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Effect of Dietary Sodium Zeolite A and Graded Levels of Calcium on Growth, Plasma, and Tibia Characteristics of Chicks[1]

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ABSTRACT Sodium zeolite A (SZA), a synthetic sodium aluminosilicate having high ion-exchange capacity, has been shown to increase eggshell specific gravity in laying hens and to improve Ca utilization in chickens. A 4 × 2 factorial arrangement of treatments was used to investigate the effect of dietary Ca (.6, .8, 1.0, and 1.2%) and SZA (0 and .75%) on growth, plasma, and tibia characteristics of chicks from 5 to 15 days of age. Increasing dietary Ca linearly increased (P<.05) Ca and alkaline phosphatase (AP) in plasma and increased tibia shearing force and percentage ash, Ca, and P in tibiae. However, dietary Ca linearly decreased (P<.05) inorganic P and Mg in plasma and Mg and Mn in tibiae. Sodium zeolite A decreased (P<.05) plasma P and AP and tibia Mg but increased (P<.05) tibia Ca, Zn, Al, and Mn concentrations. Tibia ash and shearing force were increased in chicks fed SZA receiving inadequate dietary Ca, but they were decreased in chicks fed SZA and excess Ca (Ca by SZA interaction, P<.05). Tibia density showed a similar trend, but the effect was not significant (Ca by SZA interaction, P<.12). The addition of SZA enhanced tibia ash, density, and shearing force when dietary Ca was low; however, when added to diets containing 1.2% Ca, SZA reduced many bone mineralization indices with the exception of tibia Ca.
(*Key words*: zeolite, bone ash, bone density, chicks, calcium)

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INTRODUCTION

Sodium zeolite A (SZA)[4] is a synthetic hydrated sodium aluminosilicate with a crystalline lattice structure (molecular formula, $Na_{12}[(AlO_2)_{12}(SiO_2)_{12}]\cdot 27H_2O$) having a large surface area and pore volume occupied by exchangeable sodium ions and water (Pond and Mumpton, 1984). Calcium, Zn, Mg, K, and other biologically significant cations are known to exchange readily with the Na associated with SZA (Breck, 1974). Although there are numerous synthetic and natural occurring zeolites exhibiting ion-exchange capacity, SZA is thought to have one of the highest selectivities for Ca.

The ion-exchange property of SZA may be responsible for the reported increase in eggshell specific gravity when SZA is fed to laying hens (Roland *et al.*, 1985; Miles, *et al.*, 1986; Roland, 1988; Roland and Dorr, 1989). The effect of SZA on growth, mineral balance, and bone formation, however, is less clear and often inconsistent. Although some reports have shown that SZA improves growth performance (Ingram *et al.*, 1987; Roland and Dorr, 1989; Leach *et al.*, 1990), other reports have shown no effect (Ballard and Edwards, 1988; Watkins *et al.*, 1989; Ward *et al.*, 1990), and still others have shown a reduction in growth performance (Elliot and Edwards, 1989; Daly *et al.*, 1990). Ballard and Edwards (1988) reported that male broiler chicks fed diets containing 1.0% SZA had increased absorption of orally and intramuscularly administered ^{47}Ca. Ingram *et al.* (1989) reported that medullary bone development was greater in laying hens fed SZA compared with hens not receiving SZA. However, Scheideler (1989) and Elliot and Edwards (1989) reported that SZA decreased bone ash in chicks. Edwards (1988) found that the addition of SZA to diets low in P also reduced body weight and tibia ash content in 16-day-old chicks. Watkins *et al.* (1989) reported that .75% dietary SZA increased tibia ash in chicks fed diets containing 1.0% Ca and .87% total P; however, SZA reduced tibia ash when 1.2% Ca was fed. Edwards (1988) demonstrated that SZA reduced phytate P retention and that dietary Ca and P levels

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[4]Sodium zeolite A (Ethacal Feed Component®) is a product of the Ethyl Corporation, Baton Rouge, LA 70801.

influenced the effects of SZA on mineral retention and bone ash.

Due to the increased use of sodium zeolite A in poultry feed and mounting evidence that dietary mineral concentration influences SZA efficacy, the present study was conducted to evaluate the effect of dietary Ca and SZA on the utilization and tissue distribution of various minerals. The influence of SZA, dietary Ca, and their interaction on growth, plasma, and bone mineral concentrations, and bone density and shearing force were evaluated.

MATERIALS AND METHODS

Unsexed Arbor Acres × Peterson broiler chicks from a commercial hatchery[5] were used in the current investigation. From hatching to 4 days posthatching, all chicks were fed a corn and soybean meal-based diet (Table 1) without supplemental Ca. The basal and pretest diet contained .6% Ca. After an overnight fast, chicks were weighed and randomly assigned to one of eight experimental treatments on the basis of body weight. Chicks were provided continuous fluorescent lighting and housed in heated, thermostatically controlled (mean temperature 35 C) starter batteries with raised wire floors. Four replicates of five chicks each were assigned to each treatment and fed their respective experimental diets from 5 to 15 days posthatching. Chicks were allowed *ad libitum* access to feed and water.

The basal diet (Table 1) was formulated to meet or exceed all nutrient requirements of growing chicks except for Ca, which was provided at 60% of the requirement (National Research Council, 1984). Dietary additions were made to the basal diet at the expense of silica flour.[6]

Treatments were arranged as a 4 × 2 factorial consisting of four levels of dietary Ca (.6, .8, 1.0, and 1.2%) and two levels of dietary SZA (0 and .75%). Dietary Ca levels were attained by supplementing the basal diet (Table 1) with 0, .2, .4, or .6% Ca from limestone. All diets were analyzed to contain .7% total P and were calculated to contain approximately .5% available P. At the termination of the experiment, individual chicks were weighed and pen feed consumption was determined. A blood sample (2 mL) was taken from each chick via cardiac puncture. Calcium, P, and alkaline phosphatase (AP) concentrations were determined on fresh plasma and the remaining plasma was frozen for subsequent Mg and Zn analyses. After birds were killed by cervical dislocation, both tibiae were removed for subsequent analyses.

Plasma Mg and Zn were determined by flame atomic absorption spectroscopy[7] and plasma inorganic P (Daly and Ertingshausen, 1972), Ca (o-cresolphthalein method[8]), and AP (Bowers and McComb, 1966) were determined using an automated clinical chemistry analyzer (Gilford System 203).[8]

The left tibiae were cleaned of adherent tissue, extracted (soxhlet) continuously for 48 h in 90% ethanol and then for 48 h in anhydrous diethyl ether. The fat-free tibiae

TABLE 1. *Composition of basal diet*[1]

Ingredients	Percentage
Corn	45.78
Soybean meal (44% CP)	42.50
Corn oil	5.00
Alfalfa leaf meal	2.00
Dicalcium phosphate	1.54
NaCl	.40
Vitamin mix[2]	.25
DL-methionine	.15
$MnSO_4 \cdot H_2O$	.05
$ZnCO_3$	.01
Treatment additions[3]	2.32

[1]Calculated composition (National Research Council, 1984) of the diet: crude protein, 23%; lysine, 1.37%; methionine, .52%; cystine, .37%; metabolizable energy, 3,000 kcal/kg.

[2]Roche Chemical Division, Nutley, NJ 07110. Provided the following per kilogram of diet: retinyl acetate, 6,614 IU; cholecalciferol, 1,653 IU; dl-α-tocopheryl acetate, 7 IU; vitamin B_{12}, 11 μg; riboflavin, 6.6 mg; niacin, 33.1 mg; d- pantothenic acid, 11.0 mg; choline, 551 mg; menadione, 1.5 mg; folic acid, .7 mg; pyridoxine, 1.1 mg; thiamin, 1.1 mg; d-biotin, 55 μg.

[3]The remaining 2.32% of the diet contained limestone, sodium zeolite A (SZA), or silica flour at various concentrations to provide the eight dietary treatments. Four diets containing the four different Ca levels were mixed initially. These diets were then divided and either SZA or silica flour were added to provide the 0 and .75% SZA diets. Dietary treatments were analyzed to contain: .61, .78, .99, and 1.2% Ca and .69, .70, .71, and .71% total P for the .6, .8, 1.0, and 1.2% Ca diets, respectively.

[5]Sanderson Farms, Laurel, MS 39440.

[6]Fisher Chemical Co., Pittsburgh, PA 15219.

[7]Model 3030B, Perkin-Elmer Corp., Norwalk, CT 06859.

[8]Ciba Corning Diagnostics, Oberlin, OH 44074.

TABLE 2. *Gain, feed intake, and gain:feed of chicks fed graded levels of calcium with (+) or without (–) .75% sodium zeolite A*[1]

Dietary Ca	Gain[2]		Feed[2]		Gain:feed	
	–	+	–	+	–	+
(%)	(g)				(g:g)	
.6	293	292	394	389	.745	.752
.8	288	305	383	401	.753	.762
1.0	299	297	396	390	.754	.762
1.2	287	280	382	372	.750	.752
SEM	6		6		.010	

[1]Data are means of four replicates of five chicks each during the period 5 to 15 days posthatching. Average initial chick weight on Day 5 was 82.8 g.

[2]Ca quadratic (P<.05).

were dried in a forced-air oven at 90 C, weighed, and shearing force determined using a Universal Instron Breaking Machine.[9] Shearing force was reported as kilograms force required to shear each tibia using a Warner-Bratzler shear at a crosshead speed of 20 mm/min. The fat-free sheared tibiae were dried, weighed, and dry-ashed at 590 C for 20 h. Ash content was calculated by weight loss. The Ca and Zn content of the ash were determined by flame atomic absorption spectroscopy,[7] P content was determined using an automated[10] molybdovandate method (Association of Official Analytical Chemists, 1984), and Al, Mg, and Mn were determined using an inductively coupled plasma spectrophotometer.[11] The right tibiae were cleaned of adherent tissue (fresh) and shearing force was immediately determined as previously described.

Prior to determining the shearing force, tibia density was determined using the fat-free left tibiae by the method of Barzel (1975). Tibiae were rehydrated under vacuum (65 to 75 mm Hg) for a minimum of 1 h or until all air was removed from the bone. Rehydrated tibiae were weighed in water to .001 g using an electronic balance[12] fitted with a hanging fine copper wire suspended over a beaker of distilled deionized water (weight in water). Tibiae were then removed from the beaker, blotted to remove excess water, and immediately weighed in air on the pan of the same balance (weight in air). Tibia volume was calculated using the Archimedes principle (weight in air minus weight in water) and bone density (mass per unit volume) was calculated by dividing the weight of tibia in air (grams) by tibia volume (cubic centimeters).

Data were analyzed by analysis of variance procedures appropriate for a factorial arrangement of treatments in a completely random design (Steel and Torrie, 1980). Orthogonal single degree of freedom comparisons were used to test main effect differences and interactions. Pen means (four per treatment group) were used as the experimental unit for all data. Differences in final body weight were observed and because body weight may influence bone characteristics (Brown and Southern, 1985), data were analyzed with and without adjustment for final body weight as a covariate in the model. Because no meaningful differences were observed between the two analyses, data analyzed without the covariate in the model are presented.

RESULTS

Dietary Ca supplementation reduced gain and feed intake at the 1.2% level; however, lower levels of dietary Ca (.6, .8, and 1.0%) had no effect (Ca quadratic, P<.05) on gain or feed intake (Table 2). The gain:feed ratio was not influenced by dietary Ca. Sodium zeolite A had no effect on gain, feed intake, or gain:feed ratio.

Dietary Ca linearly increased (P<.05) plasma Ca and AP and decreased (P<.05)

[9]Model 1122, Instron Corp., Houston, TX 77032.

[10]Technicon Instruments, Number 369-75A, Technicon Industrial Systems, Bran Luebbe, Elmsford, NY 10523.

[11]Series 800 Plasma AtomComp Direct-Reading Spectrometer, Thermo Jarrell-Ash Corp., Franklin, MA 02038.

[12]Model 163, Mettler Corp., Hightstown, NJ 08520.

TABLE 3. *Plasma calcium, phosphorus, magnesium, and alkaline phosphatase of chicks fed graded levels of calcium with (+) or without (–) .75% sodium zeolite A (SZA)*[1]

Dietary	Ca[2]		P[2,3,4]		Alkaline phosphatase[2,4]		Mg[2]	
Ca	–	+	–	+	–	+	–	+
(%)	(mg/dL)		(mg/dL)		(IU/dL)		(mg/dL)	
.6	10.64	10.99	5.55	4.85	175.8	136.7	1.94	1.93
.8	11.83	11.05	4.93	4.91	192.2	150.1	1.85	1.87
1.0	11.92	11.46	4.97	3.98	257.5	149.1	1.85	1.75
1.2	12.50	12.75	3.70	3.10	205.6	178.8	1.84	1.68
SEM	.48		.20		202		.07	

[1]Data are means of four replicates of five chicks each.

[2]Ca linear (P<.05).

[3]Ca quadratic (P<.01).

[4]SZA (P<.01).

plasma inorganic P and Mg (Table 3). Chicks fed the 1.2% Ca diets had the greatest decrease (Ca quadratic, P<.01) in plasma P. Dietary SZA reduced (P<.01) plasma P and AP regardless of dietary Ca level.

Tibia ash content and shearing force (Figure 1) increased as dietary Ca was increased from .6 to 1.0%; however, when excess Ca (1.2%) was fed, tibia ash was reduced (Ca quadratic, P<.01). Tibia density followed the same trend as tibia ash and shearing force (Ca quadratic, P<.07). Although force required to shear fresh tibiae was greater than that required to shear dry fat-free tibiae, the processing method did not influence treatment effects. Sodium zeolite A increased tibia shearing force and ash in chicks fed .6 or .8% Ca, but reduced these criteria in chicks fed 1.2% Ca (Ca linear by SZA interaction, P<.01). Although, a similar trend (P<.12) was observed for tibia density, the effect was not significant. Dietary SZA did not affect tibia shearing force, ash, or density in chicks fed 1.0% Ca.

Tibia Ca content (Table 4) was increased (P<.01) by dietary SZA in chicks fed 1.0 and 1.2% Ca. However, dietary Ca level had no effect on tibia Ca in chicks not fed SZA (Ca cubic by SZA interaction, P<.01). Dietary treatment had no effect (P>.10) on tibia P.

Increasing dietary Ca linearly reduced (P<.01) tibia Mg concentration (Table 4). The .2% Ca addition to the basal diet (.8% total Ca) reduced tibia Mn and Zn; however, additional Ca supplementation resulted in little or no further change in tibia Mn or Zn concentration (Ca quadratic, P<.02). Dietary SZA increased (P<.01) tibia Mn and Zn concentrations and these effects were not influenced by dietary Ca (Ca by SZA interaction, P>.10). Dietary Ca supplementation reduced tibia Al (P<.05), but SZA increased P<.02) tibia Al (Figure 2). This effect of SZA on tibia Al was most obvious at the low (.6%) and high (1.2%) dietary Ca levels.

DISCUSSION

Feeding high levels of Ca has been shown to reduce gain and feed consumption in chicks (Bafundo *et al.*, 1984; Watkins *et al.*, 1989). The growth reduction observed in the present study in chicks fed 1.2% Ca appears to have resulted from a decrease in feed intake, as excess Ca had no effect on gain:feed ratio. Gain, feed intake, and gain:feed ratio were not influenced by dietary addition of SZA. This lack of response in growth performance from SZA agrees with previous reports (Ballard and Edwards, 1988; Watkins *et al.*, 1989; Ward *et al.*, 1990), but it is not in agreement with reports that SZA improves growth performance (Ingram *et al.*, 1987; Roland and Dorr, 1989) or that SZA reduces growth performance (Elliot and Edwards, 1989; Daly *et al.*, 1990).

The addition of dietary Ca to the basal diet increased plasma Ca and AP and decreased plasma P. The inverse relationship observed in the present study between AP and plasma P has been reported previously (Njoku *et al.*, 1980) and Boyd *et al.* (1983) reported that AP was inversely related to dietary P availability. Chicks fed SZA had consistently lower AP. Although not significant, Chiang and Yeo

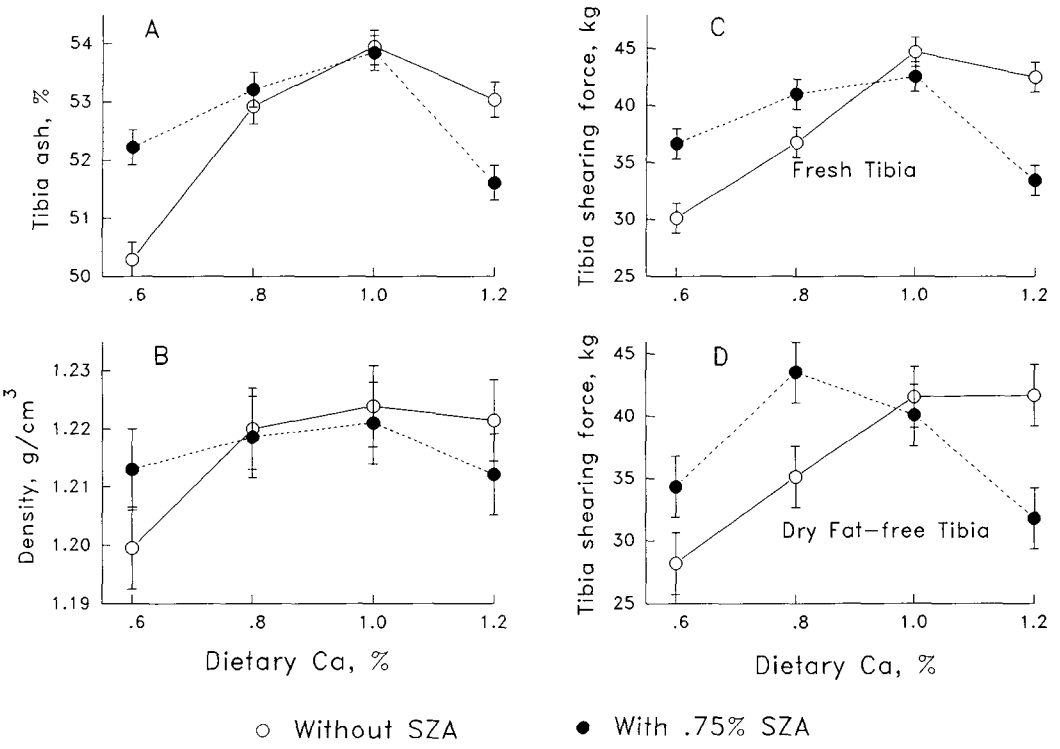

FIGURE 1. Tibia ash content (A), tibia density (B), fresh tibia shearing force (C), and dry fat-free tibia shearing force (D) of chicks fed graded levels of Ca (.6, .8, 1.0, and 1.2%) with (●) or without (○) sodium zeolite A (SZA). Each data point is represented by four replicates of five chicks each (± SEM).

(1983) found that dietary addition of 1.5 and 3.0% natural zeolite decreased AP 23 and 36%, respectively, in 40-day-old broilers fed a "low level of nutrition."

Although SZA did not affect plasma Ca levels in the current study and that of Ward *et al.* (1990), SZA had been shown to increase plasma Ca (Roland *et al.*, 1989; Leach *et al.*, 1990). The research of Roland *et al.* (1985) indicated that SZA increased plasma Ca in hens fed 4% Ca, but that SZA decreased plasma Ca in hens fed 2.75% Ca. The SZA-induced reduction in plasma P seen in the present study has been demonstrated previously (Scheideler, 1989; Leach *et al.*, 1990; Ward *et al.*, 1990).

The observed increase in tibia Ca in SZA-fed birds receiving 1 or 1.2% Ca has not been reported. This increased level of tibia Ca resulted from a decrease in tibia ash without a concurrent decrease in tibia ash Ca content. An increase in tibia Ca without a concomitant increase in P would suggest an increase in $CaCO_3$ or other Ca salts and not hydroxyapatite. A readily available form of soluble Ca not associated with hydroxyapatite might help explain this effect as well as reports that SZA increases egg specific gravity (Roland *et al.*, 1985; Miles *et al.*, 1986; Roland, 1988; Roland and Dorr, 1989) and medullary bone development (Ingram *et al.*, 1989) in laying hens. If bone provided a readily exchangeable source of Ca in addition to hydroxyapatite, one would expect less hydroxyapatite resorption and subsequently lower AP.

Dietary SZA exacerbated the Ca-induced reduction in tibia Mg. The observed decrease in tibia Mg in birds fed SZA may have resulted from an increase in Ca utilization, as high levels of dietary Ca have been shown to interfere with Mg utilization (Chicco *et al.*, 1967).

Tibia Mn was decreased as dietary Ca was increased. Smith and Kabaija (1985) reported that chicks fed high levels of dietary Ca had impaired Mn utilization. Dietary SZA in-

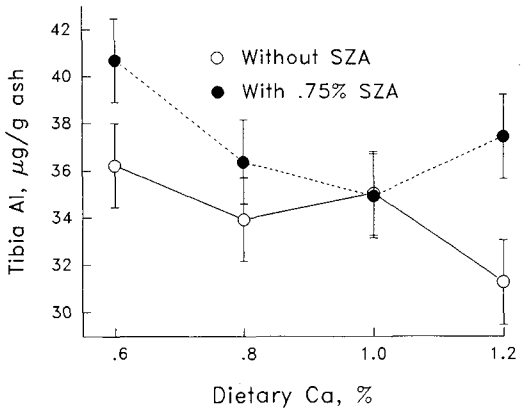

FIGURE 2. Tibia Al content of chicks fed graded levels of Ca (.6, .8, 1.0, and 1.2%) with (●) or without (○) sodium zeolite A (SZA). Each data point is represented by four replicates of five chicks each (± SEM).

creased tibia Mn. The effect of SZA on tibia Mn could be the result of mechanisms directly or indirectly related to the effects of SZA on Mn or Mn antagonists such as phytate P. Phytate and phytate-containing feedstuffs have been shown to reduce Mn utilization (Southern *et al.*, 1987) and Wedekind and Baker (1990) demonstrated that inorganic P depresses Mn utilization and is more antagonistic to Mn than is Ca. If SZA or the Al associated with SZA bound dietary inorganic or phytate P, it may have prevented the antagonism between Mn and P, resulting in an increase in bone Mn.

The increase in tibia Zn observed in chicks fed SZA has been described by Ward *et al.* (1990), and Chiang and Yeo (1983) reported that SZA increased Zn utilization in 6-wk-old broilers. Sodium zeolite A is known to exchange associated cations with Zn and Zn is ranked first on the cation selectivity profile of SZA (Zn > Sr > Ba > Ca > Co > Ni > Cd > Hg > Mg; Breck, 1974).

Sodium zeolite A increased tibia ash, shearing force, and density in chicks fed diets low in Ca. However, SZA reduced these parameters in chicks fed 1.2% Ca. The increase in shearing force is in agreement with previous reports (Hagedorn *et al.*, 1990; Kovar *et al.*, 1990). In the present study, the force required to shear fresh tibia was only 4% greater than that required to shear dry, fat-free tibia. Lott *et al.* (1980) reported that drying bones reduced breaking strength by 50%. However, their methods differed from those used here in that they did not extract the fat from the bones and that they determined breaking strength (V-shaped chisel head) as opposed to shearing force (Warner-Bratzler shear head).

The means by which SZA affects Ca or P utilization is not known. However, the Al associated with SZA has been considered as possibly influencing these observed effects (Miles *et al.*, 1986; Roland and Dorr, 1989; Watkins *et al.*, 1989). Because SZA contains approximately 15% Al (.113% dietary Al in the present study), it is possible that the observed effects in part could be attributed to the well documented antagonism between

TABLE 4. *Tibia calcium, phosphorus, magnesium, manganese, and zinc content of chicks fed graded levels of calcium with (+) or without (–) .75% sodium zeolite A (SZA)*[1]

Dietary Ca	$Ca^{2,3,4,6}$		P		$Mg^{2,3}$		$Mn^{2,3,5}$		$Zn^{2,5}$	
	–	+	–	+	–	+	–	+	–	+
	(%)				(mg/g ash)		(μg/g ash)			
.6	34.67	35.01	17.75	17.89	9.47	8.80	9.71	12.29	342.7	356.3
.8	34.82	34.90	18.22	18.15	8.87	8.15	8.78	10.26	314.3	338.4
1.0	34.87	37.28	18.03	18.34	8.14	7.60	8.85	10.64	328.0	356.8
1.2	34.84	37.29	18.09	18.09	7.74	7.30	8.85	10.69	325.2	368.2
SEM	.17		.16		.13		.34		7.9	

[1]Data are means of four replicates of five chicks each.

[2]SZA (P<.01).

[3]Ca linear (P<.01).

[4]Ca cubic (P<.01).

[5]Ca quadratic (P<.02).

[6]Ca cubic by SZA interaction (P<.01).

various Al compounds and P (Hussein *et al.*, 1989; Rossi *et al.*, 1990). Fethiere *et al.* (1990) advised that P intake be adequate when feeding SZA because feed consumption, egg production, and egg weight were decreased in the presence of SZA when hens were fed low levels of P.

The accumulation of Al in the tibiae of chicks fed SZA suggests that at least some of the Al associated with SZA is absorbed. Leach *et al.* (1990) also showed that chicks fed SZA had increased bone Al and that dietary Ca supplementation reduced bone Al content. Sodium zeolite A can be hydrolyzed to various silicate and aluminate compounds at pH 5 or less (Cook *et al.*, 1982). The presence of such compounds in the intestine of the chick could influence the utilization of other nutrients or could be absorbed. Rabon *et al.* (1991) reported that both the Al and Si associated with SZA are absorbed by the hen and suggested that both elements may influence the means of action of SZA.

The hypothesis that Al decreases P utilization may explain reports that SZA increases egg specific gravity (Roland *et al.*, 1985; Miles *et al.*, 1986; Roland, 1988; Roland and Dorr, 1989), as it has been reported that increasing dietary P levels reduces egg specific gravity (Arscott *et al.*, 1962; Holcombe *et al.*, 1976; Rossi *et al.*, 1990). Rossi *et al.* (1990) reported that the decreased egg specific gravity caused by high dietary levels of P was reversed by dietary Al supplementation.

Changing dietary concentrations of Mn, Na, Cl, or all of these have been shown to effect eggshell quality. Holder and Huntley (1978) reported that dietary Mn supplementation increased eggshell thickness, and the present authors observed that SZA increased bone Mn. Austic and Keshavarz (1988) reported that increasing the proportions of dietary Na relative to Cl increased eggshell strength and thickness. The 13% Na associated with SZA has been shown to be available to the chicken (Miles *et al.*, 1990).

Data presented here and those previously reported (Roland *et al.*, 1985; Edwards, 1988; Watkins *et al.*, 1989; Leach *et al.*, 1990) demonstrate that the effects of SZA on growth, bone formation, and eggshell quality are dependant on dietary Ca and P levels. Most research suggests that SZA enhances Ca utilization, reduces P utilization, or both. Due to the complex relationship between Ca and P, it is difficult to separate the effects of one from the other. In addition, the varied effects that have been reported and the inconsistency of results suggest that a combination of factors involving mechanisms not directly related to dietary Ca or P may also exist.

Although there are numerous studies reporting the effects of feeding SZA and other synthetic and natural occurring zeolites, no clear mode of action has been proposed. The present authors suggest that there are several concurrent factors involved. These data and those of others demonstrate that SZA influences tissue mineral distribution. Mineral metabolism and electrolyte balance can have large effects on bone and eggshell formation. It is reasonable to assume that any alterations in mineral absorption, tissue distribution, excretion, or electrolyte balance could be responsible for the reported effects of SZA on eggshell quality and bone formation. Another mode of action may be that the affinity of SZA for Ca or other cations is protecting these minerals from antagonistic factors and allowing for better utilization. Available data would seem to support a combination of mechanisms involving increased Ca utilization, decreased P utilization, and additional metabolic effects on various other minerals (Mg, Mn, Na, K, Si, and Zn).

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