Manganese requirements of broiler breeder hens

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ABSTRACT The present research was conducted to assess Mn requirements of broiler breeder hens. One hundred and twenty Cobb 500 hens, 22 wk of age, were individually allocated in cages. After fed a Mn-deficient diet (22.2 ppm), hens were randomly placed in treatments having 6 increments of 30-ppm Mn. All trace minerals were from laboratory grade sources being Mn from Mn sulfate ($\mathbf{MnSO_4\,H_2O}$). Treatments were fed for 4 periods of 28 d. There were no interactions between dietary Mn and period for any evaluated response ($P > 0.05$). Requirements of Mn for hen day egg production and settable egg production were 115.8 and 56.6 ppm and 122.1 and 63.6 ppm ($P < 0.05$), respectively, using quadratic polynomial (**QP**) and broken line quadratic (**BLQ**) models, whereas total eggs and total settable eggs per hen had Mn requirements estimated at 115.7 and 56.6 and 121.8 and 61.7 ppm ($P < 0.05$), respectively. Number of cracked, defective, and contaminated eggs decreased, whereas hatchability, hatchability of fertile eggs, eggshell percentage, and eggshell palisade layer increased when hens were fed diets having 48.5 to 168.2-ppm Mn ($P < 0.05$). Maximum responses for egg weight and eggshell percentage were 117.7 and 63.6 ppm as well as 131.6 and 71.0 ppm ($P < 0.05$), respectively, using QP and BLQ models. Breaking strength and egg specific gravity had Mn requirements estimated at 140.2 and 112.7 ppm as well as 131.3 68.5 ppm ($P < 0.05$), whereas eggshell palisade layer and eggshell thickness were maximized with 128.8 and 68.8 ppm and 140.2 134.2 ppm, respectively, for QP and BLQ models ($P < 0.05$). Maximum yolk Mn content values were obtained using 118.0- and 118.4-ppm Mn by QP and BLQ models, respectively. The average Mn requirements estimated for QP and BLQ models is 128.4 and 92.3 ppm Mn (18.7 and 13.5 mg/hen/d), respectively, which is much lower than what has been currently recommended in commercial production.

Key words: broiler breeder, micromineral, manganese

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INTRODUCTION

Manganese (**Mn**) is the fifth most abundant mineral on earth (Suttle, 2010), and it was first reported to prevent perosis in broilers by Wilgus et al. (1936). Mn deficiency in poultry is associated with structural and physiological disorders, which include cartilage and skeletal malformation as well as a reduction in the antioxidant defense system (Luo et al., 1992; Tuormaa, 1996).

As Mn was determined as an essential trace mineral for poultry, a series of involvements of Mn have been demonstrated, mainly as a constituent of metalloenzymes. For instance, Mn superoxide dismutase is involved in the control of oxidative stress in the mitochondria by converting superoxide to peroxide, which is then reduced to water afterwards (Bottje, 2018). Pyruvate carboxylase (**PC**) and arginase are also metalloenzymes, respectively, involved in the metabolism of pyruvate into oxaloacetate (Reed and Scrutton, 1974; Baly et al., 1985; Moomaw et al., 2009; Suttle, 2010) as well as in the conversion of arginine into urea and ornithine (Wu and Morris, 1998; Fernandes and Murakami, 2010).

Mn has an active role in activating glycosyltransferase, which is involved in the formation of proteoglycans; therefore, it is essential for the formation of eggshell membranes (Leach and Gross, 1983; Tuormaa, 1996; Xiao et al., 2014). Proteoglycans are major constituents of bone and eggshell extracellular matrixes that are comprised of a protein core with a large number of glycosaminoglycan side chains (Arias et al., 1993; Keen et al., 2013). Mn-deficient chicks have been observed to have skeletal and cartilage malformations with substantial reductions in the total proteoglycan bone content (Liu et al., 1994).

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In plant feedstuffs, Mn varies upon soil composition (Gupta et al., 2008). Therefore, Mn content in broiler breeder feeds is expected to vary with its dietary source. Mn content in wheat bran, which is largely used in broiler breeder feeds, varies from 88 to 163.9 mg/kg (Suttle, 2010; Rostagno et al., 2017), whereas in corn, it has been reported to vary from 5 to 15 mg/kg and in soybean meal from 36 to 48 mg/kg (NRC, 1994; Suttle, 2010; Spears and Engle, 2011). Macro mineral supplements, such as phosphates and limestone, can have high Mn content, also at variable concentrations (from 174 to 726 and 15 to 250 ppm Mn, respectively) (Reid and Weber, 1976; Lima et al., 1999; Wilkinson et al., 2013).

Availability of Mn in plant feedstuffs is dependent on its total chelation with phytic acid, similar to what happens with other positively charged minerals. Differently from Fe, Cu, and Zn, overall contents of Mn in routinely used broiler breeder feeds are low (An exception is wheat bran, which has a high proportion of phytate and, then, potentially reduces Mn availability for poultry; Mohanna and Nys, 1999; Attia et al., 2010). High dietary Ca and P has also been reported to reduce Mn absorption, probably because of the increased insolubility of phytate when reacted with these minerals (Wedekind and Baker, 1990; Spears and Engle, 2011).

Reports on Mn supplementation have been published recently with broiler chickens (Lu et al., 2006, 2016; Li et al., 2011; Pacheco et al., 2017) and laying hens (Olgun and Cufadar, 2010; Xiao et al., 2014; Zhang et al., 2017). However, limited research has been conducted with broiler breeder hens. Present recommendations for Mn as a dietary supplement for broiler breeder hens are mostly based on suggestions, which range from 70 to 90 mg Mn/kg of feed (FEDNA, 2008; Rostagno et al., 2011; Rostagno et al., 2017) to 120 mg of Mn/kg (Aviagen, 2017; Cobb-Vantress, 2018). These suggestions, however, lack representative *in vivo* research of support.

Mn is usually supplemented in broiler breeder feeds as part of the micromineral premix, frequently as a sulfate salt, but sometimes incorporated in a diversity of organic minerals. Oversupplying minerals to commercial livestock has become an environmental issue, which has been shown to contaminate groundwater. Recent regulations have stablished 150 ppm as the maximum Mn content in poultry feeds in the European Union (EFSA, 2016). In the Eastern Shore, United States, regulations have been put in place to reduce that threat (Subramanian and Gupta, 2006).

The present study was conducted using Mn sulfate monohydrate ($\mathbf{MnSO_4\,H_2O}$) as the supplemental source in a Mn-deficient feed. Evaluated responses were related to egg production, but also extended to other parameters that can affect overall hen nutrition as well as hatching chick production. The main goal of the present study is to provide Mn requirements such that daily Mn supply to broiler breeder hens does not limit competitive production and also is not excessive such that an increase in the cost of production parallels unnecessary Mn excretion, turning it into an environmental pollutant.

MATERIAL AND METHODS

All procedures performed in the present study were approved by the Ethics and Research Committee of the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Birds and Management

One hundred and twenty Cobb 500 broiler breeder hens and 30 Cobb broiler breeder male birds were obtained from a commercial breeder farm (Grupo Vibra Agroindustrial S. A, Montenegro, RS, Brazil) at 22 wk of age. Hens were individually weighed at arrival such that their coefficient of variation could be assessed. They were then individually placed in cages (0.33 m length × 0.46 m width × 0.40 m height) respecting a representative distribution of body weights throughout similar ranges in different treatments. In parallel, 30 Cobb breeder male birds were placed in 3 collective floor pens (2.0 × 1.5 m), 10 in each, for semen collection. The used experimental cages are electrostatically painted and have one stainless steel nipple drinker and a plastic trough feeder. Temperature control, lighting, and feeding programs followed the recommendations of Cobb-Vantress (2016). Semen collection and hen insemination were performed as described by Taschetto et al. (2017).

Experimental Feeds

Breeder hens were given a common preexperimental adaptation feed followed by a Mn depletion one (Table 1). The adaptation feed followed recommendations from Cobb-Vantress (2018) and was provided from the arrival of hens at the farm until 30 wk of age, whereas the Mn-deficient depletion feed (16.4 ppm formulated and 22.2 + 3.21 ppm analyzed Mn) was fed from 31 to 35 wk. Starting at 36 wk, the hens were individually weighed and assigned to the experimental treatments following a complete randomized block experimental design. Breeder male birds were fed diets providing nutrients and energy as recommended by Cobb (2018) throughout the end part of the study.

The experimental feeds were grade supplemented, at the expense of an inert (kaolin), with laboratory grade Mn sulfate monohydrate ($MnSO_4H_2O$) (Sigma Aldrich, St. Louis, MO) into the deficient feed providing increments of 0, 30-, 60-, 90-, 120-, and 150-ppm Mn. Analyses of Mn were performed in every batch of feed mixed (n = 4) using the atomic absorption spectrophotometric method of Association of Official Analytical Chemists (AOAC, 2016; No.968.08). Average analyzed contents of Mn in feeds supplemented with $MnSO_4H_2O$ throughout the treatments were 22.2 + 3.21; 48.5 + 3.44; 77.9 + 5.49; 103.1 + 1.82; 140.0 + 7.88, and 168.2 + 3.57 mg Mn/kg, respectively. The experiment was divided into 4 periods of 28 d, in 6 × 4 factorial arrangement of 6 dietary Mn contents and 4 periods. Each one of the 6 dietary treatments was replicated 20

Table 1. Experimental diets provided to breeder hens during adaptation as well as during the Mn requirement experiment.[1]

	Basal diet (22–30 wk)	Mn deficient diet (31–51 wk)
Ingredient, % as is	Adaptation phase	Preexperimental and experimental phases[2]
Corn	54.50	61.70
Soybean meal	19.82	23.44
Calcium carbonate	-	7.53
Oat hulls	-	3.87
Wheat meal	14.21	-
Limestone	8.15	-
Dicalcium phosphate	0.73	-
Soybean oil	1.44	1.00
Phosphoric acid, 85% P	-	1.36
Potassium carbonate	-	0.03
Sodium bicarbonate	0.26	0.18
Sodium chloride	0.28	0.39
Choline chloride	0.13	0.11
DL-methionine, 99%	0.18	0.18
L-threonine 98.5%	0.04	0.02
L-tryptophan, 98%	0.01	0.01
Vitamin and mineral mix[3]	0.25	0.17
Antioxidant	0.01	0.01
Total	100.00	100.00
Formulated composition, % or as shown		
AME_n, kcal/kg	2,760	
Crude protein	15.40	
Ca	2.90	
Available P	0.43	
Na	0.20	
Choline, mg/kg	1,500	
Mn, ppm[3]	175.1	16.4 (22.2)

[1]Calcium carbonate, phosphoric acid, potassium carbonate, sodium bicarbonate and sodium chloride were laboratory grade and had only trace amounts of Mn (10.0; 0.7; 0.0; 3.9 ppm, respectively).
[2]Experimental treatments resulted from feed additions with $MnSO_4H_2O$.
[3]Mineral and vitamin premix supplied the following per kg of feed: Cu, 15 mg; Zn, 110 mg; Fe, 50 mg; Se, 0.3 mg; I, 2 mg; vitamin A, 12,000 IU; vitamin D_3, 3,000 IU; vitamin E, 100 IU; vitamin C, 50 mg; vitamin K_3, 6 mg; vitamin B12, 40 µg; thiamine, 3.5 mg; riboflavin, 16 mg; vitamin B6, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4 mg; biotin, 0.3 mg. Analyzed Zn was from one pooled sample from each batch (total of four batches).

times, with one individually caged hen being the replicated experimental unit.

All ingredients and feeds were analyzed for Mn before feed formulation using the method described previously. Breeder hens were fed daily in restricted amounts as recommended by Cobb-Vantress (2016). Consumption of Mn in mg/hen/d per hen was calculated using daily feed intakes present in the feeds (Table 2). Water Mn content was analyzed using atomic absorption (ZEEnit 650 P, Analytik Jena, Jena, Germany). Averaged duplicate analysis of Mn in water was <0.006 ppm, which was not considered a significant dietary source of this mineral.

Hen Performance

Eggs were daily collected 4 times per day and classified as hatchable or not (broken, defective, or without shells). Body-checked (wrinkled equator), elongated, and rounded eggs were classified as defective eggs. The percentage of eggs in each period was calculated on the basis of the

Table 2. Supplemented, calculated, and analyzed Mn concentrations in the experimental diets, feed intake, and Mn intake per hen day in each period.[1]

	Total dietary Mn, ppm		Period, wk				
			36–39	40–43	44–47	48–51	
Supplemented Mn, ppm[1]	Calculated	Analyzed[2]	Mn intake, mg/hen/d				Average, 36–51
0	16.4	22.2 ± 3.21	3.9	3.0	2.9	3.1	3.2
30	46.4	48.5 ± 3.44	6.9	6.9	6.7	7.8	7.1
60	76.4	77.9 ± 5.49	10.8	11.3	10.8	12.5	11.4
90	106.4	103.1 ± 1.82	14.7	15.1	15.3	15.0	15.0
120	136.4	140.0 ± 7.88	20.9	19.6	19.3	21.8	20.4
150	166.4	168.2 ± 3.57	24.7	24.2	25.2	24.0	24.5
Mn intake, mg/hen/d			13.7	13.3	13.4	14.0	13.6
Feed intake, g/hen/d			145.8	144.2	145.6	147.7	145.8

[1]From laboratory grade Mn sulfate monohydrate.
[2]Analyzed Mn from one pooled sample from each feed mix batch (n = 4).

number of live hens. All hatchable eggs laid in the last week of each 28-d period were weighed and grouped in 4 replicates (Eggs of each 5 replications were added together.) per treatment and set into a single-stage incubator at 37.5°C and 65% relative humidity until 18 d. Eggs were then transferred to a hatcher set to 36.6°C and 80% RH. Incubator and hatcher were equipment commercially produced by Avicomave (Rua Edjan Barçalobre, 161, Distrito Industrial, Iracemápolis, SP, Brazil). Overall hatchability and hatchability of fertile eggs were calculated as the percentage of hatching chicks to the total and fertile eggs set, respectively. All unhatched eggs were broken, and evaluations were conducted to estimate the moment and cause of embryonic death as described by Favero et al. (2013). Contaminated eggs were those visually rotten (having uncommon green or black contents, emitting putrid odor or exploding at opening).

Hen Blood Measurements

Hematocrit (**Ht**) and hemoglobin (**Hb**) as well as alkaline phosphatase (**ALP**) concentrations were obtained from blood samples pooled from 3 hens randomly selected from each treatment per period. Blood obtained was partially transferred to 0.5-mL test tubes containing EDTA for Ht and Hb analyses. Ht was determined using microcapillaries containing blood centrifuged for 5 min from 15,650 to 18,510 × g. The cyanmethemoglobin method was used to determine Hb concentration (Crosby et al., 1954). Blood left was centrifuged to obtain the serum. Analysis of ALP was performed as described by Roy (1970), using a digital bench colorimeter (Model Labquest, Vernier Software & Technology, Beaverton, OR). Collection of blood was performed such that none of the hens were bled more than once.

Hatching Chicks

Hatching chicks were weighed and had their length measured (distance from the tip of the beak to the end of the middle toe) as described by Molenaar et al. (2008). Hb and Ht concentrations were determined with 15 chicks hatched per treatment per period. Chick blood samples were obtained from the jugular vein after euthanasia by cervical dislocation. Six hatching chicks per treatment each period were randomly selected for tibia collection: Right tibias were stripped of any adhering tissue, leaving intact cartilage bone caps, which were further submitted to fat extraction using diethyl ether overnight. Dried tibias were weighed, and their lengths were determined using a digital micrometer (Mitutoyo CSX-B, Japan). Bones were later left for 10 h at 600°C in a muffle furnace (Sanchis & Cia Ltda, Porto Alegre, RS, Brazil) to determine percent ash [(dry ash weight/dry tibia weight) × 100].

Egg Analysis

Eggs were collected in the last 4 d of each period with a total of 45 eggs per treatment. Eggs (n = 25) were used to measure egg weight, specific gravity, yolk, albumen, and eggshell percentage of the egg. Specific gravity was determined using saline solutions with concentrations ranging from 1.065 to 1.095 g/cm^3 in intervals of 0.005 units (Novikoff and Gutteridge, 1949). Eggshell weight was obtained after washing and drying shells at 105°C overnight, whereas eggshell thickness was measured using a micrometer (Model IP65; Mitutoyo Corp., Kawasaki, Japan) in the basal, equatorial, and apical regions, with these values being averaged for statistical analysis. The other 20 eggs were used to determine eggshell breaking strength, using a texture analyzer (Model TA.XT. plus; Texture Technologies Corp., Hamilton, AL) with a 75-mm (P/75) breaking probe (Molino et al., 2015). In addition, 3 eggs with the same average weight ± 10% SD per treatment obtained in the last 3 d of each period (39, 43, 47, and 51 wk) were used in the analysis of eggshell ultrastructure using scanning electron microscopy (King and Robinson, 1972). In the preparation for this analysis, eggshell samples were taken from three 0.5-cm^2 samples in the apical, equatorial, and basal areas. Eggshell samples were mounted transversely and horizontally on aluminum stubs using carbon tape, to measure the thickness of eggshell layers and the number of mammillary buttons/mm^2, respectively. These were metallized with gold at 35 nm for 3 min (BAL-TEC SCD050 Sputter Coater; Capovani Brothers Inc., Scotia, NY). Mammillary and palisade layer magnification was performed according to the study by Dennis et al. (1996). Microscopy images were analyzed in the Image-Pro Plus software (Media Cybernetics, Rockville, MD). The average eggshell layer thickness (μm) was estimated from 3 different locations per image.

Statistical Analysis

Data were submitted to the normality test and Levene's test for homogeneity of variance (Levene, 1960; Shapiro and Wilk, 1965). Data were transformed using the arcsine square root percentage (z = asin (sqrt (y + 0.5))) whenever not normally distributed (Ahrens et al., 1990). Data were submitted to ANOVA using the mixed linear model procedure of SAS (2013) using each one of the 28-d periods as repeated measures. Total egg production and settable egg production per hen at 51 wk were analyzed using the general linear models (PROC GLM). The Tukey-Kramer test was used for means comparison with differences being considered significant at $P < 0.05$ (Tukey, 1991). The covariance structures of PROC mixed linear model were chosen based on the Akaike criteria (Littell et al., 1998). Nonparametric data, such as hatching chick leg length and navel button scores, were analyzed by the generalized linear mixed model procedure, and the means were also compared by the Tukey-Kramer test ($P < 0.05$). Estimates of maximum responses to total dietary Mn were done using quadratic polynomial (QP) and broken line quadratic (BLQ) models (Robbins et al., 1979; Pesti et al., 2009). The QP model ($Y = a + b \times Mn + c \times (Mn)^2$) had Y as the dependent variable and as a function of dietary

Table 3. Broiler breeder hen performance as affected by increased dietary Mn.[1]

	Eggs[2]							Breeders			Hatching chicks		
	Hen day production	Settable	Cracked	Defective	Shell-less	Total[3]	Settable[4]	Ht, %					
Mn, ppm (mg/day)			%						Hb, g/dL	ALP, U/L	Ht, %	Hb, g/dL	ALP, U/L
22.2 (3.2)	58.5^{b}	45.9^{b}	10.4^{a}	1.64^{a}	0.33	65^{b}	51^{b}	28.4^{b}	9.4	237	30.8^{c}	9.8	3,126
48.5 (7.1)	64.0^{a,b}	57.6^{a}	4.9^{b}	0.50^{b}	0.32	72^{a,b}	65^{a}	29.1^{a,b}	9.8	262	31.2^{b,c}	10.0	3,402
77.9 (11.4)	64.1^{a,b}	58.4^{a}	4.5^{b}	0.55^{b}	0.18	72^{a,b}	66^{a}	28.7^{b}	9.7	313	31.8^{b,c}	10.3	3,391
103.1 (15.0)	64.9^{a}	60.0^{a}	4.0^{b}	0.45^{b}	0.10	73^{a}	67^{a}	30.9^{a}	10.2	322	33.8^{a}	10.8	3,208
140.0 (20.4)	64.2^{a,b}	59.9^{a}	3.5^{b}	0.59^{b}	0.09	72^{a,b}	67^{a}	30.2^{a,b}	9.9	385	31.8^{b,c}	10.0	3,380
168.2 (24.5)	64.1^{a,b}	59.6^{a}	3.2^{b}	0.46^{b}	0.05	72^{a,b}	67^{a}	30.0^{a,b}	9.9	301	32.9^{a,b}	10.8	3,438
Period, wk													
36–39	71.3^{a}	64.3^{a}	5.4^{a}	0.82	0.33	-	-	27.0^{c}	9.0^{c}	266	32.2^{a,b}	10.89^{a}	2,358^{c}
40–43	66.0^{b}	59.1^{b}	5.7^{a}	0.67	0.15	-	-	29.6^{b}	9.7^{b}	315	32.8^{a}	10.25^{a,b}	2,947^{b}
44–47	60.4^{c}	53.7^{c}	5.3^{a}	0.71	0.12	-	-	31.6^{a}	10.9^{a}	326	32.9^{a}	10.37^{a,b}	4,013^{a}
48–51	54.8^{d}	50.0^{d}	3.8^{b}	0.58	0.10	-	-	29.9^{a,b}	9.6^{b,c}	306	30.2^{b}	9.67^{b}	3,961^{a}
SEM	0.55	0.65	0.29	0.081	0.042	0.74	1.05	0.32	0.13	12.5	0.35	0.11	96.0
Probability <													
Mn	0.0289	0.0001	0.0001	0.0010	0.3024	0.0429	0.0001	0.0486	0.4032	0.0714	0.0021	0.0523	0.2526
Period	0.0001	0.0001	0.0012	0.7437	0.3076	-	-	0.0001	0.0001	0.2576	0.0139	0.0011	0.0001
Mn vs. period	0.8895	0.7715	0.1793	0.7287	0.7196	-	-	0.2882	0.2984	0.3836	0.0977	0.6206	0.3292

Means with different letters in the same column indicate significant differences ($P \leq 0.05$).

Abbreviations: ALP, serum alkaline phosphatase; Ht, hematocrit; Hb, hemoglobin.

[1]Probabilities of hen day production, settable, cracked, defective, and shell-less eggs are presented after arcsine transformation.

[2]As a percentage of total live hens at the time of measurement.

[3]Total eggs at the end of the experiment.

[4]Total settable egg at the end of the experiment.

Table 4. Broiler breeder hen incubation performance as affected by increased dietary Mn.[1]

		Hatchability, %		Embryo mortality[2], %				
Mn, ppm (mg/day)	Fertility[5], %	Total eggs[3]	Fertile eggs[4]	Early dead	Middle dead	Late dead	Pips	Contaminated eggs[6], %
22.2 (3.2)	87.3	62.0[b]	70.8[b]	5.50	1.91	6.73	6.14	7.75[a]
48.5 (7.1)	87.6	75.2[a]	85.9[a]	3.11	1.19	3.35	2.93	3.18[a,b]
77.9 (11.4)	87.8	75.8[a]	86.3[a]	3.10	0.85	3.14	3.34	2.92[a,b]
103.1 (15.0)	88.5	79.6[a]	89.9[a]	2.09	0.62	3.07	2.78	1.43[b]
140.0 (20.4)	89.5	79.8[a]	89.2[a]	2.13	0.61	3.14	2.55	1.58[b]
168.2 (24.5)	88.2	78.6[a]	89.2[a]	2.32	0.61	3.16	2.85	1.63[b]
Periods, wk								
36–39	92.4[a]	78.0[a]	84.3	4.23	1.67	3.46	3.67	2.43
40–43	88.1[a,b]	76.4[a,b]	87.0	2.15	0.51	3.85	3.13	3.03
44–47	87.9[b]	74.8[a,b]	84.6	2.72	0.98	3.80	4.06	2.92
48–51	84.2[b]	71.5[b]	84.8	3.06	0.70	3.91	2.87	3.95
SEM	0.66	1.15	1.11	0.450	0.263	0.484	0.469	0.407
Probability <								
Mn	0.9320	0.0018	0.0001	0.1841	0.8143	0.3488	0.4942	0.0039
Period	0.0001	0.0366	0.8002	0.4902	0.4277	0.9937	0.6999	0.4830
Mn vs. period	0.1807	0.1719	0.8593	0.9882	0.6833	0.7009	0.9985	0.8444

Means with different letters in the same column indicate significant differences ($P \leq 0.05$).
[1]All probabilities presented after arcsine transformation.
[2]All data were calculated as a percentage of fertile eggs; early, middle, and late dead were estimated, respectively at first, second, and third week of incubation.
[3]Hatchability as a proportion of total eggs, % = (number of chicks hatched/number of eggs set) × 100.
[4]Hatchability as a proportion total of fertile eggs, % = (number of chicks hatched/number of fertile eggs set) × 100.
[5]Fertility, % = (number of fertile eggs/numbers of total egg set) × 100.
[6]Contaminated eggs calculated as a percentage of total eggs.

level of Mn; a as the intercept; b as the linear coefficient; and c as the quadratic coefficient with the maximum response for Mn defined as Mn = $-b \div (2 \times c)$. The BLQ model ($Y = a + b \times (c - Mn)^2$) had $(c - Mn) = 0$ for Mn > c and Y as the dependent variable as a function of the dietary level of Mn, a the value of the dependent variable at the plateau, and b as the slope of the line.

RESULTS

Analyses of Mn in the dietary treatments were conducted on samples from one pool of each 4 mixed batches throughout the study and averaged 22.2 ± 3.21; 48.5 ± 3.44; 77.9 ± 5.49; 103.1 ± 1.82; 140.0 ± 7.88; and 168.2 ± 3.57 ppm (Table 2). Analyses of variance and regressions were conducted with the analyzed data. Analyses performed to check for further deviations between formulated and analyzed feeds showed no important differences that could affect the treatments with different levels of Mn (n = 24; crude protein, 16.4 ± 0.73%; Ca, 2.55 ± 0.214%; and P, 0.59 ± 0.052%).

There were no interactions between dietary Mn and period for any response. Therefore, data are presented as main factors throughout this report. Defective eggs, shell-less eggs, breeder ALP (Table 3), hatchability of fertile eggs, embryo mortality, contaminated eggs (Table 4), eggshell percentage, yolk Mn content, specific gravity, membrane thickness (Table 5), and leg scores were not affected by period (Table 6) ($P > 0.05$); however, hen day and settable egg production, hatching chick Hb (Table 3), fertility, hatchability of total eggs (Table 4), as well as albumen as a proportion of the whole egg decreased as hens aged (Table 5) ($P < 0.05$). On the other hand, egg yolk percentage (Table 5), egg weight, hatching chick weight, hatching chick length, tibia length, and hatching chick tibia ash increased as hens aged (Table 6) ($P < 0.05$). Number of mammillary buttons (Table 5) and hatching chick navel button scores (Table 6) were higher in the period of 36 to 39 wk than all other periods, whereas hen Ht and Hb were lower in the same period than all others ($P < 0.05$). Palisade layer and mammillary layer peaked highest in the period of 44 to 47 wk (Table 5) decreasing afterwards ($P < 0.05$). Eggshell breaking strength as well as eggshell thickness increased in the second period (40–43 wk) decreasing afterwards (44–51 wk) (Table 5).

Supplementing Mn at any level did not affect shell-less eggs, hen Hb and ALP, chick Hb and ALP (Table 3), egg fertility, embryo mortality (Table 4), yolk and albumen percentage, number of mammillary buttons (Table 5), egg and chick weights, hatching chick length and scores, navel button score, as well as tibia ash (Table 6) ($P > 0.05$). On the other hand, dietary increases of Mn affected ($P < 0.05$) the total and settable hen egg production, total number of eggs and settable eggs per hen, cracked eggs, defective eggs, and hen and chick Ht (Table 3). Contaminated eggs decreased, whereas total hatchability, hatchability of fertile eggs (Table 4), shell percentage, palisade and mammillary layer (Table 5), and tibia length increased as dietary Mn was higher (Table 6) ($P < 0.05$).

Estimation of Mn requirements determined using BLQ and QP regression models are shown in Tables 7 and 8. In a few cases, responses did not fit adequately; therefore, in those cases, requirement estimation is not presented. Maximum responses for hen day egg production and total egg production were the same (115.8- and 56.6-ppm Mn from QP and BLQ models, respectively), whereas requirements for percent settable eggs were

Table 5. Broiler breeder hen egg characteristics as affected by increased dietary Mn.

Mn, ppm (mg/day)	Yolk	Albumen	Shell	Yolk Mn, ppm	Specific gravity, kg/cm^3	Eggshell					
						Palisade layer	Mammillary layer	Membrane	Thickness	Breaking strength, kg/cm^2	Number of mammillary buttons/mm^2
	%[1]					μm					
22.2 (3.2)	28.7	63.2	8.12^{b}	1.81^{b}	1.080^{c}	196.3^{b}	88.4^{b}	63.6^{d}	359^{c}	3.53^{b}	213
48.5 (7.1)	29.3	62.1	8.54^{a}	2.00^{a,b}	1.084^{b}	222.5^{a}	90.0^{a,b}	70.3^{c}	377^{b}	3.84^{a,b}	206
77.9 (11.4)	29.3	62.2	8.54^{a}	2.09^{a,b}	1.084^{b}	224.0^{a}	91.7^{a,b}	70.9^{b,c}	377^{b}	3.96^{a}	195
103.1 (15.0)	29.6	61.8	8.56^{a}	2.37^{a}	1.085^{a,b}	226.5^{a}	92.8^{a,b}	74.7^{a}	391^{a}	4.00^{a}	194
140.0 (20.4)	29.5	61.7	8.77^{a}	2.29^{a}	1.086^{a}	235.1^{a}	99.9^{a}	74.3^{a,b}	391^{a}	4.06^{a}	180
168.2 (24.5)	29.5	61.8	8.65^{a}	2.12^{a,b}	1.085^{a,b}	228.6^{a}	95.0^{a,b}	75.4^{a}	390^{a}	4.07^{a}	194
Period, wk											
36–39	28.1^{c}	63.3^{a}	8.50	2.13	1.085	215.0^{b}	86.5^{b}	70.8	366^{c}	3.82^{a,b}	217^{a}
40–43	29.2^{b}	62.2^{b}	8.56	2.17	1.084	220.3^{b}	90.6^{a,b}	71.2	404^{a}	4.05^{a}	183^{b}
44–47	29.8^{a}	61.6^{c}	8.60	2.09	1.084	229.5^{a}	101.7^{a}	71.9	378^{b}	4.00^{a,b}	183^{b}
48–51	30.1^{a}	61.4^{c}	8.47	2.06	1.084	223.8^{a,b}	93.0^{a,b}	72.2	374^{b}	3.76^{b}	205^{a,b}
SEM	0.09	0.09	0.028	0.037	0.001	2.1	1.5	0.53	1.2	0.034	4.1
Probability <											
Mn	0.4979	0.0636	0.0003	0.0122	0.0001	0.0001	0.0432	0.0001	0.0001	0.0023	0.5311
Period	0.0001	0.0001	0.1789	0.5632	0.1632	0.0380	0.0449	0.6983	0.0001	0.0111	0.0059
Mn vs. period	0.3157	0.2733	0.6117	0.4424	0.9005	0.9450	0.9335	0.0646	0.2298	0.9106	0.9886

Means with different letters in the same column indicate significant differences ($P \leq 0.05$).
[1]Probabilities of yolk, albumen, and eggshell percentage are presented as arcsine transformation.

122.1- and 63.6-ppm Mn and total eggs produced were maximized at 121.8 and 61.7 ppm Mn, QP and BLQ, respectively. Cracked eggs were minimized at 129.5- and 66.4-ppm Mn using QP and BLQ regressions, respectively, whereas maximum response was obtained at 118.4 by QP on defective eggs. The highest egg hatchability and the lowest contaminated eggs were optimized at 125.7 ppm and 127.9 ppm and at 69.5 ppm and 71.9 ppm, respectively, using the QP and BLQ models. The maximum values for hatchability of fertile eggs were obtained at 124.5- and 65.8-ppm Mn by QP and BLQ models, respectively. Breeder hen Ht and ALP requirements using QP and BLQ models were estimated at 142.7- and 148.0- and 126.5- and 145.2-ppm Mn, whereas 135.5- and 122.4-ppm Mn were the highest for chick Ht, respectively.

Mn requirement for the highest egg weight was 117.7- and 63.6-ppm Mn by QP and BLQ models, respectively, while hatching chick weight using the same models were 120.4- and 85.6-ppm Mn. Requirements of dietary Mn to maximize eggshell and albumen as egg percentages were 131.6 and 127.5 ppm using the QP model and 71.0 and 113.0 ppm using the BLQ model.

Egg yolk Mn content was positively related to dietary Mn increase, with maximum responses obtained at 118.0- and 118.4-ppm Mn with QP and BLQ models, respectively, whereas yolk percentage was highest when dietary Mn was fed at 124.4 using the QP model.

Table 6. Hatching chick characteristics as affected by increased dietary Mn.

Mn, ppm (mg/day)	Egg weight	Hatching chicks[1]					
		Chick weight	Chick length[2], cm	Leg score[3]	Navel button score[4]	Tibia length, mm	Tibia ash, %
	g						
22.2 (3.2)	72.4	51.7	18.5	1.17	1.58	29.2^{b}	24.9
48.5 (7.1)	70.7	50.7	18.6	1.14	1.43	29.5^{a,b}	25.4
77.9 (11.4)	70.5	50.6	18.6	1.07	1.43	29.8^{a,b}	25.6
103.1 (15.0)	70.6	50.4	18.6	1.05	1.28	30.6^{a}	26.0
140.0 (20.4)	70.2	50.3	18.6	1.05	1.30	30.3^{a}	26.3
168.2 (24.5)	70.7	50.4	18.6	1.06	1.31	30.3^{a}	26.1
Period, wk							
36–39	69.6^{c}	48.7^{c}	18.4^{c}	1.18	1.61^{a}	28.8^{c}	24.1^{b}
40–43	70.7^{b}	50.3^{b}	18.5^{b,c}	1.04	1.14^{b}	29.5^{b,c}	26.3^{a}
44–47	71.2^{b}	51.1^{b}	18.6^{a,b}	1.05	1.31^{b}	30.2^{b}	26.1^{a}
48–51	72.3^{a}	52.6^{a}	18.8^{a}	1.02	1.36^{a,b}	31.3^{a}	26.3^{a}
SEM	0.11	0.12	0.020	0.009	0.018	0.13	0.22
Probability <							
Mn	0.6832	0.8745	0.9540	0.8219	0.0549	0.0032	0.5748
Period	0.0001	0.0001	0.0001	0.3538	0.0001	0.0001	0.0004
Level vs. period	0.9853	0.1020	0.9053	0.9999	0.9719	0.1180	0.6161

Means with different letters in the same column indicate significant differences ($P \leq 0.05$).
[1]Leg and navel scores were analyzed using proc Glimmix (variables are nonparametric data).
[2]From the tip of the beak to the end of the middle toe (third toe).
[3]Leg scores: 1, normal legs and toes; 2, signs of inflammation or redness in the legs.
[4]Navel scores: 1, completely closed and clean; 2, not completely closed and not discolored; 3, not completely closed and discolored.

Table 7. Regression equations of egg production and incubation of breeders fed with Mn supplementation.

Variable	Model	Regression equations[1]	Confidence interval, 95%	SEM	R^2	Probability <	Requirement
Total egg production[2], %	QP	$y = 56.37698 + 0.15410x\text{-}0.00066530x^2$	63.3 ± 1.08	0.55	0.03	0.0016	115.8
	BLQ	$y = 64.2859 - 0.00487\ (56.6483 - x)^2$			0.03	0.0004	56.6
Settable egg production[3], %	QP	$y = 41.23796 + 0.33202x - 0.00136x^2$	59.9 ± 1.27	0.65	0.11	0.0001	122.1
	BLQ	$y = 59.4306 - 0.00788\ (63.6067 - x)^2$			0.12	0.0001	63.6
Cracked eggs, %	QP	$y = 12.31387\text{-}0.14512x + 0.00056028x^2$	5.07 ± 0.568	0.29	0.13	0.0001	129.5
	BLQ	$y = 3.8040 + 0.00336(66.4374 - x)^2$			0.15	0.0001	66.4
Defective eggs, %	QP	$y = 1.95438 - 0.02744x + 0.00011585x^2$	0.70 ± 0.157	0.08	0.04	0.0001	118.4
Hatchability, %	QP	$y = 55.87404 + 0.39736x - 0.00158x^2$	75.2 ± 2.25	1.15	0.27	0.0001	125.7
	BLQ	$y = 78.4475 - 0.00734\ (69.4930 - x)^2$			0.29	0.0001	69.5
Hatchability of fertile eggs, %	QP	$y = 64.53912 + 0.42841x - 0.00172x^2$	85.2 ± 2.18	1.11	0.33	0.0001	124.5
	BLQ	$y = 88.6570 - 0.00942\ (65.7774 - x)^2$			0.37	0.0001	65.8
Contaminated eggs, %	QP	$y = 9.88679 - 0.13776x + 0.00053837x^2$	3.08 ± 0.804	0.41	0.28	0.0001	127.9
	BLQ	$y = 1.8898 + 0.00237\ (71.9020 - x)^2$			0.29	0.0001	71.9
Total egg production[4]	QP	$y = 63.09205 + 0.17237x - 0.00074517x^2$	71 ± 1.5	0.74	0.08	0.0124	115.8
	BLQ	$y = 71.9315 - 0.00544\ (56.5923 - x)^2$			0.09	0.0056	56.6
Settable egg production[5]	QP	$y = 46.53535 + 0.37016x - 0.00152x^2$	64 ± 2.1	1.05	0.21	0.0001	121.8
	BLQ	$y = 66.7397 - 0.00973\ (61.7100 - x)^2$			0.25	0.0001	61.7
Hen Ht, %	QP	$y = 27.48538 + 0.03812x - 0.00013359x^2$	29.5 ± 0.63	0.32	0.07	0.0900	142.7
	BLQ	$y = 30.1971 - 0.00012\ (148.0 - x)^2$			0.07	0.0917	148.0
Chick Ht, %	QP	$y = 29.61752 + 0.04676x - 0.00017260x^2$	32.0 ± 0.69	0.35	0.07	0.0856	135.5
	BLQ	$y = 32.6856 - 0.00022\ (122.4 - x)^2$			0.07	0.0778	122.4
Hen ALP	QP	$y = 168.97433 + 2.74723x - 0.01086x^2$	303 ± 24.5	12.5	0.15	0.0035	126.5
	BLQ	$y = 340.1 - 0.00717\ (145.2 - x)^2$			0.13	0.0053	145.2

Abbreviations: ALP, alkaline phosphatase; BLQ, broken line quadratic; QP, quadratic polynomial.
[1]Regression equations obtained using the increasing analyzed Mn in the diets (22.4, 48.5, 77.9, 103.1, 140.0, and 168.2 ppm).
[2]Eggs produced as a percentage of total live hens.
[3]Settable egg produced as a percentage of total live hens.
[4]Total eggs produced by live hens at the end of the experiment.
[5]Total settable eggs produced by live hens at the end of the experiment.

The highest hatching chick Ht and breeder Ht values were obtained when hens were fed dietary Mn at 103.1 ppm, whereas specific gravity increased up to 140.0 Mn ppm ($P < 0.05$). Eggshell thickness, membrane thickness, and hatching chick length were highest ($P < 0.05$) when hen dietary Mn was at 103.1 ppm or above, while eggshell breaking strength was highest at the hen dietary level above 77.9 ppm ($P < 0.05$). The QP model estimated 140.2 ppm as Mn requirement, and the BLQ regression estimated 128.0 and 134.2 ppm as the requirement for eggshell membrane layer and thickness, respectively, whereas Mn requirements for palisade layer was 128.8 and 68.8 ppm with QP and BLQ models. Requirements of Mn that maximized breaking strength using QP and BLQ models were 140.3 and 112.7 ppm, respectively, whereas

Table 8. Regression equations of egg and chick parameters of breeders fed with Mn supplementation.

Variable	Model	Regression equations[1]	Confidence interval, 95%	SEM	R^2	Probability <	Requirement
Egg weight	QP	$y = 72.99526 - 0.04801x + 0.00020389x^2$	70.8 ± 0.22	0.11	0.02	0.0001	117.7
	BLQ	$y = 63.6036 + 0.00110(63.6036 - x)^2$			0.02	0.0001	63.6
Yolk[2], %	QP	$y = 28.47918 + 0.01918x - 0.00007709x^2$	29.4 ± 0.09	0.09	0.02	0.0072	124.4
Eggshell[2], %	QP	$y = 7.94128 + 0.01178x - 0.00004474x^2$	8.6 ± 0.06	0.03	0.08	0.0001	131.6
	BLQ	$y = 8.6426 - 0.00022\ (70.9837 - x)^2$			0.08	0.0001	71.0
Albumen[2], %	QP	$y = 63.57118 - 0.03072x + 0.00012048x^2$	62.0 ± 0.09	0.09	0.04	0.0001	127.5
	BLQ	$y = 61.7111 + 0.000152\ (113.0 - x)^2$			0.04	0.0001	113.0
Yolk Mn, ppm	QP	$y = 1.51516 + 0.01306x - 0.00005533x^2$	2.11 ± 0.078	0.04	0.31	0.0001	118.0
	BLQ	$y = 2.2399 - 0.00005\ (118.4 - x)^2$			0.28	0.0001	118.4
Breaking strength, kg/cm^2	QP	$y = 3.44749 + 0.01035x - 0.00003689x^2$	3.91 ± 0.059	0.03	0.06	0.0001	140.3
	BLQ	$y = 4.0377 - 0.00006\ (112.7 - x)^2$			0.06	0.0001	112.7
Specific gravity, kg/cm^3	QP	$y = 1078.22139 + 0.11957x\text{-}0.00045540x^2$	1.084 ± 0.002	0.001	0.12	0.0001	131.3
	BLQ	$y = 1085.3 - 0.00254\ (68.4776 - x)^2$			0.12	0.0001	68.5
Eggshell membrane layer, μm	QP	$y = 59.81601 + 0.21964x - 0.00078347x^2$	71.5 ± 1.04	0.53	0.23	0.0001	140.2
	BLQ	$y = 74.7744 - 0.00095\ (128.0 - x)^2$			0.24	0.0001	128.0
Eggshell palisade layer, μm	QP	$y = 185.13330 + 0.74424x - 0.00289x^2$	222 ± 4.1	2.1	0.41	0.0001	128.8
	BLQ	$y = 228.5\text{-}0.0149(68.7501 - x)^2$			0.43	0.0001	68.8
Eggshell thickness, μm	QP	$y = 347.23371 + 0.63094x - 0.00225x^2$	381 ± 27.7	1.2	0.15	0.0001	140.2
	BLQ	$y = 390.5 - 0.00243\ (134.2 - x)^2$			0.15	0.0001	134.2
Chick body weight, g	QP	$y = 52.25175 - 0.04050x + 0.00016815x^2$	50.3 ± 0.24	0.12	0.01	0.0004	120.4
	BLQ	$y = 49.9994 + 0.000414\ (85.6426 - x)^2$			0.02	0.0002	85.6
Chick navel button score	QP	$y = 1.67508 - 0.00538x + 0.00001892x^2$	1.37 ± 0.039	0.02	0.02	0.0001	142.2
	BLQ	$y = 1.2988 + 0.000020\ (138.1 - x)^2$			0.02	0.0001	138.1
Chick tibia length, cm	QP	$y = 28.50842 + 0.02661x - 0.00009448x^2$	29.9 ± 0.26	0.13	0.09	0.0013	140.8

[1]Regression equations obtained using the increasing analyzed Mn in the diets (22.4, 48.5, 77.9, 103.1, 140.0, and 168.2 ppm).
[2]Percentage in relation to egg weight.

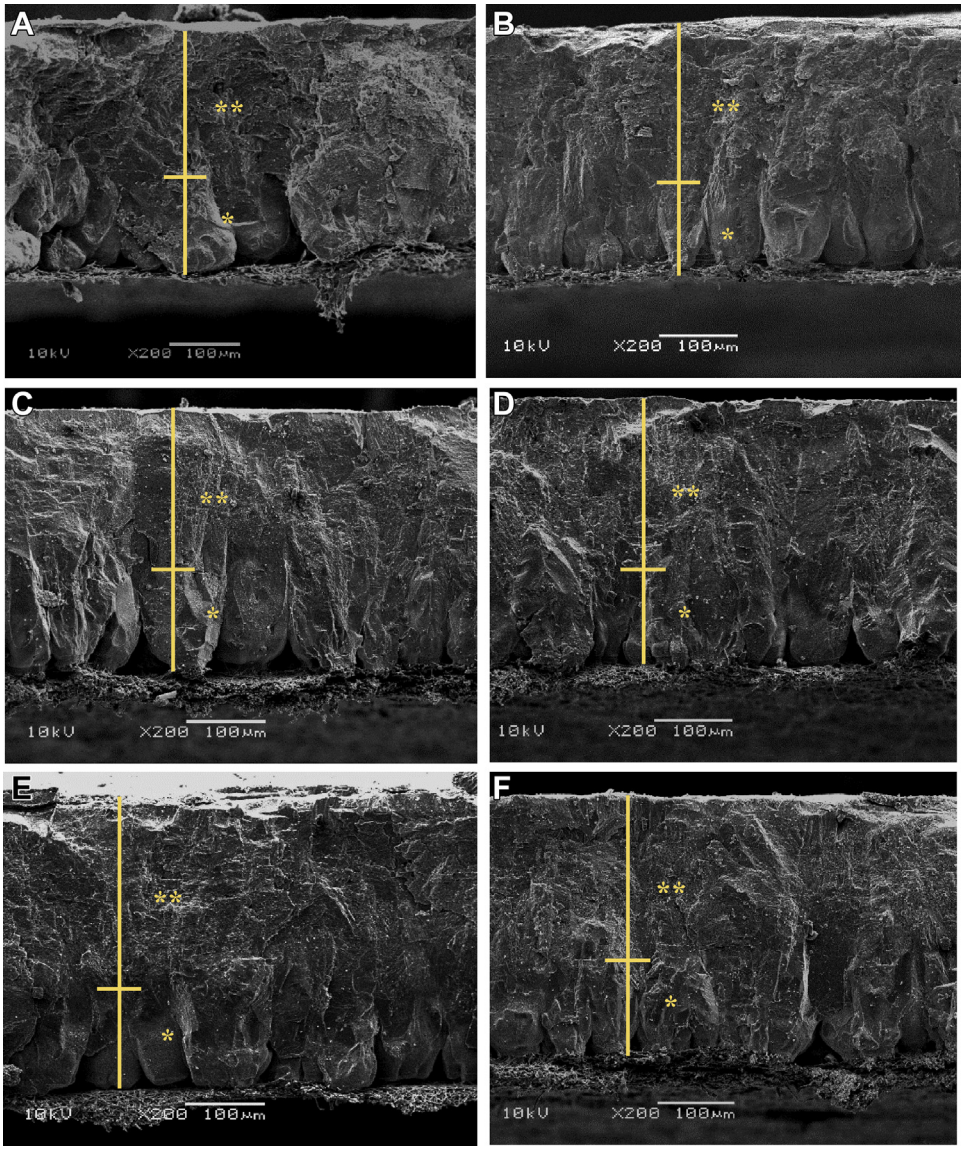

Figure 1. Scanning electron cross-sections and inner surface of the eggshell from broiler breeder hens fed a Mn-deficient diet (22.2 ppm) (A) and diets with 48.5 ppm (B), 77.9 ppm (C), 103.1 ppm (D), 140.0 ppm (E), and 168.2 ppm (F) Mn (200x). *Mammillary layer. **Palisade layer.

maximum specific gravity was obtained at 131.3 and 68.5 ppm with QP and BLQ models, respectively. In addition, close Mn requirements of 142.2 and 138.1 ppm were estimated for navel button score using QP and BLQ regressions, respectively, and 140.8 ppm by QP model for hatching chick tibia length.

DISCUSSION

In the present study, broiler breeder hens fed Mn-deficient diets demonstrated signs of deficiency throughout most of the evaluated responses. General improvements were observed as dietary Mn increased in the feeds. As Mn plays several roles in animal metabolism, observed changes in the studied responses varied depending on the amount of Mn demanded to successfully support each of its biological involvement.

Feeding feeds depleted of the studied micromineral is of utmost importance when conducting requirement studies such that adequate supplemental recommendations are presented. For instance, supplementing Mn to laying hens, broiler breeders, and broilers without previous body depletion did not allow full recovery responses (Sazzad et al., 1994; Mabe et al., 2003; Xiao et al., 2014; Zhu et al., 2015; Zhang et al., 2017).

Nutrient requirement studies are generally presented by modeling data with QP and BLQ and less frequently as exponential asymptotic or others. Data in the present study were modelled using QP and BLQ with the objective of allowing the reader to compare the majority of published results with the ones in this text, at least for these two models. Main differences, however, could be found in the estimation of Mn requirements when using these models. For instance, almost no difference was found for egg yolk, which was maximized by 118.0- and 118.4-ppm Mn using QP and BLQ, whereas estimation to optimize cracked eggs was more than doubled (129.5-ppm Mn) with QP than with BLQ (66.4-ppm Mn). Shape and sensitivity of curvature derivates from each model cause variations in points that optimize

responses. It is well known that QP tends to overestimate requirements (Runho et al., 2001) when compared to BLQ, which tends to underestimate them (Baker et al., 2002). Therefore, it is important to previously note the potential differences due to statistical models used when comparisons between different publications are made (Robbins et al., 1979).

In the present study, egg production responded with a significant increase as Mn was gradually added in the feeds. Several mechanisms that control egg production can be related to Mn. For example, decreases in egg production were observed when PC activity was low, which is associated with reduction in the use of glucose (Baly et al., 1985). Reduction in circulating estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone is also suggestive of Mn deficiency because it can affect the function of the hypothalamic-pituitary-gonadal axis (Cao and Chen, 1987; Feng and Feng, 1998). Interestingly, loss of PC activity induced by Mn deficiency in chickens can be partially alleviated by Mg (Scrutton et al., 1972; Reed and Scrutton, 1974).

Essential metal microminerals are transferred from hens to embryos to allow successful development through stored yolk phosvitin (Richards, 1997). Mabe et al. (2003) have found increases in Mn content of egg yolks in laying hens when 60.0-ppm Mn was added to a corn-soybean meal diet (24.7-ppm Mn) using Mn oxide as the supplemental source. Li et al. (2018) have reported a similar response using an organic source, where 60 ppm supplementation in a corn-soybean-wheat diet increased Mn yolk content. These results were lower than what have been found in the present study (average 118.2-ppm Mn). The higher original Mn content in their diets as well as differences in bioavailability of Mn could explain the differences.

In the present study, egg hatchability increased as the addition of Mn was increased in the deficient diet. Decreases in embryo mortality, cracked eggs, as well as in contaminated eggs were also reduced as Mn was gradually added to the feeds. Other authors also observed decreased hatchability in turkeys and Guinea fowls when Mn was supplemented at low levels (27-ppm Mn in a sorghum-corn-soybean basal diet, and 54-ppm Mn supplemented in a corn-sorghum-groundnut basal diet) (Atkinson et al., 1967; Offiong and Abed, 1980). Furthermore, Zhu et al. (2015) supplementing 120-ppm Mn on a deficient diet (14.3-ppm Mn in a corn-soybean meal diet) have observed improvements in hatchability of eggs from broiler breeder hens (88.8–95.1%). The number of settable eggs and percent hatchability are expected to be affected by eggshell integrity because well-formed membranes and eggshells protect against egg contamination (Swiatkiewicz and Koreleski, 2008).

In commercial settings, egg contamination is a major source of embryo mortality (Khabisi et al., 2012). In the present study, contaminated eggs were minimized at 127.9- and 71.9-ppm Mn using the QP and BLQ models, respectively. Cracked eggs were minimized at 129.8- and 66.5-ppm Mn using the QP and BLQ models, respectively. Venglovska et al. (2014) demonstrated that the percentage of cracked eggs was decreased when 120-ppm dietary Mn supplementation was used in a wheat-corn-soybean basal diet, whereas eggshell quality increased. The QP and BLQ adjustments occurred with maximum hen responses for eggshell breaking strength at 140.3 and 112.7 ppm and of eggshell thickness at 140.2 and 134.2 ppm dietary Mn, respectively for QP and BLQ. These results are in accordance with other published studies, where 100- and 120-ppm Mn supplementation had a positive effect on eggshell breaking strength as well as eggshell thickness in laying hens using corn-soybean meal diets (Xiao et al., 2014; Zhang et al., 2017) and increased eggshell breaking strength without affecting the thickness in broiler breeders fed with 120-ppm Mn based on a corn-soybean diet (Xie et al., 2014).

The general amelioration in eggshells as Mn was increased in the diets (eggshell breaking strength, eggshell thickness, specific gravity, and eggshell percentage) occurred in parallel with increases in the palisade and mammillary layer as well as in the eggshell membrane, even though mammillary buttons were not changed (Figure 1). Xiao et al. (2014) and Zhang et al. (2017) have shown that corn-soybean basal diets supplemented with 100- and 120-ppm Mn, respectively, increased eggshell breaking strength and thickness in laying hens. In addition, Stefanello et al. (2014) have observed improvements in eggshell percentage, thickness, and strength with 125-ppm dietary Mn added to the corn-soybean meal basal diet in laying hens. Stefanello et al. (2014) have linked these results to interferences on eggshell membrane, palisade, and mammillary layer. Glycosyltransferase is an enzyme involved in the formation of proteoglycans, components of the organic matrix (Xiao et al., 2014). Nys et al. (2004) reported that the protein matrix affects the size and orientation of the calcite crystals during eggshell build up, and increments in membrane glycosaminoglycans support the mammillary buttons to grow oriented outward forming well-structured columnar units of palisade layer (Xiao et al., 2014). Mn supplementation has been associated with improvements of glycosaminoglycan contents in membrane, which might be also responsible for increments in eggshell morphology (Ha et al., 2007).

Proteoglycans have also been associated with normal bone growth and development in chicks as the bone formation is linked to extracellular matrix formation (Velleman, 2000). It has been reported that Mn-deficient chicks have less proteoglycan in the cartilage of the tibial growth plate, which may result in chondrodystrophy and abnormal bone growth (Leach et al., 1969; Liu et al., 1994). In the present study, Mn supplementation improved hatching chick tibia length with a maximum response at 140.8 ppm when the QP model was used (Table 6). Several studies have demonstrated a reduction in ash content and length of legs and wing bones in chicks fed Mn-deficient diets (Caskey et al., 1939, 1944; Watson et al., 1971). Increases in leg abnormalities and weakness have been reported in chicks fed

Mn-deficient diets (Leach and Muenster, 1962; Watson et al., 1970, 1971; Stock and Latshaw, 1981). Hatching chick navel button score was decreased, but it was minimized at 142.2- and 138.1-ppm Mn (QP and BLQ models, respectively). Hatching chicks with unhealed navels are more likely to die during the production periods because of yolk sac infections or even gain less body weight than chicks with healthy navels (Fasenko and O'Dea, 2008). In the present study, eggs from hens fed a Mn-deficient diet led to a higher navel score in hatching chicks (Table 6).

Commercial broiler breeder feeds use supplemental microminerals regardless of their contents in macroingredients, such as corn, soy, or limestone (A corn-soybean-wheat diet has around 60-ppm Mn.). This added supplemental Mn is used as a safety margin and ranges from 100 to 120 ppm. The Mn requirements presented in this research demonstrate Mn requirements which are much lower than the total added from macroingredients plus supplemented via premix. Therefore, unnecessary excesses become obvious, which turn into increased costs and land Mn build up. The data from this study indicate Mn requirements ranging from 56.6 to 148.0 ppm dietary Mn (8.3–22.6 mg/hen/d), depending on production objectives. A few different molecular forms of Mn have been used in broiler breeder feeds, which include oxides, sulfates, as well as a diversity of organic ones. The estimated requirements provided in the present study have been obtained with Mn sulfate monohydrate, and therefore, numerical differences in these requirements are possible as Mn availability from the different sources exist. Average requirements for egg production and hatchability were 93.5 ppm (13.6 mg/hen/d) and 97.6 ppm (14.2 mg/hen/d), respectively, whereas averaged values for egg quality responses were 117.5 ppm (17.1 mg/hen/d). The average of all requirement estimates using both models (QP and BLQ) was 111.5 ppm total dietary Mn (16.3 mg/hen/d), while averaged values for QP and BLQ models are 128.4- and 92.4-ppm Mn (18.7 and 13.5 mg/hen/d), respectively.

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