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

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**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

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Received December 23, 2019.
Accepted June 19, 2020.

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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 (NaHSO₄) 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 bacteria (Line, 2002; Williams et al., 2012). Sodium bisulfate is hygroscopic, which after absorption of ambient moisture converts to sodium (Na⁺), hydrogen (H³⁻), and sulfate (SO₄⁻) 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 phosphate¹          | 1.6     | 1.8    | 1.9      |
| CaCO₃ (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 permix²               | 0.25    | 0.25   | 0.25     |
| Mineral permix³               | 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      |

¹Contained 20% P and 23% Ca.
²Supplied the following per kilogram of diet: 9,000 IU of retinyl acetate, 2,000 IU of cholecalciferol, 12.5 IU of dl-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 cyanocobalamin, 320 mg of choline chloride, 0.38 mg of folic acid, 60 mg of MnSO₄·H₂O, 80 mg of FeSO₄·7H₂O, 51.74 mg of ZnO, 8 mg of CuSO₄·5H₂O, 0.8 mg of iodized NaCl, 0.2 mg of Na₂SeO₃.
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:

$$EPEF: \frac{\text{Average BWG}}{\text{day}} \times \% \text{ Survival rate} / \text{FCR} \times 10$$

**Oocyst Counting**

The number of oocysts per gram (OPG) of faecal samples were counted on days 19 to 28 (5–14 D postinoculation [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, $\mu$ 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.
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 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.

**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.
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 all 3 supplemented groups showed a significant decrease compared with 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.

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

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

* $P$-value $< 0.05$.
2,000 ppm. Sodium bisulfate (NaHSO₃) 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 denaturized 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 (H₂S) 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 infection 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**

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

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