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**Abstract**

Commercial broiler producers and nutritionists have questioned the performance consequences of sulfur (S) from various dietary and water sources combined in current commercial production. The combination of high-S feed ingredients, including dried distillers grains with solubles, and dietary additives that contain S, such as lysine sulfate or copper sulfate, has the potential to create high S exposure, especially when combined with high-S drinking water. The tolerance of growing broiler chicks to S was determined by supplementation of a corn-soybean-5% dried distillers grains with solubles diet with up to 1% lysine sulfate or an equal amount of S from sodium sulfide. An additional diet containing copper and zinc sulfate served as a positive control for the source of S and high-S inclusion. These diets were fed to chicks provided with normal (0.008% or 80 ppm) or high water S (0.113% or 1130 ppm). We hypothesized that the addition of S sources to a commercial diet would not reduce the performance of growing chicks given access to normal or high-S water. Data showed dietary S requirements were met and excess S was easily excreted, hence, under the experimental feeding conditions, supplementation with up to 1% additional lysine sulfate (or a similar product) did not reduce performance in comparison with chicks fed a lower S diet with access to normal or high-S water. The high-S diet from copper and zinc sulfate resulted in reduced water and feed consumption, although there were no effects on chick weight gain.



**Keywords**

broiler, distillers dried grains with solubles, lysine sulfate, sulfur toxicity


**Disciplines**

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**Comments**


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# Supplemental lysine sulfate does not negatively affect the performance of broiler chicks fed dietary sulfur from multiple dietary and water sources

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**Primary Audience:** Producers, Industry, Nutritionists

## SUMMARY

Commercial broiler producers and nutritionists have questioned the performance consequences of sulfur (S) from various dietary and water sources combined in current commercial production. The combination of high-S feed ingredients, including dried distillers grains with solubles, and dietary additives that contain S, such as lysine sulfate or copper sulfate, has the potential to create high S exposure, especially when combined with high-S drinking water. The tolerance of growing broiler chicks to S was determined by supplementation of a corn-soybean-5% dried distillers grains with solubles diet with up to 1% lysine sulfate or an equal amount of S from sodium sulfide. An additional diet containing copper and zinc sulfate served as a positive control for the source of S and high-S inclusion. These diets were fed to chicks provided with normal (0.008% or 80 ppm) or high water S (0.113% or 1130 ppm). We hypothesized that the addition of S sources to a commercial diet would not reduce the performance of growing chicks given access to normal or high-S water. Data showed dietary S requirements were met and excess S was easily excreted, hence, under the experimental feeding conditions, supplementation with up to 1% additional lysine sulfate (or a similar product) did not reduce performance in comparison with chicks fed a lower S diet with access to normal or high-S water. The high-S diet from copper and zinc sulfate resulted in reduced water and feed consumption, although there were no effects on chick weight gain.

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## DESCRIPTION OF PROBLEM

The composition of commercial poultry diets may change depending on the price of ingredients and, recently, the increasing production of corn-derived ethanol has created a surplus of the by-product, dried distillers grains with solubles (**DDGS**). In 2012, the United States produced 34.4 million t of DDGS for use in livestock feed, 8% of which was used in poultry diets [1]. As corn DDGS contains all of the nutrients from the grain in a concentrated form (except most of the starch), DDGS is a rich source of CP, amino acids, phosphate, and other nutrients [2–4]. Corn naturally contains from 0.03 to 0.43% (300–4,300 ppm) sulfur (S) [5] and the fermentation

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and removal of starch from the corn during the ethanol production process concentrates that S to approximately 0.21 to 1.93% (2,100–19,300 ppm) [6, 7]. Other variables that can affect the final S content of DDGS are protein content, yeast, water source, and $H_2SO_4$ addition. For example, on an as-fed basis, sulfur content ranged from 0.3 to 1.0% when DDGS from separate sources were analyzed [6]. Additionally, S content in poultry drinking water varies across the United States. Average drinking water content is around 32 ppm, and 2,500 ppm is reported as the maximum acceptable level before high-S water causes management problems; 2,500 to 3,500 ppm is considered poor water for poultry, and 3,500 ppm and above is unacceptable [8].

Data on poultry water source S contamination are scarce; however, a USDA Animal and Plant Health Inspection Service study reported water S data from 263 cattle feedlots in 10 states (including CA, CO, ID, IA, KS, NE, NM, SD, TX, and WA). A mean concentration of 205 ppm was reported, with the lowest mean in Idaho (29 ppm) and the highest mean in South Dakota (1,007 ppm) [9]. On a total percentage basis, 77% of farms were <299 ppm, 15% were 300 to 999 ppm, 7% were 1,000 to 1,500ppm, and 1% were >1,501 ppm. A study of 54 swine farms in Ohio reported 6 to 1,629 ppm, with a mean of 232 ppm, and found a positive correlation with increased depth of well and higher sulfate concentrations, as well as higher water sulfate in the northern and western regions of Ohio [10]. Concerns in commercial poultry production regarding dietary S toxicity have arisen due to simultaneous high-S feed inputs (DDGS, lysine supplements, water) and the variable S content of these inputs.

A question about S levels in finished feeds containing lysine sulfate or lysine hydrochloride has arisen. Lysine sulfate ($[C_6H_{14}N_2O_2]2 \cdot H_2SO_4$; molecular weight = 390.4 g/mol) typically contains 8.2% S (molecular weight = 32.06). Therefore, at a 0.6% dietary inclusion rate of lysine sulfate, which is on the high end of inclusion in broiler diets today, the contribution of S is 0.05% (500 ppm) versus diets supplemented with lysine hydrochloride. Although typical feed ingredients such as corn, soybean meal, and DDGS contain lower S than lysine sulfate, they contribute over 70% of total feed S levels due to higher inclusion levels. The NRC [5] states that S toxicity in chicks arises at 14,000 ppm (reduced growth) and in laying hens at 8,100 ppm (reduced egg production). These results are based on a study where chicks were fed a purified basal diet containing up to 0.41% (4,100 ppm) S with 1.2% (12,000 ppm) added S, and growth was significantly reduced ($P < 0.01$) [11]; and 2 studies where hens were supplemented with up to 2% (20,000 ppm) and 1.2% (12,000 ppm) sodium sulfate in the drinking water, respectively, resulting in mortality and reduced egg production [12, 13]. These reports were generated over 40 years ago and current literature on multiple-source S toxicity is scarce. Therefore, the objective of this study was to determine the effects of multiple sources of dietary S on broiler chick performance using a commercial type diet fed in combination with normal or high-S water.

## MATERIALS AND METHODS

### *Chicks and Experimental Diets*

All experimental protocols were approved by the Iowa State Animal Care and Use Committee before experimentation. Four hundred and eighty female Ross 708 broiler chicks [14] were received and placed into battery cages with a 23L:1D cycle with water and diet provided ad libitum (formulated to meet or exceed industry-based nutrient requirements). Chicks were fed a nutritionally complete chick mash based on NRC guidelines [5] until d 4, when chicks were weighed, sorted, wing-banded, and randomly assigned to cages (5 chicks/cage with 6 replicate cages, 105 $in^2$ allotted per bird) for experimental treatments (average d 4 weight = 74.8 g). Experimental diets were arranged in a 2 × 8 factorial based on 2 levels of sulfate inclusion in water (normal or high S) and 8 separate corn-soybean + DDGS + supplemental sulfur source diets: control (no added S); 0.25%, 0.50%, or 1.0% of supplemental lysine-sulfate (0.014, 0.028, 0.056% supplemental S, or 140, 280, and 560 ppm, respectively); 0.03%, 0,7%, or 0.14% sodium sulfide (0.014, 0.028, and 0.056% supplemental S, respectively); or 0.077% (770 ppm) supplemental S from copper and zinc sulfate (Table 1). Lysine content of the diet was kept constant by supplementing all diets with lysine hydrochloride to match the diet supplemented with 1% lysine sulfate. For example, control and copper and zinc sulfate diets both contained 0.64% supplemental lysine from lysine hydrochloride to reach constant dietary lysine content across all diets.

Individual chick BW was measured and recorded at chick d 4 and 18, and the difference was used to calculate BW gain. The difference between feed offered (feed weight on d 4) and feed refused (feed weight on d 18) was calculated as cage feed intake. Feed conversion ratio was calculated as total cage feed intake divided by total cage weight gain including weight gain of chicks that died over the duration of the feeding period. Experimental water was provided via individual trough waterers. Water consumption was estimated daily as the difference between water provided and water refused, corrected by waterers in the same environment without chick access to account for evaporative losses. Excreta samples were collected from clean pans inserted under raised-wire cages between d 13 and 14 of the experiment for DM and S digestibility (cage basis) [15]. Up to 5 mL of blood was collected via heart puncture from 1 chick per replicate cage on d 14 for serum S determination. Digestibility of S was modified from the phosphate digestibility method as described in "Macro and micro elements in plant tissues by ICPAES" [16]. Total blood S was analyzed using a thermal combustion analyzer [17], which uses catalytic tube combustion to volatilize the sample. The target gas is converted to $SO_2$, separated from other gases using adsorption columns, and, after

**Table 1.** Experimental diets[1] formulated to contain various concentrations of sulfur (S) from different dietary sources of sulfur[2]

| Item | CON | 0.25% LS | 0.50% LS | 1.00% LS | 0.03% $Na_2S$ | 0.07% $Na_2S$ | 0.14% $Na_2S$ | Cu and Zn $SO_4$ |
|---|---|---|---|---|---|---|---|---|
| Ingredient (%) | | | | | | | | |
| Corn | 54.85 | 54.94 | 54.97 | 55.20 | 54.85 | 54.85 | 54.85 | 54.85 |
| Soybean meal (48%) | 29.60 | 29.53 | 29.52 | 29.31 | 29.60 | 29.60 | 29.60 | 29.60 |
| Distillers dried grains with solubles | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Meat and bone meal | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Animal-vegetable fat | 3.34 | 3.23 | 3.13 | 2.92 | 3.34 | 3.34 | 3.34 | 3.34 |
| Dicalcium phosphate | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Limestone | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Salt | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Choline chloride (60%) | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin and mineral premix[3] | 0.625 | 0.625 | 0.625 | 0.625 | 0.625 | 0.625 | 0.625 | 0.625 |
| Titanium marker | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Acid insoluble ash | 0.695 | 0.695 | 0.685 | 0.695 | 0.665 | 0.625 | 0.555 | 0.00 |
| DL-Methionine | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 |
| L-Threonine | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Lysine·HCl | 0.64 | 0.48 | 0.32 | 0.00 | 0.64 | 0.64 | 0.64 | 0.64 |
| Lysine sulfate | 0.00 | 0.25 | 0.50 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $Na_2S$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.07 | 0.14 | 0.00 |
| $CuSO_4$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.10 |
| $ZnSO_4$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.60 |
| Nutrient analysis | | | | | | | | |
| Added S (%) | 0.00 | 0.014 | 0.028 | 0.056 | 0.014 | 0.028 | 0.056 | 0.077 |
| Dietary S (%) | 0.20 | 0.21 | 0.23 | 0.25 | 0.21 | 0.23 | 0.25 | 0.28 |
| S percentage (analyzed) | 0.27 | 0.29 | 0.32 | 0.34 | 0.27 | 0.29 | 0.30 | 0.36 |

[1]CON = control diet without supplemental S; 0.25% LS = CON + 0.25% lysine sulfate (0.014% S addition, 140 ppm); 0.50% LS = CON + 0.50% lysine sulfate (0.028% S addition, 280 ppm); 1.00% LS = CON + 1.00% lysine sulfate (0.056% S addition, 560 ppm); 0.03% $Na_2S$ = CON + 0.03% sodium sulfide (0.014% S addition, 140 ppm); 0.07% $Na_2S$ = CON + 0.07% sodium sulfide (0.028% S addition, 280 ppm); 0.14% $Na_2S$ = CON + 0.14% sodium sulfide (0.056% S addition, 560 ppm); Cu and Zn $SO_4$ = CON + 0.10% Cu sulfate and 0.60% Zn sulfate (0.077% S addition, 770 ppm).

[2]All 8 diets were repeated factorially with normal or high-sulfur water.

[3]Vitamin and mineral premix added at 0.625% of the diet supplied the following per kilogram of diet: 250 μg of selenium; 8,250 IU of vitamin A; 2,750 IU of vitamin $D_3$; 17.9 IU of vitamin A; 1.1 mg of menadione; 12 μg of vitamin $B_{12}$; 41 μg of biotin; 447 mg of choline; 1.4 mg of folic acid; 41.3 mg of niacin; 11 mg of pantothenic acid; 1.1 mg of pyridoxine; 5.5 mg of riboflavin; 1.4 mg of thiamine; 282 mg of iron; 125 mg of magnesium; 275 mg of manganese; 275 mg of zinc; 27.5 mg of copper; 844 μg of iodine.

heating, measured using a thermal conductivity detector. Sulfadazine was used as the S standard.

### Dietary and Water S Content

Diets were formulated to add 0.25%, 0.50, or 1.0% lysine sulfate or 0.03, 0.07, or 0.14% sodium sulfide for an equivalent final added sulfur of 0.014, 0.028, or 0.056% S for both sources of S. Copper and zinc sulfate were added at concentrations used commercially for antimicrobial purposes (255 ppm of copper and 1,363 ppm of zinc) [18], and they were combined to maximize the increased S content of the diet. All diets were analyzed for S content after preparation and before feeding to chicks [16]. Final analyzed diets were as follows: the control diet contained 0.27% S and the addition of lysine sulfate increased the S content to 0.29, 0.32, and 0.34%, respectively, whereas the addition of sodium sulfide increased S content to 0.27, 0.29, and 0.30%, respectively. Copper and zinc sulfate addition resulted in 0.36% dietary S (Table 1). Water was obtained from 2 different sources, with one known to have a higher S content. The normal water source was analyzed and contained 0.008% (80 ppm) S and the high-S source contained 0.113% (1,130 ppm) S.

### Statistical Analysis

Data were analyzed using JMP statistical software [19] using a 2 × 8 factorial arrangement in a completely randomized design. Body weight gain, feed intake, FCR, and water consumption were determined over the 4 to 18-d experimental period with group cage means representing an experimental unit. Sulfur analysis and digestion were pooled samples with cage as the experimental unit. An ANOVA was performed followed by Student's *t*-test, where data were considered significantly different if $P < 0.05$.

## RESULTS AND DISCUSSION

### Dietary and Water S Content

Literature on S toxicity in chicks are scarce, and these data are especially critical in current commercial conditions where inputs such as DDGS vary in S content and the diet contains several sources of S (main ingredient, supplements, and water S). Recommendations for S toxicity in the NRC originate from 1 chick study and 1 hen study completed in 1960 [11, 12]. More recent S toxicity data through blood profiling of Fayoumi chickens (1, 2, 3, and 4% S added to diet, respectively, from 56 to 140 d of age) indicate a significantly reduced hemoglobin concentration, packed cell volume, leukocytosis, heterophilia, and lymphocytosis in birds supplemented with 2, 3, and 4% versus controls ($P < 0.01$) [20]. Producers and nutritionists have increasingly become concerned with possible S toxicity from multiple and variable dietary and water sources [21]. Lysine sulfate was added at up to 1% of the diet in an attempt to supplement S at higher than commercial levels [22]. A second source of S was added through sodium sulfide supplementation (up to 0.14%), and a third source, copper and zinc sulfate, was added as a high concentration control for the source of added sulfate (analyzed at 0.36% S, where maximum S from other diets was 0.34% in 1% lysine sulfate diet).

The use of supplemental L-lysine in commercial feed ranges from 0.05 to 0.30%, which equates to approximately 0.10 to 0.60 or 0.06 to 0.38 lysine sulfate or lysine hydrochloride, respectively. If producers added lysine sulfate at 0.60%, the supplementation would add only 0.048% S, which is a small contribution considering that DDGS contain variable levels of S, from 0.33 to 0.74%, and soybean meal varies from 0.35 to 0.44%, and both are included at higher levels in the diet [5, 23].

### Growth Performance

Past chick data indicate significantly reduced growth by 4 wk of age; therefore, the present study was carried out to 2 wk, with the assumption that any potential S toxicity would reduce early growth due to acceleration of growth starting by 7 d of age [24]. No significant interactions occurred between diet and water source for any of the response variables measured, and mortality was not significantly different among groups ($P > 0.05$). The addition of supplemental S to the control diet had no significant effects on weight gain, feed intake, or FCR (Table 2). Past research reported a reduced growth rate and disruption of hemoglobin content of blood when chicks were fed up to 1.61% dietary S in a purified diet, but the study did not examine other performance parameters of growing chicks [11]. The relative toxicities in chicks of various organic S compounds have been determined [25], yet these sources have not been used in combination. The results of the present study therefore address gaps in current literature, as studies within the last 20 yr have not focused on S content of the diet.

Water intake between groups was not significantly different versus control, with the exception of the addition of copper and zinc sulfate, where chicks drank 10% less water than controls ($P < 0.05$; Table 2). The effect of reduced water intake in conjunction with supplemental copper has been seen in the past [26]. The reduction in water intake associated with the high Cu and Zn sulfate diets did not significantly reduce feed intake from the control diet, but feed intake in this diet was reduced in comparison to several of the other experimental diets (with 0.50% lysine sulfate, 0.03% $Na_2S$, 0.07% $Na_2S$, and 0.14% $Na_2S$).

The high-S water source (0.113%, or 1130 ppm) did not significantly reduce feed intake, FCR, water intake, total sulfur intake per chick, sulfur retention (main effect of water S; $P > 0.05$), or serum S (Table 3), as compared with normal-S water (0.008%, or 80 ppm). High-S water significantly increased BW by 3%, which was an unexpected result ($P < 0.05$; main effect of S). Performance studies in young pigs and adult laying hens show that increased sodium sulfate in drinking water may cause significantly increased water intake and increased incidence of diarrhea, whereas magnesium sulfate may decrease water intake ($P < 0.05$) [27, 28]. In laying hens, 16,000 ppm of either magnesium or sodium sulfate was a lethal dose, as all hens receiving that level of either salt died during experimentation [27].

### *S Retention*

As expected, the addition of dietary supplemental S increased daily intake of S (Table 3). The addition of dietary S also significantly decreased the percentage of dietary S retained (ex-

**Table 2.** Main effects of broiler performance and water consumption of chicks consuming various concentrations of sulfur (S) from dietary and water sources

| Diet[1] | Water | BWG[2] (g/chick) | FI[3] (kg/cage) | FCR[4] | WI[5] (g/chick) |
|---|---|---|---|---|---|
| CON | | $439 \pm 6.3^{ab}$ | $3.08 \pm 0.04^{abc}$ | $1.42 \pm 0.02$ | $404 \pm 0.01^{ab}$ |
| 0.25% LS | | $438 \pm 13.9^{ab}$ | $3.07 \pm 0.08^{abc}$ | $1.40 \pm 0.01$ | $403 \pm 0.01^{ab}$ |
| 0.50% LS | | $456 \pm 7.0^{ab}$ | $3.12 \pm 0.07^{ab}$ | $1.40 \pm 0.02$ | $410 \pm 0.01^{ab}$ |
| 1.00% LS | | $429 \pm 10.3^{b}$ | $3.04 \pm 0.07^{bc}$ | $1.43 \pm 0.03$ | $402 \pm 0.01^{bc}$ |
| 0.03% $Na_2S$ | | $457 \pm 8.6^{ab}$ | $3.16 \pm 0.04^{ab}$ | $1.38 \pm 0.02$ | $423 \pm 0.01^{ab}$ |
| 0.07% $Na_2S$ | | $447 \pm 9.1^{ab}$ | $3.11 \pm 0.05^{ab}$ | $1.41 \pm 0.02$ | $408 \pm 0.01^{ab}$ |
| 0.14% $Na_2S$ | | $474 \pm 9.4^{a}$ | $3.31 \pm 0.04^{a}$ | $1.42 \pm 0.04$ | $443 \pm 0.01^{a}$ |
| Cu and Zn $SO_4$ | | $418 \pm 8.7^{b}$ | $2.83 \pm 0.07^{c}$ | $1.39 \pm 0.03$ | $364 \pm 0.01^{c}$ |
| | Normal S | $438 \pm 5.0^{y}$ | $3.09 \pm 0.03$ | $1.42 \pm 0.01$ | $405 \pm 0.01$ |
| | High S | $452 \pm 5.9^{x}$ | $3.09 \pm 0.03$ | $1.39 \pm 0.01$ | $408 \pm 0.01$ |
| | Analysis of variance | <0.01 | <0.01 | <0.01 | <0.01 |
| | Diet | <0.03 | <0.01 | 0.62 | <0.01 |
| | Water | <0.01 | 0.85 | <0.01 | 0.56 |
| | Diet × Water | 0.65 | 0.82 | 0.70 | 0.99 |

[a–c]Means ± SEM with the same superscript within columns are not significantly different ($P > 0.05$).

[x,y]Means ± SEM with the same superscript within columns are not significantly different ($P > 0.05$).

[1]CON = control diet without supplemental S; 0.25% LS = CON + 0.25% lysine sulfate (0.014% S addition, 140 ppm); 0.50% LS = CON + 0.50% lysine sulfate (0.028% S addition, 280 ppm); 1.00% LS = CON + 1.00% lysine sulfate (0.056% S addition, 560 ppm); 0.03% $Na_2S$ = CON + 0.03% sodium sulfide (0.014% S addition, 140 ppm); 0.07% $Na_2S$ = CON + 0.07% sodium sulfide (0.028% S addition, 280 ppm); 0.14% $Na_2S$ = CON + 0.14% sodium sulfide (0.056% S addition, 560 ppm); Cu and Zn $SO_4$ = CON + 0.10% Cu sulfate and 0.60% Zn sulfate (0.077% S addition, 770 ppm).

[2]Body weight gain per chick (grams) from 4 to 18 d.

[3]Feed intake per cage (kg/cage) from 4 to 18 d.

[4]Feed conversion ratio from 4 to 18 d.

[5]Water intake per chick (g/chick) from 4 to 18 d (WI).

**Table 3.** Main effects of sulfur (S) intake and retention of chicks consuming various concentrations of S from dietary and water sources

| Diet[1] | Water | TSI[2] (mg/d) | SR[3] (%) | TSR[4] (mg/d) | Serum S[5] (mg/dL) |
|---|---|---|---|---|---|
| CON | | $114 \pm 2.1^{f}$ | $52.5 \pm 1.49^{a}$ | $60 \pm 2.6^{a}$ | $609 \pm 17.4$ |
| 0.25% LS | | $120 \pm 2.1^{de}$ | $32.9 \pm 1.97^{c}$ | $38 \pm 2.6^{d}$ | $595 \pm 17.4$ |
| 0.50% LS | | $138 \pm 2.1^{c}$ | $32.8 \pm 1.51^{c}$ | $45 \pm 2.6^{c}$ | $587 \pm 19.4$ |
| 1.00% LS | | $142 \pm 2.1^{ab}$ | $39.2 \pm 1.68^{b}$ | $56 \pm 2.6^{ab}$ | $590 \pm 19.1$ |
| 0.03% $Na_2S$ | | $115 \pm 2.1^{ef}$ | $36.6 \pm 1.56^{bc}$ | $41 \pm 2.6^{d}$ | $576 \pm 19.1$ |
| 0.07% $Na_2S$ | | $121 \pm 2.1^{d}$ | $41.7 \pm 2.08^{b}$ | $51 \pm 2.6^{bc}$ | $622 \pm 21.3$ |
| 0.14% $Na_2S$ | | $131 \pm 2.1^{b}$ | $48.4 \pm 2.42^{a}$ | $63 \pm 2.6^{a}$ | $543 \pm 17.4$ |
| Cu and Zn $SO_4$ | | $145 \pm 2.1^{a}$ | $16.4 \pm 2.79^{d}$ | $24 \pm 2.7^{c}$ | $568 \pm 17.4$ |
| | Normal S | $127 \pm 1.05$ | $36.2 \pm 1.86$ | $45.47 \pm 1.30^{y}$ | $580 \pm 9.4$ |
| | High S | $129 \pm 1.05$ | $38.6 \pm 1.81$ | $49.23 \pm 1.28^{x}$ | $589 \pm 9.2$ |
| | Analysis of variance | <.001 | <0.01 | <0.01 | <0.01 |
| | Diet | <0.01 | <0.01 | <0.01 | 0.10 |
| | Water | 0.07 | 0.05 | 0.04 | 0.52 |
| | Diet × Water | 0.67 | 0.45 | 0.59 | <0.01 |

[a–f]Means ± SEM with the same superscript within columns are not significantly different ($P > 0.05$).

[x,y]Means ± SEM with the same superscript within columns are not significantly different ($P > 0.05$).

[1]CON = control diet without supplemental S; 0.25% LS = CON + 0.25% lysine sulfate (0.014% S addition, 140 ppm); 0.50% LS = CON + 0.50% lysine sulfate (0.028% S addition, 280 ppm); 1.00% LS = CON + 1.00% lysine sulfate (0.056% S addition, 560 ppm); 0.03% $Na_2S$ = CON + 0.03% sodium sulfide (0.014% S addition, 140 ppm); 0.07% $Na_2S$ = CON + 0.07% sodium sulfide (0.028% S addition, 280 ppm); 0.14% $Na_2S$ = CON + 0.14% sodium sulfide (0.056% S addition, 560 ppm); Cu and Zn $SO_4$ = CON + 0.10% Cu sulfate and 0.60% Zn sulfate (0.077% S addition, 770 ppm).

[2]Total sulfur intake per chick (mg/d) from 4 to 18 d.

[3]Sulfur retention percentage.

[4]Total sulfur retention per chick (mg/d) from 4 to 18 d

[5]Serum sulfur (mg/dL) at d 18.

cept in the highest $Na_2S$ supplementation), which indicated S requirements were met and excess S was excreted ($P < 0.05$) [29]. Total S retained per chick (mg/d) was reduced in all groups compared with the control for both water and dietary S source at all levels, with the exception of 1.00 and 0.14% lysine sulfate. Supplemental copper and zinc sulfate resulted in the lowest S retention on both a percentage and total basis, which may indicate a disruption of S metabolism due to higher copper and zinc concentrations in the diet compared with the other diets. Copper sulfate interacts with sulfur amino acids; at higher levels of sulfate inclusion, copper binds sulfur compounds and increases dietary sulfur amino acid requirements [30]. High-S water increased overall S retention ($P < 0.05$; main effect of S), but the effect was moderate in comparison to dietary effects.

No significant treatment differences were found between serum S samples, indicating the capacity to excrete excess S and an ability to maintain normal physiological concentrations; however, a significant diet × water interaction was seen (Table 3). In lysine sulfate-supplemented chicks, blood S tended to be numerically lower in high-S-water-supplemented chicks versus normal-S-water-supplemented chicks, whereas this trend was reversed in sodium sulfide and copper or zinc sulfate-supplemented chicks (high-S-water-supplemented chicks were numerically higher versus normal-S water). Although statistically significant for only 3 pairs of treatment groups ($P < 0.05$ for 0.25% lysine sulfate, 0.14% $Na_2S$, and Cu+Zn $SO_4$; Figure 1), it is an observation that may suggest a physiological difference between types of S supplemented. Both lysine sulfate and $Na_2S$ were added at the same total level of S (140, 280, or 560 ppm); however, chicks consuming high-S water with the highest S supplementation of $Na_2S$ (560 ppm) had significantly increased serum S ($P < 0.05$; Figure 1), whereas 560 ppm of lysine sulfate did not significantly affect serum S with either normal or high-S water ($P > 0.05$). Past hen work has noted differences in production and water consumption based on the type of sulfate supplementation, where magnesium sulfate

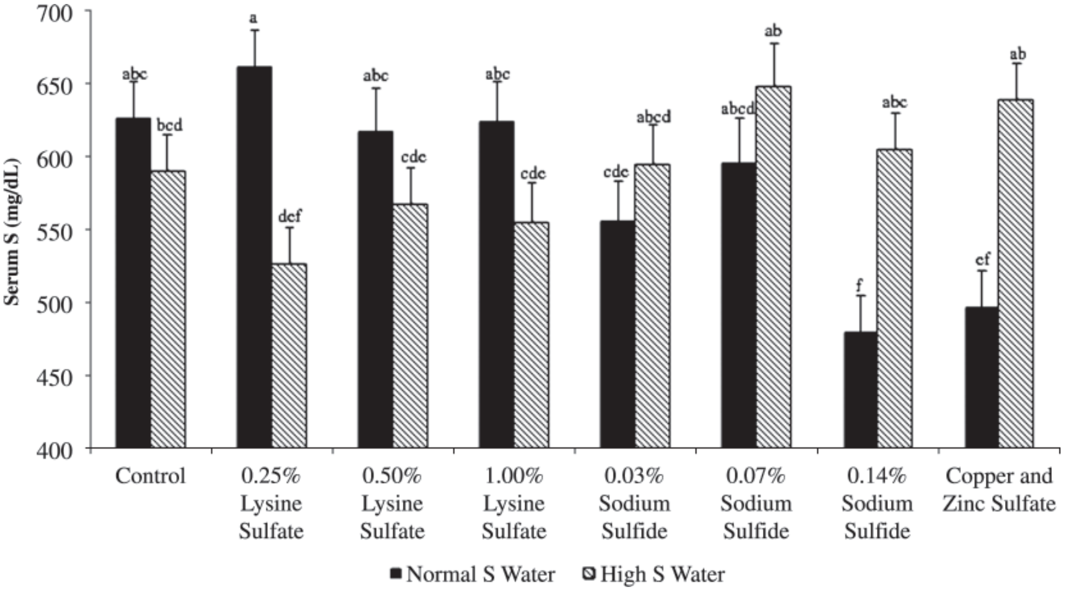


**Figure 1.** Chick serum sulfur levels (mg/dL) after 14 d on 1 of 16 combinations of dietary and water sulfur treatments (chick d 18). Results are shown as mean ± SEM for 6 chicks per diet (1 chick per replicate cage) and comparisons were made with a post-ANOVA Student's *t*-test. Normal sulfur water = 80 ppm, high sulfur water = 1,130 ppm. Differing superscript letters (a–f) denote significant difference between diets ($P < 0.05$).

tended to decrease hen-day production (versus sodium sulfate) and significantly decreased water consumption and feed intake ($P < 0.05$) [12, 27].

In the current study, chicks fed up to 560 ppm S from lysine sulfate (1.0%) or $Na_2S$ (0.14%), or 770 ppm S from Cu and Zn $SO_4$ did consume significantly more S than control ($P < 0.05$; except 140 ppm, 0.03% $Na_2S$ diet), and retained significantly more total S ($P < 0.05$; except 560ppm, 1.0% lysine sulfate and 560 ppm, 0.14% $Na_2S$), yet these chicks did not exhibit decreased BW gain or increased blood S versus control. Previous models have shown that S toxicity reduced chick growth in chicks fed 1.61% inorganic sulfate ion in a purified diet. At the levels fed in this experiment, chicks fed S from both water and dietary sources were able to metabolically and physiologically handle the excess S levels, probably through increased fecal and urinary S excretion [31].

## CONCLUSIONS AND APPLICATIONS

1. At the concentrations of S fed in this experiment, the combination of water and feed S was not found to reduce performance in growing broiler chicks.
2. Dried distillers grains and solubles were fed at a commercially relevant level (5%), hence feeding supplemental lysine sulfate at or below the concentrations found in this report (1%) should not cause reduction in performance due to S toxicity.
3. No differences were noted between lysine sulfate and sodium sulfide as a source of S.
4. The combination of zinc and copper sulfate supplemented to the diet reduced water intake and could have reduced feed intake.
5. High-S water had minimal effects on birds.

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