, droppings

[*]Corresponding author: Dr. Sándor Fekete; E-mail: safekete@univet.hu; Fax: +36 (1) 478 4128

Cereal grains constituting the highest proportion of the diet of poultry, especially corn grain, are poor in manganese (5–6 mg/kg dry matter). The manganese content of even soybean only slightly exceeds 30 mg/kg and that of fish meal and other protein sources is not higher than 10 and 20 mg/kg, respectively (Szabó et al., 1987; National Research Council, 1994). Furthermore, it was found that the extent of utilisation of manganese from these feedstuffs used in poultry diets is variable (Halpin and Baker, 1986; Halpin et al., 1986).

Inorganic Mn supplements are routinely added to conventional poultry diets to meet the Mn requirement. The Mn sources used in the practice are chosen according to economic considerations. However, differences do exist among the inorganic compounds as regards the availability of Mn for utilisation. Black et al. (1984) reported that Mn fed as $MnSO_4$ was more available than Mn from MnO and $MnCO_3$. It was found that tibial Mn increased linearly with the level of supplementation, so Mn accumulation in the bones of chicks fed diets containing varying levels of Mn was an excellent indicator for assessing relative Mn bioavailability. Similar results were obtained by Baker and Halpin (1987), who reported the biological availability of Mn from $MnSO_4$ to be superior to that of MnO and similar to that of $MnCl_2$.

In contrast, Bertechini and Hossain (1992) reported no difference in bioavailability between reagent-grade $MnSO_4 \times H_2O$, feed-grade $MnSO_4$ and MnO, and found that feed intake and body weight gain were not affected by dietary Mn levels up to 240 mg/kg.

Trace minerals that have been complexed with organic molecules have been purported to be superior to inorganic trace minerals in bioavailability in several species. The most common forms of commercially complexed manganese presently available use peptides or amino acids from hydrolysed protein or specific, individual amino acids as the organic molecules to which manganese is complexed. The alleged advantages in bioavailability of manganese from these types of manganese supplements are attributed either to superior solubility or to the unique chemical structure of the compound (Albion Laboratories, Inc., 1992; Johnson and Socha, 1998). The results of studies that addressed the bioavailability of complexed manganese are controversial. Evaluation of Mn when chelated or complexed with protein or methionine has indicated similar (Baker and Halpin, 1987; Fly et al., 1989) or superior (Henry et al., 1989) effects compared with inorganic sources. Smith et al. (1995) also reported that Mn from Mn proteinate was more available than Mn in $MnSO_4$ when measured by tibial response to dietary Mn supplementation.

In a recent evaluation of stability of trace metal–methionine complexes it was found that Mn formed significant complexes with methionine only at pH 8 and above, and the tendency to do so is even weaker with lysine (Martin and Scribante, 2000). This supports earlier suggestions that unstable trace element/amino acid complexes or chelates may dissociate in the digestive tract, al-

lowing the free element to bind with other free ligands available, creating new complexes with higher stability, which may affect their bioavailability unfavourably (Brown and Zeringue, 1994). Nevertheless, test results of trace element sources proved satisfactory for a part of the users. So it seems that the bioavailability of trace elements depends, among others, on the organic ligand to which they are attached. Thus, it may be supposed that Mn as Mn fumarate complex is more bioavailable than Mn in inorganic form, as it is well known for Fe fumarate compared to Fe sulphate in practical nutrition. As no data were found on the use of Mn fumarate in poultry diets, this study was aimed at evaluating the efficacy of Mn fumarate compared to MnO in broilers, on the basis of its effects on growth, feed efficiency, general health status, necropsy findings and tissue Mn distribution.

Materials and methods

Experimental design

Seven hundred one-day old Ross broiler cockerels, obtained from a commercial hatchery were housed in three-tier batteries for 49 days, using 60 birds/m^2. The temperature in the broiler house was controlled according to the supplier's technological recommendations, and 23 h of lighting with 1 h of dark pauses was applied. The relative humidity of air was regulated. The starter diet (Table 1) was supplied *ad libitum* until 14 days of age. During the first three days the diet was medicated with NEO-TE-SOL Pulvis A.U.V. in a dose of 90 mg/kg body weight to overcome intercurrent diseases.

To increase the immunological resistance of chickens, a water-soluble vitamin mixture (TETRAVIT Combi, Bábolna)) was added to the drinking water of the 3- to 7-day-old birds. In order to improve the health status of the broiler stock, from 9 to 14 days of age antibiotics (IMEQUIL-10% pulvis A.U.V.) was added to the drinking water (25 g/100 l). At 10 days of age the birds were marked individually and at 14 days of age they were weighed. A total of 350 cockerels were divided into seven treatments with five replicates per treatment, using 10 birds per cage in the batteries. The cockerels were randomly distributed in the cages according to their measured body weight (BW) to obtain identical average weights (172 g) with minimum variance. The basal corn-soybean grower diet (Table 2) was formulated to meet the requirements (National Research Council, 1994) for all nutrients except Mn. In one treatment group broilers were fed with the basal diet without Mn supplementation (control). The diets of the other six groups were supplemented according to the recommendations of the Hungarian Feed Codex (1990), namely 60 mg/kg Mn supplementation, as well as half (30 mg/kg) and four times that level (240 mg/kg) from MnO and Mn fumarate was used. The birds were provided with experimental diets and drinking water *ad libitum*. During the 5-week feeding experiment the BW of the birds and

their feed intake were recorded weekly, and the weight gain and feed conversion efficiency (FCE) were calculated. Chicks that died were subjected to pathological examination.

Table 1

Composition and nutritive value of starter diet

Ingredients	Starter diet 0 to 14 days
	(%)
Ground corn	43.00
Wheat	22.00
Soybean meal, extr. solv. (48% CP)	25.00
Fish meal (65% CP)	3.00
Favorit-40[1]	4.00
Limestone	1.30
Monocalcium phosphate	0.90
Salt	0.30
Trace mineral mixture[2]	0.30
Vitamin premix[3]	0.20
Nutritive value	
AME, MJ/kg	13.28
Dry matter, %	90.6
CP, %	21.3
Manganese, mg/kg	107

[1]Favorit-40 (Biofilter Kft, Budaörs), mixture of popcorn (60%) and blended animal fat (40%); [2]Trace mineral mixture provided per kilogram of diet: magnesium 125 mg, zinc 80 mg, manganese 80 mg, iron 80 mg, copper 8.0 mg, iodine 1.0 mg, selenium 0.15 mg, cobalt 0.1 mg, molybdenum 1.0 mg; [3]Vitamin mixture supplied per kilogram of diet: vitamin A (retinyl acetate) 15,000 IU, vitamin E (dl-α-tocopheryl acetate) 50 IU, cholecalciferol 5,000 IU, menadione 4.0 mg, thiamine HCl × 3.0 mg, riboflavin 8.0 mg, niacin 60 mg, pyridoxine·HCl × 6.0 mg, vitamin B_{12} 0.016 mg, Ca-d-pantothenate 20 mg, biotin 0.2 mg, L-ascorbic acid 200 mg, choline chloride 400 mg, folic acid 2.0 mg

At 49 days of age, euthanasia of 5 cockerels per treatment was carried out by inhalation of CO_2 in order to perform their histopathological examination and to determine the tissue Mn levels.

For histopathological investigation (Central Veterinary Institute, Budapest) hearts, livers, kidneys and testicles were completely recovered and weighed. Appropriate samples were taken from the femoral muscles and tibia, too. All samples were fixed in phosphate-buffered 10% formaldehyde solution, stained with haematoxylin and eosin and Fat-Red, and were examined by light microscopy.

Table 2

Composition and nutritive value of basal grower diet

Ingredients	Grower diet 15 to 49 d
	%
Ground corn	44.90
Ground wheat	14.00
Soybean meal, extr. solv. (48% CP)	28.50
Meat meal	3.00
Favorit-40[1]	5.00
Limestone	1.30
Vitamin and mineral premix (Mn-free)[2]	3.20
Starch[3]	0.10
Nutritive value	
AME, MJ/kg	13.4
Dry matter, %	89.5
CP, %	20.7
Manganese, mg/kg	23

[1]Favorit-40 (Biofilter Kft, Budaörs), mixture of popcorn (60%) and blended animal fat (40%); [2]Vitamin and mineral premix (Mn-free) provided per kilogram of diet: vitamin A (retinyl acetate) 12,000 IU, vitamin E (dl-α-tocopheryl acetate) 50 IU, cholecalciferol 5,000 IU, menadione 3.0 mg, thiamine × HCl 2.0 mg, riboflavin 6.0 mg, niacin 60 mg, pyridoxine × HCl 6.0 mg, vitamin B_{12} 0.016 mg, Ca-d-pantothenate 20 mg, biotin 0.2 mg, L-ascorbic acid 200 mg, choline chloride 400 mg, folic acid 1.8 mg, ethoxyquin (antioxidant) 100 mg, calcium 2.5 g, phosphorus 3.0 g, salt 2.4 g, magnesium 125 mg, zinc 80 mg, iron 80 mg, copper 8.0 mg, iodine 1.0 mg, selenium 0.15 mg, cobalt 0.1 mg, molybdenum 1.0 mg; [3]Manganese supplementation was added at the expense of equivalent weights of starch

Manganese concentration was measured from the above-mentioned organs as well as from feathers, blood and droppings samples of cockerels by atomic absorption spectrometry (Perkin-Elmer Model 5000 AAS and Carl-Zeiss Jena AAS-3).

Statistical analysis of data

Results were subjected to analysis of variance using the procedure of SPSS (Norušis, 1988). Mean treatment differences were determined by least square differences, the level of statistical significance being 5%.

To evaluate the effect of treatments on the Mn concentration of organs and tissues, the following aspects were considered: (1) the increase of Mn concentration caused by the supplementation in both chemical forms, compared to the

control; (2) the effect of Mn supplementation at the same level but in different chemical forms; (3) the influence of increased Mn supplementation compared to the lowest (30 mg/kg) level in the case of inorganic and organic forms.

Ethical permit

All procedures were approved by the Ethical Committee on Care and Use of Laboratory Animals, Faculty of Veterinary Science Budapest, Szent István University.

Results and discussion

Performance

During the feeding trial a total of 23 cockerels (6.6%) died in the batteries. As regards the causes of death and the number of animals that died, no causal relationship was found with the treatments. The relatively high mortality might be explained by the temperature fluctuations occasionally occurring in the fattening house.

At the beginning of the feeding trial the average body weight of the 14-day-old cockerels was 172.1 ± 12.9 g (Table 3), which shows that these birds were underweight as compared to the values guaranteed by the Ross technology. This backwardness in weight can be regarded as a result of the relatively low BW of the one-day-old chickens. On the other hand, the variance of the BWs was relatively low and very uniform. At the end of the experiment the highest average BW was found in the control group (2030 g). At each of the three different Mn levels the average BW of animals fed a diet supplemented with Mn fumarate was found to be higher than that of birds receiving MnO supplementation, although the differences were not significant ($P > 0.05$).

The lowest FCE was found in the control group (1.90 kg/kg). The differences between the treatments were not significant either (Table 3).

Pathology and histopathology

The average BWs and the absolute and relative organ weights of five cockerels selected randomly from each group are shown in Table 4. The relatively low weight of testicles may be attributed to their infantile status before the beginning of spermatogenesis. Manganese supplementation did not alter the relative weight of the liver, heart and testicles of the cockerels. Due to the absence of histopathological features, it can be stated that 240 mg/kg of supplemental Mn in either form of sources was not toxic to broiler chickens.

Table 3: Body weight of birds and feed/gain ratio

Supplemental Mn		Body weight [means ± SD (n)]						Feed/ gain (kg/kg)
Source	Level (mg/kg)	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	
		(g)						
	0	172.2 ± 12.9 (50)	361 ± 38.8 (50)	652 ± 73.1 (49)	1066 ± 126 (49)	1494 ± 117 (49)	2030 ± 236 (49)	1.90
MnO	30	172.1 ± 12.9 (50)	362 ± 44.7 (49)	638 ± 62.5 (47)	1046 ± 107 (47)	1498 ± 150 (47)	1980 ± 181 (47)	2.01
	60	171.7 ± 12.6 (50)	378 ± 36.2 (50)	654 ± 93.5 (46)	1054 ± 159 (46)	1468 ± 235 (46)	1960 ± 303 (46)	1.99
	240	172.1 ± 12.9 (50)	364 ± 40.0 (50)	648 ± 90.7 (48)	1053 ± 151 (48)	1472 ± 202 (47)	1990 ± 251 (47)	1.99
Mn fumarate	30	172.4 ± 12.8 (50)	366 ± 41.5 (49)	650 ± 74.7 (47)	1059 ± 114 (47)	1516 ± 165 (47)	2020 ± 246 (47)	1.97
	60	172.1 ± 13.0 (50)	371 ± 43.8 (50)	647 ± 91.8 (48)	1043 ± 140 (48)	1470 ± 221 (48)	1990 ± 273 (46)	1.96
	240	172.1 ± 12.9 (50)	377 ± 41.6 (50)	673 ± 66.5 (48)	1074 ± 109 (48)	1480 ± 214 (48)	2010 ± 252 (46)	1.98

Table 4: Group average values of bodyweight, and absolute and relative organ weights at 49-days of age

Supplemental Mn		Body weight[1] (g)	Liver weight[1]		Heart weight[1]		Testicle weight[1]	
Source	Level (mg/kg)		absolute (average ± SD) (g)	relative[2] (%)	absolute (average ± SD) (g)	relative[2] (%)	absolute (average ± SD) (g)	relative[2] (%)
	0	1968 ± 55	32.8 ± 3.2	1.66	6.87 ± 0.56	0.35	0.46 ± 0.089	0.023
MnO	30	2016 ± 59	34.2 ± 1.4	1.69	6.47 ± 0.54	0.32	0.45 ± 0.110	0.022
	60	2038 ± 89	40.1 ± 7.4	1.67	8.13 ± 0.83	0.39	0.53 ± 0.160	0.026
	240	2032 ± 54	38.8 ± 7.1	1.90	7.69 ± 0.39	0.38	0.53 ± 0.073	0.026
Mn fumarate	30	2000 ± 64	44.0 ± 4.5	2.19	8.05 ± 0.73	0.40	0.50 ± 0.180	0.025
	60	1970 ± 63	32.3 ± 1.7	1.63	6.13 ± 0.59	0.32	0.36 ± 0.110	0.018
	240	2062 ± 56	44.0 ± 5.3	2.13	7.45 ± 0.22	0.36	0.48 ± 0.091	0.023

[1]Data are means of five male chicks; [2]Weight of organ in percent of bodyweight

Manganese distribution

Mn supplementation induced an increase in *tibial* Mn concentration (Table 5). The differences compared to the control group, independently of the form of administration, were significant at all levels. No significant differences could be observed in the effect of the two Mn sources at similar supplementation levels ($P > 0.05$). There was no significant difference between the effects of the 30 and 60 mg/kg supplemental levels in the case of either Mn source. However, tibial Mn concentrations differed significantly between the 30 and 240 mg/kg experimental groups in the case of both chemical forms of Mn.

Table 5

Tibial manganese concentration in dry matter of 7-week-old birds as a function of manganese source and level

Supplemental Mn		Tibial Mn concentration		
Source	Level (mg/kg)	Average[1] (mg/kg)	SD (mg/kg)	CV (%)
	0	3.21[a]	0.290	9.0
MnO	30	3.71[b]	0.266	7.2
	60	3.78[b]	0.252	6.7
	240	4.44[c]	0.278	6.3
Mn fumarate	30	3.68[b]	0.392	10.6
	60	4.00[b]	0.274	6.9
	240	4.36[c]	0.467	10.7

[1]Data are means of five male chicks; a–b, a–c: $P < 0.05$; b–b, c–c: $P > 0.05$

As a result of the Mn supplementation, manganese concentration in the *liver* increased as compared to the control group, although that increase proved significant only at the levels of 60 and 240 mg/kg (Table 6). Manganese supplementation of the same level but in inorganic and organic forms resulted in remarkable differences in liver manganese only at the levels of 60 and 240 mg/kg; however, none of the differences were significant. In the case of Mn fumarate significant differences in liver manganese were found between both the 30 and 60 mg/kg (11.6 ± 1.09 and 14.0 ± 0.71 mg/kg DM, respectively) and the 30 and 240 mg/kg (11.6 ± 1.09 and 14.9 ± 0.86 mg/kg DM, respectively) levels. In contrast, in the case of the inorganic Mn source the liver manganese concentrations differed significantly only between the 30 and 240 mg/kg (11.7 ± 1.13 and 14.2 ± 1.75 mg/kg DM, respectively) levels.

Manganese supplementation also increased the manganese concentration of the *kidney* as compared to the 10.2 mg/kg DM level seen in the control group (Table 7). The differences found were significant only at the levels of 60 and

240 mg/kg independently of the Mn source. Manganese supplementation of the same level but in different chemical form caused no significant differences in kidney manganese concentrations. As to the effect of the same chemical form but different supplementation levels, significant differences in kidney manganese concentration were observed between the 30 and 60 mg/kg (11.0 ± 1.21 and 12.8 ± 0.79 mg/kg DM) and also between the 30 and 240 mg/kg (11.0 ± 1.21 and 12.8 ± 0.79 mg/kg DM) supplemental manganese levels in inorganic form, but there were no significant differences between the groups treated with Mn fumarate ($P > 0.05$).

Table 6

Liver manganese concentration in dry matter of 7-week-old birds as a function of manganese source and level

Supplemental Mn		Liver Mn concentration		
Source	Level (mg/kg)	Average[1] (mg/kg)	SD (mg/kg)	CV (%)
	0	9.8[a]	1.54	15.7
MnO	30	11.7[a]	1.13	9.7
	60	12.7[b]	1.14	9.0
	240	14.2[b]	1.75	12.2
Mn fumarate	30	11.6[a,c]	1.09	9.5
	60	14.0[b,d]	0.71	5.1
	240	14.9[b,d]	0.86	5.8

[1]Data are means of five male chicks; a–a, b–b: $P > 0.05$; a–b, c–d: $P < 0.05$

Table 7

Kidney manganese concentration in dry matter of 7-week-old birds as a function of manganese source and level

Supplemental Mn		Kidney Mn concentration		
Source	Level (mg/kg)	Average[1] (mg/kg)	SD (mg/kg)	CV (%)
	0	10.2[a]	1.04	10.3
MnO	30	11.0[a]	1.21	11.1
	60	12.8[b]	0.79	6.1
	240	12.8[b]	0.96	7.5
Mn fumarate	30	12.3[a,c]	2.1	17.1
	60	13.1[b,c]	0.71	5.4
	240	12.5[b,c]	1.67	13.3

[1]Data are means of five male chicks; a–a, b–b, c–c: $P > 0.05$; a–b: $P < 0.05$

Both Mn sources slightly increased the Mn concentration of the *heart* as compared to the control group. However, due to the high standard deviation of the individual concentrations in certain groups, the increases proved significant only at the 60 mg/kg and 240 mg/kg inorganic and 240 mg/kg organic Mn supplementation. Supplementation at the same level but in different form caused insignificant differences in heart Mn concentration. Comparing the effect of different levels but same chemical form of supplementation significant differences were found between the groups where 30 and 240 mg/kg Mn as MnO was added to the basal diet. At the same Mn fumarate administration levels there were no significant differences in heart Mn concentration.

Femoral muscle Mn concentrations in the seven experimental groups differed slightly and the variance of the individual values in each group were fairly high. Consequently, no significant differences could be observed in femoral muscle Mn concentrations.

Mn concentration of the *blood plasma* showed high variability independently of treatment group. Although the 13.2 and 13.4 μg/L concentrations found at the 240 mg/kg level (MnO and Mn fumarate, respectively) compared to the control group (9.6 μg/L) reflected the highest level of supplementation, the differences were not significant. Supplementation at the same level but in different form resulted in an almost negligible difference in the mean concentrations and the individual values fell mainly within the same range but showed high variation.

As pooled testicles, as well as feather and droppings samples of five male chicks per treatments were analysed, statistical evaluation of the results could not be performed. Mn concentration in the *testicles* increased as the level of supplementation was raised. At the same addition level the higher concentrations were always measured in the case of Mn fumarate supplementation.

As the supplemental level was raised, the Mn concentration in *feathers* increased, independently of the source of Mn supplementation. *Droppings* sensitively reflected the Mn intake. At the same level of supplementation the higher concentration was always observed after MnO addition. These results would imply that Mn fumarate is absorbed better, but the differences are slight and are within the confidence level of the analytical method used.

The bioavailability of Mn sources is generally evaluated by the measurement of tibial Mn concentration (Black et al., 1984; Baker and Halpin, 1987; Bertechini and Hossain, 1992; Ammerman, 1995; Smith et al., 1995). According to our results, the effect of the increase of Mn supplementation, independently of its chemical form, was reflected primarily by the Mn concentration of the tibia, to a lesser extent by that of the liver and kidney, followed by the heart. At the flock level, droppings proved to be a sensitive indicator of Mn intake, whereas the blood plasma and feathers showed only the extreme level of Mn loading.

Conclusion

Based on our results, it can be stated that Mn fumarate is not more efficient in broiler chickens than MnO as this Mn source did not cause significant improvement in BW gain and feed efficiency. At the same level of supplementation no significant differences were found in the Mn concentrations of organs/tissues. It is supported that the tibial Mn concentration is a proper indicator for the evaluation of Mn supply in chicks. The recently recommended supplemental level of Mn (60 mg/kg) proved to be sufficient to cover the Mn requirement in broiler chickens.

References

Albion Laboratories, Inc. (1992): Intestinal uptake of Albion chelated minerals *vs.* inorganic minerals. Research Summary. Albion Laboratories, Inc., Clearfield, Utah, USA.

Ammerman, C. B. (1995): Methods for estimation of mineral bioavailability. In: Ammerman, C. B., Baker, D. H. and Lewis, A. J. (eds) Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. 4. Academic Press, San Diego, CA. pp. 83–94.

Baker, D. H. and Halpin, K. M. (1987): Research note: Efficacy of manganese-protein chelate compared with that of manganese sulfate for chicks. Poultry Sci. **66,** 1561–1563.

Bertechini, A. G. and Hossain, S. M. (1992): Requirements and bioavailability of manganese from inorganic sources. Poultry Sci. **71 (Suppl. 1),** 32.

Black, J. R., Ammerman, C. B., Henry, P. R. and Miles, R. D. (1984): Biological availability of manganese sources and effects of high dietary manganese on tissue mineral composition of broiler-type chicks. Poultry Sci. **63,** 1999–2006.

Brown, T. F. and Zeringue, L. K. (1994): Laboratory evaluation of solubility and structural integrity of complexed and chelated trace mineral supplements. J. Dairy Sci. **77,** 181–189.

Fly, A. D., Izquierdo, O. A., Lowry, K. R. and Baker, D. H. (1989): Manganese bioavailability in a Mn-methionine chelate. Nutr. Res. **9,** 901–910.

Halpin, K. M. and Baker, D. H. (1986): Long-term effect of corn, soybean meal, wheat bran, and fish meal on manganese utilization in the chicks. Poultry Sci. **65,** 1371–1374.

Halpin, K. M., Chausow, D. G. and Baker, D. H. (1986): Efficiency of manganese absorption in chicks fed corn-soy and casein diets. J. Nutr. **116,** 1747–1751.

Henry, P. R., Ammerman, C. B. and Miles, R. D. (1989): Relative bioavailability of manganese in a manganese-methionine complex for broiler chicks. Poultry Sci. **68,** 107–112.

Hungarian Feed Codex (1990): Magyar Takarmánykódex (in Hungarian). FM-MMI, Budapest.

Johnson, B. and Socha, M. (1998): Judging trace mineral bioavailability. Feed Int., Sept. 1998, 34–38.

Martin, R. and Scribante, P. (2000): Evaluating metal-methionine complexes. Feed Int., June 2000, 10–14.

National Research Council (1994): Nutrient Requirements of Poultry. 9th revised edition. National Academy Press, Washington, D.C.

Norušis, M. J. (1988): SPSS/PC™ V3.0, Update Manual. SPSS Inc., Chicago.

Smith, M. O., Sherman, I. L., Miller, L. C., Robbins, K. R. and Halley, J. T. (1995): Relative biological availability of manganese from manganese proteinate, manganese sulfate, and manganese monoxide in broilers reared at elevated temperatures. Poultry Sci. **74,** 702–707.

Szabó, S. A., Régiusné Mőcsényi, Á., Győri, D. and Szentmihályi, S. (1987): Microelements in agriculture. I. Essential microelements (in Hungarian). Mezőgazdasági Kiadó, Budapest, p. 85.