poultry

MDPI

Article

Evaluation of Egg Quality and Performance in Late-Lay Hens Fed Different Combinations of Copper, Manganese, and Zinc Complexed with Sulfate or Amino Acid Ion

Jill R. Domel, Gabrielle M. House, Eric B. Sobotik and Gregory S. Archer *

Department of Poultry Science, Texas A&M University, College Station, TX 77843, USA; jill.domel@ag.tamu.edu (J.R.D.)
* Correspondence: gregory.archer@ag.tamu.edu

Abstract: Dietary inclusion of copper (Cu), manganese (Mn), and zinc (Zn) can improve egg shell quality through changing the membrane structure. This study aimed to compare the responses of egg shell to different mineral sources. In this study, 60-week-old laying hens (n = 378) were assigned to one of seven treatments with 18 replicates each in an RCBD. Treatments included the following: control (basal + sulfated minerals ($CuSO_4$, $MnSO_4$, and $ZnSO_4$)), and basal + amino acid complexed (AAC) minerals (AAC Cu, AAC Mn, AAC Zn, AAC Cu + Mn, AAC Mn + Zn, AAC Zn + Cu). Trace minerals were added to a basal diet containing 20 ppm $MnSO_4$ and 20 ppm $ZnSO_4$ to achieve overall target concentrations of 20 ppm Cu, 60 ppm Mn, and 60 ppm Zn. The hens were fed the treatment diet for 15 weeks, and egg production and egg quality were assessed during weeks 5, 10, and 15 of the experiment. Egg shells, egg contents, and excreta were analyzed for Cu, Mn, Zn, Ca, and P during weeks 10 and 15. No treatment differences ($p > 0.05$) were observed for production or egg quality. Differences between excreta mineral content were observed. The mineral content of egg shells and egg contents did not differ ($p > 0.05$) at any time point. The mineral source did not affect egg mineral deposition and egg quality measures ($p > 0.05$). Some AAC trace minerals enhanced retention of zinc, calcium, and manganese, although AAC Cu increased Cu excretion. Taken together, feeding AAC trace minerals does not significantly affect egg production or egg quality during the late-lay period. More research is needed to demonstrate whether Cu excretion is increased when feeding AAC Cu due to increased bioavailability or other factors.

Keywords: layer; egg quality; egg production; trace minerals

check for updates

Citation: Domel, J.R.; House, G.M.; Sobotik, E.B.; Archer, G.S. Evaluation of Egg Quality and Performance in Late-Lay Hens Fed Different Combinations of Copper, Manganese, and Zinc Complexed with Sulfate or Amino Acid Ion. *Poultry* **2024**, *3*, 36–46. https://doi.org/10.3390/poultry3010004

Academic Editors: Douglas E. Cosby and Shiping Bai

Received: 4 December 2023
Revised: 12 February 2024
Accepted: 22 February 2024
Published: 26 February 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Eggshell quality is vital in ensuring that eggs are able to withstand the washing, packaging, and transportation processes which carry them from the laying house to the grocery store. Trace minerals such as copper (Cu), manganese (Mn), and zinc (Zn) play important roles in egg production and various physiological functions in poultry. They serve as components of metalloenzymes in the formation of egg membranes and egg shell layers or directly interact with calcite crystals during shell formation [1]. Dietary supplementation of Mn affects egg shell formation by increasing the density of nucleation sites—where calcite crystals are initially deposited on the inner shell membrane and begin egg shell development—and subsequently enhances shell thickness and breaking strength [2]. Manganese may also alter calcite crystal morphology, which can affect egg shell ultrastructure and texture [1]. Copper participates in amine oxidase activity: a Cu-deficient diet results in reduced enzyme activity, which reduces elastin production, prevents adequate cross-linking of shell membrane proteins, and produces eggs with abnormal textures, shapes, and sizes, or shell-less eggs [3–5]. Zinc is a co-factor of carbonic anhydrase, which supplies carbonate ions during egg shell formation; consequently, Zn deficiency results in reduced

egg shell weight [1]. Not only do these trace minerals each play important roles in physiological function and egg formation, but they interact with other minerals as well: some may antagonize the other, which is useful during acute toxicity [6], but may also lead to deficiency. In other cases, trace minerals affect bone formation and resorption, a carefully controlled process which affects availability of Ca not only for the egg shell itself, but also for deposition in the yolk to be used in bone mineralization by a developing embryo [7]. Increasing Ca and P absorption may improve egg shell and bone strength [8].

Others have hypothesized that amino acid complexed (AAC) Zn is more nutritionally available and therefore more efficient in maintaining physiological processes than $ZnSO_4$ [9]. Indeed, Star et al. [10] found that AAC Zn is more bioavailable than $ZnSO_4$, as indicated by tibia Zn content. In hens challenged with saline drinking water, addition of Zn methionine complex increases shell weight and breaking strength [11]. When fed to dairy cows peripartum, AAC Zn, AAC Cu, and AAC Mn enhances post-partum neutrophil-mediated phagocytosis, antioxidative capability, and liver Cu concentration [12]. There has been little research examining the effects of AAC trace minerals in laying hen mineral homeostasis. The purpose of this study was to compare the effects of feeding AAC Cu, Mn, and Zn on egg quality and production and mineral retention and excretion in late-lay hens. To do so, 60-week-old HyLine W-36 hens were fed diets containing various combinations of Cu, Mn, and Zn complexed with amino acid ion or sulfate for 15 weeks and the egg quality and mineral content of egg shells, egg contents, and excreta were measured at 5-week intervals.

2. Materials and Methods

2.1. Animals and Husbandry

All procedures were carried out in accordance with the guidelines established by the Texas A&M Institutional Animal Care and Use Committee (AUP#2017-0357), and birds were cared for according to the guidelines described in the Guide for the Care and Use of Agricultural Animals in Research and Teaching [13]. Sixty-week-old Hy-Line W-36 layer hens (n = 378) were housed in wire cages in a tunnel-ventilated barn with 716.4 cm^2 per bird and had ad libitum access to feed and nipple waterers. Hens were provided with a photoperiod of 18L:6D, with lights turning on at 0530 h, and ambient temperature was maintained at 23 $\pm$ 3 °C for the duration of the experiment.

Cages were randomly assigned to treatments according to a randomized complete block design: there were seven treatments, each with 18 replicates and three hens per replicate cage (n = 54/treatment). The seven treatments correlated with an experimental diet in which trace minerals were sourced from inorganic ($CuSO_4$, $MnSO_4$, and $ZnSO_4$) or AAC (Availa-Mins, Zinpro Corporation, Eden Prairie, MN, USA) sources as indicated in Table 1. Trace minerals were added to a standard layer mash diet (Table 1). The basal diet had 20 ppm $MnSO_4$ and 20 ppm $ZnSO_4$ added on top; to that basal, additional 20 ppm Cu (either AAC Cu or $CuSO_4$), 40 ppm Mn (either AAC Mn or $MnSO_4$), and 40 ppm Zn (either AAC Zn or $ZnSO_4$) were added (Table 2). After trace minerals were added, all diets were supplemented with a total of 20 ppm Cu, 60 ppm Mn, and 60 ppm Zn, regardless of the mineral source. For the control diet, this meant 20 ppm $CuSO_4$, 60 ppm $MnSO_4$, and 60 ppm $ZnSO_4$ were supplemented on top of the basal diet.

Feed troughs were separated for each cage using solid wood barriers to prevent mixing of feed between adjacent cages, and wire barriers were attached to the front of the cages above the wood barriers to ensure hens could not access feed from neighboring troughs. Each feed trough was manually refilled with approximately 680 g of feed every other day or as needed to ensure hens had ad libitum access to feed. This provided approximately 114 g of feed per hen per day. The hens were fed the control diet for one week before the start of the experiment. On d 1 of the experiment, all feed troughs were vacuumed and filled with the appropriate treatment diet. Hens were then fed the treatment diet for the next 15 weeks.

Table 1. Dietary ingredients and nutrient composition of basal layer diet for 60- to 75-week-old layer hens.

Ingredient	%	Nutrient Content			
Corn	59.75	ME kcal/kg	2860	Fe, ppm	66.57
Soybean Meal	18.08	Protein (%)	15.58	Zn, ppm	33.20
Limestone	7.92	Crude Fat (%)	6.02	Mn, ppm	11.96
Dried Distillers Grains	5.00	Crude Fiber (%)	2.31	Cu, ppm	4.90
Soy Oil	3.36	Ca (%)	4.50	Se, ppm	0.04
Oyster Shell	3.00	P (%)	0.70	I, ppm	0.00
Biofos 16/21P	1.78	Arginine (%)	0.83		
Sodium Bicarbonate	0.44	Lysine (%)	0.75		
DL-Methionine, 98%	0.19	Valine (%)	0.62		
Lysine HCl	0.15	Threonine (%)	0.52		
Vitamin Premix *	0.15	Methionine (%)	0.42		
Salt	0.10	Cysteine (%)	0.26		
Hydrated Vitamin D3	0.05	Tryptophan (%)	0.14		
L-Threonine, 98%	0.04				

* Vitamin Premix supplied the following per kg of feed: 15.22 IU vitamin A, 5.10 IU vitamin D3, 2.07 mg vitamin K, 0.08 IU vitamin E, 6.82 mg thiamin, 9.11 mg riboflavin, 32.32 mg pantothenic acid, 80.87 mg niacin, 0.83 mg biotin, 2.83 μg folic acid, 21.48 mg vitamin B12, and 14.96 μg vitamin B6.

Table 2. Amounts (ppm) and sources (complexed with sulfate or amino acid ion [AAC]) of trace minerals included in each diet fed to seven treatment groups of 60-week-old Hy-Line W-36 layer hens for 15 weeks.

	Inorganic Sources			Amino Acid Ion Complexed Sources		
Treatment	**$CuSO_4$ (ppm)**	**$MnSO_4$ (ppm)**	**$ZnSO_4$ (ppm)**	**AAC Cu (ppm)**	**AAC Mn (ppm)**	**AAC Zn (ppm)**
Control	20	40	40	0	0	0
AAC Cu	0	40	40	20	0	0
AAC Mn	20	0	40	0	40	0
AAC Zn	20	40	0	0	0	40
AAC Cu + Mn	0	0	40	20	40	0
AAC Mn + Zn	20	0	0	0	40	40
AAC Zn + Cu	0	40	0	20	0	40

Each treatment diet included a total of 20 ppm of Cu (either AAC Cu or $CuSO_4$), 60 ppm Mn (either AAC Mn or $MnSO_4$), and 60 ppm Zn (either AAC Zn or $ZnSO_4$). The amounts and sources indicated in the table for each treatment were added to a basal diet which contained 20 ppm $MnSO_4$ and 20 ppm $ZnSO_4$.

2.2. Diet Analysis

A 450 g sample of each diet was sent to Midwest Laboratories, Inc. (Omaha, NE, USA) for proximate analysis to determine concentration (ppm) of Cu, Mn, and Zn. A 450 g sample of each diet was also sent to Zinpro Corporation (Eden Prairie, MN, USA) to determine concentration (ppm) of Cu, Mn, and Zn.

2.3. Egg Production

Eggs were manually collected once per day. All eggs were individually weighed daily and used to determine average egg weight, hen day egg production (%), and hen placed egg production (%).

Hen day egg production (%) was calculated in 5-week (35-day) intervals. For each treatment, the number of hens lost to mortality over the preceding 35 days was subtracted from the number of hens placed on d 1 of the experiment (n = 54/treatment). This provided the number of hens remaining in that treatment at the end of the 35-day period. This number was then multiplied by 35 days to yield the number of hen days. The number of eggs produced by that treatment over the preceding 35 days was divided by the number of hen days. The quotient (eggs per hen day) was then multiplied by 100 to give the hen day egg production as a percentage. The general formula is as follows:

$$\textit{Hen Day Egg Production } (\%) = \frac{\text{\#of eggs produced}}{(\text{54 hens placed on d 1 of experiment} - \text{mortality})(\text{35 days})} \times 100$$

Hen placed egg production (%) was calculated in 5-week (35-day) intervals. For each treatment, the number of hens placed on d 1 of the experiment (n = 54/treatment) was multiplied by 35 days to yield the number of hen days. The number of eggs produced by that treatment over the preceding 35 days was then divided by the number of hen days. The quotient (eggs per hen day) was multiplied by 100 to give hen placed egg production as a percentage. The general formula is as follows:

$$\textit{Hen Placed Egg Production } (\%) = \frac{\text{\# of eggs produced}}{(\text{54 hens placed on d 1 of experiment})(\text{35 days})} \times 100$$

2.4. Egg Quality

The eggs were assessed for egg quality at weeks 5, 10, and 15 of the experiment. After the eggs were weighed, all the eggs produced from a single day during the measurement week were used to determine a single measurement, with the following exception: the same eggs were used to determine Haugh units and vitelline membrane strength because both measurements were performed after the eggs were stored at 4 °C for 4 days. Egg quality measures were conducted on any eggs produced on a single day, which varied due to mortality and egg production rate. However, accounting for normal mortality (less than 5%) and a total of 21 cages per treatment with 3 hens per cage, 48 ± 6 eggs per treatment were used to assess egg weight, breaking strength, shell deformation, shell thickness, shell weight, Haugh units, and vitelline membrane strength.

Shell breaking strength was measured using the Fast-Egg-Shell-Tester (Bröring Informationstechnologie, Oldenburg, Germany), and shell deformation was measured using QC-SPA (TSS Limited, York, England). Shell thickness was measured using an Egg Shell Thickness Gauge (Israel Orka Food Technology Ltd., Ramat Hasharon, Israel). Egg shell weight was recorded after shells (and membranes) were separated from the egg contents and dried for a minimum of 2 h at 100 °C. The Haugh unit was assessed using Futura 2/A (Bröring Informationstechnologie, Germany). Vitelline membrane strength was measured using the Agrosta Texturometer (Agrosta Instruments, Serquex, France) with the following parameters: 1 kg load cell, data collection starting when grams for pull or push equals 1, high speed 10.0 mm/s, low speed 0.4 mm/s, and stroke after contact 2.0 mm/s. Specific gravity was determined using five saline solutions which yielded a specific gravity of 1.070, 1.075, 1.080, 1.085, and 1.090. The eggs were kept in a thermoneutral environment for 24 h prior to testing: an egg was then placed in the 1.070 solution and allowed to sit for a minimum of 5 s. If the egg sank, it was moved to the next highest solution. This process was repeated until the egg broke the surface of the solution and remained there for 5 s, upon which the specific gravity of that egg was recorded.

At 10 and 15 weeks into the experiment, egg shells (and membranes) and egg contents were separated for analysis of trace mineral content. There were 21 cages per treatment, and therefore 21 samples per treatment were used to assess mineral content of egg shells and egg contents. Egg shells from all eggs for a given cage were combined, dried for a minimum of 2 h at 100 °C, ground up by hand using a mortar and pestle, and then stored at −20 °C. Egg contents from all eggs for a given cage were combined and blended using an immersion blender (KBH2571, KitchenAid, Benton Harbor, MI, USA), then stored at −20 °C. The samples were then shipped using cold packs to Midwest Laboratories, Inc. (Omaha, NE, USA) for analysis of Ca, P, Cu, Mn, and Zn content (all in ppm).

2.5. Mineral Analysis and Phosphorus Retention

Excreta and eggs were analyzed at weeks 10 and 15 for Ca, P, Cu, Mn, and Zn (all in ppm), and used to determine P retention (%). All feed was removed at 2000 h the day before sampling. At 0500 h the next day, after building lights turned on, 1.8 kg of feed was added

to each feed trough, and paper was placed beneath each cage to collect excreta. At 0500 h the following day, remaining feed was removed and weighed to determine feed consumption. This would provide the amount of each mineral consumed. Excreta from each cage were collected and dried at 100 °C for 24 h. Dried excreta were weighed and sent to Midwest Laboratories, Inc. (Omaha, NE, USA) for analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) procedure. All eggs produced during this period were collected and pooled by cage (n = 21 cages/treatment). Egg shells (and membranes) and egg contents were processed as described in the previous section and sent to Midwest Laboratories, Inc. for analysis (Omaha, NE, USA). Phosphorus retention (%) was determined by subtracting the P content of the excreta, egg shells, and egg contents from the calculated P content of consumed feed for each cage.

2.6. Statistical Analysis

All data were analyzed using the PROC GLM procedure in SAS 9.1. Mean separation was performed using Fisher's least significant differences post hoc procedure, with a significant difference defined as $p < 0.05$. All percentages were transformed using arcsine prior to analysis.

3. Results

3.1. Egg Production

Average daily egg weight and egg production for weeks 5, 10, and 15 are shown in Table 3. There were no significant differences ($p > 0.05$) between treatments in hen day or hen placed egg production across the study period. There were no treatment differences ($p > 0.05$) in average daily egg weight during the first or third sampling time points. However, average egg weight differed ($p < 0.05$) during week 10 of the study: AAC Zn + Cu produced the heaviest eggs (60.8 g) during this period compared to all other treatments, with AAC Mn (59.2 g) and AAC Zn (59.1 g) being intermediate to all treatments.

3.2. Egg Quality

Egg shell quality for weeks 5, 10, and 15 are shown in Table 4. No treatment differences were observed for breaking strength, shell deformation, shell thickness, or shell weight ($p > 0.05$). Additionally, specific gravity (shown in Table 5) did not differ between treatments at any time point ($p > 0.05$). Measures of internal egg quality are also shown in Table 5. Haugh units and vitelline membrane strength did not differ between treatments at any time point ($p > 0.05$).

Table 3. Average daily egg weight, hen day production, and hen placed production measured after 5, 10, and 15 weeks of feeding 60-week-old HyLine W-36 hens a diet including trace minerals complexed with sulfate or amino acid ion (AAC).

	Egg Weight (g)			**Hen Day Egg Production (%)**			**Hen Placed Egg Production (%)**		
Treatment *	**5 wks**	**10 wks**	**15 wks**	**5 wks**	**10 wks**	**15 wks**	**5 wks**	**10 wks**	**15 wks**
Control	58.14	58.40 ^{b}	59.93	82.75	80.87	80.21	81.22	79.15	78.47
AAC Cu	57.56	58.40 ^{b}	59.59	79.95	79.41	79.21	79.95	78.04	77.51
AAC Mn	58.22	59.23 ^{ab}	61.25	82.38	83.99	83.39	81.48	80.58	79.52
AAC Zn	58.44	59.06 ^{ab}	59.81	85.82	78.34	78.94	85.82	78.41	77.35
AAC Cu + Mn	56.66	57.48 ^{b}	59.30	87.43	83.82	79.68	87.61	77.78	72.91
AAC Mn + Zn	58.28	58.95 ^{b}	59.98	85.10	81.48	84.82	84.71	79.79	81.11
AAC Zn + Cu	58.70	60.80 ^{a}	61.03	85.66	83.88	80.34	84.71	77.94	73.28
SEM	0.18	0.23	0.27	0.83	0.91	0.95	0.85	1.04	1.14

^{a,b} Values within a column with different superscripts differ significantly at $p < 0.05$. * Each treatment was fed a basal diet that contained 20 ppm MnSO4 and 20 ppm $ZnSO_4$, to which Cu (AAC Cu or $CuSO_4$), Mn (AAC Mn or $MnSO_4$), and Zn (AAC Zn or $ZnSO_4$) was added to obtain a total formulation of 20 ppm Cu, 60 ppm Mn, and 60 ppm Zn, regardless of source.

Table 4. Egg shell quality measured after 5, 10, and 15 weeks of feeding 60-week-old HyLine W-36 hens a diet including trace minerals complexed with sulfate or amino acid ion (AAC).

	Breaking Strength (g)			Shell Deformation (mm)			Shell Thickness (mm)			Shell Weight (g)		
Treatment *	**5 wks**	**10 wks**	**15 wks**	**5 wks**	**10 wks**	**15 wks**	**5 wks**	**10 wks**	**15 wks**	**5 wks**	**10 wks**	**15 wks**
Control	3309.5	3534.5	3256.6	5.86	5.78	6.06	0.43	0.41	0.41	5.52	6.37	5.06
AAC Cu	3382.7	3773.3	2979.9	5.80	5.88	5.85	0.41	0.42	0.40	5.56	6.19	5.24
AAC Mn	3489.0	3655.8	3244.1	5.86	5.86	6.12	0.42	0.42	0.40	5.55	6.30	5.14
AAC Zn	3204.8	3224.9	3150.5	5.93	6.53	6.06	0.40	0.40	0.42	5.40	5.97	5.09
AAC Cu + Mn	3464.7	3284.8	3101.8	5.91	5.33	6.21	0.40	0.42	0.42	5.59	6.24	5.22
AAC Mn + Zn	3282.1	3194.3	2768.7	5.89	5.97	6.13	0.42	0.42	0.40	5.52	6.10	5.38
AAC Zn + Cu	3543.2	3469.1	3298.0	5.64	5.82	6.12	0.43	0.43	0.44	5.62	6.39	5.35
SEM	77.0	82.4	73.9	0.04	0.15	0.04	0.00	0.00	0.01	0.06	0.10	0.06

* Each treatment was fed a basal diet that contained 20 ppm $MnSO_4$ and 20 ppm $ZnSO_4$, to which Cu (AAC Cu or $CuSO_4$), Mn (AAC Mn or $MnSO_4$), and Zn (AAC Zn or $ZnSO_4$) were added to obtain a total formulation of 20 ppm Cu, 60 ppm Mn, and 60 ppm Zn, regardless of source.

Table 5. Internal egg quality and specific gravity measured after 5, 10, and 15 weeks of feeding 60-week-old HyLine W-36 hens a diet including trace minerals complexed with sulfate or amino acid ion (AAC).

	Haugh Units			Specific Gravity			Vitelline Membrane Strength (g)		
Treatment *	**5 wks**	**10 wks**	**15 wks**	**5 wks**	**10 wks**	**15 wks**	**5 wks**	**10 wks**	**15 wks**
Control	116.07	98.84	98.75	1.09	1.08	1.08	13.27	10.56	5.90
AAC Cu	114.57	98.88	97.29	1.08	1.08	1.08	13.17	10.65	6.14
AAC Mn	114.44	98.23	100.58	1.08	1.08	1.08	14.23	10.47	6.15
AAC Zn	114.60	96.37	100.06	1.08	1.08	1.08	13.24	11.18	6.04
AAC Cu + Mn	115.90	96.88	99.62	1.09	1.09	1.08	13.22	9.38	6.30
AAC Mn + Zn	115.16	95.17	95.89	1.08	1.08	1.08	13.85	11.27	6.06
AAC Zn + Cu	115.28	96.28	99.61	1.08	1.08	1.08	13.28	10.68	6.13
SEM	0.38	0.65	0.63	0.00	0.00	0.00	0.23	0.31	0.07

* Each treatment was fed a basal diet that contained 20 ppm $MnSO_4$ and 20 ppm $ZnSO_4$, to which Cu (AAC Cu or CuSO4), Mn (AAC Mn or $MnSO_4$), and Zn (AAC Zn or $ZnSO_4$) was added to obtain a total formulation of 20 ppm Cu, 60 ppm Mn, and 60 ppm Zn, regardless of source.

3.3. Mineral Excretion and Retention

Data for Cu and Mn retention and excretion are shown in Table 6. Egg content levels of Cu and egg shell and egg content levels of Mn were below the detectable limit, and are therefore not shown. Egg shell levels of Cu did not differ between treatments ($p > 0.05$). However, excreta Cu was higher at weeks 10 and 15 ($p < 0.05$) in AAC Cu (67.5 and 69.4 ppm), AAC Cu + Mn (63.8 and 65.8 ppm), and AAC Zn + Cu (62.2 and 69.4 ppm) compared to all other treatments (average 30.5 and 26.0 ppm). Excreta Mn also differed by treatment at weeks 10 and 15: the levels were highest ($p < 0.05$) in AAC Mn (422.3 ppm) at week 10 compared to all other treatments (average 362.3 ppm). The control had higher ($p < 0.05$) excreta levels of Mn (371.2 ppm) at week 15 compared to all other treatments (average 330.8 ppm) except AAC Cu + Mn (350.7 ppm), which was intermediate with AAC Cu, AAC Mn, and AAC Mn + Zn, but higher than AAC Zn and AAC Zn + Cu.

Data for zinc and calcium retention and excretion are shown in Table 6. Zinc and calcium in egg shells and egg contents did not differ at week 10 or 15 ($p > 0.05$). Excreta zinc concentrations differed ($p < 0.05$) by treatment at week 10 and week 15: excreta zinc was higher in AAC Mn (366.8 ppm) compared to all other treatments (323.0 ppm) at week 10. Excreta zinc was lower ($p < 0.05$) in AAC Mn (284.1 ppm) and AAC Zn (283.2 ppm) compared to control (308.7 ppm) and AAC Zn + Cu (303.9 ppm), with all other treatments intermediate. Calcium concentrations in excreta differed by treatment at week 10 ($p < 0.05$) but not week 15 ($p > 0.05$): AAC Cu (9.94%) and AAC Cu + Mn (9.95%) had lower ($p < 0.05$) excreta Ca than control (10.78%), AAC Zn + Cu (10.95%), and AAC Mn (10.70%), with other treatments being intermediate.

Table 6. Egg shell, egg content, and excreta levels of Cu, Mn, Zn, Ca, and P measured after 10 and 15 weeks of feeding 60-week-old HyLine W-36 hens a diet including trace minerals complexed with sulfate or amino acid ion (AAC).

		Wk	Control	AAC Cu	AAC Mn	AAC Zn	AAC Cu + Mn	AAC Mn + Zn	AAC Zn + Cu	SEM
Cu (ppm)	Egg Shells	10	1.83	1.97	2.16	2.83	2.60	1.97	2.65	0.16
		15	1.50	1.72	1.65	1.66	1.72	1.68	1.50	0.05
	Excreta	10	28.30 ^{b}	67.46 ^{a}	31.92 ^{b}	29.99 ^{b}	63.83 ^{a}	31.87 ^{b}	62.17 ^{a}	0.91
		15	29.18 ^{b}	69.44 ^{a}	23.07 ^{b}	24.92 ^{b}	65.77 ^{a}	26.55 ^{b}	69.40 ^{a}	1.11
Mn (ppm)	Excreta	10	350.61 ^{c}	375.56 ^{bc}	422.33 ^{a}	346.05 ^{c}	390.11 ^{b}	354.00 ^{c}	357.50 ^{c}	3.78
		15	371.16 ^{a}	335.83 ^{bc}	337.88 ^{bc}	315.00 ^{c}	350.66 ^{ab}	338.88 ^{bc}	306.24 ^{c}	3.82
Zn (ppm)	Egg Shells	10	2.19	2.49	2.49	3.13	2.46	2.10	2.80	0.11
		15	1.89	2.70	1.67	2.11	1.99	1.77	2.01	0.09
	Egg Contents	10	13.48	13.46	13.58	13.04	13.47	13.18	12.87	0.19
		15	14.70	14.12	14.29	14.02	14.84	14.43	14.32	0.11
	Excreta	10	314.22 ^{bc}	331.00 ^{b}	366.78 ^{a}	308.78 ^{c}	330.61 ^{b}	329.00 ^{b}	324.50 ^{bc}	2.45
		15	308.72 ^{a}	296.78 ^{abc}	284.06 ^{c}	283.22 ^{c}	292.39 ^{bc}	292.72 ^{bc}	303.94 ^{ab}	2.09
Ca (ppm)	Egg Shells	10	28.34	29.50	29.37	29.51	28.23	44.08	41.69	1.72
		15	33.23	31.81	47.83	33.54	50.11	33.82	33.40	1.94
	Egg Contents	10	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.00
		15	0.08	0.08	0.07	0.07	0.08	0.08	0.07	0.00
	Excreta	10	10.78 ^{ab}	9.94 ^{c}	10.70 ^{ab}	10.48 ^{abc}	9.95 ^{c}	10.31 ^{bc}	10.95 ^{a}	0.08
		15	10.40	10.24	10.46	10.32	10.53	10.31	10.03	0.07
P (ppm)	Egg Shells	10	0.49	0.54	0.08	0.08	0.47	0.58	0.08	0.09
		15	0.09	0.09	0.10	0.09	0.09	0.09	0.09	0.00
	Egg Contents	10	0.22	0.21	0.21	0.21	0.22	0.22	0.21	0.00
		15	0.23	0.22	0.23	0.22	0.23	0.23	0.22	0.00
	Excreta	10	1.89 ^{a}	1.98 ^{a}	1.74 ^{b}	1.92 ^{a}	1.96 ^{a}	1.93 ^{a}	1.90 ^{a}	0.02
		15	2.17	2.13	2.07	2.12	2.17	2.07	2.16	0.02
P Retention (%)		10	47.49	49.91	43.06	48.30	59.31	48.69	52.44	2.04
		15	38.03	34.89	41.25	27.48	38.22	27.42	33.13	1.93

^{a,b,c} Different superscripts within a row indicate a significant difference at $p < 0.05$.

Data for phosphorus excretion and retention are also shown in Table 6. The phosphorus content of egg shells and egg contents did not differ by treatment at week 10 or 15 ($p > 0.05$), nor did phosphorus retention differ by treatment at either time point ($p > 0.05$). Excreted phosphorus differed ($p < 0.05$) at week 10, where AAC Mn (1.74%) had the lowest ($p < 0.05$) concentration compared to all other treatments (1.94%), but treatment differences were not observed ($p > 0.05$) at week 15.

4. Discussion

The purpose of this experiment was to evaluate the effects of feeding various combinations of Cu, Mn, and Zn complexed with amino acid ion or sulfate on egg quality and production and mineral retention in late-lay layer hens. Production values were not found to be significant, with one exception: egg weight was higher at week 10 in the AAC Zn + Cu treatment compared to all other treatments except AAC Mn and AAC Zn, which were intermediate. This effect was transient, however, because egg weight was not significant during week 15. It is unclear why the treatment diets produced significant effects on egg weight only during week 10 of the experiment, although this may be attributed to the age of the hens used and corresponding changes in mineral absorption [12]. Khoshbin, Vakili, and Tahmasbi [14] found that feeding late-lay hens a 50/50 or 25/75 ratio of $MnSO_4$ and AAC Mn increased hen day egg production and egg mass compared to feeding 100% inorganic Mn. Overall, the results of this experiment agree with several previous studies in late-phase laying hens in which feeding AAC or sulfated minerals did not affect egg production [15–19].

Egg shell structure changes as the hen ages, resulting in decreasing breaking strength over time [20]. Feeding AAC minerals has improved egg shell thickness in 28- to 36-week-

old laying hens [17] as well as 57- to 65-week-old laying hens [19]. Studies have also shown that feeding AAC minerals at approximately 30% of the typical inclusion rate resulted in smaller mammillary knobs, which could improve shell structure and increase effective shell thickness [18,19]. In this experiment, however, egg shell quality as measured by shell weight, specific gravity, Haugh units, breaking strength, and shell thickness did not differ by treatment in any of the three time points. Other researchers found no differences in egg quality in layers fed minerals complexed with amino acid ion or sulfates [16,21–23].

Favero et al. [24] proposed that the combination of AAC and sulfated trace minerals behaves in a synergistic fashion to improve egg shell calcification. The basal diet used in this study contained 20 ppm $ZnSO_4$ and 20 ppm $MnSO_4$, to which 40 ppm of each, complexed with either sulfate or amino acid ion, were added according to the given treatment. Although some of the diets contained some Zn or Mn from both sources, there were no differences in egg shell quality between treatments. Kim et al. [16] found no effect of mineral source on egg shell mineral content. Furthermore, Zhang et al. [19] found no change in egg shell mineral content from late-phase laying hens fed any combination of AAC and sulfated trace minerals compared to those fed only sulfated minerals. Perhaps the effect of mineral source on egg quality is dose-dependent or requires a longer experimental period to obtain diet-related differences in shell quality. Indeed, this study included similar levels of Zn and Mn and higher levels of Cu than previous studies, and some researchers have found that an inclusion rate of 30–40 ppm AAC Mn or AAC Zn, which is lower than that used in this study, improved performance in 85- to 95-week-old laying hens [25].

Some minerals, including Cu, Ca, and P antagonize Mn absorption in the small intestine by as much as 65% [6,21]. This effect was not observed either in Cu or Mn excretion or P excretion and retention in this experiment. In fact, AAC Mn yielded the lowest P excretion in week 10, suggesting that feeding AAC Mn increased P utilization. This agrees with Medeiros-Ventura et al. [26], who found that feeding a combination of AAC and sulfated minerals to layer chicks reduced P excretion at 35 days of age compared to feeding only sulfated minerals. Excreta Ca levels at week 10 were lowest in the AAC Cu and AAC Cu + Mn treatments, suggesting that any possible antagonistic effect between Mn and Ca may be reduced by the supplementation of AAC Cu. This effect on Ca excretion was not seen when AAC Cu was paired with AAC Zn, however. This is surprising since Zn has been shown to promote bone mineralization and prevent bone resorption and subsequent release of Ca into circulation [27]. On the other hand, previous research has shown that different sources of dietary Cu affect P retention in broilers but not laying hens, which is consistent with the results in this experiment [28]. In addition, Vohra and Heil [29] showed that Zn antagonizes both Cu and Mn. The increased bioavailability of AAC Mn may be enough to counter any impairments in intestinal absorption. Feeding AAC minerals to late-lay hens may even improve intestinal function, including absorption and utilization of said minerals [15], and antioxidative capabilities [14]. Although these parameters were not included in the design of this experiment, they may account for differences in mineral retention.

Trace mineral excretion may also be reduced by feeding AAC minerals compared to sulfated minerals [15,19]. This has been demonstrated even when AAC minerals are provided at 30% of the typical inclusion rate, without negatively affecting egg quality [30]. This study found that feeding AAC Mn reduced excreta Mn, and AAC Zn reduced excreta Zn compared to the control group. However, the same was not true for excreta Cu levels in the AAC Cu treatment. When AAC Cu was supplemented alone or in combination with AAC Zn, it increased Cu excretion at weeks 10 and 15. AAC Cu may be more bioavailable than $CuSO_4$, thereby reducing the dietary requirement for late-phase laying hens [25]. Egg shell Cu content did not differ by treatment, and Cu was below detectable limits for the majority of egg contents analyzed at weeks 10 and 15. Whether or not increased Cu excretion would have negative long-term effects on the hen's physiology might be shown by measuring other parameters, including tibia ash Cu content. Other studies have shown improvement in tibia mineral content in 35-day-old pullets [26], but not in late-phase

layers [23,30]. Future research could investigate how consistently feeding AAC minerals to layers throughout the pullet and laying phases affects mineral utilization, including changes in tibia mineral content. Additionally, a dose titration study may clarify how much AAC Cu is necessary to meet the birds' requirements. If indeed AAC minerals are more bioavailable than those complexed with sulfate, feeding the correct dose would lower the birds' dietary requirement and could even reduce mineral excretion by 67% for Zn and 81% for Mn over the course of a one-year laying cycle [21]. This carries implications for environmental safety, as there is some concern that heavy metals chelated to sulfates can build up in poultry manure [21].

There were several inconsistencies in this experiment: for example, AAC Mn increased both Mn and Zn excretion during week 10, but not thereafter. The control group, on the other hand, showed increased Mn and Zn excretion during week 15 but not week 10. These results differ from Martin et al. [31], who showed that laying hens fed AAC Zn excreted more Zn than those fed $ZnSO_4$. The different sources of minerals may interact with each other differently when fed in combination. Environmental factors such as temperature may have affected feed consumption, which was not measured in this experiment. Although not statistically significant, mortality was higher in the treatments fed AAC Zn and AAC Mn + Zn. Each of these factors, combined with the fact that certain minerals antagonize the absorption and utilization of others, can play into differences in mineral excretion. However, egg quality was not affected by the mineral source, and egg weight was only significant during week 10 of the experiment. Previous studies have analyzed the effect of AAC minerals in combination with sulfated minerals, but the treatment levels and ratios of different mineral sources vary between the studies. Further research may clarify the optimal dosage of each mineral source when fed in combination with the others to reduce mineral excretion while maintaining egg production and quality measures. In addition, assessing Haugh units and other measures of egg freshness after more than four days of storage may show treatment differences that were not observed in this experiment. In all, AAC Cu increased Cu excretion, while AAC Mn enhanced P retention, and AAC Zn + Cu increased egg weight after 10 weeks of feeding the treatment diet.

5. Conclusions

This study found that feeding AAC or sulfated trace minerals to late-lay laying hens did not significantly impact egg production or egg quality and had minor effects on mineral retention. This indicates that trace minerals from sulfated or AAC sources are equally effective in meeting hens' needs at 60 to 75 weeks of age. In addition, hens fed AAC Cu excreted more Cu than those given $CuSO_4$. This suggests that feeding AAC Cu increases the bioavailability of Cu without negatively affecting egg shell quality. However, more research is needed to confirm this and to clarify whether this trend continues over a longer experimental period.

Author Contributions: Conceptualization, G.S.A.; methodology, G.S.A.; formal analysis, G.S.A.; investigation, G.S.A., J.R.D., E.B.S. and G.M.H.; writing—original draft preparation, G.S.A. and J.R.D.; writing—review and editing, G.S.A. and J.R.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: All procedures were approved by the Texas A&M University animal care and use committee (IACUC 2017-0357).

Data Availability Statement: The data are contained within the article.

Conflicts of Interest: The authors J.R.D, E.B.S., G.H. and G.S.A. declare no real or perceived conflicts of interest.

References

1. Mabe, I.; Rapp, C.; Bain, M.M.; Nys, Y. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. *Poult. Sci.* **2003**, *82*, 1903–1913. [CrossRef] [PubMed]
2. Zhang, Y.N.; Zhang, H.J.; Wu, S.G.; Wang, J.; Qi, G.H. Dietary manganese supplementation modulated mechanical and ultrastructural changes during eggshell formation in laying hens. *Poult. Sci.* **2017**, *96*, 2699–2707. [CrossRef] [PubMed]
3. Kim, C.S.; Hill, C.H. The interrelationship of dietary copper and amino oxidase in the formation of elastin. *Biochem. Biophys. Res. Comm.* **1966**, *24*, 395–400. [CrossRef] [PubMed]
4. Chowdhury, S.D. Shell membrane protein system in relation to lathyrogen toxicity and copper deficiency. World's Poult. *Sci. J.* **1990**, *46*, 153–169.
5. Baumgartner, S.; Brown, D.J.; Salevsky, E., Jr.; Leach, R.M. Copper deficiency in the laying hen. *J. Nutr.* **1978**, *108*, 804–811. [CrossRef] [PubMed]
6. Watts, D.L. The nutritional relationships of manganese. *J. Orthomol. Med.* **1990**, *5*, 219–222.
7. Dhande, R.R.; Suryawanshi, S.A.; Pandey, A.K. Seasonal changes in plasma calcium and inorganic phosphate levels in relation to parathyroid structure of the grey quail, *Coturnix coturnix coturnix* Linnaeus. *J. Environ. Biol.* **2006**, *27*, 123–128. [PubMed]
8. Chen, Y.C.; Chen, T.C. Mineral utilization in layers as influenced by dietary oligofructose and inulin. *Int. J. Poult. Sci.* **2004**, *3*, 442–445.
9. Wedekind, K.J.; Hortin, A.E.; Baker, D.H. Methodology for assessing zinc bioavailability: Efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. *J. Anim. Sci.* **1992**, *70*, 178–187. [CrossRef]
10. Star, L.; van der Klis, J.D.; Rapp, C.; Ward, T.L. Bioavailability of organic and inorganic zinc sources in male broilers. *Poult. Sci.* **2012**, *91*, 3115–3120. [CrossRef]
11. Moreng, R.E.; Balnave, D.; Zhang, D. Dietary zinc methionine effect on eggshell quality of hens drinking saline water. *Poult. Sci.* **1992**, *71*, 1163–1167. [CrossRef]
12. Osorio, J.S.; Trevisi, E.; Li, C.; Drackley, J.K.; Socha, M.T.; Loor, J.J. Supplementing Zn, Mn, and Cu from anio acid complexes and Co from cobalt glucoheptonate during the peripartal period benefits postpartal cow performance and blood neutrophil function. *J. Dairy Sci.* **2015**, *99*, 1868–1883. [CrossRef]
13. Federation of Animal Science Societies (FASS). *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 3rd ed.; Federation of Animal Science Societies: Champaign, IL, USA, 2010.
14. Khoshbin, M.R.; Vakili, R.; Tahmasbi, A.M. Manganese-methionine chelate improves the antioxidant activity, immune system and egg manganese enrichment in the aged laying hens. *Vet. Med. Sci.* **2023**, *9*, 217–225. [CrossRef]
15. Chen, X.; Ma, X.M.; Yang, C.W.; Jiang, S.Z.; Huang, L.B.; Li, Y.; Zhang, X.; Jiao, N.; Yang, W.R. Low level of dietary organic trace elements improve the eggshell strength, trace element utilization, and intestinal function in late-phase laying hens. *Front. Vet. Sci.* **2022**, *9*, 903615. [CrossRef] [PubMed]
16. Kim, C.H.; Jeong, S.H.; Lim, S.J.; Cheon, S.N.; Kim, K.; Chun, J.; Jeon, J. Effect of organic or inorganic mineral premix in the diet on laying performance of aged laying hens and eggshell quality. *Animals* **2022**, *12*, 2378. [CrossRef]
17. Mamdooh, A.M.; Goudarzi, S.M.; Saki, A.A.; Aliarabi, H. Various zinc sources and levels supplementation on performance, egg quality and blood parameters in laying hens. *J. Livestock Sci. Technol.* **2021**, *9*, 1–9.
18. Qiu, J.L.; Zhou, Q.; Zhu, J.N.; Lu, X.T.; Liu, B.; Yu, D.Y.; Lin, G.; Ao, T.; Xu, J.M. Organic trace minerals improve eggshell quality by improving the eggshell ultrastructure of laying hens during the late laying period. *Poult. Sci.* **2020**, *99*, 1483–1490. [CrossRef] [PubMed]
19. Zhang, K.K.; Han, M.M.; Dong, Y.Y.; Miao, Z.Q.; Zhang, J.Z.; Song, X.Y.; Feng, Y.; Li, H.F.; Zhang, L.H.; Wei, Q.Y.; et al. Low levels of organic compound trace elements improve the eggshell quality, antioxidant capacity, immune function, and mineral deposition of aged laying hens. *Animal* **2020**, *15*, 100401. [CrossRef]
20. Rodriguez-Navarro, A.; Kalin, O.; Nys, Y.; Garcia-Ruiz, J.M. Influence of the microstructure on the shell strength of eggs laid by hens of different ages. *Br. Poult. Sci.* **2002**, *43*, 395–403. [CrossRef] [PubMed]
21. Leeson, S.; Caston, L. Using minimal supplements of trace minerals as a method of reducing trace mineral content of poultry manure. *Anim. Feed Sci. Technol.* **2008**, *142*, 339–347. [CrossRef]
22. Xiao, J.F.; Wu, S.G.; Zhang, H.J.; Yue, H.Y.; Wang, J.; Ji, F.; Qi, G.H. Bioefficacy comparison of organic manganese with inorganic manganese for eggshell quality in Hy-Line Brown laying hens. *Poult. Sci.* **2015**, *94*, 1871–1878. [CrossRef] [PubMed]
23. Junchang, C.; Ruangpanit, Y. Effects of organic zinc and manganese supplementation on eggshell quality and bone characteristic of laying hens during late laying cycle. *Agr. Nat. Resour.* **2023**, *57*, 145–152.
24. Favero, A.; Vieira, S.L.; Angel, C.R.; Bess, F.; Cemin, H.S.; Ward, T.L. Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. *J. Appl. Poult. Res.* **2013**, *22*, 80–91. [CrossRef]
25. Zarghi, H.; Hassanabadi, A.; Barzegar, N. Effect of organic and inorganic manganese supplementation on performance and eggshell quality in aged laying hens. *Vet. Med. Sci.* **2023**, *9*, 1256–1268. [CrossRef] [PubMed]
26. Medeiros-Ventura, W.R.L.; Rabello, C.B.V.; Barros, M.R.; Silva Junior, R.V.; Oliveira, H.B.; Faria, A.G.; Silva, A.F.; Soares, P.C.; Pereira, C.G.; Santos, M.J.B.; et al. Zinc, manganese, and copper amino acid complexes improve performance and bone characteristics of layer-type chicks under thermoneutral and cold stress conditions. *Poult. Sci.* **2020**, *99*, 5718–5727. [CrossRef] [PubMed]

27. Yamaguchi, M. Role of zinc in bone formation and bone resorption. *J. Trace Elements Exp. Med.* **1998**, *11*, 119–135. [CrossRef]
28. Banks, K.M.; Thompson, K.L.; Rush, J.K.; Applegate, T.J. Effects of copper source on phosphorus retention in broiler chicks and laying hens. *Poult. Sci.* **2004**, *83*, 990–996. [CrossRef] [PubMed]
29. Vohra, P.; Heil, J.R. Dietary interactions between Zn, Mn and Cu for turkey poults. *Poult. Sci.* **1969**, *48*, 1686–1691. [CrossRef] [PubMed]
30. Qiu, J.; Lu, X.; Ma, L.; Hou, C.; He, J.; Liu, B.; Yu, D.; Lin, G.; Xu, J. Low-dose of organic trace minerals reduced fecal mineral excretion without compromising performance of laying hens. *Asian-Australas. J. Anim. Sci.* **2020**, *33*, 588–596. [CrossRef] [PubMed]
31. Martin, K.M.; Vargas-Jurado, N.; Purdum, S.E. Prediction model for manure zinc excretion in laying hens. *Poult. Sci.* **2018**, *97*, 267–270. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.