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Excess Manganese Ingestion in the Chick

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ABSTRACT Two experiments were conducted with young chicks to investigate effects of excess manganese, (Mn) ingestion and to compare $MnCl_2 \cdot 4H_2O$, $MnSO_4 \cdot H_2O$, MnO_2, and $MnCO_3$ as sources of dietary Mn activity. Each source of Mn was added to a conventional corn-soybean meal diet to supply 3000, 4000, or 5000 mg/kg Mn. High levels of dietary Mn from $MnSO_4 \cdot H_2O$ and $MnCl_2 \cdot 4H_2O$ depressed growth slightly; MnO_2 and $MnCO_3$ did not affect chick performance. Manganese dioxide contained less Mn activity than $MnCO_3$, $MnSO_4 \cdot H_2O$, or $MnCl_2 \cdot 4H_2O$ as assessed by relative depressions in hemoglobin and hematocrit and by relative increases in bile, liver, and bone Mn concentrations. Neutralization of the chloride ion in $MnCl_2 \cdot 4H_2O$ with $NaHCO_3$ did not ameliorate the adverse effects of excess, $MnCl_2 \cdot 4H_2O$ ingestion. Bone and bile Mn concentrations reflected Mn intake better than liver Mn concentration or rate and efficiency of weight gain.

(Key words: manganese, tissue manganese, growth)

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INTRODUCTION

Practical poultry diets are routinely supplemented with manganese (Mn), usually at levels in excess of the chick's dietary Mn requirement. Supplementation leads to the possibility of errors in Mn addition, which could result in excessive intakes. The level of Mn that is toxic to the chick has not been well characterized. Gallup and Norris (1939) reported that 1000 mg/kg Mn was not toxic. Heller and Penquite (1937) observed 52% mortality in chicks fed 4779 mg/kg Mn, and Van der Hoorn *et al.* (1938) reported a depression in performance of chicks fed as little as 600 mg/kg Mn. Thus, considerable disagreement exists in the literature regarding the toxicity of Mn in the chick. In addition, the research that has been done was published in the late 1930's. Results obtained then may not be directly applicable today due to the tremendous advances in poultry nutrition (vitamin and mineral fortification of diets) that have been made since that time.

The purpose of our investigation was to determine the effect of excess Mn ingestion from four sources on chick performance, on incidence and severity of anemia, and on tissue accumulation of Mn.

MATERIALS AND METHODS

Male chicks resulting from the cross of New Hampshire males and Columbian females were used in each experiment. They were fed a corn-soybean meal diet (Table 1) from hatching to 7 days posthatching. After an overnight fast, the chicks were inspected for navel infection and fecal pasting and were then weighed, wingbanded, and randomly assigned to treatments. Three replicates of 5 chicks each were assigned to each treatment. The experimental periods were 8 to 21 days posthatching in Experiment 1 and 8 to 22 days posthatching in Experiment 2. Chicks were maintained on a 24-hr constant-light schedule in heated, thermostatically-controlled starter batteries (mean temperature of 35 C) with raised wire floors. They were allowed *ad libitum* access to the experimental diets and tap water. Weight gain and feed consumption were recorded at the end of each experiment.

The basal diet (Table 1) was a conventional corn-soybean meal diet formulated to meet or exceed the nutrient requirements of the growing chick (National Research Council, 1977). Dietary additions were made to the basal diet at the expense of cornstarch. The Mn sources ($MnSO_4 \cdot H_2O$, $MnCl_2 \cdot 4H_2O$, $MnCO_3$ and MnO_2), as well as the NaCl and $NaHCO_3$ used in Experiment 2, were reagent grade chemicals.

At the termination of each experiment, the three chicks closest in weight to the replicate mean were selected. Blood samples were taken

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TABLE 1. *Composition of corn-soybean meal basal diet*[1]

Ingredient		(%)
Cornstarch	to	100.00
Corn (8.5% CP)[2]		49.93
Soybean meal (48% CP)		37.00
Corn oil		4.00
Fish meal-Menhaden (60% CP)		2.00
Alfalfa meal, dehydrated (17% CP)		1.00
Dicalcium phosphate		2.20
Limestone, ground		1.00
Iodized salt		.40
DL-Methionine		.20
Vitamin premix[3]		.10
Choline chloride (60%)		.10
Manganese sulfate (28% Mn)		.05
Zinc carbonate (52% Zn)		.01
Lincomycin premix (44 g/kg)		.01

[1] Contained 168 mg/kg Mn by analysis.

[2] CP, crude protein.

[3] Vitamin premix provided per kilogram of diet: vitamin A, 4400 IU; vitamin D_3, 1000 IU; vitamin E, 11 IU; vitamin B_{12}, .01 mg; riboflavin, 4.41 mg; d-pantothenic acid, 10.0 mg; niacin, 22.0 mg; menadione sodium bisulfite, 2.33 mg.

by cardiac puncture, pooled by replicate, and analyzed immediately for hemoglobin content (Crosby *et al.*, 1954) and hematrocrit (Cohen, 1967). Chicks were then killed by cervical dislocation and the gallbladder-free half of liver, the bile, and the right tibia were removed, pooled by replicate, and analyzed. Liver tissue and bile were taken at the conclusion of Experiments 1 and 2, whereas bone tissue was taken only at the conclusion of Experiment 2. The dry, fat-free tibia was ashed at 650 C for 24 hr. The liver segment and bile were dried at 100 C for 24 hr, weighed, and then wet ashed with HNO_3 and H_2O_2 (30%).[3] Manganese was determined in all tissues by atomic absorption spectrophotometry (Perkin-Elmer, Model 306).

In Experiment 1, effects of excess Mn ingestion from $MnSO_4 \cdot H_2O$, $MnCl_2 \cdot 4H_2O$, $MnCO_3$, and MnO_2 were assessed. Each Mn source was added to a corn-soybean meal diet (Table 1) in amounts to supply 3000, 4000, or 5000 mg/kg Mn. In Experiment 2, 3000 mg/kg Mn from $MnSO_4 \cdot H_2O$ or from $MnCl_2 \cdot 4H_2O$ was compared. In this experiment, however, the effect of the chloride (Cl) ion on the adverse effect of $MnCl_2 \cdot 4H_2O$ was assessed. Excess NaCl was added to the basal diet to provide Cl at a level isomolar to the Cl present in the $MnCl_2 \cdot 4H_2O$ addition. Also, $MnCl_2 \cdot 4H_2O$ and $NaHCO_3$ were fed in combination to supply isomolar additions of Cl and Na.

All data were analyzed by analysis of variance procedures (Steel and Torrie, 1980). Treatment means were compared by orthogonal as well as meaningful nonorthogonal single degree-of-freedom comparisons. In Experiment 1, differences among Mn sources were tested by the least significant difference procedure only if the F-value for treatment was significant ($P<.05$).

RESULTS

Results of Experiment 1 are shown in Table 2. The $MnCl_2 \cdot 4H_2O$ compound was the only Mn source to depress weight gain; none of the Mn sources affected efficiency of feed utilization. Blood hemoglobin was significantly depressed by $MnCl_2 \cdot 4H_2O$, $MnCO_3$, and $MnSO_4 \cdot H_2O$ but not by MnO_2. The depression in hemoglobin obtained from $MnSO_4 \cdot H_2O$ and $MnCO_3$, however, was not as severe as that obtained from $MnCl_2 \cdot 4H_2O$. Hematocrit values were significantly depressed by both $MnCl_2 \cdot 4H_2O$ and $MnCO_3$, but $MnCl_2 \cdot 4H_2O$ resulted in the greater depression. Hematocrit was reduced, but not significantly, by $MnSO_4 \cdot H_2O$ or MnO_2. Bile Mn concentration was increased dramatically by all Mn sources. This is consistent with the fact that absorbed Mn is primarily excreted in the bile (Underwood, 1977). The $MnSO_4 \cdot H_2O$ and $MnCl_2 \cdot 4H_2O$ compounds resulted in the greatest increase in bile Mn concentration. Smaller increases were obtained from $MnCO_3$ and MnO_2. Liver Mn concentration was increased by all Mn sources, but the increase was not as great as that observed in bile Mn concentration. The greatest increase in liver Mn concentration resulted from $MnSO_4 \cdot H_2O$ with smaller increases coming from the other three Mn sources. Iron concentration in the liver was depressed by all Mn compounds with no statistical differences among sources. $MnCl_2 \cdot 4H_2O$, however, appeared to result in the greatest depression.

Although differences were observed among

[3] Diets were analyzed for Mn using similar wet-ashing procedures.

TABLE 2. *Performance, blood and tissue data of chicks fed high levels of Mn from four different sources (Experiment 1)*

Dietary addition			Performance[1]		Blood analyses[2]		Tissue analyses[2]		
Mn Source	Mn Level		Gain	Gain/feed	Hemoglobin	Hematocrit	Bile Mn[3]	Liver Mn	LIver Fe
	(mg/kg)		(g)	(g/kg)	(g/100 ml)	(%)	(μg/g dry tissue)		
None	...		233^{b}	631	9.1^{d}	30.6^{c}	20^{a}	15^{a}	382^{a}
$MnSO_4 \cdot H_2O$	3000		234	650	8.4	29.1	150	28	212
	4000		239	675	8.3	29.3	204	29	268
	5000		232	660	7.9	28.0	268	32	218
		Mean	235^{b}	662	8.2^{bc}	28.8^{bc}	207^{d}	30^{c}	233^{b}
$MnCO_3$	3000		226	650	8.0	28.4	116	22	197
	4000		237	663	7.6	28.5	163	30	287
	5000		229	645	7.5	28.1	173	26	176
		Mean	231^{ab}	653	7.7^{ab}	28.3^{b}	151^{c}	26^{bc}	220^{b}
$MnCl_2 \cdot 4H_2O$	3000		237	658	7.6	27.5	163	24	183
	4000		202	632	6.9	24.8	182	22	186
	5000		211	642	6.8	24.8	188	26	169
		Mean	217^{a}	644	7.1^{a}	25.7^{a}	178^{cd}	24^{b}	179^{b}
MnO_2	3000		236	655	8.6	29.7	63	19	224
	4000		216	638	8.3	28.3	65	25	290
	5000		237	662	8.7	28.5	60	24	223
		Mean	230^{ab}	652	8.5^{cd}	28.8^{bc}	63^{b}	23^{b}	246^{b}
Pooled SEM			5.8	11.0	.28	.76	14.9	2.3	32.2

^{a,b,c,d}Means not sharing a common superscript differ significantly ($P<.05$).

[1] Data are means of three replicates of five male chicks during the assay period 8 to 21 days posthatching; average initial weight was 79 g.

[2] Data are means of three samples, each sample representing pooled tissue or blood from three uniform chicks within a replicate.

[3] Manganese linear effect within $MnSO_4 \cdot H_2O$ and $MnCO_3$ was significant ($P<.01$).

Mn sources, very few differences among Mn levels were seen. Bile Mn concentration, however, was increased linearly by graded increments of $MnSO_4 \cdot H_2O$ and $MnCO_3$. In some instances, Mn level effected quadratic responses that were difficult to explain. Weight gain, for example, was not affected by 3000 or 5000 mg/kg Mn from MnO_2, but the intermediate level of Mn (4000 mg/kg) depressed gain. We have no explanation for this unusual response.

The results of Experiment 2 are presented in Table 3. Both $MnSO_4 \cdot H_2O$ and $MnCl_2 \cdot 4H_2O$ depressed weight gain, hemoglobin, and hematocrit and increased liver, bile, and bone Mn concentrations compared to chicks fed the basal diet. No differences were observed between the two Mn sources. Bone ash percent and efficiency of feed utilization were not affected by excess Mn in the form of either $MnSO_4 \cdot H_2O$ or $MnCl_2 \cdot 4H_2O$. Bone Mn concentration was dramatically increased by high dietary Mn: concentration of Mn in bone ash was similar to the Mn concentration in bile.

Addition of NaCl to the basal diet did not depress chick performance, hemoglobin, or hematocrit and did not affect tissue Mn. When the combination of $MnCl_2 \cdot 4H_2O$ and $NaHCO_3$ was fed to provide isomolar levels of Cl and Na, the results were very similar to those obtained when $MnCl_2 \cdot 4H_2O$ was fed alone. Two exceptions, however, were evident: bile Mn content was increased and liver Mn content was decreased as a result of adding $NaHCO_3$ to the diet containing $MnCl_2 \cdot 4H_2O$. This suggests an apparent redistribution of absorbed Mn as a result of the $NaHCO_3$ addition, more being excreted and less being deposited in the liver.

TABLE 3. *Comparative toxicity of Mn from $MnSO_4 \cdot H_2O$ and $MnCl_2 \cdot 4H_2O$ and the influence of the Cl ion on the toxicity of $MnCl_2 \cdot 4H_2O$ (Experiment 2)*

Diet no. and description	Mineral level		Performance[1]		Blood analyses[2]		Tissue Mn concentration[2]			Bone ash[2]
	Mn	Cl	Gain[3]	Gain/feed	Hemoglobin[3]	Hematocrit[3,4]	Bile[3,4,5]	Liver[3,4,5]	Bone[3,4]	
	(mg/kg)		(g)	(g/kg)	(g/100 ml)	(%)	(μg/g dry tissue)		(μg/g ash)	(%)
1. None	0	0	267	675	9.2	28.9	13	13	17	52.9
2. .92% $MnSO_4 \cdot H_2O$	3000	0	250	667	8.6	27.7	121	26	111	52.9
3. 1.08% $MnCl_2 \cdot 4H_2O$	3000	3872	251	650	8.3	26.7	104	24	95	51.6
4. .64% NaCl	...	3872	264	672	8.8	29.9	15	15	17	51.2
5. As 3 + .92% $NaHCO_3$	3000	3872	254	667	8.3	26.7	136	21	97	52.3
Pooled SEM			5.8	9.0	.28	.76	9.4	.8	8.3	.40

[1] Data are means of three replicates of five male chicks during the assay period 8 to 22 days posthatching; average initial weight was 72 g.

[2] Data are means of three samples, each sample representing pooled tissue or blood from three uniform chicks within a replicate.

[3] The mean of Diets 1 and 4 differs from the mean of Diets 2, 3 and 5 ($P<.04$).

[4] Diet 4 different from Diet 5 ($P<.01$).

[5] Diet 3 different from Diet 5 ($P<.04$).

DISCUSSION

Exceptionally high (3000 to 5000 mg/kg) Mn depressed growth, but only slightly, and caused mild anemia in chicks. Similar results have been reported in pigs (Leibholz *et al.*, 1962) and poults (Vohra and Kratzer, 1968). Earlier work with chicks by Heller and Penquite (1937) however, had indicated that Mn levels similar to those we used were toxic. The likely explanation for this discrepancy is the nutritional adequacy of the diet we used compared to the diet used by Heller and Penquite (1937). Their diet contained no vitamin or trace mineral fortification. This diet would increase the chick's susceptibility to Mn toxicity not only because of its nutritional de-

ficiencies, but also because it probably contained fewer minerals such as Co, Ca, and P that would directly decrease Mn availability and absorption (Underwood, 1977).

In Experiment 1, $MnCl_2 \cdot 4H_2O$ appeared to provide a more bioavailable source of Mn than $MnSO_4 \cdot H_2O$ as determined by depressed growth and by altered hematological parameters; $MnSO_4 \cdot H_2O$, however, resulted in liver and bile Mn concentrations higher than those observed for $MnCl_2 \cdot 4H_2O$. Thus, it was difficult to ascertain which compound provided the most available Mn. We thought that the observed differences between the two compounds may have been caused by the high Cl present in the diet when $MnCl_2 \cdot 4H_2O$ was fed. In Experiment 2, however, $MnCl_2 \cdot 4H_2O$ and $MnSO_4 \cdot H_2O$ elicited nearly identical responses. Neutralization of the Cl ion by Na did not ameliorate the adverse effects of $MnCl_2 \cdot 4H_2O$, and NaCl addition to the diet to supply an isomolar level of Cl did not adversely affect chick performance. Therefore, we concluded from these results that $MnSO_4 \cdot H_2O$ and $MnCl_2 \cdot 4H_2O$ were equally growth depressing and that the growth depression was due to the Mn portion of the compound.

High levels of dietary Mn decreased hemoglobin, hematocrit, and liver iron levels. Similar observations have been made previously in mamalian species (Underwood, 1977). The lowered iron uptake results from an iron-manganese antagonism at the absorptive surface of the intestine (Thomson and Valberg, 1972). High levels of dietary Mn interfere with iron absorption producing a mild iron-deficiency anemia.

Watson *et al.* (1970, 1971) have reported a difference in the bioavailability of Mn from different Mn sources. The results of our investigation confirm their findings. The Mn in MnO_2 was less toxic and hence, in all likelihood, less biologically available than the Mn in $MnCO_3$, $MnSO_4 \cdot H_2O$, or $MnCl_2 \cdot 4H_2O$.[4] High levels of MnO_2, for example, had less of an effect on tissue Mn levels and had no effect on blood hemoglobin or hematocrit levels.

The results of this investigation indicate that high levels of Mn produce a mild anemia and depress growth slightly in chicks. Also, bone and bile Mn concentrations reflect Mn intake better than liver Mn content or performance.

[4]Multiple regression of bile Mn concentration on Mn intake (using 0, 3000, and 4000 mg/kg Mn levels) in Experiment 1 gave the equation: $Y = 18.6 + .1275X_1$ (sulfate) $+ .0984X_2$ (carbonate) $+ .1302X_3$ (chloride) $+ .0369X_4$ (oxide), $r^2 = .995$. Using slope-ratio, and setting $MnSO_4 \cdot H_2O$ as 100%, gives availability estimates of 77.2% for $MnCO_3$, 102.1% for $MnCl_2 \cdot 4H_2O$, and 28.9% for MnO_2.

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