2018

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ABSTRACT The objective of this research trial was to compare 2 sources of zinc and different levels of supplementation on manure zinc excretion in laying hens. Bovan White Leghorn hens were used in this study and fed one of 6 dietary treatments. Treatments were in a 2 × 3 factorial arrangement with 2 sources of zinc (zinc sulfate or Availa®Zn) and 3 levels (40, 80, or 120 ppm) and were randomly assigned to 48 cages with 5 hens/cage and 8 replicates/treatment. Hens were housed in a tiered manure-belt housing system providing 627.1 cm^2/hen and were given access to 110 g/hen/d of feed. Manure samples were collected, and manure zinc content was calculated at 10-week intervals. Data were analyzed using the Glimmix procedure in SAS. There was a significant overall effect of source ($P < 0.0001$) for zinc content, such that hens fed zinc sulfate had lower amounts of zinc excretion. There was also a significant level effect ($P < 0.0001$) in which hens fed 120 ppm zinc excreted the greatest amount of zinc. A significant source by level interaction was observed ($P < 0.0001$) for both the overall and individual analyses, such that 120 ppm Availa®Zn showed the highest zinc excretion, and both 40 ppm zinc sulfate and Availa®Zn showed the lowest zinc excretion. Based on these results, a prediction equation was written for an estimated amount of zinc excretion based on the amount of zinc provided in the diet.

Key words: zinc, laying hen, nutrition, mineral

2018 Poultry Science 97:267–270
http://dx.doi.org/10.3382/ps/pex295

INTRODUCTION

Zinc is an essential trace mineral in poultry diets. It is a required component for over 200 enzymes that regulate bone resorption and DNA replication (Olhaberry et al., 1983; MacDonald, 2000). The Poultry NRC (1994) recommends 35 mg zinc per kg of feed in the diet for White Leghorn laying hens. A concern many producers have is their impact on the environment through ammonia losses in poultry manure. Zinc supplementation at levels higher than normal has been shown to decrease manure total nitrogen loss (Kim and Patterson, 2004). Zinc sulfate has previously been shown to inhibit the activity of microbial uricase, an enzyme that breaks down uric acid, thereby increasing nitrogen retention. The objective of this study was to compare 2 sources of zinc at 3 different levels of supplementation on manure zinc excretion in laying hens. In this trial, it was hypothesized that an organic form of zinc would be more efficiently utilized by the hen compared to a traditional inorganic source. A previous study showed higher bioavailabilities for organic trace minerals when compared to inorganic forms (Star et al., 2012). Another study showed no difference between bioavailability of organic and inorganic trace minerals (Cao et al., 2000). Supplementation of a form of zinc that is more readily available to the hen should help decrease excretion in the manure, keeping toxic levels of heavy metals out of fertilizers.

Availa®Zn is an organic zinc supplement produced by Zinpro Corporation (Eden Prairie, MN). It is a metal non-specific amino acid complex. It contains many essential amino acids, each bound to a zinc ion in a 1:1 ratio. A previous study done with the Availa®Zn product reported significantly higher bioavailability when compared to zinc sulfate (Star et al., 2012). It is hypothesized that producers may be able to supplement zinc at higher levels to achieve increased quality of production parameters without excess waste in the manure.

MATERIALS AND METHODS

Birds and Housing

Two-hundred-forty Bovan White Leghorn laying hens were used in this trial from 19 to 60 wk of age. The hens were provided access to 110 grams of feed per hen per day. Water was also provided ad libitum through a nipple-drinker system in each cage unit. The trial was conducted in a tunnel-ventilated building with evaporative cooling pads. The trial began in June of 2014 and

Received October 12, 2016.
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Table 1. Composition of diets.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Corn (%)	55.40	55.40	55.40	55.40	55.40	55.40
Soybean meal (%)	27.70	27.70	27.70	27.70	27.70	27.70
Oil (%)	4.45	4.45	4.45	4.45	4.45	4.45
Dical phosphate (%)	1.60	1.60	1.60	1.60	1.60	1.60
Ca carbonate (%)	4.96	4.96	4.96	4.96	4.96	4.96
Limestone (%)	4.96	4.96	4.96	4.96	4.96	4.96
Salt, white (%)	0.46	0.46	0.46	0.46	0.46	0.46
Lysine (%)	0.02	0.02	0.02	0.02	0.02	0.02
Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20
Trace min premix[1] (%)	0.10	0.10	0.10	0.10	0.10	0.10
Vit premix[2] (%)	0.05	0.05	0.05	0.05	0.05	0.05
Zinc sulfate (ppm)	40.00	80.00	120.00	0.00	0.00	0.00
Availa®Zn[3] (ppm)	0.00	0.00	0.00	40.00	80.00	120.00
Nutrient analysis[4]	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Protein (%)	18.50	19.00	19.00	19.30	19.40	19.20
Phosphorus (%)	0.60	0.69	0.65	0.71	0.62	0.61
Calcium (%)	4.64	4.91	4.66	4.51	4.16	4.66
Zinc (ppm)	89.30	115.00	162.00	86.60	131.00	160.00

[1]Trace mineral premix provided the following per kilogram: Cu ($CuSO_4$, 3180 mg); I (I_2, 480 mg); Fe ($FeSO_4$, 26,700 mg); Mn ($MnSO_4$, 10,000 mg); Se (Na_2O_3Se, 380 mg); and limestone (59,260 mg).
[2]Vitamin premix provided the following per kilogram: Vitamin A (1322,751.3 IU); vitamin D3 (1322,7751.3 IU); vitamin E (2204.6 IU), vitamin B12 (1.75 mg); biotin (11 mg); menadione (110.25 mg); thiamine (275.55 mg); riboflavin (771.6 mg); D-Pantothenic acid (1102.3 mg); vitamin B6 (220.45 mg); niacin (4850.1 mg); folic acid (44.1 mg); and phytase (60,000 FYT).
[3]Zinpro Corporation, Eden Prairie, MN.
[4]Conducted at Midwest Labs, Omaha, NE.

ran through March of 2015. The hens were maintained on a 16-hour light:8-hour dark photoperiod. Hens were housed in a Big Dutchman stacked manure-belt housing unit (Big Dutchman, Inc., Holland, MI). The cage dimensions were 45.7 cm tall in the front by 40 cm tall in the back by 61 cm wide by 51.4 cm deep, providing 627.1 cm^2/hen. Hens were distributed 5 to a cage in 48 total cages. Each cage unit was randomly assigned to one of 6 treatment diets with 8 replicates per treatment. Hens were fed the experimental diets from 19 to 60 wk of age. Feed intake was measured weekly, and egg production was measured daily and calculated as percent production. All procedures were approved by the University of Nebraska Institutional Animal Care and Use Committee (IACUC).

Diets

Diets were formulated based on the Poultry NRC recommendations for laying hens (1994). Zinc was excluded from the vitamin and mineral premix and added in separately based on the source and amount for each treatment. The diets were organized in a 2 × 3 factorial arrangement. The variables were source: inorganic zinc (zinc sulfate; International Nutrition, Omaha, NE) or organic zinc (Availa®Zn; Zinpro Corporation, Eden Prairie, MN) and supplemental level: 40, 80, or 120 ppm. Diet formulation for the trial is shown in Table 1.

Measurements

Manure samples were taken at 10-week intervals and analyzed for zinc content for a total of 4 sample sets. Samples were taken by sectioning off the manure belt by cage using spray paint and advancing the manure belt one cage at a time to collect the samples. The samples were then dried in an oven at 100 °C, ground, and sifted to eliminate feathers. Finally, the samples were sent to Midwest Labs (Midwest Laboratories, 13,611 B St., Omaha, NE 68144) for analysis of zinc content.

Data Analysis

Data were analyzed using the Glimmix procedure in SAS for a randomized complete block design with a 2 × 3 factorial arrangement. For all tests, P-values < 0.05 were considered indicative of significant differences among means. Analysis was performed for the factors: source and level, as well as any interactions between the factors. Repeated measures were used to evaluate treatment effects over time and to look at any treatment by time interactions. The covariance structure was assumed to be first-order auto-regression because of the use of repeated measures. A linear regression analysis also was performed to predict manure zinc content based on the dietary level of zinc using Proc Reg. The model fitted was:

$$f_i = \beta_0 + \beta_1 Z_i$$

Where: f_i is the zinc concentration in the manure (ppm), β_0 is the intercept, β_1 is the slope, and Z_i is the zinc concentration for the i^{th} observation. Since the interaction between source and dose was found to be significant, the regression was fitted separately for each source.

Table 2. Dietary effects of zinc source and level on manure zinc content.

Source	**Diets supplemental** level (ppm)	**Zinc level (ppm)** Month 3	Month 6	month 8	Month 10	Average
Zinc sulfate	40	285.63	243.63	268.13	238.75	259.03
Zinc sulfate	80	418.00	366.13	344.00	344.12	368.06
Zinc sulfate	120	494.50	465.63	463.88	420.00	461.00
Organic zinc[1]	40	326.88	255.50	245.63	230.12	264.53
Organic zinc[1]	80	416.50	404.13	369.63	334.00	381.06
Organic zinc[1]	120	647.13	618.50	591.00	556.00	603.16
SEM[2]		14.39	8.22	8.21	8.97	5.43
Main effects						
Source						
Zinc sulfate		399.38	358.46	358.67	334.29	362.70
Organic zinc[1]		463.50	426.04	402.08	373.37	416.25
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001
Level						
40 ppm		306.25	249.56	256.87	234.44	261.78
80 ppm		417.25	385.13	356.81	339.06	374.56
120 ppm		570.81	542.06	527.44	488.00	532.08
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001
Time						
P-value						<0.0001
Interactions				***P*-value**		
Source x Level		< 0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001
Source x Time						0.072
Level x Time						0.059
Source x Level x Time						0.083

[1]Availa®Zn; Zinpro Corporation, Eden Prairie, MN.
[2]Standard error of means.

RESULTS

Significant treatment effects were observed for manure zinc content (Table 2). There was a significant source by level interaction ($P < 0.0001$), such that hens fed 40 ppm zinc sulfate had the lowest zinc excretion values, while hens fed 120 ppm organic zinc had the highest zinc excretion values. For both the low and moderate levels of supplementation, both sources of zinc had similar excretion values. At the highest level of supplementation, there was a significant difference between the 2 sources with hens fed the organic zinc source having significantly greater zinc excretion in the manure. Both the source by time and level by time interactions approached significance ($P = 0.072$ and $P = 0.059$, respectively), indicating that both sources and all levels had decreased zinc excretion over time. Hens fed 40 ppm and 80 ppm zinc from both sources had similar excretion levels throughout the study, while there was significant difference between hens fed 120 ppm zinc sulfate and those fed 120 ppm organic zinc throughout the study. There was also a significant effect of time ($P < 0.0001$). For all treatments, overall zinc excretion decreased during the study. When analyzed separately by month, the source effect, level effect, and source by level interactions were all significant at each month ($P < 0.0001$). Hens fed 120 ppm organic zinc had the highest zinc excretion each month. Hens fed 40 ppm zinc sulfate had the lowest zinc excretion for months 3 and 6, while hens fed 40 ppm organic zinc had the lowest zinc excretion for months 8 and 10. Results of the linear regression equation showed a significant linear effect of dietary zinc on level of zinc in the manure for both zinc sulfate ($P < 0.0001$; $R^2 = 0.832$) and organic zinc ($P < 0.0001$; $R^2 = 0.885$). The analysis resulted in the following prediction equations:

$$\begin{aligned}\text{Manure Zn (ppm)} &= (20.462 \pm 16.224) \\ &\quad + (2.760 \pm 0.127) \\ &\quad * \text{Diet Zn Sulfate (ppm)} \\ \text{Manure Zn (ppm)} &= (-160.576 \pm 21.909) \\ &\quad + (4.652 \pm 0.172) \\ &\quad * \text{Diet Organic Zn (ppm)}\end{aligned}$$

The estimates of the slope in these equations do not include zero, indicating that there is a strong relationship between manure zinc concentration and dietary zinc. More importantly, the slope for the organic zinc source is 1.69 times higher than that for zinc sulfate, suggesting that more zinc is excreted when hens are fed organic zinc as compared to zinc sulfate. These equations allow for future prediction of the amount of zinc excretion given the amount of zinc provided in the diet, based on source, with good accuracy.

DISCUSSION

In this study, the use of an inorganic zinc supplement was compared to the use of an organic zinc supplement in laying hen diets. The hypothesis was that the organic source of zinc (Availa®Zn) would be more efficiently utilized by the hen and would, therefore, show decreased manure zinc excretion.

A concern associated with increasing levels of zinc supplementation is excess zinc excretion in the manure. Since poultry manure is commonly used as a fertilizer, increased levels of certain trace minerals, such as zinc, can be a concern. Significant differences were found between treatments for manure zinc content, with hens fed 40 ppm zinc sulfate having the lowest excretion levels and hens fed 120 ppm organic zinc having the highest excretion levels. The highest level of supplementation (120 ppm) was correlated to a high zinc excretion for both sources. This indicates that the hens are not retaining a large portion of the zinc that is fed. Since absorption was not measured in this study, conclusions about the overall bioavailability are difficult. Further study in this area to measure absorption and storage in tissues would help give a better view of how the hens are utilizing high levels of dietary zinc. Overall, the zinc content of the manure decreased for all treatments during the trial period. This could indicate that, as the hen's body adapts to the diet, it can better utilize the nutrients in the feed. These results partially agree with previous studies by Burrell et al. (2004) and Kim and Patterson (2004) who reported that increasing levels of supplemental zinc in the diet resulted in increased levels of zinc excretion in the manure. The linear regression analysis showed a strong linear relationship between zinc level in the diet and zinc level in the manure for both sources and indicates that zinc from the organic source is excreted at higher levels than zinc from zinc sulfate. The prediction equations will be valuable to producers who may be interested in feeding higher levels of zinc for the proposed benefits, but also want to monitor zinc levels in the manure. This will provide them with an accurate indicator of zinc excretion in the manure based on the zinc source and content of the proposed diet fed. The prediction equations generated from this study will be a useful tool for producers and agronomists.

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