Influence of dietary *dacitic tuff breccia* on laying hen performance and egg quality parameters and bone structure at 85 weeks of age after a non-anorexic molt program at 73 to 77 weeks

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ABSTRACT The objective of the study was to assess the efficacy of AZOMITE (**AZM**), a *dacitic tuff breccia*, in laying hens through egg quality and production parameters. A total of ninety six 73-wk-old Hy-Line W-36 commercial laying hens were randomly assigned to 2 dietary treatments, a control diet and the same diet containing 0.25% AZM, with 24 replicates of 2 hens/replication. From 73 to 77 wk, hens went through nonanorexic molt, and, from 77 to 85 wk, the hens were evaluated for egg production, eggshell quality, and bone health. At wk 85, tibiotarsi were collected for ash and mineral composition, ileal contents were collected for calcium, phosphorus, apparent metabolizable energy corrected for N (**AMEn**), and apparent nitrogen retention (**ANR**) evaluation. AZM-fed hens tended to have higher body weight ($P = 0.07$) from 82 to 83 and 84 to 85 wk, and higher hen day egg production than control (90.54 vs. 79.51%, $P = 0.005$) from 84 to 85 wk. In general, no differences were reported in feed intake, eggshell color, egg weight, albumen height, Haugh units, or eggshell thickness ($P > 0.05$). However, shell strength and elasticity were improved ($P < 0.02$) and yolk color was decreased ($P = 0.03$) in AZM-fed hens than control. Moreover, the digestibility of Ca, AMEn, and ANR was increased with 0.25% AZM compared to control ($P < 0.01$). Tibiotarsi P and Ca percentage were lower in AZM-fed birds than control ($P < 0.01$), without affecting bone strength and mineral density ($P > 0.36$). Therefore, the use of 0.25% AZM showed a potential in improving egg production and eggshell strength, while maintaining bone quality in post-molt laying hens.

Key words: poultry, nutrition, calcium, tibiotarsus, AMEn, leghorn, egg production

2022 Poultry Science 101:101718
https://doi.org/10.1016/j.psj.2022.101718

INTRODUCTION

In 2020, per capita egg consumption was estimated to be 285 eggs (USDA 2020), indicating that each person consumed eggs in excess of 3 of every 4 d. Conway (2015) predicted that egg consumption would continue to rise as the global population continues to expand and the desire for animal protein increses in developing countries. At the same time, commercial egg production is altering management strategies as consumer preferences encourage change, including the use of more square feet of house allocated to each bird (Doyon et al., 2016) and moving to cage-free production (Lempert, 2016). As producers adapt to these changes, maximizing productivity of each hen will help compensate for the loss of bird numbers due to increase space allocation. Moreover, cracked or broken eggs account for 80 to 90% of the eggs that are routinely downgraded (Mabe et al., 2003). Therefore, tools that allow producers to meet consumer demands while improving hen productivity and eggshell quality need to be identified and explored as this transition advances.

Minerals, essential and nonessential, are important to food production animals, companion animals, and humans, and their importance in promoting general health, improving growth performance, egg production, and bone metabolism has been extensively reviewed (Spears, 1999; Richards et al., 2010; Soetan et al., 2010; Nys et al., 2018; Gaffney-Stomberg, 2019). AZOMITE (**AZM**, AZOMITE Feed Grit., AZOMITE Mineral Products, Nephi, UT), a *dacitic tuff breccia* (**DTB**), has been shown to contain over 70 different macro and trace minerals, including Fe, Mg, Mn, Se, Zn, Cu, and rare earth elements (**REE**), including lanthanides. This product of volcanic origin is used as an anticaking agent in feed mills to aid in pellet manufacturing, and it is labeled as a hydrated sodium calcium aluminosilicate (**HSCAS**).

Received May 20, 2021.
Accepted December 30, 2021.

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Previous research has been conducted to evaluate the effects of AZM on the performance in food animal production systems (Liu et al., 2009, 2011; Tan et al., 2014; Musthafa et al., 2016; Juzaitis-Boelter et al., 2021), and preliminary reports suggest that AZM inclusions in laying hen diets may influence marketable egg production (Juzaitis-Boelter et al., 2021) and some eggshell characteristics (unpublished data). Therefore, the objective of this study was to evaluate the effects of supplementing 0.25% of AZM in the diet on the egg production, quality, and bone health of laying hens post-molt.

MATERIALS AND METHODS

General Procedures

All animal procedures were in accordance with the Institutional Animal Care and Use Committee of North Carolina State University (Raleigh, NC). A total of 96 commercial HyLine W36 laying hens were used in the assessment, and were randomly distributed to 2 dietary treatments (with or without AZM), 24 replicates with 2 hens/replication, from 73 to 85 wk of age. The hens were allocated to a 2-tier full stair-step cage system following the "Guide for the Care and Use of Agricultural Animals in Research and Teaching" (FASS, 2020). The cages provided 696.9 cm^2 per bird (2 hens/cage - 30.5 × 45.7 cm), with 30.5 cm of feeder space and 2 nipple drinkers, which are both greater allocations than commercial standards. Feed and water were provided ad libitum throughout the experiment. The multilevel caged systems were kept in a 9 × 4.6 × 2.5 m room at the Poultry Entomology Research Unit (**PERU**). The room was tunnel ventilated by one 61 cm fan with an airflow rate of 155.7 cmm, at 1.27 mm H_2O of static pressure.

Before being assigned to the experimental diets, the hens were allowed a 2-wk adaptation period for acclimation to the cages (71−73 wk of age), in which they were fed a standard 18% protein laying hen diet and given 16 h of light. Subsequently, the hens were randomly assigned to two treatments with 0 (control, **CON**) or 0.25% AZM.

From 73 to 77 wk of age, all hens went through induced molting using the non-anorexic molt procedure as described by Anderson (2018). During the molt period, 2 diets were provided: 1) non-anorexic molt, a low protein/energy diet, formulated to provide nutrition for body maintenance but allow a loss of body weight and 2) the resting diet, which provided layers with the nutrients needed to maintain static bodyweight with no egg production (Table 1). A basal diet was formulated, and mixed for each feeding phases during the molting, resting and subsequent laying phases. Diets were split then mixed separated with or without the inclusion of AZM. The basil diets were submitted to the NC Department of Ag & Consumer Services, Food and Drug protection Division, Feed/Forage Testing Laboratory Crude Protein, Energy, Ca and P. In AZM supplemented molt diets, 0.25% of AZM displaced the same amount of soy hulls. During the molt period, the hens were given 10 h of light and 14 h of dark and were weighed every other d to assess weight loss. When the target bodyweight reduction was achieved (20% reduction), the molt diet was discontinued at 75 wk of age, and the hens were placed on the resting diet until 77 wk of age. At this time, the hens were returned to the laying diet.

The laying diet was based on corn and soybean meal and formulated to meet or exceed the levels for all nutrients according to the breeder guideline (Hy-Line W36, 2016), mirroring the current industry recommendations. A single basal laying diet was mixed then split, and in the AZM supplemented diets, 0.25% of AZM displaced the same amount of corn gluten meal (Table 1). The diets were manufactured at the North Carolina State University (**NCSU**) feed mill under the supervision of the feed mill manager and were offered in the mash form.

Samples and Analysis

Egg number and weights were collected and recorded daily. Body weights (**BW**) and feed intake (**FI**) were recorded biweekly for the duration of the experiment, and the FI was given as g/hen/d. The egg weight and FI were used to calculate the egg to feed ratio (egg:feed), given as g of egg/g of feed. Internal and external egg quality parameters were evaluated bi-weekly, from 6 eggs/replicate (144 eggs/treatment). Parameters included physical measurements for Haugh unit, yolk color, shell thickness, strength, and elasticity. Eggs were broken onto plate glass and the Haugh unit was measured using a TSS QCD system Haugh unit-measuring device (Technical Services and Supplies, Dunnington, York, UK). Subsequently, yolk color was evaluated by TSS QCD system (Technical Services and Supplies). The eggshells were washed, air dried at 21°C, and shell thickness was assessed in 3 different locations at the equatorial region of the egg, using Origin SpeedMic (iGaging, San Clemente, CA). The eggshell strength and elasticity were determined on intact eggs with an exterior USDA grade of A to ensure uniform egg shape, by a TA-HD Plus instrument (Stable Micro Systems, South Hamilton, MA), following the methodology described by Jones et al. (2010). Shell elasticity is the deflection in mm of the shell prior to breaking.

At the end of the experiment (85 wk), 48 hens were individually housed, and collection pans were placed under each cage to collect 2 days of excreta from each hen. The crude Protein (**CP**) was determined using the Leco Nitrogen Analyzer (Truspec N, Leco Corporation, St Joseph, MI) as published in (Mihaljev et al., 2015). The excreta was used to determine the apparent metabolizable energy corrected for N (**AMEn**) and Apparent Nitrogen Retention (**ANR**). For that, 2% of Celite (Celatom, EP Minerals, Reno, NV) was included in the feed at 85 wk as a acid insoluble ash marker, according with Scott and Boldaji (1997). Using this method, it is

Table 1. Diets formulation for all phases (molt and layer diet, As-fed basis/ % Diet).

	Molt diets (73−77 wk)				Layer diets (77−85 wk)	
Ingredient	Low ME control	Low ME azomite[1]	Resting control	Resting azomite	Control	Azomite
Corn	30.10	30.10	63.70	63.70	46.45	46.45
Soybean hulls	63.15	62.90	10.15	9.90	-	-
Soybean meal 48%	-	-	11.15	11.15	21.45	21.45
Wheat bran	0.90	0.90	-	-	6.00	6.00
Corn gluten meal	-	-	-	-	5.00	4.75
Coarse limestone	0.70	0.70	7.73	7.73	10.81	10.81
Mono-dicalcium phosphate	3.70	3.70	2.07	2.07	1.56	1.56
Salt	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.21	0.21	0.10	0.10
Lysine	0.34	0.34	-	-	-	-
Azomite	-	0.25	-	0.25	-	0.25
Vit. premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Min. premix[3]	0.20	0.20	0.20	0.20	0.20	0.20
Choline HCl 60%	0.21	0.21	0.10	0.10	0.17	0.17
Poultry fat	-	-	4.25	4.25	7.81	7.81
Propionic acid 505	0.05	0.05	0.05	0.05	0.05	0.05
Selenium premix 0.06%[4]	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100
Calculated analysis						
Crude protein (%)	9.92	9.92	11.75	11.75	18.0	18.0
Metabolizable energy (kcal/kg)	1,650	1,650	2,859	2,859	2,904	2,904
Calcium (%)	1.33	1.33	3.80	3.80	4.05	4.05
Available phosphorus (%)	0.76	0.76	0.44	0.44	0.40	0.40
Lysine (%)	0.42	0.42	0.55	0.55	0.96	0.96
Total sulfur amino acids (%)	0.35	0.35	0.49	0.49	0.69	0.69
Analyzed values[5]						
Crude protein (%)	10.9		11.0		17.0	17.0
Metabolizable energy (kcal/kg)	1,589		2,750		2,899	2,842
Calcium (%)	1.11		3.24		4.12	4.13
Total phosphorus (%)	0.47		0.46		0.75	0.75

[1]Azomite Feed Grit (0.25%) was added displacing the same amount of soybean hulls in the molt diets and corn gluten meal in the layer diet: Composition 284 mg of Ca, 20 mg of P, 0.029 mg of Se, 13.1 mg of Na, 0.03 mg of Cu, 100 mg of Mn, 0.0012 mg of Zn

[2]Vitamin premix supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B12, 0.08 mg; and ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL-α-tocopheryl acetate.

[3]Mineral premix supplied the following per kilogram of feed: 120 mg of Zn as ZnSO4H2O, 120 mg of Mn as MnSO4H2O, 80 mg of Fe as FeSO4H2O, 10 mg of Cu as CuSO4, 2.5 mg of I as Ca(IO3)2, and 1.0 mg of Co as CoSO4.

[4]Selenium premix provided 0.3 ppm Se from sodium selenite.

[5]Analysis conducted by the NC Dept of Ag and Consumer Services, FDPD Laboratory, Feed/Forage Office.

necessary to know only the percentage of the marker and the nutrient present in the food and the excreta to determine the nutrient retention. The nutrient retention was calculated with the formula described by Scott and Boldaji (1997).

$$AMEn = GE_{feed} - \left(GE_{faecal} \times \frac{AIA_{feed}}{AIA_{feacal}}\right) - \left(8.22 \times \frac{CP_{feacal}}{6.25}\right)$$

With:

AME_n = Apparent Metabolizable Energy, corrected for nitrogen
GE = Gross Energy (Bomb Calorimeter)
AIA = Acid Insoluble Ash recovery

$$ANR = 100 \times \left(1 - \left(\left(\frac{AIA_{feed}}{AIA_{feacal}}\right) \times \left(\frac{CP_{faecal}}{CP_{feed}}\right)\right)\right)$$

With:

ANR = Apparent nitrogen retention
CP = Crude protein (Leco Nitrogen Analyzer)

Additionally, at 85 wk of age, 40 hens were randomly selected from each treatment group (total of 80 hens), euthanized by the American Veterinary Medical Association (AVMA, 2020) approved method of cervical dislocation, and 80 tibiotarsi were collected from each treatment (right and left tibiotarsi/hen). The right tibiotarsi were subjected to a biomechanical assay using a TA-HD Plus insturment (Stable Micro Systems, South Hamilton, MA), using a three-point bending test, with 7.5 cm of distance between the supports and a 50 Kg load cell. The EXPONENT software (Stable Micro Systems) recorded the values of breaking strength, determined by the maximum load supported before failure, and bending moment evaluation, based on the structural element of the shaft flexure induced by the external force or moment when applied to the shaft. Bone mineral density (**BMD**) was evaluated (right tibiotarsi) by a dual-energy X-ray absorptiometry optical densitometry analysis (**DXA**) using Model 7100 (Schick Technologies, Inc., Long Island City, NY). Furthermore, the same tibiotarsi were defatted (72 h in ether), dried, weighed, and ashed at 600°C for 10 h. The ash was weighed and used to calculate the ash percentage relative to the dry

deffated bone weight. Subsequently, the ash was used to determine the Ca and P content, following the methodology described by Brenes et al. (2003), with the use of ICP-OES (Perkin Elmer Optima 8000, Waltham, MA).

At the same time, after 5 d that the hens were fed with a feed with a marker, Celite included, the ileum content from the middle ileum up to the cecal junction was collected from each hen, to evaluate the nutrient digestibility, with the use of Celite as ileum content indigestible marker. The Ca and P content was determined using the same method as the bone using, the methodology described by Brenes et al. (2003), with the use of ICP-OES (Perkin Elmer Optima 8000). The digestibility was calculated according of the formula below, discribed previously by Vogtmann et al. (1975).

$$\textit{Digestibility}\ \% = 100 \times \left(1 - \left(\frac{z\ \textit{ileum}\ \%/\textit{AIA ileum}\ \%}{z\ \textit{diet}\ \%/\textit{AIA diet}\ \%}\right)\right)$$

with:

z = one of the measured elements such as protein, fat, etc.
AIA = Acid Insoluble Ash recovery

Table 2. Average body weight (BW), feed intake (FI), hen day egg production, and egg to feed ratio (egg:feed) of post molt laying hens fed the control (0% AZM[1]) or Azomite (0.25% AZM) supplemented diet according to the experimental timepoints[2].

Treatments	78−79 wk	80−81 wk	82−83 wk	84−85 wk
BW (g)				
Azomite	1,298	1,394	1,490	1,364
Control	1,288	1,359	1, 432	1,319
SEM	15.45	14.79	15.52	12.48
P-value	0.739	0.249	0.070	0.072
FI (g/hen/d)				
Azomite	84.60	93.80	92.80	126.40
Control	82.90	90.40	86.90	118.20
SEM	2.80	3.20	2.20	3.90
P-value	0.761	0.602	0.179	0.307
Hen day egg production (%)				
Azomite	0.74	14.21	51.50	90.54[a]
Control	1.19	16.83	46.04	79.51[b]
SEM	0.40	3.65	3.57	1.91
P-value	0.577	0.721	0.449	0.005
Egg:feed (g of egg/g of feed)				
Azomite	0.001	0.07	0.22	0.55
Control	0.001	0.10	0.21	0.51
SEM	0.01	0.02	0.01	0.01
P-value	0.730	0.521	0.860	0.143

[1]Abbreviation: AZM, Azomite.
[2]Values are calculated from means of the 24 replicate groups of 2 hens, from each dietary treatment.
[ab]Means with different superscript letters differ by F-test ($P < 0.05$).

Statistical Analysis

The data was summarized by replicate then means were determined for each cage for each of the determined trait measured. All data based on replicate means were analyzed using one-way ANOVA in JMP SAS Institute (JMP version 14, SAS Inst. Inc., Cary, NC). The level of probability was set at $P < 0.05$ and trends were considered at $P < 0.10$.

RESULTS

The post molt BW of hens initially assigned to CON and AZM were statistically similar (1.18 kg ± 0.016) at 77 wk of age (data not shown). No differences in BW were found between the treatments from 78 to 79 and 80 to 81 wk of age ($P > 0.25$; 1.30 vs. 1.29 and 1.39 vs. 1.36 kg, by period, AZM vs. CON, respectively) (Table 2). However, a trend was observed from 82 to 83 and 84 to 85 wk ($P = 0.07$), in which birds fed AZM showed higher BW than CON (1.49 vs. 1.43 and 1.36 vs. 1.32, by period, AZM vs. CON, respectively). Moreover, no differences were observed among the diets for FI ($P > 0.18$) and egg:feed ($P > 0.14$) at any timepoint. From 84 to 85 wk, hens fed AZM had greater egg production than the control group (90.54 vs. 79.51%, $P = 0.005$), whereas as for the earlier timepoints, no differences between treatments were observed ($P > 0.44$).

Differences among treatments were not detected for egg weight ($P > 0.76$), Haugh unit ($P > 0.15$), or shell thickness ($P > 0.21$) in any of the evaluated timepoints (Table 3). From 80 to 81 wk of age, the control group had a darker egg yolk color value compared to the AZM group ($P = 0.03$). In the same time interval, AZM eggs had greater shell strength ($P = 0.009$) and elasticity ($P = 0.02$) than the control group. At 85 wk of age, Ca ileal digestibility ($P = 0.01$), P ileal digestibility (P, 0.05), AMEn ($P < 0.001$), and ANR ($P < 0.001$) were superior in birds fed AZM than the control-fed birds (Table 4). Furthermore, at the same age, hens that received AZM had lower percentage of calcium ($P < 0.001$) and phosphorus ($P < 0.001$) in the tibiotarsus compared to the control group (Table 5). Despite the differences in mineral content, no differences were reported for bone dry weight ($P = 0.87$), ash percentage ($P = 0.29$), and the biomechanical and structural integrity of the bones, evaluated as bone strength ($P = 0.37$), bending moment ($P = 0.90$), and BMD ($P = 0.71$).

DISCUSSION

In the present study, the trending increase in BW of birds fed AZM during the last 4 wk of the study (82−85 wk), without increase in FI, suggests that these birds were able to utilize the nutrients more efficiently. Indeed, there was observed an increase in the Ca (79.67 vs. 61.98%), P (76.90 vs. 59.94%), AMEn (2,999 vs. 2,741 kcal/kg), and ANR (47.83 vs. 31%) digestibility when birds received AZM compared to CON, at 85 wk of age. Considering the unique composition of AZM, it is hypothesized that the presence of complex of components, including REE, influenced, indirectly, the

Table 3. Average egg weight, Haugh unit, yolk color, shell strength, shell thickness, and shell elasticity of post molt laying hens fed the control (0% AZM[1]) or Azomite (0.25% AZM) supplemented diet according to the experimental timepoints[2].

Treatments	78−79 wk*	80−81 wk*	82−83 wk*	84−85 wk*
Egg weight (g)				
Azomite	64.53	61.62	57.83	58.85
Control	64.21	61.58	57.96	59.13
SEM	0.60	0.46	0.39	0.45
P-value	0.784	0.966	0.871	0.758
Haugh unit				
Azomite	89.28	91.72	87.51	94.00
Control	90.89	91.42	88.08	92.33
SEM	0.79	0.74	0.65	0.57
P-value	0.312	0.842	0.657	0.149
Yolk color (DSM Scale)				
Azomite	5.00	4.17[b]	4.60	5.53
Control	5.40	4.47[a]	4.36	5.51
SEM	0.13	0.07	0.09	0.11
P-value	0.150	0.036	0.209	0.927
Shell strength (N)[3]				
Azomite	46.85	39.86[a]	37.26	37.95
Control	45.44	36.30[b]	37.55	38.01
SEM	1.17	0.66	0.83	0.69
P-value	0.546	0.009	0.86316	0.963
Shell thickness (mm)				
Azomite	0.35	0.33	0.31	0.31
Control	0.34	0.32	0.31	0.31
SEM	0.006	0.003	0.004	0.003
P-value	0.364	0.211	0.623	0.782
Shell elasticity (mm)				
Azomite	0.60	0.54[a]	0.52	0.52
Control	0.60	0.51[b]	0.54	0.52
SEM	0.010	0.006	0.008	0.008
P-value	0.833	0.026	0.111	0.748

[1]Abbreviation: AZM, Azomite.
[2]Values are calculated from means of the 24 replicate groups of 2 hens, from each dietary treatment.
[3]N = Newtons (1 Kg = 9.80665 N).
[ab]Means with different superscript letters differ by F-test ($P < 0.05$).
*Ninety six eggs were evaluated in each analyses, at each time point.

nutrient digestibility mainly by acting as cofactors of enzymes involved in the digestive process.

Studies have shown that the inclusion of 0.2 to 0.4% of AZM led to the increased intestinal activity of protease, lipase, and amylase in grass carp (Liu et al., 2011) and white shrimp (Tan et al., 2014). Moreover, levels ranging from 0.25 to 0.50% of AZM not only increased the protease activity and digestibility of protein and dry matter, but also led to greater intestinal villi height, width, and density and a higher number of *Lactobacillus* in tilapia (Liu et al., 2009). In broilers, the supplementation of 0.5% of AZM was shown to increase the Ca and P digestibility at 21 d (Juzaitis-Boelter et al., 2021). Therefore, it is possible that this mineral combination could also affect intestinal cell development and modulate the microbiota. However, it is important to further investigate these effects on intestinal health in poultry and other animal species.

Table 4. Apparent ileal Ca and P digestibility, apparent metabolizable energy corrected for N balance (AMEn), and apparent nitrogen retention (ANR) of post molt laying hens fed the control (0% AZM[1]) or Azomite (0.25% AZM) supplemented diet at 85 wk of age[2].

Treatments	Ca (%)	P (%)	AMEn (kcal/kg)	ANR (%)
Azomite	79.67[a]	76.90[a]	2,999[a]	47.83[a]
Control	61.98[b]	59.94[b]	2,741[b]	31.00[b]
SEM	3.50	5.69	26.16	1.98
P-value	0.019	0.048	<0.001	0.001

[1]Abbreviation: AZM, Azomite.
[2]Values are calculated from means of the 24 replicate groups of 2 hens, from each dietary treatment.
[ab]Means with different superscript letters differ by F-test ($P < 0.05$).

Furthermore, the possible energy surplus in AZM-fed hens due to the increased AMEn digestibility relative to the control group, might explain why they reached production peak (above 80% of egg production) earlier than the control-fed hens. As this was the last time interval evaluated in the experiment, it is unknown how the production curves would have progressed in the following weeks and the effects of AZM on the persistency of egg production. However, if the egg production difference had persisted throughout the subsequent weeks, that would represent 11 extra eggs per 100 hens, which is economically relevant.

In the current study, because the egg weight and shell thickness were not different between the treatments, the positive effects of AZM supplementation in improving shell strength and elasticity, from 80 to 81 wk, might have been due to a change in the shell ultrastructure. The hen eggshell is composed of inorganic (approximately 96%) and organic (approximately 4%) portions (Hamilton, 1986), which interact with one another and influence the eggshell structure and biomechanical properties (Nys et al., 2004). Even though $CaCO_3$ constitutes most of the inorganic portion of the eggshell, P and other trace minerals are also important to its formation and integrity.

For instance, Cu, Zn, and Mn, minerals present in AZM, are cofactors of key enzymes involved in the synthesis of the eggshell membrane and organic matrix structure (Baumgartner et al., 1978; Xiao et al., 2014; Zhang et al., 2017a). Moreover, Zn, Mn, and Li have also been shown to influence $CaCO_3$ crystals deposition and orientation (Rajam and Mann, 1990; Mann et al., 1993; Elzinga and Reeder, 2002; Xiao et al., 2014; Zhang et al., 2017b,2018), which could affect the eggshell mechanical properties. The supplementation with Cu, Zn, and Mn was shown to increase the egg elastic modulus, breaking strength, and fracture toughness in hens from 60 to 73 wk (Mabe et al., 2003). Additionally, Mn supplementation increased glycosaminoglycan content in the eggshell membrane in hens from 50 to 62 wk (Xiao et al., 2014), which has a strong positive correlation with the shell breaking strength (Young et al., 2007). Therefore, it is believed that, during the first weeks post-molting, AZM supplementation could potentially decrease the egg breakage, which accounts for up to 10% of the total egg production (Ketta and Tůmová, 2016), preventing economic losses. However, the analysis of the eggshell mineral composition is needed to provide a complete explanation for these findings.

Regarding the egg yolk color, it is hypothesized that the decreased yolk color intensity in AZM-fed hens

Table 5. Average tibiotarsi dry weight, ash, Ca, P, bone mineral density (BMD), bone strength, and bending moment of post molt laying hens fed the control (0% AZM[1]) or Azomite (0.25% AZM) supplemented diet at 85 wk of age[2].

Treatments	Dry weight (g)	Ash (%)	Ca (%)	P (%)	BMD (g/cm^2)	Bone strength (N)[3]	Bending Moment (N)
Azomite	5.43	46.14	38.02[b]	17.54[b]	0.472	158.08	0.481
Control	5.48	47.56	40.47[a]	18.52[a]	0.481	168.28	0.500
SEM	0.13	0.66	0.20	0.11	0.01	5.55	0.019
P-value	0.869	0.292	<0.001	<0.001	0.709	0.366	0.896

[1]Abbreviation: AZM, Azomite.
[2]Values are calculated from means of the 40 tibiotarsi from each treatment group.
[3]N = Newtons (1 Kg = 9.80665 N).
[ab]Means with different superscript letters differ by F-test ($P < 0.05$).

compared to the control group from 80 to 81 wk of age might have occurred due to the oxidation of feed carotenoids during feed manufacture or lower % corn gluten meal. This observation was momentary and exclusive to this timepoint, suggesting something punctual rather than an effect of feeding AZM. In future studies, however, it is important to investigate how AZM influences the deposition of minerals in the yolk and access its oxidative status, especially focusing on lipid oxidation.

In addition, to maintain egg production and quality, it is imperative to support adequate bone health in laying hens. After the onset of egg production, there is a shift in bone formation from the structural bone (trabecular and cortical) towards the medullary bone, which is a labile reservoir of Ca for eggshell formation (Whitehead, 2004). However, the bone resorption during egg production is not limited to the medullary portion, which could result in the development of osteoporosis (Whitehead and Fleming, 2000; Whitehead, 2004). Osteoporosis, a metabolic disease characterized by the progressive loss of structural bone, ultimately leads to fractures and it is a welfare and economic concern (Whitehead and Fleming, 2000; Webster, 2004). At the end of the experiment, a reduction in tibiotarsi Ca and P percentage was observed in birds fed AZM compared to the control group. Because the mineral analysis cannot distinguish between the different portions of the layer bone (Kim et al., 2012), the lower tibial mineralization in AZM-fed birds may be related to less Ca deposited as medullary rather than a loss in the structural bone. Indeed, AZM-fed birds laid significantly more eggs and showed higher Ca digestibility (79.6 vs. 61.98%) at the end of the study, thus reducing the Ca storage in the medullary bone. It has been established that medullary bone also contributes to bone strength (Fleming et al., 1998). However, in the current study, the BMD, bone strength, and bending moment were not different among the treatments, indicating that the skeletal integrity port-molt was not compromised in AZM-fed birds even with the increased egg production.

In summary, post molt hens appear to utilize nutrients more efficiently when fed 0.25% of AZM in the diet, evidenced by the increased Ca, AMEn, and ANR digestibility. Additionally, hens fed 0.25% of AZM reached an early egg production peak and showed improved eggshell strength and elasticity at one timepoint, while supporting bone health. However, AZM supplementation reduced the yolk color index from 80-81 wk of age. These findings suggest a potential increase in eggs/ housed hen and a decrease in egg breakage, which is economically significant, and evidenciate the need to further understand the mechanisms behind AZM`s effects on post-molting laying hens.

DISCLOSURES

All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere. There were no set of conditions in which professional judgment concerning these findings affected the validity of research, nor influenced by a secondary interest. All authors have no conflicts of interest.

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