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Effects of sodium bisulfate or sodium sesquicarbonate as a sodium source in broiler chicken diets with or without a coccidiosis challenge

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ABSTRACT

Broilers commonly experience stressors such as coccidiosis, a parasitic infection that results in intestinal damage, malabsorption, and performance losses. The poultry industry is exploring alternatives to anticoccidials for controlling coccidiosis, especially through the enhancement of gut health. Sodium bisulfate ($NaHSO_4$; **SBS**), an acid salt, has been used for many years as a litter acidifier to reduce aerial ammonia in poultry housing. More recently, SBS has shown promise as a feed additive that improves growth performance through enhancing gut health in poultry. Two trials of 1,920 broiler chicks each were raised to 42 d in floor pens. Chicks were randomly divided into 1 of 4 treatments (480 chicks/treatment) on d 0 that utilized SBS (0.3 or 0.4% of diet) or control diets using sodium sesquicarbonate (0.2 or 0.3% of diet) as a sodium source. In Trial 2, birds were challenged with a 2 × dose of coccidiosis vaccine on d 0. Growth performance was measured on d 0, 14, 28, and 42. Each trial was analyzed independently to yield results for non-challenged or coccidiosis-challenged conditions. All data were analyzed with means separation using a Duncan's test. SBS and control treatments with equivalent sodium contributions were also directly compared. Significance was accepted at $P \leq 0.05$. On d 28, broilers fed 0.4% SBS weighed more ($P < 0.05$) than broilers from either control, regardless of challenge status. This effect was sustained in the challenged broilers at d 42 but was not different ($P > 0.05$) in non-challenged broilers. No differences ($P > 0.05$) in feed intake or mortality were observed in either trial. Birds fed 0.4% SBS had lower feed conversion than both control treatments for d 0-42, regardless of challenge status. Collectively, these results indicate that SBS promoted growth performance with and without a coccidiosis challenge more than that of sodium sesquicarbonate as a supplemental sodium source. Because experimental treatments were balanced for mineral content, it is possible the sulfate content of SBS influenced these results.

Introduction

Broiler chickens are commonly exposed to environmental stressors during rearing. One highly prevalent stressor is coccidiosis, a parasitic infection of the digestive tract that results in intestinal damage, malabsorption of nutrients, and losses in growth performance (Levine, 1973; McDougald and Fitz-Coy, 2013; Deplazes et al., 2016; Teng et al., 2020). Due to legislation and negative consumer perception of traditional anti-microbial methods of controlling this disease, the industry has been exploring other routes for the prevention and treatment of coccidiosis (Kadykalo et al., 2018). One such method and a key topic of current research is the promotion of gut health, which can consist of incorporating novel feed ingredients (e.g., probiotics or minerals) or optimizing nutrient densities, among others (Rochell et al., 2017; Abdul Rasheed et al., 2020; Jespersen et al., 2024a; Jespersen et al., 2024b).

Sodium bisulfate ($NaHSO_4$; **SBS**), a dry granular acid salt, has been traditionally used in the poultry industry for over 30 years as a litter amendment to reduce litter pH and ammonia levels in livestock housing (Baxter, 1997; Johnson et al., 2011; Payne et al., 2019). SBS has also been investigated as a feed additive for poultry and swine due to its status as generally recognized as safe (GRAS) by the FDA and its potential as a chloride-free source of sodium in feed formulations. Recent research on SBS as a feed additive in broilers yielded promising results where SBS improved growth performance and intestinal morphology measurements during coccidiosis challenges (Chadwick et al., 2020; Suarez et al., 2021). However, these trials incorporated SBS at the

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expense of dietary NaCl, which lowered the overall chloride content of the diets. Previous research has shown that reducing chloride levels in the diet can improve litter quality and performance in chickens, especially during periods of heat stress when the dietary electrolyte balance (**DEB**) is altered (Ahmad et al., 2006; Enting et al., 2009; Mushtaq et al., 2013). For this reason, other chloride-free sources of sodium are available and are often incorporated into poultry diets, including sodium bicarbonate and sodium sesquicarbonate (**SSC**; Ahmad et al., 2006; Enting et al., 2009; Mushtaq et al., 2013). Both ingredients, however, are basic in nature and do not contain a sulfate group. In fact, it is thought that the sulfate group unique to SBS is the major driver of improved performance and intestinal measurements observed in previous research (Chadwick et al., 2020). This is attributed to the property of sulfated mucins (i.e., sulfomucins) in the intestine being more resistant to enzymatic degradation compared with mucins not containing sulfate groups (Roberton and Wright, 1997; Amerongen, 1998; Struwe et al., 2015); however, more research is necessary to substantiate this conclusion. As such, this research was conducted to assess the effect of SBS on growth performance in broilers with and without a coccidiosis challenged compared to another chloride-free source of sodium in diets with equivalent sodium, chloride, and DEB content.

Materials and methods

Birds, husbandry, and diets

This experiment was conducted by Poultry Research Partners, LLC (Hoschton, GA) under the supervision of Dr. Bradley Turner, DVM, MAM. All research conducted followed guidelines approved by the Institutional Animal Care and Use Committee. In 2 separate trials, a total of 3,840 one-d-old Cobb 500 by-product male broiler chicks, consisting of 1,920 chicks per trial, were obtained from a commercial hatchery, transported to Poultry Research Partners, LLC, (Hoschton, GA), and housed in floor pens for the duration of each trial (42 d). Chicks were distributed randomly into pens resulting in a stocking density of ~38 kg/m^2 or 13.5 birds/m^2 at d 42. Chicks were observed daily to monitor general health, clinical signs of disease, adverse vaccine reactions, and mortality until trial completion. The lighting and temperature program followed common industry practices for broiler chickens.

All experimental diets were corn and soybean meal-based and formulated to meet the Cobb 500 nutrient specification guidelines (Cobb-Vantress, Inc., 2022). Ingredient compositions and nutrient densities of diets are provided in Tables 1-3. Diets were similar in both trials and dietary phases were fed as follows: starter diets d 0 to 14 (crumbled), grower diets d 14 to 28 (pelleted), finisher diets d 28 to 42 (pelleted). Feed and water were provided *ad libitum* throughout each trial. Feed grade SBS (AFG®, Jones-Hamilton Co., Walbridge, OH) or SSC (S-Carb®, Genesis Alkali, Houston, TX) was added to experimental diets according to dietary treatment. Because SBS and SSC contain approximately 20% and 30% sodium, respectively, diets were formulated for equivalent sodium levels rather than equal inclusion rate of ingredient (SBS or SSC). To achieve this, inclusions of the sodium ingredient, corn, salt, and choline chloride were adjusted accordingly to keep sodium, chloride, and DEB constant across diets for each phase.

Experimental design

In each trial, chicks were weighed and randomly divided into 4 experimental treatments with 12 replicate pens each containing 40 birds (480 chicks/treatment) on d 0. Experimental treatments were identified according to diet and were as follows: 0.2% SSC (**Control-L**), 0.3% SSC (**Control-H**), 0.3% SBS (**SBS-L**), 0.4% SBS (**SBS-H**). Both trials utilized randomized complete block designs. In Trial 1, there was no coccidiosis challenge applied to the birds, whereas in Trial 2 all birds were challenged with a 2 × dose of a commercially available live coccidiosis vaccine (Advent®; Huvepharma, Inc., Peachtree City, GA) prior to placement in pens on d 0. Birds were reared to 42 d of age in both trials.

Table 1
Composition of experimental starter phase diets (d 0-14) in both trials[1].

Items	Experimental diets			
	Control-L	SBS-L	Control-H	SBS-H
Ingredients, % as-fed				
Corn	53.83	53.65	53.68	53.49
Soybean meal	39.44	39.47	39.46	39.49
Soybean oil	2.35	2.41	2.40	2.46
Dicalcium phosphate	2.03	2.03	2.03	2.04
Limestone	0.95	0.95	0.95	0.95
Salt	0.26	0.26	0.18	0.22
MHA[2], 88%	0.38	0.38	0.38	0.38
L-Lysine HCl, 78.8%	0.21	0.21	0.21	0.21
L-Threonine, 98.5%	0.09	0.09	0.09	0.09
Choline chloride, 60%	0.13	0.12	0.19	0.15
Vitamin premix[3]	0.05	0.05	0.05	0.05
Trace mineral premix[4]	0.08	0.08	0.08	0.08
Sodium sesquicarbonate	0.20	-	0.30	-
Sodium bisulfate	-	0.30	-	0.40
Calculated content[5]				
AMEn, kcal/kg	3,008	3,008	3,008	3,008
CP	22.00	22.00	22.00	22.00
Digestible Lys	1.18	1.18	1.18	1.18
Digestible Met	0.61	0.61	0.61	0.61
Digestible Met+Cys	0.88	0.88	0.88	0.88
Choline	2,474	2,413	2,833	2,592
Na	0.19	0.19	0.19	0.19
Cl	0.26	0.26	0.25	0.25
Electrolyte balance, mEq/kg	243	243	246	246

[1] Birds in Trial 1 were non-challenged, whereas all birds in Trial 2 were challenged with a 2× dose of coccidiosis vaccine on d 0.
[2] MHA = methionine hydroxyl analog (Novus Inc. Saint Charles, MO).
[3] Vitamin mix provided the following (per kg of diet): Vitamin A, 8818 IU; Vitamin D3, 2480 IU; 25-hydroxyvitamin D3, 69 μg; Vitamin E, 35 IU; vitamin B_{12} (cobalamin),15.5 μg; Biotin, 0.17 mg; Menadione, 1.98 mg; Thiamine, 1.87 mg; Riboflavin, 7.7 mg; d-Panthothenic Acid, 13.23 mg; Vitamin B6, 3.3 mg; Niacin, 44.1 mg; Folic Acid, 1.1 mg
[4] Provided per kg of complete diet: 16 mg Cu from $CuSO_4$; 30 mg Fe from $FeSO_4$; 1.50 mg I from $Ca(IO_3)_2$; 120 mg Mn from $MnSO_4$; 110 mg Zn from $ZnSO_4$; 0.30 mg Se from a Se premix.
[5] Reported as a percentage (%) unless otherwise specified.

Data and sample collection

Bird and feeder weights were collected on d 0, 14, 28, and 42 and were averaged per replicate pen. Mortality was recorded throughout each trial. Average BW, FI, mortality-corrected feed conversion ratio (**FCR**), and FCR corrected for BW and mortality (**FCRBW**) were calculated.

Statistical analysis

Each trial was analyzed independently to yield results for non-challenged or coccidiosis-challenged conditions. The experimental unit for each analyzed outcome was pen of birds. All data were subjected to an ANOVA using XLSTAT in Microsoft Excel. Observed means for each treatment and the pooled SEM for each outcome were reported. Additional means separation was conducted across treatments in each trial using a Duncan's multiple range test. For each trial, the SBS and control treatments with equivalent sodium contributions (i.e., Control-L vs SBS-L and Control-H vs SBS-H) were also directly compared. For all data, significance was accepted at $P \leq 0.05$.

Results

Growth performance

Body Weights. Results for BW in Trials 1 and 2 are presented in

Table 2
Composition of experimental grower phase diets (d 14-28) in both trials[1].

Items	Experimental diets			
	Control-L	SBS-L	Control-H	SBS-H
Ingredients, % as-fed				
Corn	58.11	57.92	57.98	57.80
Soybean meal	34.58	34.61	34.60	34.62
Soybean oil	3.22	3.28	3.26	3.31
Dicalcium phosphate	1.88	1.89	1.88	1.89
Limestone	0.91	0.91	0.91	0.91
Salt	0.26	0.27	0.18	0.22
MHA[2], 88%	0.33	0.33	0.33	0.33
L-Lysine HCl, 78.8%	0.18	0.17	0.17	0.17
L-Threonine, 98.5%	0.06	0.06	0.06	0.06
Choline chloride, 60%	0.16	0.15	0.20	0.16
Vitamin premix[3]	0.05	0.05	0.05	0.05
Trace mineral premix[4]	0.08	0.08	0.08	0.08
Sodium sesquicarbonate	0.20	-	0.30	-
Sodium bisulfate	-	0.30	-	0.40
Calculated content[5]				
AMEn, kcal/kg	3,086	3,086	3,086	3,086
CP	20.00	20.00	20.00	20.00
Digestible Lys	1.05	1.05	1.05	1.05
Digestible Met	0.55	0.55	0.55	0.55
Digestible Met+Cys	0.80	0.80	0.80	0.80
Choline	2,565	2,505	2,802	2,563
Na	0.19	0.19	0.19	0.19
Cl	0.28	0.28	0.26	0.26
Electrolyte balance, mEq/kg	217	217	222	222

[1] Birds in Trial 1 were non-challenged, whereas all birds in Trial 2 were challenged with a 2× dose of coccidiosis vaccine on d 0.
[2] MHA = methionine hydroxyl analog (Novus Inc. Saint Charles, MO).
[3] Vitamin mix provided the following (per kg of diet): Vitamin A, 8818 IU; Vitamin D3, 2480 IU; 25-hydroxyvitamin D3, 69 μg; Vitamin E, 35 IU; vitamin B_{12} (cobalamin),15.5 μg; Biotin, 0.17 mg; Menadione, 1.98 mg; Thiamine, 1.87 mg; Riboflavin, 7.7 mg; d-Panthothenic Acid, 13.23 mg; Vitamin B6, 3.3 mg; Niacin, 44.1 mg; Folic Acid, 1.1 mg
[4] Provided per kg of complete diet: 16 mg Cu from $CuSO_4$; 30 mg Fe from $FeSO_4$; 1.50 mg I from $Ca(IO_3)_2$; 120 mg Mn from $MnSO_4$; 110 mg Zn from $ZnSO_4$; 0.30 mg Se from a Se premix.
[5] Reported as a percentage (%) unless otherwise specified.

Table 3
Composition of experimental finisher phase diets (d 28-42) in both trials[1].

Items	Experimental diets			
	Control-L	SBS-L	Control-H	SBS-H
Ingredients, % as-fed				
Corn	62.68	62.49	62.55	62.36
Soybean meal	29.43	29.46	29.45	29.48
Soybean oil	4.06	4.12	4.10	4.16
Dicalcium phosphate	1.67	1.67	1.67	1.67
Limestone	0.85	0.85	0.85	0.85
Salt	0.26	0.27	0.19	0.22
MHA[2], 88%	0.30	0.30	0.30	0.30
L-Lysine HCl, 78.8%	0.19	0.19	0.19	0.19
L-Threonine, 98.5%	0.08	0.08	0.08	0.08
Choline chloride, 60%	0.15	0.14	0.20	0.16
Vitamin premix[3]	0.05	0.05	0.05	0.05
Trace mineral premix[4]	0.08	0.08	0.08	0.08
Sodium sesquicarbonate	0.20	-	0.30	-
Sodium bisulfate	-	0.30	-	0.40
Calculated content[5]				
AMEn, kcal/kg	3,167	3,167	3,167	3,167
CP	18.00	18.00	18.00	18.00
Digestible Lys	0.95	0.95	0.95	0.95
Digestible Met	0.50	0.50	0.50	0.50
Digestible Met+Cys	0.74	0.74	0.74	0.74
Choline	2,412	2,351	2,711	2,470
Na	0.19	0.19	0.19	0.19
Cl	0.28	0.28	0.26	0.26
Electrolyte balance, mEq/kg	193	193	199	199

[1] Birds in Trial 1 were non-challenged, whereas all birds in Trial 2 were challenged with a 2× dose of coccidiosis vaccine on d 0.
[2] MHA = methionine hydroxyl analog (Novus Inc. Saint Charles, MO).
[3] Provided per kg of complete diet: Vitamin A, 8818 IU; Vitamin D3, 2480 IU; 25-hydroxyvitamin D3, 69 μg; Vitamin E, 35 IU; vitamin B_{12} (cobalamin),15.5 μg; Biotin, 0.17 mg; Menadione, 1.98 mg; Thiamine, 1.87 mg; Riboflavin, 7.7 mg; d-Panthothenic Acid, 13.23 mg; Vitamin B6, 3.3 mg; Niacin, 44.1 mg; Folic Acid, 1.1 mg
[4] Provided per kg of complete diet: 16 mg Cu from $CuSO_4$; 30 mg Fe from $FeSO_4$; 1.50 mg I from $Ca(IO_3)_2$; 120 mg Mn from $MnSO_4$; 110 mg Zn from $ZnSO_4$; 0.30 mg Se from a Se premix.
[5] Reported as a percentage (%) unless otherwise specified.

Tables 4 and 5, respectively. There was no influence ($P > 0.05$) of sodium source on d 14 in either trial. However, by d 28, broilers fed SBS-H weighed more ($P < 0.05$) than broilers from both control treatments, regardless of challenge status. The effect of increased BW was sustained in the challenged broilers in Trial 2 through d 42 (Table 5), but the difference for BW between SBS and control treatments was lost ($P > 0.05$) in the non-challenged birds in Trial 1 (Table 4). At d 42, the challenged birds in Trial 2 fed SBS-H were heavier than all other treatments, including SBS-L.

Feed Intake. Results for FI in Trials 1 and 2 were corrected for mortality and are presented in Tables 6 and 7, respectively. No differences ($P > 0.05$) in FI were observed across treatments for any phase or challenge status during these trials.

Feed Conversion Ratio. Results for FCR in Trials 1 and 2 were corrected for mortality and are presented in Tables 8 and 9, respectively. FCR was also adjusted to similar BW (i.e., FCRBW) and these results yielded trends similar to FCR (Tables 10 and 11**)**. Birds provided SBS had better (i.e., lower) FCR during d 0-14 than control birds, although this effect was only significant ($P < 0.05$) for coccidiosis-challenged birds in Trial 2. Provision of SBS-H yielded the numerically lowest FCR during d 0-28 and was significantly lower ($P < 0.05$) than all other treatments in non-challenged birds in Trial 1. FCR for SBS-H was significantly lower than both control treatments, regardless of challenge status, for d 0-42. Birds fed SBS-L numerically outperformed both control treatments during the 0-42 d period, but this was only significant ($P < 0.05$) in non-challenged birds.

Mortality. Results for mortality in Trials 1 and 2 are found in Tables 12 and 13**,** respectively. No differences ($P > 0.05$) were observed across treatments for mortality, regardless of challenge status.

Table 4
Results of BW in non-challenged birds (Trial 1)[1].

Dietary treatment	Day of study			
	0	14	28	42
Control-L	42	442	1,485[b]	2,948
SBS-L	42	447	1,507[ab]	2,971
Control-H	42	447	1,504[b]	2,953
SBS-H	42	455	1,535[a]	2,982
SEM	0.1	3.4	10.5	35.4
P-value	0.69	0.07	**0.01**	0.45
Control-L vs SBS-L				
SEM	0.1	3.3	10.4	15.8
P-value	0.79	0.30	0.15	0.31
Control-H vs SBS-H				
SEM	0.1	3.6	10.6	17.7
P-value	0.36	0.11	**0.05**	0.26

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Abbreviations: SBS, sodium bisulfate.
[ab] Means lacking a common superscript within a column differ ($P < 0.05$).

Discussion

In this research, the growth performance of broilers was assessed when feeding a chloride-free sodium ingredient when diets were balanced for chloride content, with or without the added complication of a coccidiosis challenge. The overall average ending BW on d 42 was

Table 5
Results of BW in coccidiosis-challenged birds (Trial 2)[1].

	Day of study			
Dietary treatment	0	14	28	42
Control-L	43	461	1,483[c]	2,695[c]
SBS-L	43	465	1,534[ab]	2,719[bc]
Control-H	43	460	1,514[b]	2,745[b]
SBS-H	43	468	1,554[a]	2,786[a]
SEM	0.1	3.1	10.1	12.1
P-value	0.29	0.28	**<0.001**	**<0.001**
Control-L vs SBS-L				
SEM	0.1	2.8	9.9	12.8
P-value	0.07	0.31	**0.001**	0.21
Control-H vs SBS-H				
SEM	0.13	3.4	10.3	11.4
P-value	0.68	0.13	**0.01**	**0.02**

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Chicks received a 2× dose of coccidiosis vaccine on d 0. Abbreviations: SBS, sodium bisulfate.
[abc]Means lacking a common superscript within a column differ ($P < 0.05$).

Table 6
Results of feed intake in non-challenged birds (Trial 1)[1].

	Days		
Dietary treatment	**0-14**	**0-28**	**0-42**
Control-L	532	2,140	4,597
SBS-L	530	2,145	4,569
Control-H	535	2,146	4,604
SBS-H	537	2,156	4,549
SEM	2.7	9.7	22.9
P-value	0.37	0.68	0.31
Control-L vs SBS-L			
SEM	2.8	10.4	23.3
P-value	0.72	0.75	0.39
Control-H vs SBS-H			
SEM	2.6	8.9	22.4
P-value	0.65	0.42	0.10

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Feed intake calculated as BW times feed conversion adjusted by mortality. Abbreviations: SBS, sodium bisulfate.

Table 7
Results of feed intake in coccidiosis-challenged birds (Trial 2)[1].

Dietary treatment	**Days**		
	0-14	**0-28**	**0-42**
Control-L	534	2,086	4,474
SBS-L	528	2,101	4,456
Control-H	531	2,081	4,522
SBS-H	530	2,108	4,496
SEM	2.6	13.0	31.6
P-value	0.45	0.43	0.49
Control-L vs SBS-L			
SEM	2.6	14.8	28.2
P-value	0.11	0.48	0.21
Control-H vs SBS-H			
SEM	2.7	10.78	34.6
P-value	0.92	0.09	0.60

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Feed intake calculated as BW times feed conversion adjusted by mortality. Chicks received a 2× dose of coccidiosis vaccine on d 0. Abbreviations: SBS, sodium bisulfate.

2,964 g and 2,736 g for Trials 1 and 2, respectively. Therefore, the coccidiosis-challenged birds (Trial 2) weighed approximately 8% less than their non-challenged counterparts (Trial 1) at study conclusion, providing evidence that the 2 × dosage of coccidiosis vaccine on d 0 was successful in inducing a mild challenge resulting in bird performance reduction at the end of the growth period. Additionally, no differences in mortality were observed across treatments in either trial, and mortality rates were similar to those reported in the commercial broiler industry

Table 8
Results of feed conversion ratio in non-challenged birds (Trial 1)[1].

Dietary treatment	Days		
	0-14	0-28	0-42
Control-L	1.20	1.44[a]	1.56[a]
SBS-L	1.19	1.42[b]	1.54[b]
Control-H	1.20	1.43[ab]	1.56[a]
SBS-H	1.18	1.41[c]	1.53[b]
SEM	0.007	0.006	0.007
P-value	0.07	**0.001**	**0.003**
Control-L vs SBS-L			
SEM	0.006	0.005	0.008
P-value	**0.01**	**0.03**	0.06
Control-H vs SBS-H			
SEM	0.008	0.007	0.006
P-value	0.09	**0.02**	**0.001**

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Feed conversion is corrected by mortality. Abbreviations: SBS, sodium bisulfate.
[abc]Means lacking a common superscript within a column differ ($P < 0.05$).

Table 9
Results of feed conversion ratio in coccidiosis-challenged birds (Trial 2)[1].

Dietary treatment	Days		
	0-14	0-28	0-42
Control-L	1.16[a]	1.41[a]	1.66[a]
SBS-L	1.13[b]	1.37[b]	1.64[ab]
Control-H	1.15[a]	1.38[b]	1.65[a]
SBS-H	1.13[b]	1.36[b]	1.61[b]
SEM	0.005	0.007	0.009
P-value	**0.003**	**<0.001**	**0.01**
Control-L vs SBS-L			
SEM	0.005	0.007	0.008
P-value	**0.003**	**0.001**	0.07
Control-H vs SBS-H			
SEM	0.006	0.007	0.011
P-value	**0.02**	0.09	**0.04**

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Feed conversion is corrected by mortality. Chicks received a 2× dose of coccidiosis vaccine on d 0. Abbreviations: SBS, sodium bisulfate.
[abc]Means lacking a common superscript within a column differ ($P < 0.05$).

Table 10
Results of feed conversion ratio in non-challenged birds (Trial 1) adjusted by BW[1].

Dietary treatment	Days		
	0-14	0-28	0-42
Control-L	1.20	1.45[a]	1.56[a]
SBS-L	1.19	1.42[b]	1.54[bc]
Control-H	1.20	1.43[ab]	1.56[ab]
SBS-H	1.18	1.40[c]	1.52[c]
SEM	0.007	0.007	0.009
P-value	0.07	**0.001**	**0.006**
Control-L vs SBS-L			
SEM	0.006	0.007	0.009
P-value	0.07	**0.03**	0.07
Control-H vs SBS-H			
SEM	0.008	0.008	0.008
P-value	0.09	**0.02**	**0.004**

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Feed conversion adjusted by mortality and similar BW as follow: [(average BW - individual BW)/1000]×0.1455 + individual FCR. Average BW is the average of BW at d 14, 28, or 42. Individual FCR is the feed conversion corrected by mortality. Abbreviations: SBS, sodium bisulfate.
[abc]Means lacking a common superscript within a column differ ($P < 0.05$).

Table 11
Results of feed conversion ratio in coccidiosis-challenged birds (Trial 2) adjusted by BW[1].

Dietary treatment	Days		
	0-14	0-28	0-42
Control-L	1.16[a]	1.41[a]	1.67[a]
SBS-L	1.13[b]	1.37[bc]	1.64[a]
Control-H	1.15[a]	1.38[b]	1.65[a]
SBS-H	1.13[b]	1.35[c]	1.61[b]
SEM	0.006	0.008	0.010
P-value	**0.003**	**<0.001**	**0.001**
Control-L vs SBS-L			
SEM	0.005	0.007	0.008
P-value	**0.003**	**0.001**	0.07
Control-H vs SBS-H			
SEM	0.006	0.007	0.011
P-value	**0.02**	0.09	**0.04**

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Chicks received a 2× dose of coccidiosis vaccine on d 0. Feed conversion adjusted by mortality and similar BW as follow: [(average BW - individual BW)/1000]× 0.1455 + individual FCR. Average BW is the average of BW at d 14, 28, or 42. Individual FCR is the feed conversion corrected by mortality. Abbreviations: SBS, sodium bisulfate.

[abc]Means lacking a common superscript within a column differ ($P < 0.05$).

Table 12
Cumulative mortality (%) in non-challenged birds (Trial 1)[1].

	Days		
Dietary treatment	0-14	0-28	0-42
Control-L	0.42	2.29	3.54
SBS-L	0.83	2.50	4.38
Control-H	0.21	1.88	2.50
SBS-H	1.04	2.50	4.17
SEM	0.311	0.645	0.856
P-value	0.23	0.89	0.42
Control-L vs SBS-L			
SEM	0.320	0.704	0.802
P-value	0.37	0.84	0.47
Control-H vs SBS-H			
SEM	0.301	0.581	0.906
P-value	0.06	0.46	0.21

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Abbreviations: SBS, sodium bisulfate.

Table 13
Cumulative mortality (%) in coccidiosis-challenged birds (Trial 2)[1].

Treatments	Days		
	0-14	0-28	0-42
Control-L	1.46	2.92	5.42
SBS-L	1.04	1.88	3.96
Control-H	1.25	2.71	5.00
SBS-H	1.25	1.88	4.79
SEM	0.374	0.523	0.684
P-value	0.89	0.36	0.50
Control-L vs SBS-L			
SEM	0.372	0.574	0.663
P-value	0.44	0.21	0.13
Control-H vs SBS-H			
SEM	0.377	0.466	0.704
P-value	1.00	0.22	0.84

[1] Means represent 12 replicates/treatment of 40 male birds/replicate. Chicks received a 2× dose of coccidiosis vaccine on d 0. Abbreviations: SBS, sodium bisulfate.

(National Chicken Council, 2020), indicating that the coccidiosis infection was of low virulence as expected. Conversely, it is possible that the 8% difference in BW may have been due to variability across batches of chicks rather than coccidiosis (Jespersen et al., 2024a), as these trials were not run concurrently. However, inducing a coccidiosis challenge through a high dosage of a coccidiosis vaccine containing live *Eimeria* oocysts is a common and reliable disease model in published poultry research (Williams and Catchpole, 2000; Chadwick et al., 2020). Additional evidence may have been helpful to substantiate this conclusion further through oocyst shedding or lesion scoring, however, such measurements are often subjective, variable, and do not necessarily indicate severity of infection (Williams and Catchpole, 2000; Jordan et al., 2011; Soutter et al., 2020; Jespersen et al., 2024a; Jespersen et al., 2024b). Nevertheless, the growth performance patterns observed in the current study yielded similar results as other trials utilizing vaccine-challenge models (Suarez et al., 2021). Therefore, we conclude that the reduced growth performance observed in this study was indicative of a successful coccidiosis challenge.

The experimental diets in this trial were formulated to contain equivalent sodium contributions from the product (SBS or SSC) associated in the diet at 2 inclusion levels. Because SBS contains approximately 20% sodium, whereas SSC contains approximately 30% sodium, this meant that the inclusion rate of SSC in the control diets needed to be slightly lower than the SBS inclusion rate to contribute an equal amount of sodium to the diet. In this way, 0.2% SSC (Control-L) and 0.3% SBS (SBS-L) were paired diets at the lower test product inclusion rate, and 0.3% SSC (Control-H) and 0.4% SBS (SBS-H) were paired diets at the higher test product inclusion rate. The formulations were then adjusted so that each treatment 'pair' contained nearly identical nutrient levels. In all instances that were significant, the birds provided SBS outperformed the control birds fed diets containing SSC. Because these ingredients were contributing equal amounts of sodium and the resulting diets were equivalent in mineral concentrations, it is likely that the differences in chemical compositions of the sodium sources were the drivers of these differences in growth performance.

The chemical formula for SSC is $Na_3H(CO_3)_2$, and it is considered an equimolar mixture of sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3). Sodium bicarbonate is a widely used ingredient in animal feed as a sodium source and a basic pH buffer (Damron et al., 1986). Individually, these sodium compounds are reported to improve performance in heat-stressed broilers, with sodium bicarbonate having a more pronounced positive effect than sodium carbonate (Ahmad et al., 2006). Conversely, SBS ($NaHSO_4$) is an acidic granular salt that dissasociates into Na^+, H^+, and SO_4^{2-} and contributes sodium, acidification, and sulfate to the diet. Traditionally, sulfates have had a negative perception by consumers. Sulfur content is often attributed to foul odor and taste in drinking water (World Health Organization, 2004) and many shampoos and soaps are labeled as 'sulfate-free,' with a perception by consumers that these shampoos are healthier options (Cline et al., 2018). Additionally, sulfate is part of the chemical compound dextran sodium sulfate (i.e., DSS) that is used to experimentally-induce colitis in animal research (Poritz et al., 2007). However, it has been shown that the DSS molecule does not disassociate into its ionic components within the intestine, and therefore the sulfate group is not considered the driver of DSS-associated intestinal inflammation (Araki et al., 2012).

On the contrary, sulfates are reported to have a beneficial effect of re-enforcing the intestinal mucosal layer, where they bind to mucin proteins and make them more resistant to degradation from bacterial and endogenous enzymes (Roberton and Wright, 1997; Duangnumsawang et al., 2021). In fact, research has shown that chickens have a higher concentration of these sulfated mucins (i.e., sulfomucins) in their intestine compared to humans, and it is thought that these higher levels aid in reducing pathogenic bacteria from colonizing, such as *Campylobacter jejuni* (Struwe et al., 2015). As such, this may explain the ability of SBS to improve growth performance in broilers during and without a coccidiosis challenge, where dietary sulfate groups are incorporated into mucins to re-enforce the mucosal layer against pathogens and endogenous enzymes. This conclusion corroborates results from previous research on SBS (Chadwick et al., 2020; Suarez et al., 2021), where performance and gut health measurements were improved when SBS was incorporated into broiler diets. The current study further establishes this relationship by removing the unequal dietary chloride levels and DEB as a possible confounding factor, as these variables are known to influence growth performance in poultry (Mushtaq et al., 2013). In fact, the 'paired' dietary treatments in the current study were almost identical in nutrient content for each phase with the exception of choline, where choline chloride inclusion was increased in the Control-H diet in order to balance the DEB after the replacement of NaCl with SSC.

In conclusion, incorporating SBS as a sodium source for broiler chickens elicited higher BW and improved FCR than SSC when both ingredients are formulated to contribute equal sodium levels in the diet. This effect was established in FCR through d 42 in both coccidiosis-challenged and non-challenged birds, and in BW to d 28 and 42 for non-challenged and challenged birds, respectively. Because these experimental diets were balanced for nutrient content, with special emphasis on sodium, chloride, and DEB, we hypothesize the 80% sulfate content of SBS to be the driver of improved growth performance in broiler chickens observed in the current study. Additional research to determine the fate of dietary sulfates from SBS would be necessary to establish this relationship further.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Bradley Turner reports financial support was provided by Jones-Hamilton Co. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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