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

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ABSTRACT  Sodium zeolite A (SZA), a synthetic sodium aluminosilicate having a high ion exchange capacity, has been shown to influence Ca and P utilization in chickens. A $3 \times 2 \times 2$ factorial arrangement of treatments was used to investigate the effect of dietary P (.41, .55, and .69% total P), Ca (.6 and 1%), and SZA (0 and .75%) on growth, plasma, and tibia characteristics of chicks from 5 to 15 days of age. Growth, feed intake, gain:feed ratio, and tibia characteristics were influenced by dietary Ca and P in a manner consistent with dietary recommendations for these macro minerals. The addition of Ca, SZA, or both exacerbated the adverse effects of feeding low-P diets, yet alleviated the adverse effects of feeding a low-Ca, high-P diet. Dietary SZA had no effect ($P > .5$) on plasma Ca or alkaline phosphatase; however, SZA reduced ($P < .01$) plasma P. Dietary SZA increased ($P < .02$) tibia Mn, Zn, Cu, and Al. The SZA-induced increase in tibia Al was most evident in chicks fed low levels of P (SZA by P interaction, $P < .02$). The overall response to dietary SZA addition paralleled the response observed from Ca supplementation, indicating that SZA increased Ca utilization, reduced P utilization, or contributed to both of these effects. These data demonstrate that the effects of SZA are influenced by the dietary concentration of Ca and P and that the addition of SZA to diets low in P results in bone Al accumulation.

(Key words: zeolite, chicks, calcium, phosphorus, aluminum)


INTRODUCTION

The use of zeolites in animal agriculture has been investigated for many years (Mumpton and Fishman, 1977). Although there are numerous naturally occurring zeolites, sodium zeolite A (SZA$^4$) is a synthetic hydrated sodium alumino-silicate with a crystalline lattice structure (molecular formula; $\text{Na}_12\text{(AlO}_2\text{)}_12\text{(SiO}_2\text{)}_12\cdot27\text{H}_2\text{O}$). Calcium, Zn, Mg, K, and other biologically significant cations can exchange readily with the Na associated with SZA without disrupting its molecular structure (Breck, 1974).

Sodium zeolite A has been reported to affect eggshell quality, egg production, growth performance, Ca absorption, femur medullary development, bone composition and strength, P utilization, and incidence of tibial dyschondroplasia. These effects have been attributed to the ion exchange properties of SZA, the Al associated with SZA, or both of these factors combined. Ballard and Edwards (1988), Ingram et al. (1989), and Watkins et al. (1989) reported that SZA increased the absorption of Ca, increased medullary bone development, and accentuated the adverse effects of excess Ca, respectively. However, Edwards (1988), Fethiere et al.

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(1990), and Moshtaghian et al. (1991) reported that SZA affected P nutriture of chickens, and Watkins and Southern (1991), along with others (Fethiere et al., 1990; Roland et al., 1990; Latshaw and Turner, 1991; Moshtaghian et al., 1991), speculated that at least a portion of the effects of SZA may be due to its influence on P utilization by chickens.

Therefore, the present study was conducted to further evaluate the effect of feeding SZA when both dietary Ca and P vary. The influence of dietary P, Ca, and SZA and their interactive effects on growth, plasma, and mineral concentrations, density, and shearing force of tibia were evaluated.

MATERIALS AND METHODS

Unsexed Peterson × Arbor Acres broiler chicks from a commercial hatchery were used in the present investigation. From hatching to 4 days posthatching, all chicks were fed a corn and soybean meal basal diet (Table 1) without supplemental Ca or P. The pretest diet was formulated to contain 0.41% total P (0.16% nonphytate P; National Research Council, 1984) and 0.6% Ca. After an overnight fast, chicks were weighed and randomly assigned to 1 of 12 experimental diets on the basis of body weight (average initial weight was 80.4 g).

Chicks were provided continuous fluorescent lighting and penned in heated, thermostatically controlled (mean temperature was 35 C) starter batteries with raised wire floors. Four replicates of five chicks each were assigned to each treatment and fed their 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 and P, which were provided at 60 and 36% of the requirements (National Research Council, 1984), respectively. Dietary treatment additions were made to the basal diet at the expense of silica flour.

Treatments were arranged as a 3 × 2 × 2 factorial consisting of three levels of total dietary P (0.41, 0.55, and 0.69%), two levels of dietary Ca (0.6 and 1.0%), and two levels of dietary SZA (0 and 0.75%). Dietary P levels were attained by supplementing the basal diet (0.41% total P) with 0, .53, and 1.06% ammonium phosphate monobasic (26.93% P) providing 0, .14, and .28% total P, respectively. The diets were calculated (National Research Council, 1984) to contain 0.16, 0.31, and 0.45% nonphytate P. Dietary Ca levels were attained by supplementing the basal diet (0.6% Ca) with 0 and 1.0% limestone, providing 0 and 0.4% Ca, respectively.

At the termination of the experiment, individual chicks were weighed and penned. Feed consumption was determined. A blood sample (2 mL) was taken from each chick via cardiac puncture at trial termination. Calcium (o-cresolphthalein method), inorganic P (Daly and Ertingshausen, 1972), and alkaline phosphatase (Bowers and McComb, 1966) concentrations were determined on fresh plasma using an automated Gilford System 203 clinical chemistry analyzer.

After bleeding, the chicks were killed by cervical dislocation and both tibiae removed for subsequent analyses. 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 were dried in a forced-air oven at 90 C, weighed, and shearing force was determined using a Universal Instron Breaking Machine. 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. The Ca, Cu, and Zn content of the ash was determined by flame atomic absorption spectroscopy. P content was determined using an automated molybdovanadate method (Association of Official Analytical Chemists, 1984), and Mn and Al were
TABLE 1. Dietary composition

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<thead>
<tr>
<th>Ingredients</th>
<th>Percentage</th>
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<td>Corn</td>
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<td>Soybean meal (44% CP)</td>
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<td>Alfalfa leaf meal</td>
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<td>Vitamin mix</td>
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<tr>
<td>DL-methionine</td>
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<td>Dietary treatment additions</td>
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1Calculated composition (National Research Council, 1984) of the basal diet: crude protein, 23%; lysine, 1.37%; methionine, .52%; cystine, .37%; metabolizable energy, 3,000 kcal/kg; Ca, .6%; total P, .41% (.16% nonphytate P).

2Roche 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 B₁₂, 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.

3Ammonium phosphate monobasic (26.93% P) and limestone were added at the expense of silica flour in order to provide the desired dietary Ca and P levels. Six diets containing the six different combinations of Ca and P were mixed initially. These diets were then divided and either .75% sodium zeolite A (SZA) or .75% silica flour was added to provide the 0 and .75% SZA diets. Dietary Ca and total P levels were confirmed with actual chemical analyses and did not differ from calculated levels.

RESULTS

Multiple main effects and interactions were present for several of the criteria studied; however, in some cases only the highest order interactions or pertinent, biologically significant main effects are discussed. All data are presented in graphic form to facilitate the interpretation of multiple interactions. Readers are referred to Tables 2 and 3 for a complete summary of all data and statistics, respectively.

Gain, feed intake, and gain:feed ratios were affected by dietary P, Ca, and SZA and by the interaction of these dietary additives (Figure 1). Incremental additions of P to the basal diet improved (P < .01) gain, feed intake, and gain:feed ratios of
<table>
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<tr>
<th>Response variables</th>
<th>P, %</th>
<th>Ca, %</th>
<th>SZA, %</th>
<th>Ca:P ratio</th>
<th>Gain, g/14 days</th>
<th>Feed intake, g/14 days</th>
<th>Gain:feed, g/kg</th>
<th>Plasma Ca, mg/dL</th>
<th>Plasma P, mg/dL</th>
<th>Plasma AP, IU/L</th>
<th>Tibia ash, %</th>
<th>Tibia density, g/cm³</th>
<th>Tibia shearing force, kg</th>
<th>Tibia ash composition, %</th>
<th>Ca, %</th>
<th>P, %</th>
<th>Cu, µg/g</th>
<th>Zn, µg/g</th>
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1 SZA = sodium zeolite A.
2 AP = alkaline phosphatase.

TABLE 2. Treatment means and standard errors for all response variables.
TABLE 3. Probability values for all main effects and interactions

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<th>Variable</th>
<th>SZA&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P lin&lt;sup&gt;2&lt;/sup&gt;</th>
<th>P quad&lt;sup&gt;3&lt;/sup&gt;</th>
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<th>Ca x SZA</th>
<th>P lin x SZA</th>
<th>P quad x SZA</th>
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<sup>1</sup>SZA = sodium zeolite A.
<sup>2</sup>P lin = P linear effect.
<sup>3</sup>P quad = P quadratic effect.
<sup>4</sup>AP = alkaline phosphatase.

chicks fed diets supplemented with SZA or Ca. This improvement in performance was greater between .41 and .55% P than between .55 and .69% P (quadratic, P < .01), especially in gain:feed ratio. The incremental addition of P to diets without supplemental Ca and SZA also resulted in a quadratic response in these performance variables. The .55% P level improved gain, feed intake, and gain:feed ratio compared with the .41% P level. However, the subsequent addition of .15% P to the .55% P diet reduced these performance variables (P linear by SZA by Ca, P < .01).

The incremental addition of P to the basal diet linearly reduced plasma Ca in chicks fed 1% Ca (Figure 2), but dietary P had no effect on plasma Ca levels of chicks fed .6% Ca (P linear by Ca, P < .02). Dietary SZA had no effect (P > .9) on plasma Ca.

Dietary P supplementation generally increased (P < .01) plasma P (Figure 2). However, the increase in plasma P was dependent on the level of P in the diet and on the presence of SZA, Ca, or both (P quadratic by SZA by Ca, P < .01). Without SZA or additional Ca in the diet, plasma P was maximized at the .55% dietary P level and no further increase in plasma P was observed at the .69% level. With only SZA in the diet, plasma P was increased slightly at the .55% dietary P level and was maximized at the .69% level. With only the additional Ca in the diet, plasma P was not increased at the .55% dietary P level but was maximized at the .69% P level. With the combination of SZA and Ca in the diet, plasma P was not increased at the .55% dietary P level, and although plasma P was increased at the .69% P level, it was not maximized.

Plasma alkaline phosphatase was not affected (P > .5) by either SZA or Ca (Tables 2 and 3). Plasma alkaline phosphatase decreased (P < .06) nonsignificantly as dietary P level increased (2,995, 2,659, and 2,458 IU/L for the .41, .55, and .69% dietary P levels, respectively).

Tibia ash, density, and shearing force responded similarly to dietary treatment (Figure 3) and not unlike the response observed with plasma P. Dietary P supplementation linearly increased (P < .01) each
of these bone parameters. The addition of Ca, SZA, or both decreased tibia ash and density in chicks fed diets containing .41 or .55% P. However, the addition of Ca, SZA, or both increased tibia ash and density in chicks fed .69% P (P linear by Ca by SZA, P < .01). Dry, fat-free shearing force followed trends similar to that observed in tibia ash and density (P linear by Ca by SZA, P < .07). Although both dry, fat-free, and fresh tibia shearing force responded similarly to dietary treatment, approximately 11 kg (47%) more force was required to shear fresh tibia than to shear dry, fat-free tibia.

Dietary Ca had no effect (P > .8) on the Ca content of tibia ash (Figure 4). The incremental addition of P to the basal diet linearly decreased the Ca content of tibia ash in chicks not fed SZA but increased tibia Ca in chicks fed SZA (P linear by SZA, P < .01). This interaction also demonstrated that SZA decreased tibia Ca in chicks fed low-P diets. Phosphorus supplementation tended to increase tibia P in chicks fed .6% Ca without added SZA and in chicks fed 1.0% Ca regardless of SZA addition. However, dietary P supplementation decreased tibia P in chicks fed .6% Ca with added SZA (P linear by Ca by SZA, P < .03). Tibia Ca and P content

FIGURE 1. Growth (A), feed intake (B), and gain: feed ratio (C) ± SEM of chicks fed graded levels of P (.41, .55, and .69%) with: .6% Ca without SZA (sodium zeolite A; o), 1% Ca without SZA (●), .6% Ca with SZA (▴), or 1% Ca with SZA (▼).

FIGURE 2. Plasma Ca (A) and P (B) content ± SEM of chicks fed graded levels of P (.41, .55, and .69%) with: .6% Ca without SZA (sodium zeolite A; o), 1% Ca without SZA (●), 6% Ca with SZA (▴), or 1% Ca with SZA (▼).
FIGURE 3. Tibia ash content (A), tibia density (B), fresh shearing force (C), and dry, fat-free shearing force (D) ± SEM of chicks fed graded levels of P (.41, .55, and .69%) with: .6% Ca without SZA (sodium zeolite A; ○), 1% Ca without SZA (●), .6% Ca with SZA (▽), or 1% Ca with SZA (▼).

expressed as a percentage of dry, fat-free tibia (as opposed to tibia ash), mimicked the treatment responses observed for tibia ash and density (data not shown).

Selected trace mineral composition of tibia ash is presented in Figure 5. Tibia ash Cu, Zn, and Mn concentrations were increased ($P < .01$) by SZA, but incremental addition of dietary P reduced ($P < .01$) these trace elements. The dietary P-induced decreases in tibia Cu and Mn concentrations were greater in chicks fed SZA than in those not fed SZA ($P$ linear by SZA, $P < .01$) and this effect was most evident with the initial supplementation of P to the basal diet. A similar trend was observed for tibia Zn concentration ($P$ quadratic by SZA, $P < .07$). Dietary Ca did not affect ($P > .7$) tibia Cu and Zn concentrations. Dietary Ca increased tibia Mn in chicks not fed SZA but decreased tibia Mn in chicks fed SZA (Ca by SZA, $P < .02$).

Dietary P supplementation linearly decreased ($P < .01$) tibia ash Al content, but dietary SZA increased ($P < .02$) tibia Al content (Figure 5). The SZA-induced increase in tibia Al content was much greater in chicks fed .41% P than in those fed higher levels of P ($P$ linear by SZA, $P < .02$).

DISCUSSION

Gain, feed intake, and gain:feed ratio followed expected trends related to Ca, P, and the Ca:P content of the respective dietary treatments (Table 2). These data support the generally recognized recommendations for Ca, P, and Ca:P ratio recently reviewed by Waldroup (1989). As expected, the adverse effects of feeding a low-P diet were exacerbated by Ca supplementation. The adverse effects observed from supplementing low-P diets with Ca were mimicked by SZA. The data of Moshtaghian et al. (1991) showed that chicks fed P-deficient diets with added SZA required more supplemental P to maximize weight gain and feed efficiency than chicks fed P-deficient diets without SZA. The present study showed that feeding a diet deficient in P depressed growth and feed efficiency. Moreover, this
Plasma alkaline phosphatase levels decreased nonsignificantly as plasma and dietary P increased. An inverse relationship between P and alkaline phosphatase has been reported previously (Njoku et al., 1980; Boyd et al., 1983). The SZA-induced reduction in plasma alkaline phosphatase previously reported (Watkins and Southern, 1991) was not observed in the present study.

The influence of dietary treatment on tibia ash and density was nearly identical to that observed for gain and feed efficiency, indicating that these bone characteristics also responded to alterations in dietary Ca, P, and Ca:P ratio. The addition of SZA, supplemental Ca, or both of these ingredients exacerbated the adverse effects of feeding low-P diets but alleviated the adverse effects of feeding high-P, low-Ca diets. Edwards (1988) also reported that the addition of SZA to diets low in P reduced bone ash.

In the present study, 43% less force was required to shear dry, fat-free tibia compared with fresh tibia; however, treatment responses were nearly identical between the two processing methods. Lott et al. (1980) also reported that drying bones reduced breaking strength by approximately 50%. However, Orban et al. (1991) reported that processing method had no effect on bone mineral content, density, and strength, and in a previous study in the authors’ laboratory (Watkins and Southern, 1991) only 4% more force was required to break fresh tibia compared with dry, fat-free tibia. Because identical procedures were used to prepare and shear bones, the differences between these two studies remain unexplained.

The increased tibia Mn content of chicks fed SZA was observed previously (Watkins and Southern, 1991). In addition, the present study demonstrated that tibia Mn content was inversely related to die-

| FIGURE 4. Tibia ash Ca (A) and P (B) content ± SEM of chicks fed graded levels of P (.41, .55, and .69%) with: .6% Ca without SZA (sodium zeolite A; ○), 1% Ca without SZA (■), .6% Ca with SZA (◇), or 1% Ca with SZA (◆).

Effect was exacerbated by the dietary addition of SZA, Ca, or the combination of both, indicating that SZA either inhibited P utilization or enhanced Ca utilization.

Although SZA had no effect on plasma Ca in the current study and in previous studies in the authors’ laboratory (Ward et al., 1990; Watkins and Southern, 1991), SZA has been shown to increase plasma Ca (Roland et al., 1989; Leach et al., 1990). The incremental addition of P linearly reduced plasma Ca in chicks fed 1% Ca but had no effect on chicks fed .6% Ca. Plasma Ca was directly related to dietary Ca:P ratio, although all chicks fed diets with a Ca:P ratio below 1.6 had similar plasma Ca levels (Table 2).

The supplementation of the basal diet with P increased plasma P in a manner similar to that reported by Gardiner (1962). The addition of SZA, Ca, and both SZA and Ca to diets containing .55% P reduced plasma P and this effect was most evident when SZA was added to diets containing 1% Ca. Others also have reported that SZA reduces plasma P (Leach et al., 1990; Ward et al., 1990; Watkins and Southern, 1991).
Dietary P. Wedekind and Baker (1990) also reported that P depresses Mn utilization. Therefore, the increased tibia Mn observed in chicks fed SZA could merely be a function of less P available to inhibit Mn utilization.

The increase in tibia Zn observed in chicks fed SZA has been described previously by Ward et al. (1990) and Watkins and Southern (1991), and Chiang and Yeo (1983) reported that SZA increased Zn utilization in 6-wk-old broilers. However, Laurent et al. (1991) reported that 6-wk-old broilers receiving SZA had lower liver Zn levels. Zinc is ranked first on the cation selectivity profile of SZA and SZA is known to exchange associated cations with Zn (Breck, 1974). Therefore, the binding of Zn by intact SZA may have allowed for enhanced absorption. Tibia Cu was increased in chicks fed SZA and this effect was most evident in chicks fed low levels of P. Copper is not listed in the divalent selectivity profile of SZA. However, binding of Zn, a divalent cation known to antagonize Cu utilization (Davis and Mertz, 1987), by SZA might have allowed for better Cu utilization. Interactions among SZA, Ca, phytate P, and various trace minerals also may have contributed to the observed treatment effects on tibia Zn and Cu.

The accumulation of Al in tibia of chicks fed SZA suggests that at least some of the Al associated with SZA is absorbed. Chicks fed low levels of P had the greatest SZA-induced increase in tibia Al, but chicks fed recommended (National Research Council, 1984) Ca and P levels did not accumulate Al. 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 is hydrolyzed to various silicate and aluminate compounds at pH 5 or less (Cook et al., 1982). Such components in the intestine of the chick would be available to influence the utilization of other nutrients or to be absorbed. The fact that increasing amounts of Al were retained in the tibia of chicks fed decreasing amounts of P further supports the theory that the effects of SZA

![FIGURE 5. Tibia ash Cu (A), Mn (B), Zn (C), and Al (D) concentrations ± SEM of chicks fed graded levels of P (.41, .55, and .69%) with: .6% Ca without SZA (sodium zeolite A; O), 1% Ca without SZA (●), .6% Ca with SZA (V), or 1% Ca with SZA (▼).](image-url)
on mineral utilization and bone and eggshell formation might be mediated through the well-documented antagonism between Al and P (Hussein et al., 1989; Rossi et al., 1990). In P-supplemented diets, more P would be available to bind with Al, possibly creating insoluble aluminophosphate compounds less available for absorption. Without excess P present in the gut of chicks fed low levels of P, more of the Al liberated during the gastric hydrolysis of SZA could be available for absorption.

In addition to the release of Al, Si(OH)₄ is formed during the hydrolysis of SZA and Si has been proposed to be a basis for activity (Raban et al., 1991; Wiegand et al., 1991). Carlisle demonstrated that Si is required for normal growth (Carlisle, 1972) and bone and cartilage formation (Carlisle, 1980) in chicks, and has suggested that dietary Si is involved with P during bone formation and influences the Ca:P ratio of the bone (Carlisle, 1986).

Although the present data support reports that the effects of SZA are influenced by dietary Ca, P, or the combination of both (Roland et al., 1985; Edwards, 1988; Watkins et al., 1989; Leach et al., 1990; Watkins and Southern, 1991), no mode of action has been proposed that would completely explain the reported effects of SZA on growth, bone formation, eggshell quality, and tissue mineral partitioning. The varied and often conflicting reports suggest that SZA has more than one biological influence. The effects of various Si and Al compounds formed during the hydrolysis of SZA or the intact molecular binding properties of SZA have the most potential for involvement. Knowing to what extent SZA breaks down in the gastrointestinal tract of the chicken would be beneficial in elucidating these mechanisms. If little or none of the SZA molecule is left intact, then the ion exchange properties would probably not be the basis of activity and either biological effects attributed to Si or Al, or their interaction with other luminal components would likely be responsible. The paralleled response observed in the current study between Ca supplementation and SZA inclusion supports reports that SZA either increases Ca utilization, decreases P utilization, or both. Although it is difficult to differentiate the effects of one from the other, the increased tibia Al accumulation in chicks fed low-P diets supports the theory that Al from SZA decreases P utilization.

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