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Supplementation of Feed Grade Sodium Bisulfate in Broiler Diets Improves Feed Efficiency

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Abstract: The effects of Sodium Bisulfate (SB) supplementation on growth, intestinal integrity, blood gas chemistry and litter microbiology of broiler chickens were evaluated. Birds were fed a corn-soybean meal diet meeting all of the NRC (1994) requirements. In Exp. 1 birds were fed diets supplemented with 0 (control diet, CTL), 0.25, 0.5, or 0.75% (w/w) of feed-grade SB (SB25, SB50 and SB75, respectively); in Exp. 2 and 3 only the CTL, SB25 and SB75 diets were evaluated. In Exp. 4 the chicks (n = 920) were placed in 20 pens and fed the CTL diet, or the SB25 diet offered during the first 21, 35, or 49 d (21D, 35D and 49D, respectively); the CTL diet was offered until the end of experiment (d 49) for treatments 21D and 35D. The data was analyzed as a two way ANOVA (diet and gender as main effects). In Exp. 1 birds fed diets with SB had lower FC than birds fed the CTL diet, but BW was not different among treatments. In Exp. 2 females fed the SB25 diet were heavier ($p<0.05$) at d 21 than females fed the CTL or the SB75 diet; the FC was similar among groups. In Exp. 3 chickens fed the SB25 or SB75 diets were consistently heavier and had a lower FC ($p<0.05$) than birds fed the CTL diet. In Exp. 4 birds fed the SB25 diet had lower BW than birds fed the CTL diet; however birds in the 35D or 49D treatments had a better FC than birds fed the CTL diet. The litter of birds fed the SB25 diet had lower levels of Salmonella at wk 4 and 6 in Experiment 3 and at wk 4 in Exp. 4. Neither the duodenum villus height nor the blood gas composition was affected by the dietary treatments. Our results show that SB supplementation improves productive performance and reduces the environmental levels of *Salmonella*, with variable efficacy, perhaps due to seasonal conditions.

Key words: Broiler, sodium bisulfate, productive performance, *Salmonella*

INTRODUCTION

Poultry is the fastest-growing livestock industry in the world. Modern strains of birds are intensively raised and genetically selected to maximize output at high stocking densities under environmental conditions that are often less than optimal (Monreal and Paul, 1989). These factors often lead to lower immune levels, increasing the risk of disease (Van der Zijpp, 1983); thus, clinical and subclinical infections are a persistent problem in poultry production, causing significant economic losses and affecting animal welfare. Moreover, the contamination of poultry products with bacteria such as *Salmonella* and *Clostridium*, that could affect human health, are a constant concern. For this reason, the prophylactic use of antibiotics (low or preventative levels) in poultry production has become standard practice. Indeed, it has been estimated that in the U.S., the animal agriculture sector uses more than 11 million kg of antibiotics every year. The widespread use of prophylactic antibiotics in animal production has been associated with the development of antibiotic-resistant bacteria of human interest. Because of this problem, the European Union banned the use of prophylactic antibiotics in animal feeds in 2006. However, this ban was associated with an increase in disease occurrence, compromising animal welfare and an increase in the use of antibiotics at therapeutic levels (Phillips *et al*., 2004). Clearly, there is a critical need to ensure animal health and productivity, particularly if we want to effectively reduce or eliminate the use of antibiotics in animal agriculture and at the same time ensure a safe product for the consumer.

Diet acidification through the use of feed additives that modify the environment of the intestinal tract has been used to reduce the prevalence of Salmonella (Wales *et al*., 2010). Acids disrupt cellular pH gradients and intracellular regulation of pH affecting vital microbial metabolic processes (Cherrington *et al*., 1990) as well as having direct toxic effects on membrane structure and macromolecule synthesis (Ricke, 2003). Acids may also interfere with the expression of virulence genes, reducing the ability of *Salmonella* to penetrate the intestine. The use of acidifying additives in the feed of broiler chickens has been shown to reduce *Salmonella* colonization at the intestinal and systemic phases (Fernandez-Rubio *et al*., 2009), although other authors

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have reported limited success (Heres *et al*., 2004). Sodium Bisulfate (SB), also known as sodium hydrogen sulfate ($NaHSO_4$), has been used as an agent to reduce pH in several industrial and agricultural applications and as an acidifier in pet diets and has been classified as a general purpose food additive under Association of American Feed Control Officials (AAFCO) Feed Ingredient Definition. Addition of Na^+ into the diet has been used as a means to counteract blood alkalinization resulting from heat stress. During heat stress there is an increase in respiratory rate with concomitant decreases in tidal volume, which leads to an excessive loss of CO_2 and increases in blood pH and bicarbonate levels (respiratory alkalosis). One of the mechanisms to maintain pH at normal values is through the renal excretion of bicarbonate (HCO_3^-) in association with cations such as potassium (K^+) or sodium (Na^+), which may lead to the depletion of those electrolites. Several reports indicate that the addition of sodium salts improve productive performance and reduce rectal temperature of broilers grown under heat stress (Ahmad *et al*., 2008; Borges *et al*., 2003, 2004).

Thus, we hypothesize that the supplementation of SB in poultry diets will change the acidity of the intestinal tract in birds, reducing the proliferation and shedding of *Salmonella* and will improve the productive performance of broiler chickens by reducing the effects of heat stress. The objectives of these studies were to evaluate the effects of different levels of sodium bisulfate (0, 0.25, 0.5, or 0.75%) on productive performance, blood gas parameters, litter *Salmonella* loads and intestinal integrity, of broiler chickens grown under commercial conditions and under different environmental conditions.

MATERIALS AND METHODS

Grow out conditions: Four trials were conducted. For each trial, 1-d old chicks were obtained from a local hatchery, wing-sexed and identified by gender and placed in identical rearing pens (10.6 m^2 of rearing space per pen) at a placement density of 0.24 m^2/bird. Pens included one 13.5 kg tube feeder and nipple drinker system. Broilers were reared on litter consisting of 50% built-up litter from a commercial broiler house and 50% fresh pine shavings, resembling current industry conditions. Immediately upon placement, chicks received age-appropriate supplemental heat and were brooded following commercial standards. All the birds were fed starter (0-21 d), grower (21-35 d) and finisher diets (35-49 d) formulated to meet all of the requirements set by the NRC (1994). Feed and water was provided on an *ad libitum* basis. During heat stress periods in Experiment 1, feed was withdrawn in the morning (by 9:00 a.m.) and was returned in the evenings after the birds had stopped panting (10-12 p.m.).

Treatments and experimental design: In experiment 1 there were 4 feed treatments: 0, 0.25%, 0.5%, or 0.75% (w/w) of feed-grade sodium bisulfate (SB; Jones-Hamilton Inc., Walbridge OH 43465) supplementation in the feed. Only in Experiment 1 sodium was balanced to meet the specific requirements (NRC, 1994) by using variable levels of common salt, sodium bisulfate and an inert filler (builder sand). Basal rations of broiler starter, grower and finisher were used to develop the treatment feeds. The chicks (Ross 708) were sexed at hatching and reared by sex in separate pens. There were 8 total treatments: 4 diets x 2 sexes. Each treatment was replicated 4 times while each feed treatment was replicated 8 times across sex, for a total of 32 pens with 25 birds in each pen. In Experiments 2 and 3, the birds were randomly assigned to one of three different dietary treatments: diets supplemented with 0 (control diet, CTL), 0.25, or 0.75% (w/w) of feed-grade SB (SB25 and SB75, respectively). For each dietary treatment there were 6 pen replicates with 23 male and 23 female chicks under a complete block design (6 blocks by location in the house to compensate for micro-environmental conditions). For Experiment 4, one day old chicks (n = 920) were placed in 20 pens (23 males and 23 females per pen) and the dietary treatments were distributed randomly under a complete block design, with 4 treatments, 5 replicates per treatment and 5 blocks. Dietary treatments were as follow: control diet (CTL, basal diet without SB supplementation), or a diet supplemented with 0.25% SB offered during the first 21, 35, or 49 d of the growing experiment (treatment 21D, 35D and 49D, respectively). The basal diet was offered until the end of experiment for treatments 21D and 35D. For Experiments 2, 3 and 4, the diets were not balanced for sodium content (Table 1). The data was analyzed as a two way ANOVA with diet and gender as main effects (SigmaStat, Systat Software, Inc., San Jose, CA 95110); when treatments effects were significant we used the Student-Newman-Keuls for the separation of means.

Data collection

Body weight and feed conversion: In trial 1 bird weight and feed consumption, both by pen, were determined at 2, 5 and 7 wk of age. The weight of each pen of birds was also determined at placement. In trial 2 through 4, BW by sex was recorded at day 1, 21, 35 and 49. Feed consumption by pen was determined by subtracting the feed left in the feeder from the feed offered at day 21, 35 and 49.

Blood gas analysis: In experiments 2 and 3 venous blood pH and gas composition was measured at day 21, 35 and 48. Two birds per pen were randomly chosen, 2 mL of blood were drawn from the wing vein using heparinized syringes, sealed and immediately submerged in iced water. The blood samples were transported to the Animal Physiology lab for gas measurements using an automatic gas-blood analyzer (Radiometer ABL 77 Blood Gas Analyzer). The time from blood collection to analysis was less than one hour.

Table 1: Composition of the basal diets and calculated nutrient content of the experimental diets

Ingredient (%)	Starter	Grower	Finisher
Corn	58.36	60.66	67.28
Soybean meal	34.06	29.64	24.46
Biophos[1]	1.54	1.40	1.28
Limestone	1.55	1.57	1.57
Blended fat	2.6	4.74	3.42
Salt	0.20	0.31	0.39
DL-Methionine	0.26	0.24	0.19
Lysine HCl	0.17	0.14	0.12
Mineral Premix[3]	0.05	0.05	0.05
Vitamin Premix[2]	0.25	0.25	0.25
Filler[4]	1.00	1.00	1.00
Calculated nutrient content			
Crude protein (%)	21.80	19.80	17.80
ME energy (kcal/kg)	3018.00	3168.00	3149.00
Calcium (%)	0.94	0.91	0.88
Available phosphorous (%)	0.45	0.41	0.37
Methionine	0.58	0.54	0.48
Cystine (%)	0.35	0.33	0.30
Lysine (%)	1.29	1.14	1.00
Threonine (%)	0.81	0.73	0.67
Sodium (%)[5]	0.1, 0.15, 0.24	0.14, 0.19, 0.28	0.17, 0.22, 0.31

[1]Biophos: 15.9% Ca, 21.2% P (Marshall Minerals Inc., Marshall, TX).

[2]Vitamin Premix (kg): Vitamin A 14,000,000 IU, vitamin D3 5,000,000 IU, vitamin E 60,000 IU, vitamin B12 24 mg, riboflavin 12,000 mg, niacin 80,000 mg, d-pantohenic acid 20,500 mg, K 2,700 mg, folic acid 1,800 mg, vitamin B6 5,000 mg, thiamine 4,000 mg, d-biotin 150 mg (Sanderson, DSM Nutritional Products, Inc. Parsippany, NJ).

[3]Mineral Premix (kg): Ca 1.20%, Mn 30.0%, Zn 21.0%, Cu 8500 mg, I 2100 mg, Se 500 mg, Mo 1670 mg (Tyson Poultry 606 Premix, Tyson Foods, Springdale, AR).

[4]Filler was a combination of rice bran (1.0, 0.75, or 0.25%) and sodium bisulfate (0.0, 0.25, or 0.75%) for the control, SB25 and SB75 diet, respectively.

[5]The three sodium content values correspond to the CTL, the SB25 and the SB75 diet, respectively

Histology: In Experiment 3 two male chickens per pen were killed at days 14, 21 and 35. A 1-cm segment of the jejunum (2 cm from the end of duodenum) was excised, washed in physiological saline solution and fixed in 10% buffered formalin. The tissue samples were later embedded in paraffin and a 2-µm section of each sample was placed on a glass slide and stained with hematoxylin and eosin. Histological sections were examined with a Nikon phase contrast microscope coupled with a MicroComp integrated digital imaging analysis system (Nikon Eclipse 80i, Nikon Corp., Tokyo, Japan). Villus height was measured from the top of the villus to the top of the lamina propria (Baurhoo *et al.*, 2007). Ten measurements were taken per bird for each variable; for purposes of statistical analysis, the average of these values was used.

Litter parameters: In trial 2 and 3, litter samples were obtained once a wk from five random areas of the pen and mixed into a Ziploc-type bag and the samples shaken to ensure a representative sample of the whole pen. Twenty five g of the sample was weighed and placed into Whirl-Pak bags and diluted with 50 ml of Butterfield's Buffer solution. Litter samples from the solution were direct plated (10^0 to 10^{-2} dilutions) onto XLT4 agar for quantitative analysis. A portion of the sample (1 mL) was dispersed into a 9 mL tube of RV for enrichment purposes, incubated for 24 h at 42°C and then re-plated onto XLT4 agar for a qualitative analysis. Plates were incubated for 24 h at 37°C. Positive plates from enrichment were assigned a log10 value of 1.5 (Corrier *et al.*, 1993). Similar serial dilutions were made and samples were direct plated onto Plate Count Agar; these plates were incubated for 18-24 h at 35-37°C, then the colonies were counted and recorded. Litter samples (3 g) were mixed with 50 mL of deionized water for determination of pH. Microbial colony counts, pH readings and moisture data were analyzed using SAS v9.1 for windows with PROC GLM and means separated using the Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Broiler growth performance: In experiment 1 the BW of male and female broilers, mortality and litter pH was not affected by the dietary treatments. However, chickens fed CTL diet had a higher ($p<0.05$) cumulative feed conversion (1.80±0.05 and 1.89±0.05 for males and females, respectively) than birds fed the diets with SB; furthermore, the cumulative FC was not different among SB supplementation levels (1.72, 1.64, 1.71±0.05 for males and 1.71, 1.77, 1.74±0.05 for females, fed 0.25, 0.5, or 0.75% SB, respectively). In experiment 2 birds fed the SB supplemented diets had a consistent tendency (both male and females) to be heavier than birds fed the

Table 2: Average body weight (g) by sex (M, males; F, females) and cumulative Feed Conversion Rate (FCR) of broiler chickens fed different levels of sodium bisulfate in experiments 2 and 3

	Experiment 2						
	BW d 21		BW d 35		BW d 49		
Treatment[1]	M	F	M	F	M	F	FCR
CTL	829	770[b]	1925	1680	3190	2780	1.84
SB25	852	795[a]	1920	1733	3251	2831	1.81
SB75	830	763[b]	2025	1760	3296	2782	1.80
SEM	7	6	44	28	37	16	0.02
	Experiment 3						
	BW d 21		BW d 35		BW d 49		
Treatment	M	F	M	F	M	F	FCR
CTL	695[b]	678[b]	1461[c]	1363[c]	2512[b]	2253[c]	2.06[a]
SB25	748[a]	720[a]	1637[b]	1486[b]	2640[a]	2344[b]	1.98[ab]
SB75	749[a]	717[a]	1731[a]	1560[a]	2726[a]	2418[a]	1.93[b]
SEM	20	20	40	30	60	50	0.06

[1]Birds were fed diets formulated to meet all of the NRC requirements supplemented with 0 (CTL), 0.25 (SB25), or 0.75% (SB75) sodium bisulfate in the diet.

[a,b]Means within the same column and with different superscript are statistically different ($p<0.05$)

Table 3: Average body weight (g) and cumulative Feed Conversion (FC) for male and female broiler chickens fed diets supplemented with 0.25% sodium bisulfite or a control diet during different periods of time

	CTL[1]	21D	35D	49D
Day 21				
Male	662±9[a]	575±10[b]	542±10[b]	554±10[b]
Female	651±10[a]	569±10[b]	536±9[b]	566±10[b]
FC	1.34±0.03	1.36±0.02	1.36±0.02	1.35±0.04
Day 35				
Male	1832±23[a]	1697±24[b]	1675±23[b]	1690±25[b]
Female	1686±18[a]	1569±19[b]	1528±19[b]	1574±19[b]
FC	1.54±0.01	1.56±0.02	1.52±0.01	1.55±0.01
Day 49				
Male	3133±33[a]	2974±32[b]	3001±31[b]	2989±33[b]
Female	2634±21[a]	2509±24[b]	2549±23[b]	2535±23[b]
FC	1.94±0.06[a]	1.87±0.04[ab]	1.74±0.01[b]	1.75±0.02[b]

[1]Birds in the CTL group were fed a diet free of Sodium Bisulfate (SB) during the whole growing period (day 1-49); birds in the 21D and 35D group were fed a diet with 0.25% SB during the first 21 or 35 days, respectively and a diet free of SB afterwards. Birds in the 49D group were fed a SB supplemented diet from day 1-49.

[a,b,c]Means within the same row with different letter are statistically different ($p<0.05$)

CTL diet, although only at d 21 female chickens fed the SB25 diet were heavier ($p<0.05$) than birds fed the CTL or the SB75 diet; the FC was similar among the three treatment groups. In experiment 3 however male and female chickens fed the SB-supplemented diets were consistently heavier ($p<0.05$) than birds fed the non-supplemented diet (Table 2); furthermore, there was a dose dependent increase in BW in males and females at d 35 and in females at d 49. In experiment 3 the FC was also improved with increasing levels of SB-supplementation and was lower in chickens fed the SB75 diet compared with the CTL diet. In experiment 4 birds fed a diet with SB, regardless of the length of supplementation, were consistently lighter in BW than birds fed the CTL diet, but conversely, at the end of the experiment birds fed the SB-supplemented diet for 35 or 49 d had a better FC than birds the CTL diet (Table 3). Thus, our results show that the effects of SB supplementation on BW were not very consistent across the experiments and this could be attributed to the environmental conditions; Experiment 2 and 4 were conducted under thermoneutral conditions in the late winter and early spring in central Texas, with SB supplementation causing marginal improvements (non statistically significant) in BW and FC (Experiment 2) or small reductions in BW associated with improvements in FC (Experiment 4); Experiment 1 was conducted under a more severe heat stress (May and June) and SB supplementation caused small (but statistically significant) improvements in FC; whereas Experiment 3 was conducted under moderate heat conditions in the early fall, with SB supplementation resulting in consistent improvements in both BW and FC. The positive effects of Na^+ supplementation (as sodium bicarbonate, sodium carbonate, or sodium sulphate) in birds grown under heat stress have been attributed to a lower body temperature mediated by an increase in water consumption (Ahmad *et al*., 2008; Borges *et al*., 2003), although the same authors indicate that very high levels of carbonate addition can exacerbate the alkalotic conditions associated with panting during heat stress. In this regard, the acidifying effects of the bisulfate anion (HSO_4^-) in SB could help to maintain the blood pH during heat stress. Thus, the beneficial effects of SB ($NaHSO_4$) on productive performance during heat stress could be associated with the increased water consumption due to the extra Na and with decrease in arterial pH due to the bisulfate anion.

Blood gas variables and intestinal integrity: However, dietary treatments did not affect the venous blood gas variables measured (pH, pCO_2, pO_2, sO_2 and HCO_3; data not shown) in experiments 2 and 3. The lack of effects could be due to the fact that in Experiment 2 the environmental temperature was comfortable and panting did not occurred. In Experiment 3, however, the birds were exposed to cyclic heat stress and since the measurements were taken at 14, 28 and 49 d, the birds could have adapted to the cyclic heat stress; also, bleeding was performed during the cooler morning hours. Furthermore, blood gas variables, particularly pH and pCO_2, have to be maintained within a very narrow range to avoid alkalosis during heat stress, thus birds adapt to heat stress by reducing metabolic demands, including reductions in cardiac output, ventricular work and mean arterial pressure (Whittow *et al*., 1966), or modulation in respiratory rate and tidal volume (Arad and Marder, 1983). Thus the beneficial effects of SB in stabilizing the pH in the blood of birds exposed to heat stress may be hard to document under these experimental conditions. Borges *et al*. (2003) did not find differences in blood pH or blood pCO_2 when broilers were fed different levels of electrolytes (0 to 240 mEq/kg of feed; Na+K-Cl) and grown under moderately high temperature, whereas Ahmad *et al*. (2008) found reduction in blood pH when broilers where fed sodium salts and grown under severe heat conditions; we did not measure blood gas variables in Experiment 1, when heat stress was more pronounced.

Intestinal integrity: Another effect of acidifying agents in the feed is to modify intestinal environment and microbial populations (Van Immerseel *et al*., 2005; Fernandez-Rubio *et al*., 2009; Heres *et al*., 2004) and improve intestinal integrity and function (Senkoylu *et al*., 2007; Adil *et al*., 2010). The effects of SB supplementation on villus height (VH, an important parameter of gut health and absorption capacity) evaluated in Experiment 3 were not consistent (Fig. 1). At d 14 birds fed the SB25 diet had a lower VH than those

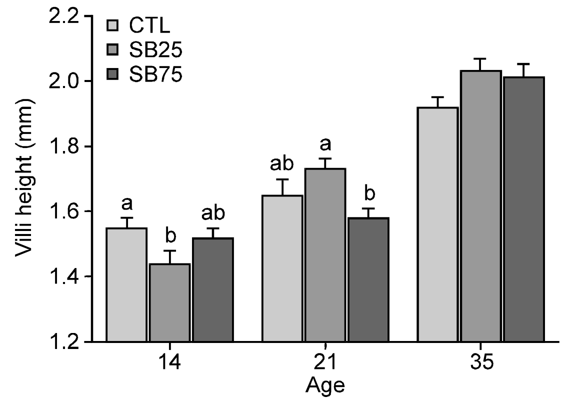

Fig. 1: Villi height in the duodenum of broiler chickens fed different levels of sodium bisulfate. Birds were fed diets formulated to meet all of the NRC requirements supplemented with 0 (CTL), 0.25 (SB25), or 0.75% (SB75) sodium bisulfate in the diet.
[a,b]Columns within the same age group and without common letter are statistically different ($p<0.05$)

of the CTL group, whereas birds fed the SB75 diet had an intermediate VH value; at d 21 birds fed the SB25 diet had a higher VH than birds fed the SB75 diet, but not different of the VH of birds fed the CTL diet; at d 35 the dietary treatments did not have effects on the VH of chickens. These results do not correlate with productive performance, suggesting that the beneficial effects of SB on growth performance and feed efficiency may not be related with improvements in intestinal integrity, which agrees with the reports of Hernandez *et al*. (2006) in which supplementation of diets with formic acid did not improve intestinal morphology, but had slight improvements in ileal digestibility of nutrients.

Litter microbiology: Lower levels of pathogenic intestinal microbial populations will result in lower levels of bacteria in the environment, improving the safety of poultry products. As previously discussed, diet acidification has the potential to reduce the number of pathogenic bacteria, such as *Salmonella*, in the gastrointestinal tract of chickens and consequently reduces bacterial shedding (Van Immerseel *et al*., 2005). The supplementation of SB did not have effects on the total aerobic plate count (data not shown) in any of the two experiments in which this parameter was measured. In both experiments, the amount of *Salmonella* recovered in the litter and the litter pH was not different among treatments at the beginning of the experiment and during the first three first weeks of growth (data not shown). In Experiment 2 the litter of the birds fed the SB75 diet had higher levels of *Salmonella* ($p<0.05$; Table 4) compared with the litter from birds fed

Table 4: Litter *Salmonella* recovery ($\log_{10}$), pH and humidity (%; only in Experiment2) from pens of broiler chickens fed different levels of sodium bisulfate in experiments 2 and 3, from week 4 through week 7

	Experiment 2								
	Salmonella				Litter pH				Humidity
Treatment[1]	Wk4	Wk5	Wk6	Wk7	Wk4	Wk5	Wk6	Wk7	Wk7
CTL	1.10^{b}	1.64	3.01^{a}	2.26	8.42^{a}	8.85^{a}	8.65^{ab}	8.94	29.90^{ab}
SB25	0.93^{b}	1.69	2.08^{b}	1.92	7.95^{b}	8.80^{ab}	8.78^{a}	8.90	26.70^{b}
SB75	1.50^{a}	2.27	2.49^{ab}	2.65	8.48^{a}	8.71^{b}	8.49^{b}	8.77	36.55^{a}
SEM	0.60	1.68	2.22	2.17	0.01	0.03	0.04	0.04	1.78

	Experiment 3							
	Salmonella				Litter pH			
Treatment	Wk4	Wk5	Wk6	Wk7	Wk4	Wk5	Wk6	Wk7
CTL	2.29^{b}	2.25	2.28	2.25	8.16^{b}	8.56^{b}	8.55	8.88
SB25	2.50^{b}	2.79	2.48	1.63	8.70^{a}	8.88^{ab}	8.84	8.86
SB75	3.10^{a}	2.99	2.79	1.82	8.72^{a}	9.01^{a}	8.88	8.99
SEM	0.22	0.24	0.28	0.36	0.16	0.13	0.14	0.14

[1]Birds were fed diets formulated to meet all of the NRC requirements supplemented with 0 (CTL), 0.25 (SB25), or 0.75% (SB75) sodium bisulfate in the diet.

[a,b]Means within the same column and different superscript are statistically different ($p<0.05$)

the SB25 or CTL diet at wk 4, with a similar tendency at wk 5; however by wk 6 the litter from birds fed the SB25 diet had lower levels of *Salmonella* than the litter from CTL birds, whereas the litter from birds fed the SB75 diet had intermediate levels of *Salmonella* and by wk 7 the litter from birds fed the SB25 had numerically lower (although not statistically significant due to variability) counts of *Salmonella* compared with the other two groups. In Experiment 3 the *Salmonella* counts in the litter of birds fed the SB75 diet was higher than that of the other groups at wk 4, consistent with the results in Experiment 2 (Table 4); however, at wk 5, 6 and 7 the dietary treatments had no significant effects on litter *Salmonella* levels, although by wk 7 the litter of birds fed the SB25 diet had numerically lower counts, similar to the findings in Experiment 2. Other researchers have reported mixed effects of diet acidification on *Salmonella* control; Heres *et al*. (2004) reported that chickens fed a diet acidified with lactic and acetic acid were less susceptible to infection with *Campylobacter*, but not with *Salmonella*, whereas Fernandez-Rubio *et al.* (2009) reported that diet acidification with sodium butyrate (a sodium salt of butyric acid) partially protected with vegetable fats effectively reduced *Salmonella* infection in the crop and ceca of broiler chickens. Line (2011) reported that litter acidification, using either aluminum sulfate or sodium bisulfate, reduced *Campylobacter* colonization in the ceca, but it had no effect on *Salmonella* levels. The results obtained in our experiments, along with the ones reported in the literature, suggest that SB supplementation at 0.25% may have the potential to reduce *Salmonella* levels on the litter, particularly if used as a component of a more complex pre-harvest strategy to reduce microbial loads.

The effects of SB supplementation on litter pH were not consistent in the two experiments in which this parameter was measured. In Experiment 2 the litter of birds fed the SB25 diet had lower pH compared with the other two treatments at wk 4, whereas at wk 5 and 6 the lowest pH was recorded in the litter of birds fed the SB75 diet and by wk 7 the litter pH was not different among treatments. In Experiment 3 the litter from the CTL birds had the lowest pH at wk 4 and at wk 5 the litter from the CTL fed birds was lower than that of the SB75-fed, whereas the litter pH from birds fed the SB25 diet had an intermediate value. However at wk 6 and 7 the litter pH was not different among treatment groups. The inconsistent effects of SB supplementation on the litter pH may be explained by the differences in environmental conditions, which have a direct impact on drinking behavior, ventilation and litter humidity. Also, the mild effects of SB on litter acidification could be attributed to the low supplementation levels used in the feed as compared with the levels used in direct litter applications.

Litter humidity (measured only in Experiment 2) was not affected by the dietary treatments during the course of the experiment (wk 1 through 6), but at wk 7 the litter from birds fed the SB25 had lower humidity compared with the litter of birds fed the SB75 diet, although it was not different when compared with the litter of CTL birds (Table 4). Thus, although higher levels of Na in the diet may elicit a higher intake of water (Ahmad *et al*., 2008; Borges *et al*., 2003), under these environmental conditions litter humidity was not increased.

In conclusion, the supplementation of SB in broiler diets improves growth performance and feed efficiency, but the beneficial effects of SB are more noticeable under moderate hot conditions than under cool conditions. The supplementation of SB had inconsistent effects on the microbiology of the litter, reducing the levels of *Salmonella* during some periods, which could be related with environmental variables. Thus, the supplementation of SB could improve broiler productivity and could be used as a component of a pre-harvest strategy to improve poultry product safety. Further research is warranted to establish the mechanisms by which SB improves broiler performance.

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