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Effects of a dry acidulant addition to prevent *Salmonella* contamination in poultry feed

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ABSTRACT

Salmonella subs. serovar Enteritidis is a potential biological pathogen of concern in the poultry industry. Contamination of the bacterium on eggshells has led to human illnesses. With the implementation of new regulations, animal feed manufacturing continues to be under more stringent requirements. Specifically, there is zero tolerance for *Salmonella* Pullorum, Gallinarum, or Enteritidis in poultry feed. For this reason, it is important to determine an effective method of reducing or preventing *Salmonella* contamination in feed for poultry. Therefore, the objective of this study was to evaluate the impact of sodium bisulfate (SBS; Jones-Hamilton, Co., Walbridge, OH) added to poultry mash to reduce or prevent *Salmonella* growth over time. A single, commercially produced all-flock poultry mash was mixed with four different levels of SBS: 0.0%, 0.25%, 0.50%, and 0.70%. After SBS addition, the treated mash was inoculated with *Salmonella enterica* subsp, *enterica* serovar Enteritidis (ATCC 13076) and enumerated for *Salmonella* on days 0, 1, 2, 7, and 14 post-inoculation by plating on xylose lysine deoxycholate agar. There was no significant effect of SBS inclusion level on the reduction of *Salmonella* ($P = 0.23$); however, there was a significant effect of time across treatments ($P < 0.0001$). Additionally, there was no inclusion level × time interaction ($P = 0.68$). These results suggest that while SBS inclusion has no effect on *Salmonella* concentrations, storage time is effective at reducing or eliminating *Salmonella* contamination in poultry feed.

Key words: acidifier, feed, poultry, *Salmonella*, sodium bisulfate

INTRODUCTION

Non-typhoidal *Salmonella* species are estimated to globally cause 93.8 million infections and 155,000 deaths each year (Varga et al., 2013). Approximately, 11% of *Salmonella* infections are attributed to contact with infected animals annually (Mead et al., 1999; Hale et al., 2012). Animal food is at the beginning of the food safety chain and may be contaminated through cross-contamination during manufacturing at the feed mill (Crump et al., 2002). The contaminated animal feed may, in turn, lead to infection of food-producing animals, including poultry. Pathogens such as *Salmonella* can then be transmitted through the food chain to humans, resulting in foodborne illness.

Salmonella is a Gram-negative bacterium that can be found in the intestinal tracts of many animals, including poultry (Heymann, 2008; Behravesh et al., 2014). Poultry are well recognized as carriers of *Salmonella* and may appear healthy while infected (Behravesh et al., 2014), thus posing the risk for zoonotic disease transfer to humans from birds that appear healthy. Some strains of non-typhoidal *Salmonella* have great survivability in animal feed (Andino et al., 2014), making feed a possible disease vector. By reducing *Salmonella* contamination in animal feed, the number of *Salmonella* infections in poultry flocks may be reduced, therefore reducing the risk to the human food supply.

There are several methods to control *Salmonella* contamination in animal food. High temperatures during pelleting and conditioning have been shown to reduce or eliminate *Salmonella* contamination (Cox et al., 1986), but this does not prevent post-processing contamination. The addition of chemicals such as formaldehyde, medium-chain fatty acids, organic acids, and essential oils to ingredients and finished swine feed have been shown to reduce *Salmonella* contamination to varying degrees of effectiveness (Cochrane et al., 2016).

It has been well established that acidifiers reduce pH and can prevent *Salmonella* growth and viability (Humphrey and Lanning, 1988; Matlho et al., 1997; Koyuncu et al., 2013). Acidifiers are also an attractive option to utilize as a feed safety mechanism because many are currently included in poultry diets to improve growth performance (Hamid et al., 2018), improve digestibility (Abdollahi et al., 2020), and induce changes in the gastrointestinal tract (Hamid et al., 2018). However, many of these acidifiers are hazardous to handle, cause equipment corrosion, and require specialized equipment for inclusion.

Sodium bisulfate (SBS) is an acidifier that is generally recognized as safe (GRAS) by the Food and Drug Administration. It is a weak acid that is commonly available in a bulk, dry, powdered form and approved for inclusion in poultry feed

to reduce pH. Sodium bisulfate is hygroscopic (Sun et al., 2008) and dissociates into sodium, hydrogen, and sulfate when moisture is absorbed. It has been shown to induce a considerable reduction in the populations of various bacteria when applied to poultry litter (Pope and Cherry, 2000). Despite this, SBS has not been evaluated for its effectiveness in reducing bacterial populations in poultry diets. Sodium bisulfate is an attractive alternative to traditional acidifiers due to its ease of use, reduced equipment corrosion, and improved palatability (European Food Safety Authority, 2014). Therefore, the objective of this study was to evaluate the ability of SBS to reduce or prevent *Salmonella* growth over time in poultry mash diets. The hypothesis of this experiment is that SBS inclusion will decrease *Salmonella* concentrations in contaminated feed, with the greatest effect occurring at 0.7% inclusion.

MATERIALS AND METHODS

SBS Inclusion

A single, commercially produced all-flock poultry mash (Country Lane, Moberly, MO) was coated with powdered SBS (Jones Hamilton Co., Walbridge, OH). The manufacturer recommends an inclusion rate of 0.5%, so this experiment targeted inclusion rates below, equal to, and above these recommendations. The untreated nutritional analysis and ingredient list of the poultry mash are presented in Table 1. SBS was added at the inclusion levels of 0.0%, 0.25%, 0.50%, and 0.70% w:w to mimic industrial coating levels typically used in poultry mash formulations. For each level of inclusion, SBS was mixed thoroughly for 5 min for an even distribution.

Table 1. Formulation and ingredient list[a] of the all-flock mash

Ingredient	Guaranteed analysis, %
Crude protein (min)	16.00
Lysine (min)	0.60
Methionine (min)	0.30
Crude fat (min)	3.00
Crude fiber (max)	9.00
Calcium (min)	1.50
Calcium (max)	2.00
Phosphorous (min)	0.50
Salt (min)	0.25
Salt (max)	0.75
Sodium (min)	0.15
Sodium (max)	0.65
Vitamin A (min)	3,000 IU/lb
Vitamin E (min)	20 IU/lb

[a]Processed grain by-products, grain products, plant protein products, calcium carbonate, sodium bentonite, salt, l-lysine, dl-methionine, ferrous carbonate, ferrous sulfate, copper sulfate, manganous oxide, manganese sulfate, zinc oxide, zinc sulfate, cobalt carbonate, sodium selenite, vitamin A supplement, vitamin D_3 supplement, vitamin E supplement, menadione dimethylpyrimidinol bisulfite, thiamine mononitrate, riboflavin supplement, niacin supplement, choline chloride, calcium pantothenate, pyridoxine hydrochloride, folic acid, biotin, vitamin B_{12} supplement, and propionic acid (a perservative).

Salmonella Inoculum Preparation and Inoculation

Salmonella enterica subsp, *enterica* serovar Enteritidis (ATCC 13076) stock stored at –80 °C was transferred to fresh tryptic soy broth (TSB; Difco, Franklin Lakes, NJ, USA) and incubated at 35 °C for 48 h. Following incubation, the cultured TSB was added to 100 g of previously sterilized, uncoated poultry mash and allowed to dry overnight in the biosafety cabinet. The prepared inoculum was then mixed with the previously treated poultry mash at each of the four inclusion levels. Treated and inoculated poultry mash was shaken vigorously for 2 min to evenly distribute the inoculum. Following inoculation, the mash was allowed to sit at room temperature (20 °C based on room thermostat settings) for 1 h prior to the collection of day-0 enumeration. Additionally, all inoculated treatments were stored at room temperature throughout the 14-d sampling period.

Sample Plating and Enumeration

Enumeration of *Salmonella* was carried out on days 0, 1, 2, 7, and 14 post-inoculation according to the methods described by Andrews et al. (2018). For each day of enumeration, subsamples were collected, diluted with buffered peptone water (BPW; Difco, Franklin Lake, NJ), and stomached for 30 s in a stomacher (Seward 400, Davie, FL). Following sample stomaching, serial dilutions in BPW were performed and spread plated onto xylose lysine deoxycholate (XLD) agar (Difco, Franklin Lake, NJ). All inoculated XLD plates were incubated at 35 ± 2 °C for 24 ± 2 h. After incubation, black colonies typical for *Salmonella* were counted and total colony forming units (CFU) per gram calculated.

Statistical Analysis

Data were analyzed using the GLIMMIX procedure of SAS version 9.3 (SAS Inst. Inc., Cary, NC) and were $\log_{10}$ transformed prior to analysis because the data spanned a large range in order of magnitude and because bacterial population dynamics are exponential in nature (Gao et al., 2020). The model included the fixed effects of SBS inclusion level, sampling day, and the interaction between inclusion level and sapling day, and sampling day served as a repeated measure. The first-order autoregressive covariance structure was used for all repeated measures analyses because the correlation between subsequent samplings was assumed to decline exponentially with time. There were three experimental units of each SBS inclusion level. Differences were considered statistically significant at $P < 0.05$, and means were separated using pairwise comparisons with least significant difference when the analysis of variance revealed a significant difference.

RESULTS AND DISCUSSION

Sodium bisulfate, regardless of inclusion level, did not impact ($P = 0.23$) *Salmonella* concentration in the current experiment (Fig. 1). Previous research has shown that SBS does not affect the prevalence of *Salmonella* in broiler houses (Line and Bailey, 2006) or poultry litter (Williams et al., 2012). It is possible that the inclusion rate of SBS in the current experiment was not great enough to evenly coat all the feed particles, therefore reducing the probability that SBS will interact with *Salmonella* and reduce the population of bacteria. Additionally, the mash form of the diet in

this experiment may have also contributed to the growth of *Salmonella* as the particle size would be smaller and, therefore, provide greater surface area for *Salmonella* to attach. Although pH was not measured in the current experiment, it is possible that the SBS did not decrease the pH of the feed to the point that it would affect *Salmonella* concentrations. The *Salmonella* spp. is well adapted for survival in mildly acidic conditions (Álvarez-Ordóñez et al., 2012), and Payne et al. (2007) demonstrated that *Salmonella* populations can be reduced in poultry litter below detectable limits at a pH of 4.

While SBS did not impact *Salmonella* concentration, time substantially reduced ($P < 0.0001$) the concentration regardless of the inclusion level of SBS (Figs. 2 and 3). At the end of the 14-d storage period, *Salmonella* was undetectable in all treatments. These results are supported by Williams et al. (2012) who demonstrated that *Salmonella* concentrations declined over time in poultry litter. This effect may be explained by changes in water activity over time. Payne et al. (2007) demonstrated that *Salmonella* populations can be reduced through
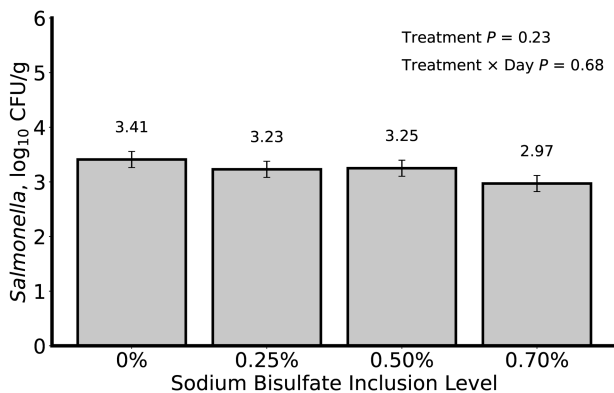

Figure 3. Effects of dry acidulant inclusion level on *Salmonella* Enteritidis over time. A single, commercially produced all-flock poultry mash was treated with a dry acidulant, sodium bisulfate, at 0.0%, 0.25%, 0.50%, and 0.70%. On day 0, samples were inoculated with *Salmonella* and enumerated for *Salmonella* on XLD agar. Stored samples were enumerated for *Salmonella* Enteritidis on days 1, 2, 7, and 14 post-inoculation. $P < 0.05$.
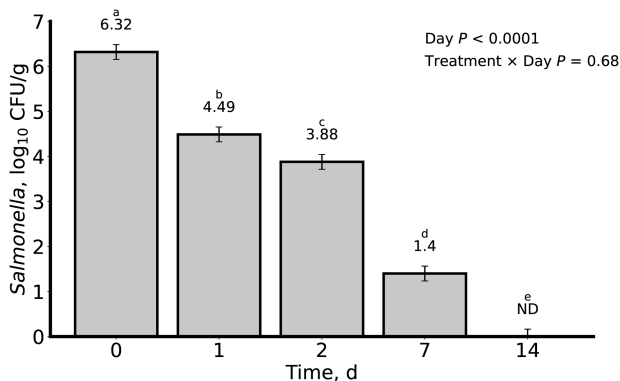
a reduction in water activity. As feed is stored, water activity decreases, which may explain the reduction in *Salmonella* concentration during feed storage in this experiment. Therefore, feed storage may be an effective step to reducing bacterial contamination, and the results of this experiment suggest that storing feed for 14 d may reduce *Salmonella* contamination in feed. However, practical considerations limit this as an effective control measure, as most poultry feed in integrated systems is fed within days of manufacturing.

In conclusion, dry acidulant addition had no effect on the concentration of *Salmonella* Enteritidis in mash poultry diets, regardless of inclusion level. However, *Salmonella* concentrations declined over time, reaching an undetectable level after 14 d of storage. Practical considerations regarding feed manufacturing and feeding limit the use of storage as an effective solution to feed contamination; therefore, further research is needed to identify effective and practical methods to reduce *Salmonella* Enteritidis contamination in poultry feed.

Figure 1. Effect of dry acidulant inclusion level on *Salmonella* Enteritidis level. A single, commercially produced all-flock poultry mash was treated with a dry acidulant, sodium bisulfate, at 0.0%, 0.25%, 0.50%, and 0.70%. On day 0, samples were inoculated with *Salmonella* and enumerated for *Salmonella* on XLD agar. Stored samples were enumerated for *Salmonella* Enteritidis on days 1, 2, 7, and 14 post-inoculation. $P < 0.05$.

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Figure 2. Effect of day on *Salmonella* Enteritidis level. A single, commercially produced all-flock poultry mash was treated with a dry acidulant, sodium bisulfate, at 0.0%, 0.25%, 0.50%, and 0.70%. On day 0, samples were inoculated with *Salmonella* and enumerated for *Salmonella* on XLD agar. Stored samples were enumerated for *Salmonella* Enteritidis on days 1, 2, 7, and 14 post-inoculation. Means lacking a common superscript letters (a–e) differ; $P < 0.05$.
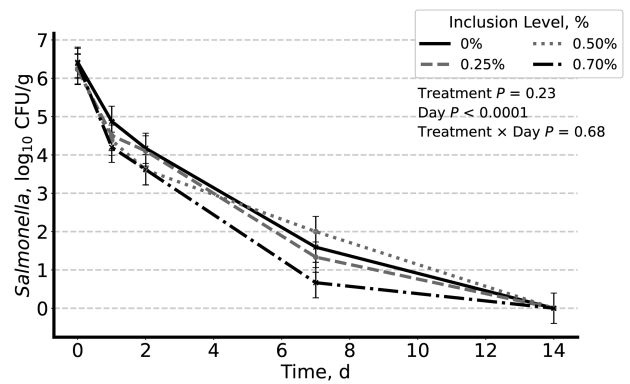
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