Dietary inclusion of AZOMITE improves feed efficiency in broilers and egg production in laying and broiler breeder hens

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ABSTRACT The dietary inclusion of aluminosilicates has been reported to enhance pellet quality, improve feed mill throughput, bind toxins, improve feed efficiency, and promote immunological function across a variety of production systems. AZOMITE is a product marketed as a hydrated sodium calcium aluminosilicate containing macro and trace minerals, and rare earth elements and the potential benefits of its dietary inclusion in broiler, layer, and broiler breeder diets was investigated. In a battery study, broilers were fed diets containing 0, 0.125, 0.250, or 0.500% AZOMITE from 0 to 21 d of age. Laying hens were fed a control diet or this diet supplemented with 0.25% AZOMITE from 54 through 98 wk of age, with the hens fed a standard molting diet or this diet supplemented with 0.25% AZOMITE from 71 to 72 wk of age. Broiler breeder hens were fed a control diet or this diet supplemented with 0.25% AZOMITE from the onset of photostimulation at 21 wk of age through 65 wk of age. All 3 dietary inclusion rates of AZOMITE improved ($P < 0.05$) the feed to body weight gain ratio in broilers fed these diets relative to broilers fed the control diet. In laying hens total marketable eggs, and in broiler breeder hens total settable eggs were increased ($P < 0.05$) with the dietary inclusion of AZOMITE by 8 eggs per hen. The inclusion of dietary AZOMITE also improved apparent Ca and P digestibility in broilers and tibia ash content in laying hens. The results indicate the dietary inclusion of AZOMITE in poultry diets improves bird performance.

Key words: tibia ash, molt, egg production, broiler

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INTRODUCTION

AZOMITE (**AZ**; Azomite Mineral Products, Nephi, UT), can be described as a lightly altered vitric, poorly welded dacitic tuff of solidified volcanic igneous rock origination. Volcanic tuff is a rock made from the consolidated volcanic ash of small particles, mainly volcanic glass, and other eruptive debris, such as large mineral fragments. Alteration by exposure to water near the surface of the Earth can also produce minerals such as aluminosilicate clays and calcite (calcium carbonate), in place of some of the original material. Thus, AZ is a complex mixture of different kinds of mineral matter and has been shown to contain measurable amounts of some 74 different elements. Therefore, AZ is a hydrated sodium calcium aluminosilicate that differs from other absorbent aluminum phyllosilicate clays such as calcium bentonite which only consists of 15 additional elements. All of the rare earth elements (**REE**), but promethium which is not typically found in the Earth's crust (Hu et al., 2006), are found in AZ. The concentration of REE in AZ is 530 mg/kg.

Previous research indicates that aluminosilicate-based clays can bind dietary toxins (Kubena et al., 1998; Chen et al., 2014), slow gastrointestinal transit time for better digestion (Quisenberry, 1968), increase intestinal villi surface area (Wawrzyniak et al., 2017), enhance immunity (Jarosz et al., 2017), and decrease ammonia emissions in poultry litter (Prasai et al., 2017). Luna et al. (2016) and Wlazlo et al. (2016) reported that the addition of aluminosilicate clays as a litter amendment also reduced ammonia production. Previous research in poultry with REE indicates that their dietary addition will improve egg production, hatchability of eggs, and feed efficiency in laying hens as reviewed by Lei and Xueying (1997). The addition of REE to broiler diets has been reported to improve body weight gain (BWG; He et al., 2010).

Although the dietary inclusion of AZ might provide the benefits seen previously with the individual addition of aluminosilicate clays or REE in poultry production, research involving AZ in poultry is limited. Within feed mills, AZ is used for its anticaking properties and

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recently Tillman et al. (2020) reported that the inclusion of AZ at 0.25% and 0.50% improved pellet production rates when meat and bone meal (4%) and/or distillers dried grains with solubles (8%) were used in the diet. The objectives of the current research was to evaluate the potential production benefits of including dietary AZ when feeding broilers from d of hatch until 21 d of age, laying hens from 54 to 98 wk of age with a molting diet fed from 71 through 72 wk of age, and broiler breeder hens from 21 to 65 wk of age.

MATERIALS AND METHODS

All animal procedures were approved by the University of Georgia Animal Care and Use Committee.

Experiment 1

The aim of the first experiment was to evaluate the performance of broilers from 0 to 21 d of age fed a starter diet supplemented with AZ at 0, 0.125, 0.250, and 0.500% of the diet (Table 1). The 2 highest inclusion rates were selected because in feed manufacturing, concentrations of AZ between 0.25 and 0.500% are commonly used for its anticaking properties (Tillman et al., 2020). Given the possibility that the dietary inclusion of AZ might be beneficial to broiler performance, the 0.125% inclusion rate was chosen to determine if this potential benefit might occur at a lower inclusion rate than what is used for the feed manufacturing benefits of AZ. Five hundred male broiler chicks from a female parent stock (Cobb 500 fast feathering) obtained from the Cobb hatchery in Cleveland, GA, were raised in thermostatically controlled, electrically heated battery brooder cages with wire floors. Chicks had free access to water and the starter diet. Before placement, the chicks were sorted according to weight profiles and those with extreme weights or physical abnormalities such as open navels were discarded. The remaining chicks were assigned to experimental groups to achieve similar weight distributions among all pens and minimize variation.

The dietary treatments were equally distributed and randomized across 3 battery brooders each equipped with 24 pens to create 18 replicate pens for each dietary treatment. Each replicate pen contained 5 chicks. Individual pens measured 98 cm long by 35 cm wide by 23 cm high. A computerized controller for the room housing the batteries regulated a gas-fired furnace, exterior evaporative cooling system for intake air, 46-cm ceiling circulation fan, and 2 exhaust fans, one measuring 53-cm, and the other one measuring 26-cm, at the end of the room for heating, cooling, and ventilation. Ambient temperature was set to 34°C on d 0 and decreased by 0.28°C/d. For the duration of the study, light intensity was 20 lux for 24 h/d. Bodyweight and total feed consumption on a pen basis were determined every 7 d.

To determine apparent calcium and phosphorus digestibility, the amount of feed consumed during the last 48 h of the experiment was determined and total feces were also collected in clean stainless steel pans for each pen during this time. To ensure clearance of the digestive tract of food, the feeding troughs were removed 12 h before the start of this feeding period and 12 h before the end of the study when the feces were collected. On the final day of the experiment, after weighing the birds and feed, blood samples were collected from the brachial vein of the control and the 0.500% AZ-supplemented birds to obtain serum samples. Serum was frozen at -80°C. The serum samples were collected to subsequently determine if the 0.500% dietary supplement of AZ depressed serum alpha-1-acid glycoprotein (**AGP**) concentrations which would indicate a decreased acute phase response in broilers fed this diet.

Experiment 2

This experiment aimed to evaluate the potential production benefits of the dietary inclusion of AZ at 0.25% of the diet in laying hens from 54 through 98 wk of age, with the additional stress of a molting period from 71 through 72 wk of age. The molting stress was incorporated into this experiment based on AZ reducing serum AGP concentration in broilers in experiment 1. Without any published research on potential beneficial or detrimental effects of long-term feeding of AZ-supplemented diets, the lowest level (0.25%) of AZ that has feed manufacturing benefits was selected for the dietary inclusion level in this long duration experiment. Egg production and hen BW were monitored in 120 individually caged, 50-wk-old, Hy Line W-36 White Leghorn laying hens for a pre-experimental period of 4 wk. Cages were in 3 tiered batteries with each battery containing 48 cages. Each cage had a sloped floor for egg displacement from the interior of the cage; thus, the height of the cage was 41 cm in the back and 46 cm in the front, while cage width and depth were 33 cm and 46 cm, respectively. Each cage was equipped with a nipple drinker and individual trough feeder. The hens were housed in an environmentally controlled room equivalent to the one described in Experiment 1 with a set temperature of 22.5°C. The daily lighting schedule provided 16 h of light and 8 h of dark. During the 2 wk molting period, the daily lighting schedule was 8 h of light and 16 h of dark.

At the end of wk 3, the 96 birds with the greatest egg production and egg specific gravity values were selected and divided into 2 treatment groups and 2 batteries. Each treatment group had 3 replicate rows of 8 individually caged hens per battery ($n = 6$ replicate rows per treatment). The hens had a 1-wk period to re-assimilate to this distribution prior to the commencement of the experiment at 54 wk of age. The hens were distributed such that the 12 rows of hens did not differ in body weight profile, egg production, or egg specific gravity at the start of the experiment.

The laying hens were fed a standard corn-soy diet supplemented with 0.25% Solka-Floc (control diet; International Fiber Corporation, North Tonawanda, NY) or

Table 1. Composition of the broiler diet (Experiment 1), laying hen diets (Experiment 2) and broiler breeder diets (Experiment 3).

Ingredient	Experiment 1 Broiler diet[1]	Experiment 2 Layer diet[2]	Molting diet[3]	Experiment 3 Developer diet[4]	Layer diet[5]
			%		
Corn	57.62	56.12	23.00	59.75	62.05
Soybean meal (46% CP)	32.32	27.75	0.00	14.00	16.38
Corn distillers dried grains with solubles	3.00	0.00	0.00	0.00	0.00
Soybean oil	2.59	3.78	0.00	0.54	2.62
Wheat middlings	0.00	0.00	42.95	21.00	7.71
Soy hulls	0.00	0.00	22.00	0.00	0.00
Calcium carbonate	0.65	9.79	9.78	1.00	7.37
Defluorinated phosphate	1.24	0.00	0.00	1.77	0.00
Dicalcium phosphate	0.00	1.36	1.36	0.00	1.73
Salt	0.35	0.24	0.24	0.16	0.19
Sodium carbonate	0.00	0.09	0.09	0.00	0.00
L-Lysine HCl (78.8%)	0.24	0.02	0.00	0.07	0.26
DL- Methionine (99%)	0.30	0.25	0.00	0.50	0.30
L-Threonine (98%)	0.08	0.02	0.00	0.08	0.26
L-Tryptophan (98%)	0.00	0.01	0.00	0.00	0.00
Choline chloride (60%)	0.04	0.02	0.02	0.00	0.00
Vitamin mix[6]	0.39	0.23	0.23	0.80	0.80
Mineral mix[7]	0.08	0.08	0.08	0.08	0.08
Solka-Floc[8]	0.99	0.25	0.25	0.25	0.25
Phytase[9]	0.06	0.01	0.01	0.00	0.00
NSP-enzyme[10]	0.01	0.00	0.00	0.00	0.00
Monensin[11]	0.05	0.00	0.00	0.00	0.00
Calculated analysis					
AMEn (kcal/kg)	2,964	2,867	1,875	2,836	2,864
Crude protein (%)	20.09	17.26	10.44	14.48	13.92
Calcium (%)	0.95	4.41	4.44	1.08	3.43
Available phosphorus (%)	0.48	0.51	0.54	0.45	0.42
Digestible total sulfur amino acids (%)	0.86	0.71	0.23	0.87	0.67
Digestible lysine (%)	1.13	0.82	0.38	0.64	0.78
Digestible threonine (%)	0.73	0.59	0.27	0.52	0.68

[1]Broiler starter diet was fed from d 0 to 21 d of age.

[2]Layer diet was fed from 54 wk of age to commencement of molting period at 70 wk of age. Hens returned to their respective layer diet from 73 wk of age to 98 wk of age.

[3]Layer molting diet was fed from 71 through 72 wk of age.

[4]Broiler breeder developer diet was fed from wk 21 (onset of photostimulation) until wk 24 of age.

[5]Broiler breeder layer diet was fed from wk 24 until wk 65 of age.

[6]Vitamin premix (DSM custom vitamin premix, DSM Nutritional Products, Inc. Parsippany, NJ) provided the following per kilogram of broiler diet (Experiment 1): vitamin A, 8,598 IU; vitamin D_3, 1,720 IU; vitamin E, 17 IU; vitamin B_{12}, 0.02 mg; riboflavin, 6.9 mg; niacin, 69 mg; d-panthotenic acid, 17 mg; choline chloride, 299 mg; menadione sodium bisulfate, 1.7 mg; folic acid, 0.86 mg; pyridoxine HCl, 3.4 mg; thiamin mononitrate, 3.5 mg; d-biotin, 0.17 mg; and ethoxyquin, 195 mg. The vitamin premix provided the following per kilogram of layer diet (Experiment 2): vitamin A, 5,071 IU; vitamin D_3, 1,014 IU; vitamin E, 10 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.1 mg; niacin, 41 mg; d-panthotenic acid, 10 mg; choline chloride, 176 mg; menadione sodium bisulfate, 1.0 mg; folic acid, 0.51 mg; pyridoxine HCl, 2.0 mg; thiamin mononitrate, 2.0 mg; d-biotin, 0.10 mg; and ethoxyquin, 115 mg. The vitamin premix provided the following per kilogram of the broiler breeder diet (Experiment 3): vitamin A, 17,637 IU; vitamin D_3, 3,527 IU; vitamin E, 35 IU; vitamin B_{12}, 0.04 mg; riboflavin, 14 mg; niacin, 141 mg; d-panthotenic acid, 35 mg; choline chloride, 612 mg; menadione sodium bisulfate, 3.5 mg; folic acid, 1.8 mg; pyridoxine HCl, 7.1 mg; thiamin mononitrate, 7.1 mg; d-biotin, 0.35 mg; and ethoxyquin, 400 mg.

[7]Trace mineral premix (Southeastern Minerals custom trace mineral mix. Southeastern Minerals Inc. Bainbridge, GA) provided the following in milligrams per kilogram of diet: manganese, 60; zinc, 50; iron, 30; copper, 5.0; iodine, 1.5; selenium, 0.3. Trace mineral forms were manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, calcium iodate, and sodium selenite.

[8]AZOMITE (Azomite Mineral Products, Nephi, UT) was added at the expense of Solka-Floc (International Fiber Corporation, North Tonawanda, NY). The experimental diets were made from a common minimal basal diet that only lacked Solka-Floc. The individual dietary treatments were made by adding either Solka-Floc, AZ or a combination of Solka-Floc and AZ to the minimal basal diet.

[9]Quantum Blue (5,000 FTU/g, AB Vista, Plantation, FL), added to supply 3,000 FTU/kg finished feed in Experiment 1 or 5,000 FTU/kg finished feed in Experiment 2.

[10]Econase XT (AB Vista, Plantation, FL), added to supply 16,000 Birch Xyan Units/kg finished feed.

[11]Coban 90 (Elanco Animal Health, Greenfield, IN), added to supply Monensin at 0.1 g/kg finished feed.

0.25% AZ (Table 1) from 54 to 71 and 73 through 98 wk of age. From 71 through 72 wk of age, the hens were fed a molting diet (Table 1) containing 0.25% Solka-Floc or 0.25% AZ.

Every 4 wk during the 44 wk experimental period, all hens were individually weighed and total feed consumption per replicate row of hens was determined. In addition, blood was collected from the brachial vein of the hens from 3 replicate rows per dietary treatment (n = 24 individually caged hens) on a rotating basis every 4 wk. Serum from these blood samples was stored at -80°C. Daily egg production and weekly egg weights were recorded for each bird and each replicate row of hens, respectively. Hen-housed and hen-day egg

production were calculated weekly from daily egg counts. Eggs were manually collected once/d, and at the time of egg collection, all eggs were classified as normal (marketable eggs), cracked, double-yolked, misshapen, membrane, or dirty. Every 4 wk, all eggs produced over a 48-h period were collected for specific gravity measurement following procedures similar to Phillips and Williams (1944).

At the conclusion of the experiment, a segment of duodenum from the top of the duodenal loop and the right tibia were collected from 24 hens from each treatment with the greatest egg production for histology and bone ash determination, respectively. These hens were selected because they would have had the greatest Ca demands for egg production. Each collected segment of the duodenum was flushed with 10% formalin (3.7% formaldehyde) prior to being submerged in 10% formalin. After formalin fixation for 72 h, the samples were embedded in paraffin wax, sectioned at 4 microns, routinely stained with hematoxylin and eosin and coverslipped. Slides were examined by light microscopy and photomicrographs were taken using a Leica (Wetzlar, Germany) DF550 model camera with LAS V4.8 imaging software. For each sample, 3 villi and crypts were measured with the values obtained averaged. Tibias were cleaned of adhering tissue, defatted, and dried before ash determination following the AOAC (2011) method.

Experiment 3

Given the increased egg production associated with feeding laying hens a diet supplemented with AZ, the aim of this experiment was to evaluate the influence of the dietary addition of AZ on the performance of broiler breeder hens from the time of photostimulation at 21 wk of age through 65 wk of age. From 1 d of age, Cobb 500 fast feathering pullets and cockerels were reared as previously described (Gibson et. al., 2008).

At 20 wk of age, all pullets were individually weighed. The pullets were matched by weight into 38 categories for placement into the laying pens. To ensure that the weight distribution in each pen was similar, a pullet from each of the 38 weight categories was then randomly selected and distributed to a laying pen. There were 18 laying pens, and each contained 38 pullets and 4 roosters. Each pen measured 3.65 × 2.75 m, and the floor space of each pen consisted of two-thirds pine shavings litter and one-third elevated slats. Each pen had one 6-hole nest box located on the slatted area and was equipped with 10 nipple drinkers. Hens and roosters were hand-fed with plastic feeder pans in the laying pens. Each pen contained 3 hen feeder pans that were fitted with rooster exclusion grills. The feeding system provided 8.4 cm of feeder space per hen. Males were provided their own feeder pan, which was elevated in height in order to prevent females from consuming their feed. Each rooster had 25.9 cm of feeder space. The male-to-female ratio was maintained throughout the experiment by replacing dead males from a pool of extra males. Photostimulation occurred at 21 wk of age by providing 14 h of light (lights on at 06:30 h), and this photoperiod was maintained until the end of the experiment when the birds completed their 65 wk of age.

Prior to the start of the experiment at 21 wk of age, the broiler breeders were being fed a developer diet. At the initiation of the experiment, 9 of the replicate breeder pens continued on this developer diet supplemented with 0.25% Solka-Floc while the birds in the remaining 9 pens were fed this diet supplemented with 0.25% AZ (Table 1). At 24 wk of age, the broiler breeders were switched to laying diets for the rest of the experiment that contained 0 or 0.25% AZ (Table 1). During the entire experimental period, the birds were fed on a daily basis at 06:30 h. The amount of feed provided to the birds during the breeding period was based on BW and egg production, as suggested by the guidelines of the primary breeder. All of the hens were individually weighed at the start of the experiment and at wk 26, 30, 35, 39, 43, 51, and 61 wk of age. For the other weeks of the experimental period, the hens from 3 of the 9 pens for each treatment were weighed on a rotating basis every week.

Eggs were manually collected 2 times/d, and egg production was calculated weekly from daily egg counts. At the time of egg collection, all eggs were classified as normal (settable eggs), cracked, double-yolk, misshapen, membrane, or dirty. Every 4 wk, collected eggs were saved for specific gravity analysis. Settable eggs produced in each room were weighed every other week. The specific gravity of settable eggs was measured following procedures similar to Phillips and Williams (1944), when the hens were 30, 35, 39, 44, 48, 53, 58, and 65 wk of age.

Ninety settable eggs from each pen were incubated (Natureform Hatchery Systems, Jacksonville, FL) every 3 wk when the hens were 26 to 59 wk of age. Eggs were collected and stored between 18.3 and 19.9°C for up to 7 d before each incubation period. Eggs were candled on d 14 of incubation, transferred for hatching on d 19 of incubation, and hatched on d 21 of incubation. Eggs were incubated at 37.8°C with 53% relative humidity from d 0 through 18, and then at 37.2°C with 70% relative humidity from d 19 to 21. During candling and transfer and after hatching, eggs were characterized as being infertile, cracked, contaminated, or containing early dead embryos (less than 14 d), or late-dead embryos (15 to 21 d). Eggs that were cracked during transfer were removed from the data set.

Alpha-1-Acid Glycoprotein and IgY ELISA

The AGP content of serum samples collected at the end of broiler experiment (Experiment 1) and when the laying hens (Experiment 2) were 54, 70 and 73 wk of age, was determined using the ABCAM Chicken Alpha-1-acid Glycoprotein Sandwich ELISA (Cambridge, MA) following the manufacturer's protocol. The IgY content of serum samples collected from the laying hens at 54

and 70 wk of age was determined utilizing an ABCAM Chicken IgY Sandwich ELISA Kit (Cambridge, MA). For both assays, serum was added to polystyrene microliter wells that were impregnated at the surface with either chicken AGP or IgY antibodies. Following washing and removal of unbound protein, anti AGP or anti IgY antibodies conjugated to horseradish peroxidase were added to react with the bound AGP or IgY in the wells. After another wash step, the amount of enzyme bound in complex to either AGP or IgY was determined by adding the chromic substrate 3,3'5,5'-tetramethylbenzidine. A SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA) was used for the colorimetric analysis of the samples for each ELISA.

Statistical Analysis

Data from each experiment were subjected to ANOVA using the General Linear Model (**GLM**). For BW, BWG, feed to gain and feed intake data, individual comparisons were made between the control treatment (0% AZ), and each of the individual AZ treatments using a Bonferroni contrast procedure (Neter et al., 1990) in Experiment 1. In Experiments 2 and 3, an *F*-test (Neter et al., 1990) was used to detect significant weekly or overall experimental period differences between the control and AZ treatment. In addition, in Experiment 2, Tukey's multiple-comparison procedure was used to detect significant differences for AGP concentrations based on age of hens (Neter et al., 1990). Differences were considered significant when $P < 0.05$. The Minitab statistical software package (Release 16, State College, PA) was used for all statistical procedures.

RESULTS

Experiment 1

The addition of 0.125, 0.250, and 0.500% AZ to a control broiler starter diet improved the feed to gain ratio at 21 d of age (Table 2). Apparent calcium and phosphorus digestibility values were improved, and serum AGP concentrations were reduced in broilers fed a diet containing 0.50% AZ relative to broilers fed the control diet (Table 2). Total mortality were 1, 4, 3 and 2 birds in the control, 0.125, 0.250, and 0.500% AZ treatments, respectively.

Experiment 2

Throughout the experimental period, feed intake, BWG, and BW loss during the molting period did not differ between the hens fed the control or molting diets, and the hens fed these diets supplemented with AZ (data not shown). For the hens fed the control diets, mean feed intake was 99, 38, and 101 g/hen/d prior to molt, during molt and after molt, respectively, while mean feed intake for the hens fed the AZ-supplemented diet during the corresponding periods was 100, 37 and 102 g/hen/d. For the hens fed the control diets, mean BW was 1.561, 1.734, 1.375 and 1.831 kg/hen at the start of the experiment, prior to molt, after molt, and at the end of the experiment, respectively. The corresponding BW values for the hens fed the AZ-supplemented diets were 1.561, 1.747, 1.386 and 1.843 kg/hen.

Over the entire experimental period, the addition of AZ improved total egg and marketable egg production (Table 3). Egg production completely ceased 10 d after the onset of feeding the molting diets in both treatment groups. A hen in the AZ treatment resumed egg production 24 d after the initiation of the molt (or 10 d after the end of the molting period), while the first hen in the control treatment resumed egg production 31 d after the onset of the molt (17 d after the end of the molting period). At 20 d after the end of the molting period, 18 of the AZ−fed hens had resumed egg production compared to only 8 control hens, and this resulted in the AZ hens producing significantly ($P < 0.05$) more eggs (8 versus 4% total hen housed egg production, SEM 1%) than the control hens in the 3 wk after the end of the molting period. Weekly percent hen housed egg production did not differ between the 2 dietary treatments from 4 wk after molt to the conclusion of the experiment.

Throughout the experiment, weekly egg weight was unaffected by the addition of AZ to the laying hen diets (data not shown, except for select wk in Table 3). The

Table 2. Body weight, body weight gain, feed efficiency, apparent Ca and P digestibility coefficients, and serum alpha 1 acid glycoprotein concentration of broilers fed diets containing 0.000, 0.125, 0.250, or 0.500 % AZOMITE from 0 to 21 d of age (Experiment 1).[1]

Parameter	Dietary treatment				SEM
	0.000% AZ	0.125% AZ	0.250% AZ	0.500% AZ	
BW (g/bird)	970	1,004*	991	987	7.4
BWG[2] (g/bird)	927	961*	948	944	7.4
Feed to gain	1.356	1.316***	1.313***	1.318***	0.0073
Feed intake (g/bird)	1,241	1,253	1,243	1,254	10.6
Apparent P digestibility (%)	51	ND	ND	55*	1.0
Apparent Ca digestibility (%)	49	ND	ND	56**	1.5
AGP (ug/mL)	255	ND	ND	224*	9.9

[1]The values are means, n = 18 replicate pens for each dietary treatment with each pen containing 5 chicks. *Values differ from the corresponding control value for a given parameter (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

[2]BWG = body weight gain, AGP = alpha 1 acid glycoprotein, AZ = AZOMITE, and ND = not determined.

Table 3. Total egg and marketable egg production per hen housed, egg weight and specific gravity of eggs of laying hens fed a diet supplemented with 0.00 or 0.25% AZOMITE from 54 to 98 wk of age (Experiment 2)[1].

Parameter and hen age (wk)	Dietary treatment		SEM
	0.00% AZ[3]	0.25% AZ	
Egg production (eggs/hen)			
Before molt (54–71)	99	101	1.4
After molt (73–98)	136	142	2.2
Total production (54–98)	235	242*	2.0
Marketable egg production[2] (eggs/hen)			
Before molt (54–71)	98	100	1.3
After molt (73–98)	135	141	2.4
Total production (54–98)	233	241*	2.1
Egg weight (g)			
54	59.09	58.87	0.448
57	60.73	60.85	0.430
62	60.88	61.25	0.371
66	61.37	61.20	0.633
70	61.92	62.85	0.670
76	63.98	63.37	0.627
81	63.97	64.26	0.545
85	65.38	64.91	0.561
88	67.10	66.17	0.658
92	66.75	66.55	0.664
96	66.37	66.27	0.611
Specific gravity			
54	1.075	1.074	0.0010
57	1.082	1.079***	0.0004
62	1.080	1.079	0.0007
66	1.075	1.072*	0.0009
70	1.076	1.075	0.0006
76	1.079	1.077	0.0007
81	1.081	1.078*	0.0009
85	1.078	1.077	0.0007
88	1.077	1.074*	0.0008
92	1.069	1.065**	0.0009
96	1.074	1.072	0.0008

[1]The values are the means, n = 6 replicate groups of hens per treatment with each group containing 8 individually caged hens. *Values differ from the corresponding control value for a given parameter (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).
[2]Marketable egg production excluded cracked, soft-shelled and double yolk egg production.
[3]AZ = AZOMITE.

Table 4. Tibia ash percent, duodenum villi height and crypt depth, and serum alpha 1 acid glycoprotein and IgY concentrations in laying hens fed a diet supplemented with 0.00 or 0.25% AZOMITE from 54 to 98 wk of age (Experiment 2)[1].

Parameter and hen age	Dietary treatment		SEM (diet)
	0.00% AZ	0.25% AZ	
Tibia ash at 98 wk (%)	52.4	54.5**	0.48
Villi height at 98 wk (nm)	1,822	1,747	69.7
Crypt depth at 98 wk (nm)	167	145	7.8
Villi height to crypt depth ratio at 98 wk	11	12	0.5
AGP[2] (mg/mL)			
Start of experiment (54 wk)	1.12[b]	1.37[b]	0.204
Before start of molt (70 wk)	1.38[b]	1.18[b]	0.178
End of molt (73 wk)	2.75[a]	2.54[a]	0.232
SEM (age)	0.201	0.211	
IgY (mg/mL)			
Start of experiment (54 wk)	109	131	12.0
Before start of molt (70 wk)	106	114	10.7
SEM (age)	11.6	11.1	

[1]The values are means for 24 individual hens for each treatment. *Values differ from the corresponding control value for a given parameter (**$P < 0.01$). [a,b]Values within a column with different superscripts differ for a given age, ($P < 0.001$).
[2]AGP = alpha 1 acid glycoprotein, AZ = Azomite.

specific gravity of eggs produced by the hens fed a diet containing AZ was sometimes less than the eggs produced by the control hens (Table 3). However, the total of cracked and softshell eggs produced throughout the experiment did not differ between the 2 treatments with the control and AZ hens producing a mean of 2 and 1 eggs/hen (SEM 0.68), respectively. Tibia ash percent was greater at the conclusion of the experiment in the hens that had been fed diets supplemented with AZ (Table 4). Blood AGP levels were not affected by dietary AZ supplementation, but AGP levels were greater at the end of the molting period than prior to molt in both the control and AZ hens (Table 4). The only mortality during the experiment was one control hen at 94 wk of age.

Experiment 3

Throughout the experiment, there was no difference in BW between the broiler breeder hens fed the control diet or this diet supplemented with AZ (data not shown). Overall, settable egg production was significantly greater for the broiler breeder hens fed the AZ-supplemented diet (Table 5). Egg weight did not differ throughout the experiment between the two dietary treatments (data not shown). For both dietary treatments, mean egg weight was 54 and 72 g/egg at 29 and 65 wk of age, respectively. Specific gravity values of the eggs only differed at 53 wk of age when the eggs from the hens fed the diet supplemented with AZ had a lower value ($P < 0.001$) than the eggs produced by the control hens. Overall, hatchability and fertility did not differ between the eggs produced from the hens fed the control diet or this diet supplemented with AZ (Table 5). However, the overall number of chicks that pipped, but did not hatch (in shell mortality) was greater for the eggs from the hens fed the diet supplemented with AZ (Table 5). At the start of the experiment, the average number of hens per pen was 38 for each treatment. With subsequent mortality, the average number of hens was 34 and 32 hens per pen for both the control and AZ treatment at 49 and 65 wk of age, respectively.

DISCUSSION

The current research indicates that adding AZ to broiler, laying hen and broiler breeder diets has positive effects on bird performance, but the specific mechanisms by which AZ improves performance was not determined. In experiment 1, the apparent digestibility of Ca and P was improved in broilers fed a diet supplemented with 0.5% AZ. In a subsequent experiment (not presented), the true digestibility coefficients of Ca and P were determined in broilers at 42 d of age in broilers that had been fed from d of age diets containing either 0 or 0.125% AZ (20 broilers per treatment). The digestibility coefficient

Table 5. Total egg and settable egg production per hen housed, and overall fertility and hatchability of eggs produced by broiler breeder hens fed a diet supplemented with 0.00 or 0.25% AZOMITE from 21 (onset of photostimulation) through 65 wk of age (Experiment 3)[1].

Parameter	Dietary treatment		SEM
	0.00% AZ[5]	0.25% AZ	
Total egg production (eggs/hen)	158	166	5.0
Total settable egg production (eggs/hen)	132	140*	4.3
Total cracked egg production (eggs/hen)	6	5	0.3
Total misshaped egg production (eggs/hen)	2	2	0.2
Total double-yolked egg production (eggs/hen)	1	1	0.1
Total dirty egg production (eggs/hen)	12	11	0.7
Total floor egg production (eggs/hen)	6	5	0.6
Fertility (%)	81.0	84.3	1.73
Hatchability[2] (%)	75.4	77.7	1.82
Hatch of fertile (%)	93.0	92.2	0.40
Early dead embryos[3,4] (%)	2.2	1.9	0.21
Late dead embryos[3,4] (%)	1.9	1.7	0.23
In-shell[3,4] (%)	1.6	2.9***	0.14

[1]The values are means, n = 9 replicate pens per treatment with 38 hens per pen. Ninety eggs from each replicate pen were incubated and hatched at 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, and 59 wk of age. *AZ value for a given parameter differs from the corresponding control value, (*$P < 0.05$, ***$P < 0.001$).

[2]Hatch of eggs set

[3]Embryo mortality was classified as early dead (less than 14 d) or late dead (15−21 d of incubation) embryos. In shell included both live and dead −in-shell at the time of hatch.

[4]Calculated as a percentage of fertile eggs.

[5]AZ = AZOMITE.

of Ca was 49 and 68 percent ($P < 0.05$) in the control and AZ broilers, respectively while the digestibility coefficient of P was 57 and 68 percent ($P = 0.07$), respectively. If the increased Ca digestibility with AZ supplementation in broilers also occurs in laying hens, it may have contributed to the laying hens fed diets supplemented with AZ having increased tibia ash values at the end of the experiment relative to control-fed hens.

Although the specific gravity of eggs produced by the laying hens fed a diet containing AZ was at times significantly less than the eggs produced by the control hens (Table 3), it is important to keep in mind these hens produced over 8 more marketable eggs per hen than the control hens without a reduction in egg size. Thus, the AZ-fed hens had a greater calcium demand for egg shell formation, and although the specific gravity of their eggs was decreased at times, they did not produce more cracked eggs or soft shelled eggs relative to the control-fed hens. Similarly, the broiler breeder hens fed the diet supplemented with AZ produced about 8 more settable eggs than the control fed hens, with no decrease in egg size and only a decrease in egg specific gravity detected at one-time point in the experiment. Reka et al. (2018) fed laying hens a control diet or this diet supplemented for 2 mo with either a combination of 100 mg/kg lanthanum and 150 mg/kg cerium or 200 mg/kg lanthanum and 200 mg/kg cerium. The combination of these 2 specific REE at the low dose increased blood Ca and P levels in the laying hens when measured at 4 and 8 wk after the start of the experiment. Laying hens fed the high dose of these 2 specific REE had increased blood Ca and P levels at 4 wk, but not at 8 wk. Bölükbaşı,et al. (2016) reported that the addition of cerium oxide to laying hen diets at 100 mg/kg, but not at 200, 300, or 400 mg/kg, increased blood Ca and P levels. However, laying hens fed diets containing 0, 100, 200, 300 or 400 mg/kg lanthanum oxide did not differ in serum Ca and P levels (Durmuş and Bölükbaşı, 2015). This previous research suggests that the REE content of AZ may contribute to improving Ca absorption and warrants subsequent research in laying hens where improved Ca absorption can impact egg shell quality and bone health.

Based on previous research, the REE contained in AZ might also be playing a role in the increased egg production seen in the current experiment with laying hens and broiler breeder hens. As reviewed by Lei and Xueying (1997), the addition of REE to laying hen diets has improved egg production. Subsequently, specific REE have been investigated for their potential role in improving egg production in laying hens. Egg production in laying hens was increased relative to control hens when cerium oxide was added to the diet at 100, 200, 300, or 400 mg/kg (Bölükbaşı et al., 2016). For laying hens fed diets containing 0, 100, 200, 300, or 400 mg/kg lanthanum oxide egg production relative to the control hens was only increased in the hens fed the 400 mg/kg dose (Durmuş and Bölükbaşı, 2015). The combination of 100 mg/kg lanthanum and 150 mg/kg cerium and 200 mg/kg lanthanum and 200 mg/kg cerium each improved egg production in laying hens (Reka et al., 2019).

Acute-phase proteins are primarily produced and secreted by the liver. As reviewed by O'Reilly and Eckersall (2014) these serum factors mediate the acute phase response to systemic or local perturbations such as inflammation, infection, and stress. One of the major acute-phase proteins is AGP and its serum concentration increases during an acute phase protein response and its production is regulated by a host of mediators such as cytokines and glucocorticoids as reviewed by Fournier et al. (2000). Given the lower serum concentration of AGP in the broilers fed a diet supplemented with AZ in experiment 1, it was hypothesized that AZ supplementation of the laying hen diet might modify the expected rise in serum AGP in laying hens undergoing the stress of a molt. Shakeri et al. (2014) reported that the stress associated with high stocking density was associated with an increase in serum AGP levels. Food deprivation in broilers (Najafi et al., 2016, 2018) increased the serum AGP concentration and corticosterone levels, but chemically inhibiting the rise in corticosterone during the food deprivation prevented the rise in AGP (Najafi et al., 2018). In the current research, there was an equal rise in the serum AGP concentration in the

control and AZ-fed hens from the start to the conclusion of the molting period. However, the hens fed the AZ supplemented diet did return to lay at a quicker initial rate than the control hens.

Mounting an acute phase protein response requires energy for maintenance that otherwise could be used for growth in boilers and this may be playing a role in why the control broilers had a greater feed to gain ratio than the broilers fed diets supplemented with AZ in the current research. The improvement in feed efficiency in the AZ birds could be the result of a combination of mechanisms as a result of the complex components inherently within the material. Previous research indicates that various clays or aluminosilicates classified as hydrated sodium calcium aluminosilicate can bind toxins (Kubena et al., 1998; Chen et al., 2014), slow gastrointestinal transit time for better digestion (Quisenberry, 1968) and increase intestinal villi surface area (Wawrzyniak et al., 2017) for potential better absorption of nutrients. Similarly, as reviewed by Panichev (2015) and Wakabayashi et al. (2016), REE has antibacterial and antiviral activities, influences immunity (including anti-inflammatory), and alters hormone production and enzyme activity. In the current research, based on villi height and crypt depth measurements in laying hens, duodenum surface area was not increased by dietary AZ-supplementation. The serum concentration of IgY, was also unaffected in laying hens after being fed a diet supplemented with AZ for 16 wk. As recently reviewed by Tariq et al. (2020) in swine and poultry production and previously by Lei and Xueying (1997) in poultry, the use of individual or combinations of specific REE has been reported to improve feed utilization efficiency. However, the results are not uniform and supplements of individual REE can have negative effects on animal performance which may be related to the over-supplementation and associated toxicity of elevated REE (Pagano et al., 2015; Panichev, 2015).

In summary, the addition of AZ to broiler diets improved feed to gain values through 21 d of age, and the supplementation of laying hen and broiler breeder hen diets improved marketable egg and settable egg production, respectively. The results from the current research also suggest that dietary supplementation of poultry diets with AZ may improve Ca and P absorption and utilization. Further research is needed to elucidate the mechanisms by which AZ supplementation of diets improves poultry production efficiency.

DISCLOSURES

The authors declare no conflicts of interest.

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