The effect of sodium bisulfate and coccidiostat on intestinal lesions and growth performance of *Eimeria* spp.–challenged broilers

Mariam Talghari,[*] Alireza Behnamifar,[*] Shaban Rahimi,[*] Mohammad Amir Karimi Torshizi,[*] Robert Beckstead,[†] and Jesse L. Grimes[†,1]

*Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran 1411713116, Iran; and †Prestage Department of Poultry Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC 27695-7608, USA

ABSTRACT Coccidiosis is a high-prevalence disease that annually entails huge costs for the poultry industry. Control of coccidiosis in poultry production is based on the use of coccidiostats and vaccines. However, along with the problem of drug resistance, there is a concern about food safety and drug residues in poultry products. The objective of this study was to evaluate the effect of sodium bisulfate (**SBS**) in comparison with monensin (**M**) and their combination (**SBSM**) effects on controlling coccidiosis in broilers. In a randomized design, 300 chickens (Ross 308) were divided into 5 treatments and 4 replications (15 birds per replicate). All birds, except the negative control (**NC**), were orally inoculated with 4 *Eimeria* species on 14 D of age. Treatments included were as follows: NC, an unsupplemented basal diet, nonchallenged; positive control, a basal diet unsupplemented, challenged with *Eimeria* spp; a basal diet supplemented with 5 g/kg of SBS; a basal diet supplemented with 1 g/kg of M; and a basal diet supplemented with 5 g/kg SBS and 1 g/kg M (SBSM). Oocyst shedding per gram (**OPG**) of the faecal sample from each experimental unit was counted on 5 to 14 D after inoculation. Two chicks from each experimental unit were euthanized to investigate intestinal lesions on day 5 after inoculation. The NC birds showed the highest BW gain and the lowest feed conversion ratio. The birds in the SBSM group had improved feed consumption compared with the M group in the prechallenge period ($P < 0.05$). All supplemented treatments resulted in a significant decrease in OPG. The M and SBSM treatments showed more efficacy than the SBS group ($P < 0.05$) in reducing OPG. There was a significant reduction in cecal lesions owing to supplementation with SBS, but the effect of SBS in the upper part of the intestine was lower than the M and SBSM groups ($P < 0.05$). Based on the results of this study, SBS has protective effects against coccidiosis in ceca, and the combination of M and SBS (SBSM) did not show any further improvement effect compared with M alone on the control of coccidiosis.

Key words: broiler, coccidiosis, lesion score, monensin, sodium bisulfate

INTRODUCTION

Coccidiosis is a gastrointestinal infectious disease created by different species of *Eimeria* and has a direct life cycle between the external and internal environment of the host (Lillehoj and Lillehoj, 2000). Seven *Eimeria* species *Eimeria acervulina, Eimeria brunetti, Eimeria praecox, Eimeria maxima, Eimeria mitis, Eimeria necatrix,* and *Eimeria tenella* cause disease in chicken; however, the severity of the disease depends on the pathogenicity of *Eimeria* species (Morris and Gasser, 2006). Chickens in all ages are sensitive to coccidiosis, but the most sensitivity occurring between 3 and 5 wk of age (Yun et al., 2000).

Generally, ionophores such as salinomycin, narasin, monensin (**M**), lasalosid sodium, maduramicin, and semduramicin are used for prevention and control of coccidiosis. However, it has been found that some resistance to most of anticoccidial drugs is developed (Blake and Tomley, 2014), and new anticoccidials have not been marketed in recent years through pharmaceutical companies (Allen and Fetterer, 2002). In addition to drug resistance, there are some concerns about drug residues in poultry products and its dangers to human health and the environment (Olejnik et al., 2009). Consumers'

demand for prohibition of using drugs on animal feed has caused the European Union withdrawal of several brands of anticoccidial medicines that were used to control coccidiosis in broilers (Williams, 2002). All of the items mentioned indicate the need to use safe and harmless substances for treatment of this disease.

Sodium bisulfate (**SBS**) or sodium hydrogen sulfate ($NaHSO_4$) is an acid salt that is generally recognized safe by the Food and Drug Administration and as an agent to reduce pH in food without a sour taste. Sodium bisulfate is exerted in several industrial and agricultural applications such as meat and poultry processing, in browning prevention of fresh-cut produce, and as an acidifier in pet diets (Ruiz-Feria et al., 2011). It has been suggested that SBS in food may affect the gastrointestinal microbiota and promote the growth of the chickens (Line, 2002; Williams et al., 2012). Sodium bisulfate is hygroscopic, which after absorption of ambient moisture converts to sodium (Na^+), hydrogen (H^+), and sulfate (SO_4^-) constituents.

Historically, SBS has been used commercially as an acidifier on poultry litter. When used as a litter amendment at high doses, SBS reduced ammonia volatilization from poultry litter and also reduces the presence of *Salmonella* (Payne et al., 2002). In addition, the dietary inclusion of SBS decreased the shedding of *Salmonella* in the litter (Ruiz-Feria et al., 2011). Pertinent to poultry processing, SBS was used as an antimicrobial rinse agent on apples to reduce artificially inoculated *Listeria monocytogenes* (Kim et al., 2018). The Environmental Protection Agency has declared SBS as a safer choice as an antimicrobial and processing aid (EPA, 2018). In addition, as per the World Health Organization, the use of SBS is approved with no restrictions on allowable daily intake in more than 150 countries that recognize the World Health Organization codex (WHO, 2007). Owing to all of the preliminary data and the Environmental Protection Agency designation, it is evident that SBS could be a valid agent for reducing pathogenic microorganisms during multiple stages of poultry production, including processing. There are conflicting reports about the antimicrobial effect of SBS as a feed additive in chickens. Ruiz-Feria et al. (2011) reported that adding SBS to the diet in broiler chickens reduced the levels of *Salmonella* in the litter; however, Kassem et al. (2012) observed a diet supplemented with SBS did not significantly reduce *Salmonella* Enteritidis shedding rate. It has been reported that sodium sources can improve ionophores' efficiency (Hooge et al., 1999a). Supplementation feed with M and different types of sodium sources (sodium bicarbonate [**SBC**], chloride, and sulfate) significantly improved weight uniformity, feed efficiency, mortality, breast meat yield, and reduced coccidial lesion scores in broiler chickens (Hooge et al., 1999b).

As little research has been carried out on anticoccidiosis effects of SBS and the interactions between a sodium source and a coccidiostat, this study was conducted to evaluate the effect of SBS as a new and safe feed additive in comparison with a coccidiostat (M) and their combined (**SBSM**) effects on broilers challenged with *Eimeria* spp.

MATERIALS AND METHODS

Experimental Treatments/Coccidia Species

The experiment was conducted in the Poultry Research Center, Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. All procedures were approved by the Animal Care and Use Committee of Tarbiat Modares University.

Through a completely randomized design, a total of 300 1-day-old broiler chicks (Ross 308) were divided into 5 treatments and 4 replications (15 birds per replicate) in floor pens with litter. Treatments included were as follows: noninfected and nonmedicated negative control and infected and nonmedicated positive control (**PC**), where both of these groups were fed only corn and soy–based diet; SBS, 5 g/kg in the basal diet; M, 1 g/kg in the basal diet; and SBS + M (SBSM), 5 g/kg + 1 g/kg, respectively, in the basal diet.

The lighting program was applied in accordance with the Ross 308 guidelines (www.Aviagen.com) throughout the experiment. The temperature of the poultry house was maintained at 33°C at the arrival of chicks for the initial 3 D and then reduced 2.5°C per week until a temperature of 23°C was achieved. Feed and water were provided ad libitum throughout the trial. The basal diet (Table 1) was a corn and soybean meal–based diet

Table 1. Nutrient content of diets of broilers (as-fed basis): starter (day 1–14), grower (day 15–28), and finisher (day 29–42).

Item	Starter	Grower	Finisher
Ingredients (%)			
Corn	42	50	50
Soybean meal (44%)	34	28	25.5
Wheat	18	16	17.5
Soybean oil	2.0	2.0	2.0
Dicalcium phosphate[1]	1.6	1.8	1.9
$CaCO_3$ (38%)	1.4	1.1	0.9
Sodium chloride	0.34	0.34	0.34
L-Lysine HCl	0.05	0.18	0.18
DL-methionine	0.10	0.14	0.14
Vitamin permix[2]	0.25	0.25	0.25
Mineral permix[3]	0.25	0.25	0.25
Contents by calculation			
ME (kcal/kg)	2,950	3,000	3,050
CP (%)	21	19	18
Met (%)	0.48	0.45	0.42
Met + Cys (%)	0.91	0.87	0.81
Lys (%)	1.21	1.14	1
Available phosphorus (%)	0.72	0.71	0.69
Calcium (%)	1.05	0.95	0.9

[1]Contained 20% P and 23% Ca.
[2,3]Supplied the following per kilogram of diet: 9,000 IU of retinyl acetate, 2,000 IU of cholecalciferol, 12.5 IU of dl-α-tocopheryl acetate, 1.76 mg of menadione sodium bisulfite, 0.12 mg of biotin, 1.2 mg of thiamine, 3.2 mg of riboflavin, 6.4 mg of calcium d-pantothenate, 1.97 mg of pyridoxine, 28 mg of nicotinic acid, 0.01 mg of cyanocobalamine, 320 mg of choline chloride, 0.38 mg of folic acid, 60 mg of $MnSO_4.H_2O$, 80 mg of $FeSO_4.7H_2O$, 51.74 mg of ZnO, 8 mg of $CuSO_4.5H_2O$, 0.8 mg of iodized NaCl, 0.2 mg of Na_2SeO_3.
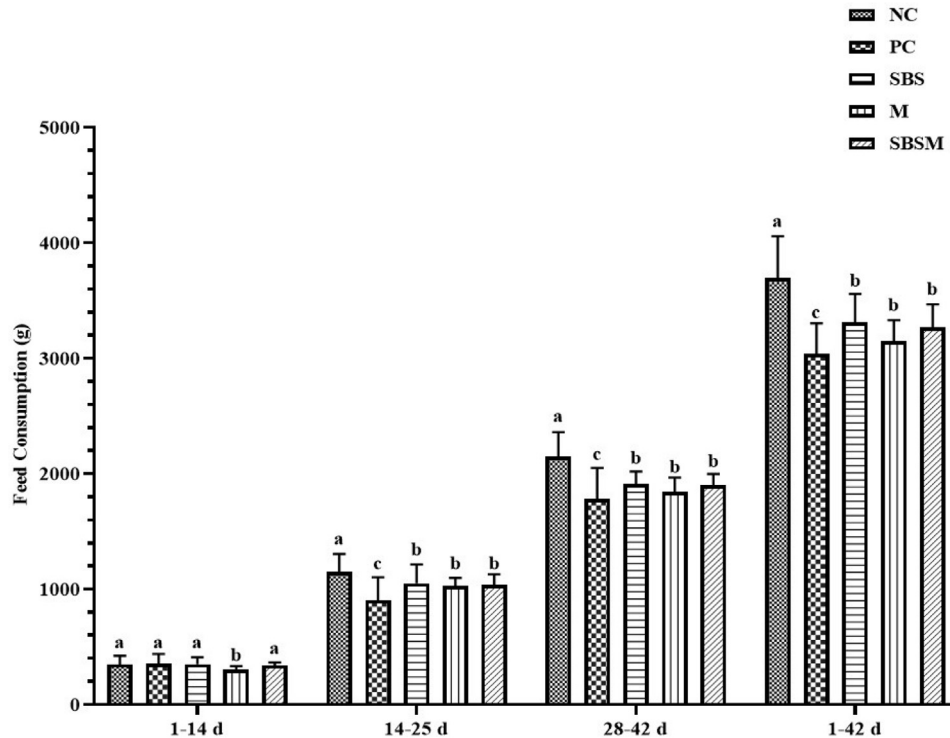
Figure 1. Effect of treatments on feed consumption (g) ±SD of broiler chickens at different periods of the experiment. [a–c] Different letters within each period of the experiment show significant differences among the groups ($P < 0.05$). Abbreviations: M, monensin; NC, negative control; PC, positive control; SBS, sodium bisulfate; SBSM, sodium bisulfate + monensin.

with a nutrient content based on Aviagen's recommendation (www.Aviagen.com).

All birds except the negative control group were orally inoculated by a mixed proportion of *Eimeria* oocysts isolated from the litter of a commercial broiler farm in Iran (70% *E. tenella*, 15% *E. maxima*, and 15% equal blend of *E. necatrix* and *E. acervulina*; 50,000 oocysts per bird) on 14 D of age.

Performance Parameters

For evaluation of the performance indicators, BW of all chickens of each replication and their feed consumption were determined weekly. BW gain (**BWG**), feed consumption (**FC**), and feed conversion ratio (**FCR**) were measured on a weekly bases and at end of the experiment. The European Production Efficiency Factor (**EPEF**) was measured by using the following formula:

EPEF: (Average BWG / day × % Survival rate) /

FCR × 10

Oocyst Counting

The number of oocysts per gram (**OPG**) of faecal samples were counted on days 19 to 28 (5–14 D postinnoculation [**PI**]), by using the modified McMaster counting chamber technique of Hodgson (1970). The faecal samples were kept in separate airtight plastic bags. A suspension of 10% (w/v) faeces in saturated salt solution was prepared. The samples were shaken, and 1 mL of

the suspension was mixed with 9 mL of saturated salt solution. Specific amount of the suspension was put on the McMaster chamber, and the number of the oocysts was counted under 10× magnitude using a light microscope (Peek and Landman, 2003).

Coccidiosis Lesion Scoring

Five days PI, 2 birds from each experimental unit were euthanized to evaluate the lesion scores on different segments of the small intestine and ceca using the Johnson and Reid method (1970).

Statistical Analysis

The data obtained through the experiment were analyzed using the ANOVA procedure in SAS 9.1 software, and means of experimental groups were compared using Duncan's multiple range test at 5% level of significance (SAS Inst, 1989). The following statistical model was used:
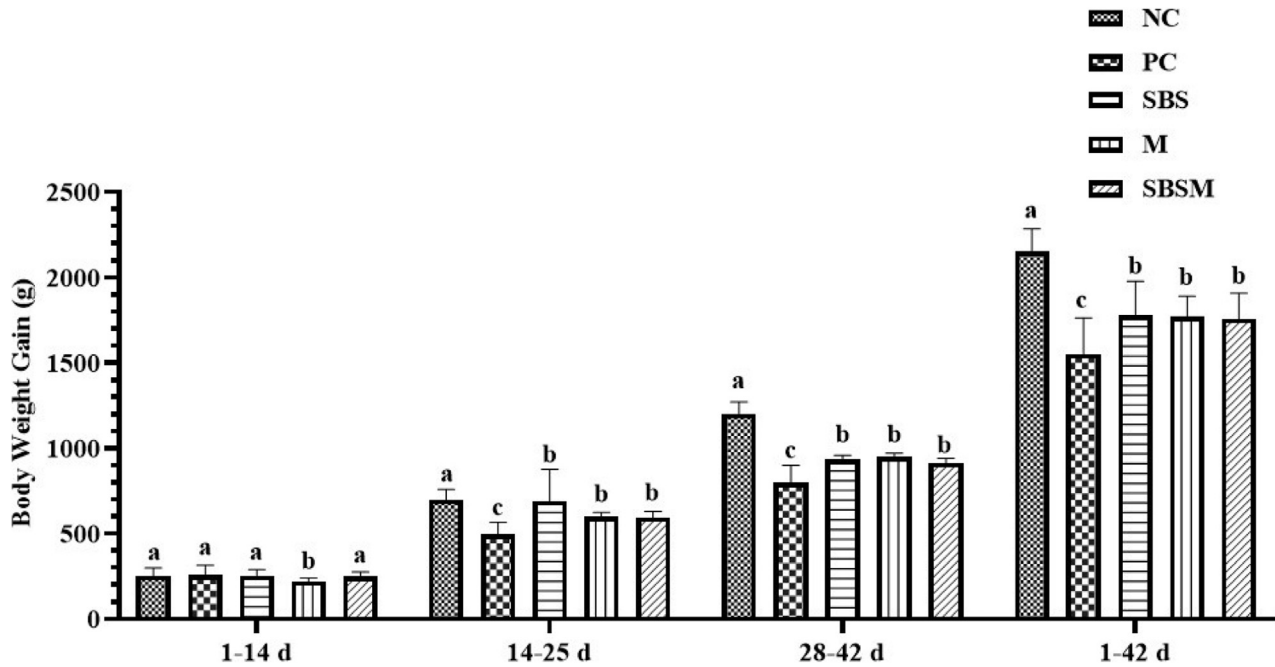
$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the mean of the jth observation of the ith treatment, μ is the sample mean, T_i is the effect of the ith treatment, and e_{ij} is the effect of the error.

RESULTS

Performance Parameters

The effects of treatments on FC (g), BWG (g), FCR, and EPEF are presented in Figures 1–4, respectively.
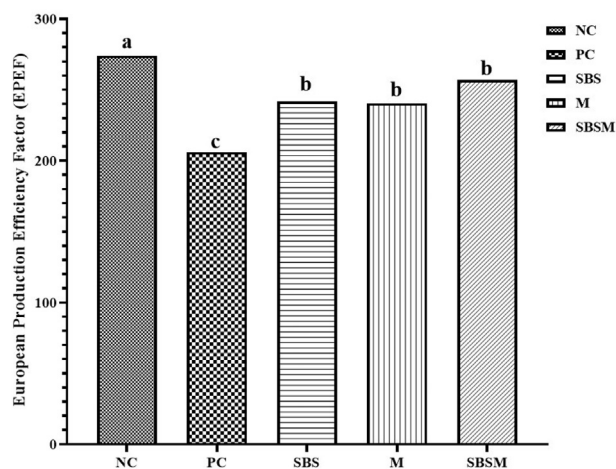
Figure 2. Effect of treatments on BWG (g) ±SD of broiler chickens at different periods of the experiment. [a–c] Different letters within each period of the experiment show significant differences among the groups ($P < 0.05$). Abbreviations: M, monensin; NC, negative control; PC, positive control; SBS, sodium bisulfate; SBSM, sodium bisulfate + monensin.

Based on the result of the prechallenge period, M had a downward effect on FC, and subsequently, the BWG was also reduced ($P < 0.05$). However, consumption of SBS and M simultaneously (SBSM) compensated the negative FC effect of M. After the challenge, FC of the M group was less in comparison with that of other supplementary groups. However, with BWG that decreases under the same statistical category, this reduction in FC is offset, and M had the best FCR between the challenged groups ($P < 0.05$). Sodium bisulfate significantly improved the overall FCR, FC, BWG, and EPEF compared with the PC group ($P < 0.05$). The combination of M and SBS did not show any further improvement effect on performance parameters.
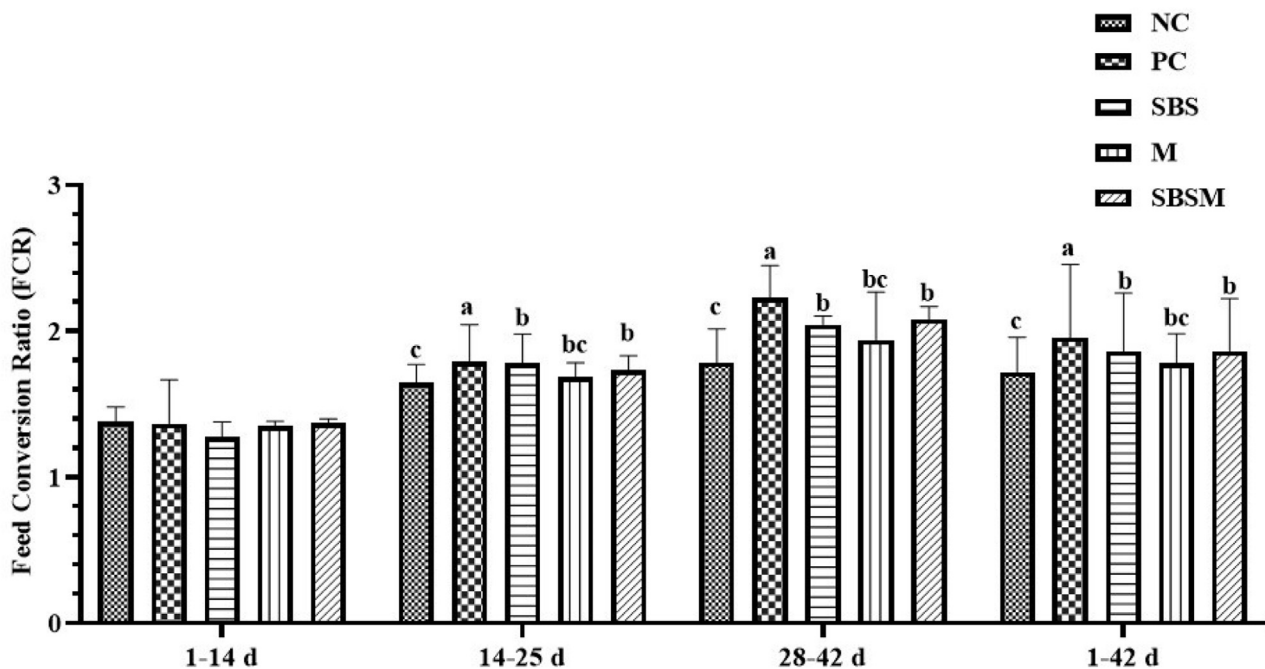
Figure 3. Effect of treatments on feed conversion ratio ±SD of broiler chickens at different periods of the experiment. [a–c] Different letters within each period of the experiment show significant differences among the groups ($P < 0.05$). Abbreviations: M, monensin; NC, negative control; PC, positive control; SBS, sodium bisulfate; SBSM, sodium bisulfate + monensin.

Figure 4. Effect of treatments on European production efficiency factor (EPEF) of broiler chickens. [a–c] Means within a column with no common superscript differ significantly ($P < 0.05$). Abbreviations: M, monensin; NC, negative control; PC, positive control; SBS, sodium bisulfate; SBSM, sodium bisulfate + monensin.

Lesion Scoring

Duodenal, jejunal, and cecal lesions on the fifth day after challenge in different experimental groups are presented in Table 2. In the duodenum, M and SBSM had the same and fewer lesion scores between challenged experimental groups, and SBS group showed fewer lesions than the PC group ($P < 0.05$). Only the PC group showed signs of disease in the ileum, and other experimental groups had no lesions ($P < 0.05$). Lesion scores in the ceca were not significantly different between the M, SDSM, and SBS groups and were less than that of the PC group ($P < 0.05$). In the jejunum, the M and SBSM groups showed the same performance and had a better therapeutic effect than the SBS group ($P < 0.05$). The combination of M and SBS did not show any improvement effect than M on intestinal lesions.

The Oocyst Shedding per Gram of Faeces

As observed in Figure 5, the faecal OPG shedding of the logarithm [Log $(x + 1)$] in different postchallenge sampling day in the all 3 supplemented groups showed a significant decrease compared with that of the PC group ($P < 0.05$) that M and SBSM treatments had better efficiency than the SBS group. Thus, the SBSM

Table 2. Lesion scores in 3 segment of intestine at 5 D after inoculation.

Treatments	Duodenum	Jejunum	Ileum	Cecca
Negative control	0.00[c]	0.00[c]	0.00[b]	0.00[c]
Positive control	1.37[a]	1.62[a]	0.50[a]	1.56[a]
Sodium bisulfate	0.37[b]	1.00[a,b]	0.00[b]	0.31[b]
Monensin	0.00[c]	0.25[b]	0.00[b]	0.37[b]
Sodium bisulfate + monensin	0.00[c]	0.22[b]	0.00[b]	0.29[b]
P-value	*	*	*	*
SEM	0.17	0.27	0.25	0.24

[a–c] Means within a row with no common superscript differ significantly (*$P < 0.05$).
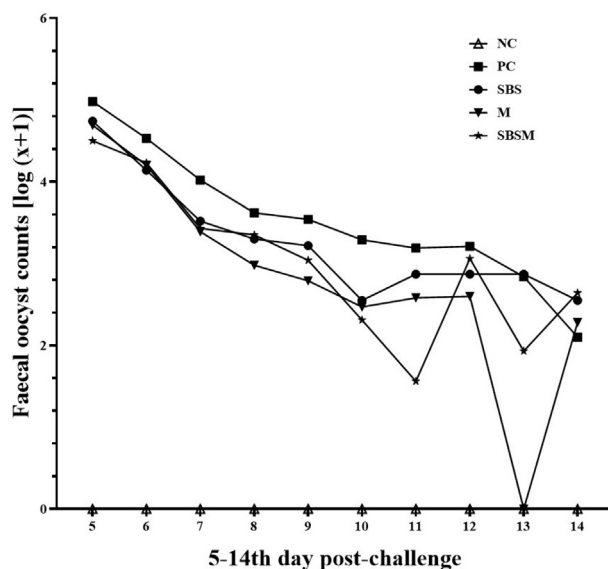
Figure 5. Effect of treatments on faecal oocyst counts [log $(x + 1)$] in different sampling day (5–14 D after challenge). Abbreviations: M, monensin; NC, negative control; PC, positive control; SBS, sodium bisulfate; SBSM, sodium bisulfate + monensin.

group had no amelioration effect compared with the M group on oocyst shedding. There was a significant difference between SBSM treatment on days 10 to 11 PI and M treatment on days 12 to 13 PI compared with the other challenged groups ($P < 0.05$).

DISCUSSION

Because of the adverse effects of M on the growth performance of chickens in hot weather, using this ionophore in poultry farms decreases during the summertime period. The main reason for this is the belief that M reduces FC during the warm months, and therefore, in the shuttle program, M is replaced with other ionophores in summer and fall seasons (Chapman et al., 2010). Consistent with this, the M group had a lower FC during the preinoculation period ($P < 0.05$). As shown in Figures 1–3, SBS, before the coccidiosis challenge, did not affect the FC, BW, and FCR, which is inconsistent with results of Ruiz-Feria et al. (2011) who reported that SBSM might offset the reducing effect of M alone on FC, especially, when chickens have not been exposed to coccidiosis agent.

As shown in Table 2, the lesions were observed throughout the intestine of birds in the PC group. The SBS treatment reduced lesions in all segments of the intestine and oocyst shedding compared with the PC group. Sodium bisulfate showed a less therapeutic effect than M and SBSM in different intestinal segments. However, SBS has been able to demonstrate similar function to M and SBSM in reducing of cecal lesions. Anticoccidial activity of hydrochloric acid (HCl) against *E. tenella* in broiler chickens has been reported by Abbas et al. (2011). In that study, the authors demonstrated that HCl in drinking water reduced the pH of the ceca and resulted in lower *E. tenella* lesion scores at 1,000 and

2,000 ppm. Sodium bisulfate (NaHSO$_4$) may function similarly. Future research could determine if SBS treatment affects the pH of the ceca. It is unlikely that acidic properties of SBS have a supportive role against the *Eimeria* infections in the upper parts of the intestine. In *E. acervulina*–infected birds (lesions in the upper third of the small intestine), protein at the mucosal layer in the affected parts of the intestine is denatured because of low pH caused by the *Eimeria* infection (Kouwenhoven and van der Horst, 1972; Stephens, 1965; Stephens et al., 1967). It seems that the protective effect of SBS in the upper parts of the intestine is related to modifying the intestinal environment and improving intestinal integrity and villus height (Ruiz-Feria et al., 2011), which may result from its antioxidant effects. The role of SBS as an antioxidant in the intestine is also not clear. For several chemicals such as SBS, hydrogen sulfide, and organosulfur compounds that may be derived from SBS in the digestive system, antioxidant properties are attributed. Sodium bisulfite is an inorganic compound commonly used as an antioxidant in pharmaceutical formulations (Trivedi and Patel, 2011). Hydrogen sulfide (H$_2$S) is an antioxidant gas transmitter that protects endothelial cells against oxidative stress. It has a protective effect against oxidant damage in the endothelium both in vitro and in vivo (Xie et al., 2016). It also decreases mitochondrial reactive oxygen species production and protects neuronal cells against stress-induced senescence (Xie et al., 2014). In various studies, the beneficial effects of dietary antioxidant supplements against *Eimeria* infection have been reported. For example, Bun et al. (2011) indicated that organic Zn supplementation reduced oxidative stress and improved some immune responses irrespective of whether birds were healthy or challenged with *E. tenella*. It is reported that plant extracts with antioxidant activity significantly decreased the *Eimeria* oocyst production in the birds and essential oils, as natural products, obtained from aromatic plants have the potential to serve as an alternative to anticoccidials (Naidoo et al., 2008; Idris et al., 2018).

The protective effect of different sources of sodium, SBC, chloride, or sulfate, with a coccidiostat in coccidial-challenged chickens was investigated. Based on those results, corn–soy diets with either of the 3 sodium sources resulted in significantly lower 21-D coccidial lesion scores than M alone (Hooge et al., 1999b). In the present study, the combination of M and SBS (SBSM) did not show any further improvement effect compared with M alone. In a 2 × 5 factorial trial using built-up litter pens (8 replicate pens; 88 chicks per pen) vs. each coccidiostat alone, 0.20% dietary SBC with M significantly improved BW uniformity and feed efficiency; 0.20% SBC with halofuginone, lasalocid, M, or salinomycin significantly reduced mortality; and 0.20% SBC with lasalocid, M, or salinomycin significantly increased breast meat yield (Hooge et al., 1999b). It has been mentioned that enhancement of gut health by SBS during coccidial invasion was associated with reduced coccidial lesions and improved performance of chicks, suggesting a possible stimulation or acceleration of immunity and appearing to improve the efficacy of the coccidiostats (Hooge et al., 1999b). However, in the present study, the differences between the M and SBSM groups were not significantly different except for the improvement of FC in the prechallenge period, which may be related to possible moderately increased water intake owing to sodium consumption (Borges et al., 2004).

Overtime, anticoccidial drug development has increased in response to the urgent need to control avian coccidiosis. Today, there are several strategies available, many of which are currently widely used in the broiler industry. Moreover, new alternatives are emerging, as is the case with anticoccidials obtained from plants, fungi, or microorganisms (Lillehoj et al., 2011). One of the advantages of using natural extracts is the lower risk of developing resistance, such as that observed with chemical drugs (Blake and Tomley, 2014). It is well known that the availability of raw materials and the cost of production could be high in the development of natural extract alternatives. However, the cost may be well worth if one considers that these alternatives are friendly to the environment, producers, and consumers (Quiroz-Castañeda and Dantán-González, 2015).

It is concluded that consumption of SBS alone in *Eimeria*-challenged broilers has somewhat protective effects against coccidiosis although it is not as effective as M. Supplementation with the combination of M and SBS can be recommended to improve the FC in broilers especially for offsetting the reducing effect of M on feed consumption. The therapeutic effect and mechanism of action of SBS against coccidiosis in broiler chickens should be researched further.

ACKNOWLEDGMENTS

REFERENCES

Abbas, R. Z., Z. Manzoor, S. H. Munawar, Z. Iqbal, M. N. Khan, M. K. Saleemi, M. A. Zia, and A. Yousaf. 2011. Anticoccidial activity of hydrochloric acid (HCl) against *Eimeria tenella* in broiler chickens. Pesqui. Vet. Bras. 31:425–429.

Allen, P. C., and R. H. Fetterer. 2002. Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin. Microbiol. Rev. 15:58–65.

Blake, D. P., and F. M. Tomley. 2014. Securing poultry production from the ever-present *Eimeria* challenge. Trends Parasitol. 30:12–19.

Borges, S. A., A. V. Fischer da Silva, A. Majorka, D. M. Hooge, and K. R. Cummings. 2004. Physiological responses of broiler chickens to heat stress and dietary electrolyte balance (sodium plus potassium minus chloride, milliequivalents per kilogram). Poult. Sci. 83:1551–1558.

Bun, S. D., Y. M. Guo, F. C. Guo, F. J. Ji, and H. Cao. 2011. Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with *Eimeria tenella*. Poult. Sci. 90:1220–1226.

Chapman, H. D., T. K. Jeffers, and R. B. Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. Poult. Sci. 89:1788–1801.

Hodgson, J. N. 1970. Coccidiosis: oocyst counting technique for coccidiostat evaluation. Exp. Parasitol. 28:99–102.

Hooge, D. M., K. R. Cummings, J. L. McNaughton, C. L. Quarles, and B. A. George. 1999a. Dietary sodium bicarbonate, coccidial challenge, and ionophore coccidiostats in broiler chickens. J. Appl. Poult. Res. 8:89–99.

Hooge, D. M., K. R. Cummings, and J. L. McNaughton. 1999b. Evaluation of sodium bicarbonate, chloride, or sulfate with a coccidiostat in corn-soy or corn-soy-meat diets for broiler chickens. Poult. Sci. 78:1300–1306.

Idris, M., R. Z. Abbas, S. Masood, T. Rehman, U. Farooq, W. Babar, R. Hussain, A. Reza, and U. Riaz. 2017. The potential of antioxidant rich essential oils against avian coccidiosis. Worlds Poult. Sci. J. 73:89–104.

Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 28:30–36.

Kassem, I. I., Y. M. Sanad, R. Stonerock, and G. Rajashekara. 2012. An evaluation of the effect of sodium bisulfate as a feed additive on *Salmonella enterica* serotype Enteritidis in experimentally infected broilers. Poult. Sci. 91:1032–1037.

Kim, S. A., S. H. Park, C. Knueven, R. Basel, and S. C. Ricke. 2018. A decontamination approach using a combination of bisulfate soda and peracetic acid against *Listeria innucua* inoculated on whole apples. J. Food Cont. 84:106–110.

Kouwenhoven, B., and C. J. van der Horst. 1972. Disturbed intestinal absorption of vitamin A and carotenes and the effect of a low pH during *Eimeria acervulina* infection in the domestic fowl (*Gallus domesticus*). Z. Parasitenkd. 38:152–161.

Lillehoj, H. S., and E. P. Lillehoj. 2000. Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Dis. 1:408–425.

Lillehoj, H. S., D. K. Kim, D. M. Bravo, and S. H. Lee. 2011. Effects of dietary plant-derived phytonutrients on the genome-wide profiles and coccidiosis resistance in the broiler chickens. BMC Proc. 4:34.

Line, J. E. 2002. *Campylobacter* and *Salmonella* populations associated with chickens raised on acidified litter. Poult. Sci. 81:1473–1477.

Morris, G. M., and R. B. Gasser. 2006. Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in *Eimeria*. Biotechnol. Adv. 24:590–603.

Naidoo, V., L. J. Mcgaw, S. P. Bisschop, N. Duncan, and J. N. Eloff. 2008. The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. Vet. Parasitol. 153:214–219.

Olejnik, M., T. Szprengier-Juszkiewicz, and P. Jedziniak. 2009. Multi-residue confirmatory method for the determination of twelve coccidiostats in chicken liver using liquid chromatography tandem mass spectrometry. J. Chromatogr. A. 1216:8141–8148.

Payne, J. B., E. C. Kroger, and S. E. Watkins. 2002. Evaluation of litter treatments on *Salmonella* recovery from poultry litter. J. Appl. Poult. Res. 11:239–243.

Peek, H. W., and W. J. M. Landman. 2003. Resistance to anticoccidial drugs of Dutch avian *Eimeria* spp. field isolates originating from 1996, 1999 and 2001. Avian Pathol. 32:391–401.

Quiroz-Castañeda, R. E., and E. Dantán-González. 2015. Review article control of avian coccidiosis: future and present natural alternatives. Biomed. Res. Int. 2015:11.

Ruiz-Feria, C. A., E. Larrison, M. Davis, M. Farnell, J. Carey, J. L. Grimes, and J. Pitts. 2011. Supplementation of feed grade sodium bisulfate in broiler diets improves feed efficiency. Int. J. Poult. Sci. 10:670–676.

SAS Inst. 1989. SAS/STAT User's Guide, Version 6, 4th ed, Vol. 2. SAS Inst., Cary, NC.

Stephens, J. F. 1965. Some physiological effects of coccidiosis caused by Eimeria necatrix in the chicken. J. Parasitol. 51:331–335.

Stephens, J. F., L. M. Kowalski, and W. J. Borst. 1967. Some physiological effects of coccidiosis caused by *Eimeria maxima* in young chickens. J. Parasitol. 1:176–179.

Trivedi, H. K., and M. C. Patel. 2011. A stability indicating method for the determination of the antioxidant sodium bisulfite in pharmaceutical formulation by RP-HPLC technique. Sci. Pharm. 79:909–920.

United States Environmental Protection Agency (EPA). 2018. Safer chemical ingredients list. Accessed Sept. 2018. https://www.epa.gov/saferchoice/safer-ingredients.

Williams, R. B. 2002. Anticoccidial vaccines for broiler chickens: pathways to success. Avian Pathol. 31:317–353.

Williams, Z. T., J. P. Blake, and K. S. Macklin. 2012. The effect of sodium bisulfate on *Salmonella* viability in broiler litter. Poult. Sci. 91:2083–2088.

World Health Organization (WHO). 2007. Compendium of food additive specifications. FAO JECFA monographs *4*. Accessed Dec. 2018. http://www.fao.org/3/a-a1447e.pdf.

Xie, L., H. Feng, S. Li, G. Meng, S. Liu, X. Tang, Y. Ma, Y. Han, Y. Xiao, Y. Gu, Y. Shao, C. M. Park, M. Xian, Y. Huang, A. Ferro, R. Wang, P. K. Moore, H. Wang, and Y. Ji. 2016. SIRT3 mediates the antioxidant effect of hydrogen sulfide in endothelial cells. Antioxid. Redox Signal. 24:329–343.

Xie, Z. Z., M. M. Shi, L. Xie, Z. Y. Wu, G. Li, F. Hua, and J. S. Bian. 2014. Sulfhydration of p66Shc at cysteine59 mediates the antioxidant effect of hydrogen sulfide. Antioxid. Redox Signaling 21:2531–2542.

Yun, C. H., H. S. Lillehoj, and E. P. Lillehoj. 2000. Intestinal immune responses to coccidiosis. Dev. Comp. Immunol. 24:303–324.