

Research Note: Evaluation of manganese hydroxychloride in 45-wk-old white leghorn layers using yolk and shell manganese content

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ABSTRACT The objective of the current study was to evaluate increasing levels of manganese hydroxychloride (MHC) in 45-wk-old white leghorn laying hens, using yolk and shell manganese (Mn) content as a potential marker for Mn concentration. A total of 80, 45-wk-old white leghorns were assigned to 6 dietary treatments, each consisting of 14 individually caged laying hens, with the exception of the reference diet containing 10 individually caged laying hens. The experiment consisted of a reference diet that contained 70 ppm of supplemental inorganic Mn in the form of Mn oxide and 5 experimental treatments each containing 0, 15, 30, 60, and 90 ppm supplemental MHC. Experimental birds were subjected to a 21 D depletion phase in which no supplemental Mn was included in the diet; however, during this time reference fed birds were fed the control diet (70 ppm Mn). After

the 21 D depletion phase, the depleted birds were fed experimental diets for a 35 D evaluation period. Yolk and shell Mn content were analyzed at the end of the depletion phase and during the experimental phase on day 5, 10, 15, 25, and 35. During the experimental phase, Mn was replenished in the yolk and shell in all experimental treatments containing supplemental Mn; however, dose and time impacted the rate of replenishment. The yolk tended to be more sensitive to variations in Mn level as increases in Mn inclusion significantly ($P < 0.05$) increased concentration. These data demonstrate the ability to deplete and replenish Mn, and the use of egg yolk Mn concentration as measurement for determining changes in dietary Mn. At the conclusion of the experiment at 35 D, 60 ppm of Mn hydroxychloride seemed to be adequate in replenishing Mn to the level of the reference.

Key words: manganese, layers, egg, yolk, depletion, replenish

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INTRODUCTION

Dietary manganese (Mn) requirement for white-egg laying strains is 20 ppm during egg production as stated by the NRC (1994). Since the establishment of these requirements, other Mn sources, in addition to traditional inorganic mineral sources, have been researched and implemented in commercial production. Alternative mineral sources may allow for reductions in inclusion rates without negatively impacting performance or cost and reduce environmental impact. The target for egg producers differ, as breeders remain concerned with shell quality (Jackson et al., 1987; Roberts, 2004) production rate, and nutrients available to the developing embryo during incubation. Novel feed ingredient developments and additives may offer innovative methods to alter dietary formulation to benefit the animal, producer, and consumer. The implication of different sources of min-

erals have raised question regarding bioavailability, biological effects, and recommended inclusion rates.

Manganese is important for a myriad of pathways and functions in developing and advanced stages of growth. In poultry, reductions in egg production and hatchability were observed by Gallup and Norris (1939), diets containing 13 ppm Mn reduced lay rate, egg weight, fertility, embryo mortality, and hatch of fertile compared to hens fed diets containing 200 or 53 ppm Mn respectively. The negative impacts on performance and reproduction imply the importance of Mn for early development and physiological maintenance. The absorption of trace minerals in the egg is mediated by the yolk sac membrane which is responsible for the regulation of trace minerals from the liver to the embryo (Richards, 1997). Thus, impairment and malformation of embryonic growth and development is controlled by the developing yolk and can indicate a deficiency in Mn. The objective of the current experiment was to evaluate the effect of increasing levels of dietary hydroxychloride Mn in white leghorn hens on yolk and shell Mn level of eggs at different time points, for determination of repletion.

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MATERIALS AND METHODS

Experimental Design

The experimental design of the current trial consisted of 5 experimental treatments containing increasing levels of Mn in the form of manganese hydroxychloride (MHC) at 0, 15, 30, 60, and 90 ppm. A control diet containing an adequate level of Mn (70 ppm) was fed to 10 replicate pens; the reference diet represented the sixth treatment. Prior to feeding the experimental treatments containing MHC, 70 layers (5 experimental treatments) were fed a diet absent of supplemental Mn for a 21 D period. This period was defined as the depletion phase. During the depletion phase, layers fed the reference diet (treatment 6) remained on a diet adequate in Mn. All experimental treatments contained 14 replicates per treatment, the reference diet (treatment 6) contained 10 replicates, each containing 1, 45-wk-old Hyline W-36 white-leghorn layer placed in battery cages for a 21 D depletion period followed by a 35 D experimental period (56 total days). Birds were acquired from the Texas A&M Poultry Science Research Center established fertile flock, which were originally obtained from Hy-line hatcheries.

Experimental Diets

Diets were corn and soybean meal based and contained distillers dried grains with solubles at 5.00% during the entirety of the experiment (Table 1). The depleted diet contained no supplemental Mn and experimental treatments contained supplemental Mn in the form of hydroxychloride at 0, 15, 30, 60, and 90 ppm. The reference diet contained 70 ppm Mn in the form of feed grade Mn oxide (minimum of 60% Mn) during the depletion and experimental phase of the experiment to provide a reference for Mn inclusion. Manganese inclusions for each treatment during the experimental phase were 0, 15, 30, 60, 90, and 70 ppm, Mn levels were analyzed at 65, 73, 93, 129, 151, and 117 ppm respectively. Diets were manufactured from 1 large basal, and a custom premix containing Mn was added to achieve target Mn level. All diets were fed as a mash.

Animals and Management Practice

In the experiment, a total of 80, 45-wk-old Hy-line leghorn layer were placed in battery style layer cages at day 0 of the experiment. The study consisted of a total of 80 pens, each containing 1 layer at the start of the trial. Pens were 1587 cm² cages equipped with one nipple drinker and a trough style feeder. Water was available ad libitum, however, feed allocation was restricted to no more than 115 g/day to avoid compensative eating in an effort to meet Mn requirement. Layers were provided age appropriate environmental temperature of

Table 1. Ingredient profile and nutrient content for basal diet fed 45-week-old white leghorn hens, evaluating the manganese hydroxychloride requirement.

Ingredient	Experimental
Corn	55.44
Soybean meal	21.48
DL-methionine	0.19
Lysine HCl	0.09
L-threonine	0.02
Soy oil	4.25
Limestone	7.85
Oystershell	3.00
Monocalcium phosphate	1.90
Salt	0.21
Sodium bicarb	0.30
Vitamins ¹	0.25
Mineral premix ²	0.05
Distillers dried grains with solubles	5.00
Calculated nutrient content (%)	
Crude protein	16.80
Calcium	4.50
Available phosphorus	0.51
AME poultry (kcal/kg)	2880
Dig methionine	0.43
Dig lysine	0.79
Dig TSAA	0.66
Dig tryptophan	0.16
Dig threonine	0.55
Dig arginine	0.93
Sodium	0.20
Analyzed nutrient content (%)	
Protein	15.20
Crude fat	6.80
Crude fiber	3.30
Calcium	4.58
Phosphorus	0.78
Sodium	0.21
Manganese (ppm)	65
Copper (ppm)	15
Zinc (ppm)	90

¹Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D₃, 46 IU vitamin E, 0.0165 mg B₁₂, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, and 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

²Mineral premix added at this rate (453.6 g per 907.2 kg or 1.0 lbs. per ton) yields 60 ppm zinc, 30 ppm iron, 8 ppm copper, 0.3 ppm selenium, and 0.8 ppm iodine.

approximately 23°C and were subject to an industry type lighting program. The lighting program consisted of 16 h of continuous light (2-foot candles) and 8 h of dark for the duration of the study.

On day 5, 10, 15, 25, and 35 eggs were collected for the determination of Mn content of the yolk and shell. The yolk portion of the egg was separated from the albumen and freeze-dried using a Labconco free-zone freeze dryer (Kansas City, MO). Manganese content of yolk and shell were determined at a third-party lab (ATC Scientific, North Little Rock, AR) using microwave digestion followed by inductively coupled plasma—optical emission spectrometry. Shell and yolk Mn concentration were selected to evaluate multiple time points in layer production, without the destruction of birds used from the previous time point. This sampling method allowed for the evaluation of the Mn deficiency impact over the duration of the experiment.

Table 2. Yolk manganese (Mn) concentration (ppm) in 45-wk-old white leghorn hens fed manganese hydroxychloride at the end of the depletion phase and throughout the experimental phase.

Mn	Day 0	5	10	15	25	35
0	1.10 ^b	0.97 ^d	1.16 ^d	1.25 ^d	1.30 ^b	1.27 ^c
15		1.06 ^{c,d}	1.21 ^{c,d}	1.55 ^c	1.57 ^a	1.59 ^b
30		1.16 ^{b-d}	1.35 ^{c,d}	1.86 ^b	1.64 ^a	1.68 ^{a,b}
60		1.31 ^{b,c}	1.46 ^{b,c}	1.89 ^b	1.65 ^a	1.63 ^b
90		1.38 ^b	1.63 ^{a,b}	2.14 ^a	1.81 ^a	1.91 ^a
Ref.	1.59 ^a	1.83 ^a	1.83 ^a	1.87 ^b	1.80 ^a	1.78 ^{a,b}
ANOVA						
SEM	0.085	0.069	0.058	0.064	0.055	0.050
<i>P</i> -value	0.036	<0.001	<0.001	<0.001	0.003	<0.001
Regression						
Linear						<0.001
Quadratic						<0.001

^{a-d}Means within a column with different superscripts differ at $P < 0.05$.

All animal husbandry procedures were conducted in accordance with an approved animal use protocol (IACUC).

Statistical Analysis

All data were subject to a one-way ANOVA using the GLM (SPSS software). Means were deemed significantly different at a P -value of ≤ 0.05 and were further separated by Duncan's multiple range test. Linear and quadratic regression analysis was conducted on MnHCl treatments only to determine the correlation between increasing levels of MHC on target parameter at termination of the study.

RESULTS AND DISCUSSION

At the termination of the depletion phase layers consuming the depleted diet absent of supplemental Mn, significantly reduced yolk Mn concentration compared to layers consuming the reference diet (Table 2). These results were expected and provided a baseline for the experimental phase of the study. Replenishment of yolk Mn to the reference diet was achieved as quickly as 10 D in hens consuming experimental treatments; however, dependent on level of dietary Mn. Similar to the current study, Mabe et al. (2003) reported increases in yolk Mn concentration in relation to elevations in dietary Mn concentration after a 4 wk depletion phase. Venglovska et al. (2014) observed elevations in yolk Mn in layers consuming Mn-glycine chelate compared to the basal diet absent of supplemental Mn. Li et al. (2018) also reported elevations in yolk Mn content when increasing dietary Mn concentrations; however, Mn values in this study seemed to be low (~ 0.20 ppm) compared to the current study (~ 1.00 to 2.15 ppm), Mabe et al. (2003) (0.60 to 0.93 ppm), and Venglovska et al. (2014) (1.65 to 2.40 ppm). The drastic differences in Mn

Table 3. Shell manganese (Mn) concentration (ppm) in 45-wk-old white leghorn hens fed manganese hydroxychloride at the end of the depletion phase and throughout the experimental phase.

Mn	Day 0	5	10	15	25	35
0	0.13	0.61 ^c	0.90 ^c	1.31	1.17 ^{a,b}	0.74 ^b
15		0.51 ^c	1.08 ^{b,c}	1.17	1.15 ^{a,b}	0.84 ^b
30		1.26 ^a	1.13 ^{a-c}	1.14	1.07 ^b	1.04 ^a
60		1.02 ^b	1.23 ^{a-c}	1.10	1.08 ^b	1.10 ^a
90		0.67 ^c	1.28 ^{a,b}	1.16	1.25 ^a	1.06 ^a
Ref.	0.12	0.98 ^b	1.46 ^a	1.10	0.93 ^c	1.06 ^a
ANOVA						
SEM	0.006	0.055	0.063	0.027	0.027	0.031
<i>P</i> -value	0.532	<0.001	0.027	0.06	0.002	<0.001
Regression						
Linear						<0.001
Quadratic						<0.001

^{a-c}Means within a column with different superscripts differ at $P < 0.05$.

yolk composition between studies could be contributed to the amount of background Mn in the basal diets or may indicate a Mn depletion phase exacerbates the impact of Mn repletion in yolk. In the current study, layers consuming 90 ppm MnHCl for 15 D produced the highest yolk Mn concentration compared to all other treatments, with a similar stair-step elevation in yolk Mn with increases in dietary Mn being observed on subsequent days. Conflicting of the previous studies measuring yolk Mn content, Inal et al. (2001) did not observe differences in yolk Mn concentration between birds fed a control diet or a diet absent of trace minerals for 10 or 12 wk. At termination of the current study, linear and quadratic correlations were observed indicating a direct relationship between increasing dietary MnHCl (0 to 90 ppm) and yolk Mn deposition.

Different from yolk Mn, shell Mn composition was less sensitive to dietary changes in Mn and seemed sporadic during the early stages of the experimental phase. However, layers consuming higher levels of Mn deposited more Mn overall at termination of the experimental phase (Table 3). An initial increase in Mn shell deposition is observed once birds are fed supplemental Mn through day 10, however normalize as supplementation continues through day 35. This may be due to deficient birds depositing shell Mn more aggressively in response to dietary Mn supplementation, and normalizing Mn deposition over time. Similarly, Xiao et al. (2015) reported increases in shell Mn composition at dietary inclusion greater than 50 ppm for inorganic and 20 ppm for organic Mn, though no further rise in shell Mn was observed. Mabe et al. (2003) reported the differences associated with eggshell Mn did not resemble dietary Mn concentrations. Though the results of these experiments do not correlate level of dietary Mn with deposition of Mn in the shell, at termination of the current study linear and quadratic correlations were observed for shell Mn deposition in laying hens consuming 0 to 90 ppm MnHCl. The lack of differences in shell

Mn in previous studies may be due to the duration of which these biological processes occur. The yolk forms and matures from the ovaries when the hen reaches maturity to lay; however, the shell is constructed in the oviduct in less than 24 h. The large differences in duration between shell and yolk could contribute to the reduced impact dietary Mn has on shell Mn content.

Manganese deposition in the both the egg yolk and shell responded to different levels of dietary Mn. However, the egg yolk demonstrated the highest sensitivity to changes in dietary Mn concentration in the current study compared to the eggshell. Replenishment of yolk Mn to the reference diet level was achieved as quickly as 10 D in hens consuming 90 ppm MnHCl, and 15 D in hens consuming 60 and 30 ppm, indicating MnHCl supplementation between these levels is adequate to meet the layers Mn requirement based on Mn replenishment of the egg yolk observed. The egg yolk sampling technique used in this study provided a non-destructive method for determining Mn requirement. This method will allow researchers to evaluate different sources of Mn and their deposition into the yolk at different time points, and could be used in the future to analyze and evaluate availability of different sources and concentrations of Mn fed in egg producing poultry.

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