IMPACT OF LIVE COCCIDIOSIS VACCINE ON INTESTINAL MORPHOLOGY, PERFORMANCE, AND VITAMIN D STATUS OF BROILERS: USE OF DIETARY SUPPLEMENTATION OF ANIMAL FEED GRADE SODIUM BISULFATE AND 25-HYDROXYCHOLECALCIFEROL

A Dissertation

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ABSTRACT

Studies were conducted to determine the extent to which animal feed grade sodium bisulfate (SBS) can help ameliorate the detrimental effects of coccidiosis in broilers. Four trials were conducted to establish a working foundation for SBS mode of action and differences between twenty-five hydroxycholecalciferol (25-OH-D_3) vs D_3 in broilers subjected to a live coccidiosis vaccine challenge. Previous research indicates the use of 25-OH-D_3 as replacement or partial replacement of cholecalciferol (D_3) in broiler diets can result in significant differences in performance and bone mineralization. This series of studies analyzed the impact of a 2X recommended dose coccidiosis vaccine challenge on vitamin D status, intestinal morphology, performance and bone mineralization in broilers. Studies revealed a consistent performance advantage from the dietary inclusion of 0.3 and 0.4% of SBS. Increasing SBS in the diet reduced ($P < 0.001$) feed pH and increased sodium and sulfur content ($P < 0.001$) of the diet. SBS didn't appear to modulate intestinal pH and similar performance advantages (higher body weights, improved feed conversion) can be expected when using animal feed grade sodium bisulfate or potassium bisulfate vs Control. Intestinal morphology was negatively modulated under the current coccidiosis vaccine challenge. SBS fed to broilers under coccidiosis vaccine challenge significantly reduced crypt depth compared to Control suggesting improved intestinal integrity. Vitamin D status can significantly ($p<0.05$) be impacted by a 2X coccidiosis vaccine challenge. Malabsorption syndrome associated with coccidiosis challenge was observed during the trial which resulted in significantly lower bone mineralization, and serum 25-OH-D_3 concentration in birds fed a control diet. The results suggest the use of

dietary 25-OH-D_3 compared to inclusion of D_3 alone provides a significantly improved safety margin for performance and bone mineralization. The results of this research effort provide practical information for the poultry industry as it relates to nutritional strategies that could be further studied as means to maintain performance under coccidiosis vaccine and offer flexibility in feed formulation.

DEDICATION

I dedicate this work to my wife Katie, my mom, dad, brother, and friends for they have always supported my goals and dreams.

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1 INTRODUCTION

The poultry industry has evolved to become an effective, high yield, and economically feasible industry through the development of better ventilation, housing, animal care, and intense research. However, due to high levels of production and in combination with consumer demand and media pressure the poultry industry has been forced to develop new strategies for pathogen control, animal health, and food safety without the use of antibiotics as growth promoters. In recent years, many changes have transpired in the poultry industry in regards to the use of antibiotics, which has resulted in major impacts on gut health, specifically concerning coccidiosis and necrotic enteritis.

Coccidiosis is a major concern within the poultry industry for its detrimental effects in poultry health and substantial economic losses that exceed $3 billion dollars worldwide (Anon, 2013). Coccidiosis is caused by intestinal infection with coccidia protozoan parasites, which are commonly found in broiler grow out houses (Merck Veterinary Manual, 2016).

Major focus is put in the first stages of the growth curve of a broiler in order to supply the nutrients that can support the fast growth curve. The industry has looked at several strategies to maintain flock health and performance without the use of antibiotics. Current strategies that have received more attention include: coccidiosis vaccines, probiotics, prebiotics, essential oils and acidifiers. Animal feed grade sodium bisulfate has been used as an acidifier in poultry feed before.

In today's commercial poultry industry, balancing cost and performance is an everyday challenge that requires an understanding of the interrelated nature of many production factors. Nutrition plays a critical role in maintaining production and economic feasibility. There are many strategies and additives aimed at maximizing performance, and maintaining animal health and welfare while keeping costs within acceptable margins. Currently, poultry nutritionists are concerned about the negative effects of coccidiosis vaccine on vitamin D status and how to better implement nutritional strategies in order to increase the safety margin to guarantee maximum genetic potential. The need to develop successful nutritional strategies that can maintain gut integrity under coccidiosis challenge is imperative in commercial poultry production.

There is great concern in the industry that the shift that has happened in commercial production in regards to the absence of antibiotics will increase coccidiosis problems as most enteric related issues (Cervantes, 2015). As enteric health in commercial broilers decreases, the risk for a loss in performance increases. Nutritionists have to be aware of field issues that can hinder the ability of the flock to utilize all the nutrients carefully formulated in a diet. Multiple strategies exist in order to combat problems associated with antibiotic free production. Enteric health has received major focus, as it is necessary to maintain production standards.

Animal feed grade sodium bisulfate has been used in poultry feed before as an acidifier and continues to be studied as a potential nutritional strategy to maintain intestinal integrity during conditions of disease challenge. In the literature, villi height to crypt depth ratio is a well-accepted parameter to measure gut integrity and nutrient

absorption capacity. A higher villi height to crypt depth ratio indicates an improved intestinal nutrient absorption capacity, and their measurements are significant indicators of intestinal integrity (Awad et al., 2008).

Multiple studies have reported positive effects on broiler performance from dietary supplementation of SBS (Ruiz-Feria et al., 2011; Kassem et al., 2012; Chadwick et al., 2020). This compound of acidic nature has shown positive effects on poultry health and grow-out conditions due to its ability to effectively bind to ammonia in order to improve air and litter quality, while having various effects on bacteria found in the environment (Pope and Cherry, 2000). It is currently used in pet food as an effective feed acidifier. It is worth mentioning that most of the studies with SBS reported in the literature were conducted in the absence of a coccidiosis vaccine challenge.

Sodium bisulfate is composed of sodium, hydrogen, and sulfate ions. All these ions play key roles in gut function such as acid–base balance, electrolyte homeostasis, and in the case of sodium absorption of sugars and fluids. (Gennari, and Weise 2008). Sulfate serves a key component for the maintenance of tissues (Hooge, et al., 1999; Ahmad, et al., 2006). Therefore, increased availability of these compounds during conditions of stress could provide a benefit to the host.

In previous research conducted at our lab and published data from North Carolina State University, significant difference in short chain fatty acids (SCFA) production in broilers fed SBS was observed. SCFA are carboxylic acids of 1–6 carbons formed by the host's own microbiota fermentation of undigested dietary carbohydrates and dietary fibers in the intestine entering the distal gut (Topping and Clifton, 2001). These organic fatty

acids produced within the intestinal lumen by bacterial fermentation allow for the salvage of energy mainly from carbon sources that are not digested in the small intestine. It has been estimated that SCFA can contribute 5% to 15% of the total caloric requirements of humans (Bergman, 1990) and between 20 to 30% the maintenance energy requirement in pigs (Rerat, et al., 1987; Yen et al, 1991).

The SCFA that are most abundant in the gastrointestinal tract (GIT) are acetate (C2), propionate (C3), and butyrate (C4). SCFA play a significant role in energy metabolism, they serve as energy substrates for multiple host cells, and very importantly participate in different host-signaling mechanisms; butyrate for example is considered an epigenetic substance. SCFA play an important role in maintaining gut homeostasis, and energy metabolism. Further research has shown evidence of their potential in reducing the risk of developing digestive disorders.

Vitamin D status has been shown to have a role in immune function. The development of the different effector mechanisms of the chicken adaptive immune system have been further studied in recent decades. In order to maintain a high level of production while upholding animal health at all levels of the production cycle one has to understand the way the early development of the chicken adaptive immune system will be able to effectively combat pathogens at a commercial production setting. Modern poultry production results in birds being exposed to multiple immunological challenges. Malabsorption syndrome is a common observation in birds challenged with coccidiosis, (Allen and Fetterer, 2002).

Previous research indicates the use of twenty-five hydroxycholecalciferol (25-OH-D_3), as replacement or partial replacement of cholecalciferol (D_3) in broiler diets, can result in significant differences in performance and bone mineralization. However, in order to better comprehend the benefits and limitations of vitamin D metabolites as a nutritional strategy especially in antibiotic free production, future research should aim at evaluating these nutritional strategies at different dietary concentrations and in the presence of a coccidiosis challenge. It has been reported that Vitamin D may have a role in modulating the morphological and functional development of intestinal villus mucosa (Shinki et al., 1991).

Commercial broilers often are exposed to stressful conditions and field challenges that can generate a malabsorption syndrome, and as a result have an impaired absorption of nutrients or liver hydroxylation of vitamin D_3. In our lab, vitamin D status in broilers can be limited because of vitamin D source, dosage, and vaccine challenge. As enteric health in commercial broilers decreases, the risk for a loss in performance increases. Nutritionists must be aware of the impact on gut function from commonly used vaccines and management practices, which can ultimately hamper the ability of the flock to utilize all the nutrients carefully formulated in a diet.

The use of dietary 25-OH-D_3, as replacement or in addition to vitamin D_3 is effective in promoting performance, enhancing bone mineralization (Leyva-Jimenez, et al. 2019), and modifying avian immunity (V.G. Gómez, et. al. 213). Measuring vitamin D absorption in serum concentration of 25-OH-D_3 could give us an indication of an improved health status of birds challenged with coccidiosis vaccine. Multiple reports in

the literature identify serum 25-OH-D_3 concentration as an effective indicator of the vitamin D status of a broiler. The half-life of 25-OH-D_3 is close to three weeks making it ideal to test vitamin D status.

Among the different objectives of this study was to further study practical nutrional strategies like dietary supplementation of animal feed grade sodium bisulfate and 25-hydroxycholecalciferol 25-OH-D_3 at different inclusion levels to combat common detrimental effects from coccidiosis. To establish a robust scientific foundation for the generation of new avenues of research in applied poultry and swine nutrition. Assess the impact on performance, intestinal morphology, and vitamin D status in broilers administered a 2X recommended dose live coccidiosis vaccine challenge at day of placement. Analyze the effect of SBS dietary supplementation and 25-OH-D_3 on broilers subjected to coccidiosis vaccine vs unvaccinated birds.

2 STUDY ON THE FEED MIXING UNIFORMITY OF ANIMAL FEED GRADE SODIUM BISULFATE AND ITS IMPACT ON INTESTINAL PH, AND PERFORMANCE OF BROILERS

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Summary

Two experiments were conducted with the objective to analyze the effects on intestinal pH and performance of broilers fed animal feed-grade sodium bisulfate (SBS) as well as determine changes in feed composition, and feed mixture uniformity when adding SBS. At day 0, 288-day-old-male Ross708 broiler chicks were randomly assigned to one of four dietary treatments: Control, Encapsulated SBS (EnSBS), SBS or animal feed-grade potassium bisulfate (KBS). Broiler chicks were placed in battery brooders for an 18-day trial period. SBS and KBS were included in the experimental ration at 0.3%, while EnSBS at 0.15%. Replicates were arranged in a completely randomized block design with six broilers per replicate and twelve replicates per treatment, a 4x1 factorial arrangement. Body weights (BW), feed consumption (FC), intestinal pH (pH), and feed:gain (FCR) were calculated at day 7, 14, and 18. No significant differences ($p>0.05$) in pH, or FC reported but BW, and FCR were significantly ($P<0.05$) different at day 14 and 18. SBS and KBS showed higher BW and lower FCR. Experiment two treatments consisted of a 2x3 factorial design with main effects of corn particle size (700vs 1,000µm) and SBS inclusion of 0, 0.25, and 0.50%. Increasing SBS in the diet reduced ($P < 0.001$) pH and increased sodium and sulfur content ($P < 0.001$) of the diet. For CV sulfur, there was a diet×particle size interaction ($P = 0.029$) in the 0.50% SBS inclusion compared to all other treatments. However, all treatments had an acceptable (< 10% CV) CV for sulfur content.

Key words: sodium bisulfate, broiler, uniformity, pH, sulfur

Description of the problem

In commercial poultry production, feed and feed manufacture represent a considerable production cost for an integrator. Research has consistently demonstrated significant benefits associated with correct feed manufacturing including pelleted diets (Behnke, 1996). The uniformity of a feed mixture is determined from the coefficient of variation (CV) from feed samples collected when mixing a batch of feed. The feed industry standard for many years has been a % CV of less than 10 using a single source tracer such as salt or trace mineral; the uniformity of a feed mix can be affected by many factors including equipment design, ingredient properties, mixing time, and sample preparation (Clarke, 2007). Measuring accurately the uniformity of a feed mix when using a feed additive is of utmost importance and can be a determining factor in whether the feed additive can be of practical use for an integrator or not. Therefore, one of the objectives of this study was to determine if a uniform mixture can be achieved when sodium bisulfate is added.

In the poultry industry, many changes have transpired in regards to the use of antibiotics. These changes have resulted in an increase incidence of enteric related issues that affect performance and poultry health. There is great concern in the industry that the shift that has happened in commercial production in regards to the absence of antibiotics will increase coccidiosis problems as most enteric related issues (Cervantes, 2015). In commercial poultry production, flock performance can be significantly impacted from commonly detrimental enteric conditions. The use of antibiotics in the majority of commercial poultry operations has been reduced or eliminated because of concerns with

the development of antibiotic-resistant bacteria of human interest. In 2006, the European Union banned the use of prophylactic antibiotics in animal feeds and soon after the United States will follow their direction. Undoubtedly, it has become critical to find effective strategies to ensure animal health and productivity without the use of antibiotics.

Feed grade acids used in water and feed have been studied in animal nutrition for many years in part to control pathogens and establish a pH conducive for beneficial bacteria. Acids can affect pathogens by disrupting cellular pH gradients and intracellular regulation of pH as well as having direct toxic effects on membrane structure and macromolecule synthesis (Ricke, 2003). Animal feed grade sodium bisulfate has been used in poultry feed before. It is currently used in pet food as an effective feed acidifier.

Multiple studies report positive effects on broiler performance from dietary supplementation of sodium bisulfate (Ruiz-Feria et al., 2011; Kassem et al., 2012; Chadwick et al 2020). It has been reported that diet acidification with feed additives can reduce the prevalence of Salmonella (Wales et al., 2010). It is known that nutrient bioavailability and mineral absorption can be altered due to pH changes in the GI tract. One of the objectives of this study was to determine if intestinal pH changes could be attributed to SBS and therefore serve as the base in explaining the performance advantages when used in poultry diets.

In recent years, there has been an increased interest in the use of encapsulated feed additives in an effort to increase product effectiveness by delivering the product further down the gastrointestinal (GI) tract. One of the objectives of this study was to determine if the use of an encapsulated process SBS could be used at a lower concentration or impact

performance of broilers more efficiently. The current study focused on the use of SBS and included the evaluation of animal feed grade potassium bisulfate in order to identify the effects of the sulfate portion of the product when coupled with sodium vs potassium. Sulfate is an anion that serves as an important component for the maintenance of tissues (Markovich, 2001). It is also essential for sulfonated carbohydrates in mucin, a protective barrier of the gastrointestinal tract (Dawson et al, 2009).

SBS is a compound of acidic nature that has shown positive effects on poultry health and grow-out conditions due to its ability to effectively bind to ammonia in order to improve air and litter quality, while having various effects on bacteria found in the environment (Pope and Cherry, 2000). It is well known that commercial broilers often are exposed to stressful conditions, pathogens, and field challenges that can generate an impaired absorption of nutrients.

The nutritional status of a bird can determine the level and efficacy of its response to stress, for example coccidiosis (Yun, et al., 2000). SBS may promote performance of broilers under coccidiosis vaccine challenge by supporting intestinal integrity and function. Therefore, this study aimed to analyze the effect of dietary supplementation of animal feed grade sodium bisulfate on performance, and intestinal pH of broilers subjected to a 1X dose coccidiosis vaccine.

Materials and methods

Experiment I

All animal handling and care practices were in accordance with an approved animal use protocol by the Texas A&M institutional animal care and use committee (IACUC). Broilers were monitored daily with regard to general flock condition, temperature, lighting, water, feed, and any unanticipated events for the rearing facility. At day 0, broiler chicks were placed in two Petersime battery brooders environmentally controlled at Texas A&M Poultry Research Center. A total of 48 (24 per room) individual brooding cages were used to allocate the broilers; each treatment was represented at least one time in each level of the battery brooders. Six randomized Ross 708 male day-old-broilers (Gonzalez, Texas) were placed into each cage. All broiler chicks received a 1x dose of coccidiosis vaccine (B52, Merck Animal Health, Madison, New Jersey) via spray cabinet at the hatchery prior to pick up. Twelve replicates per dietary treatment in a completely randomize block design were used (6 birds per treatment * 4 treatments * 12 replicates per treatment = 288) a 4*2 factorial arrangement. All broilers were weighed individually and grouped together so that broilers could be allocated to each cage with nearly identical initial body weights and variance (±5 g). Cage average was the unit of measurement for all performance variables. Individual broilers were the unit of measure for intestinal pH.

Diets

A basal corn-soy broiler starter diet was formulated using a standard mineral-vitamin premix. Dietary treatments consisted of a control diet and diets supplemented with either animal feed grade sodium bisulfate (encapsulated vs not encapsulated) or animal feed

grade potassium bisulfate (KBS) manufactured by Jones-Hamilton Co (Walbridge, OH) at the different levels indicated in Table 1 and fed from 0-18d. SBS was added at the expense of sodium chloride. Feed samples were collected prior to the start of the trial in order to analyze the formulated nutrient composition of each diet by Near Infrared Reflectance spectroscopy (NIR). Samples were sent to Feed and Water Environmental Lab UGA (Athens, Ga). Mineral analysis was conducted to determine the extent to which sodium bisulfate can modulate the sulfur levels in the feed. Feed and water was *ad libitum* during the entire experimental period. No antibiotics or coccidiostats were used during the study. Feed samples of all diets were tested for pH levels at day of feed mixing.

A composite sample was collected from three different individual feed samples collected randomly from each of the finish dietary treatments. Following feed sample collection, 10 g from each of the composite samples was used and placed individually in 50 mL of deionized water. Using a pH meter *Oakton pH Testr 5* (Oakton Co, Vernon Hills, Illinois) the pH level was recorded.

Performance

Feed consumption and body weights were recorded on day 0, 7, 14 and 18. Weight gain, feed: weight ratio, and mortality were calculated weekly. The experimental broilers were observed daily for any clinical signs and mortality. Mortality and body weight of dead birds were recorded daily.

Intestinal sampling

On day 7, 14, & 18, one randomly selected bird per cage was euthanized by cervical dislocation. The small intestine (duodenum, jejunum, & ileum) was analyzed by inserting a pH probe (Waterproof meat pH meter, Hanna Instruments, Woonsocket, RI) directly in situ immediately post euthanasia. The fine tip of the pH probe was dip in 70% ethanol prior to each reading of each intestinal section; the pH was recorded twice, and the mean of the two measurements was used for statistical evaluation (Jimenez-Moreno, et al. 2009). The duodenum readings were taken at the middle of the duodenal loop; the jejunum readings were taken in the middle point between the bile ducts and the Meckel's diverticulum; the ileum samples were taken 8 cm proximal to the ileocecal junction.

Statistical analysis

Collected data was analyzed as one way- ANOVA using the GLM Procedure of SPSS (SPSS Inc., Chicago, IL, USA) for a completely randomized design where treatment diets were used as blocks in the model. If significance, means were separated by Duncan's Multiple Range Test. Significance was accepted at $P \leq 0.05$.

Experiment II

Feed was manufactured in accordance with CGMPs at the Kansas State University OH Kruse Feed Technology Innovation Center (Manhattan, KS). Experimental treatments consisted of a 2 × 3 factorial design with main effects of corn particle size (700 and 1,000 µm) and inclusion rate of animal feed grade sodium bisulfate (Jones-Hamilton, Walbridge, OH) at 0, 0.25, and 0.50 %. Each of the six treatments (Table 4) were mixed in three separate batches to provide three replications per treatment.

A typical corn and soybean meal diet were used for this study. The major ingredients (corn and soybean meal) were added to the mixer first, followed by the micro ingredients including SBS. Diets were then mixed in 200 lb batches for five minutes using a 200 lb mixer (Davis paddle mixer SS-S1; 6 cubic ft). Approximately 20 lbs was discharged from the mixer. During this discharge step, a feed sample taken from the stream line was collected. This process was repeated until the mixer was empty, allowing collection of ten samples per 200 lb batch of feed. These samples were then analyzed for sodium, sulfur, zinc, and copper.

A coefficient of variation was calculated for each marker by dividing the standard deviation by the mean of the ten samples. In addition, a composite feed sample was used for analysis of pH measured the same day of feed mixing.

Statistical analysis

Data was analyzed as 2 x 3 factorial using the PROC-GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) batch serving as the experimental unit. Contrast statements were used to separate treatment means with comparison of diet (swine vs poultry) and linear

and quadratic polynomials were used to test increasing SBS within each diet. Results were considered significant $P \leq 0.05$, and marginally significant at $P \leq 0.10$.

Results and discussion

Experiment I

Results of all performance parameters are summarized in Table 5. There were differences ($P<0.05$) at day 7 and 18 between dietary treatments with SBS-fed broilers having higher body weights compared to Control broilers. KBS vs SBS fed birds had no significant body weight differences throughout the experimental period. The birds fed the encapsulated SBS finished with higher ($P<0.001$) body weights by day 18 compared to Control. There were no significant differences in body weights between encapsulated SBS and the dietary treatments KBS and SBS except by day 18 when KBS-fed broilers had higher ($P<0.001$) body weights. Several studies in the past have shown a coccidiosis vaccine effect on body weights (Peek, 2011, Chapman, et al. 2013, Ahmad, et al. 2016).

Feed conversion ratio (FCR) was consistently less efficient in Control compared to all other dietary treatments. At the end of the trial, all dietary treatments had improved ($P<0.05$) cumulative FCR when compared to Controls. Studies have indicated a positive correlation with broiler growth if given sodium bisulfate in the diet with or without a disease challenge (Line, 2002; Ruiz-Feria et al 2011; Kassem et al 2012).

A dietary effect was more noticeable in the concluding part of the study, which suggests that the difference became apparent as coccidia-induced gut damage increased. Sodium bisulfate is composed of sodium, hydrogen, and sulfate ions. All these ions play key roles in gut function such as acid–base balance, electrolyte homeostasis, and in the

case of sodium absorption of sugars and fluids. (Gennari, and Weise 2008). Sulfate serves a key component for the maintenance of tissues (Hooge, et al., 1999; Ahmad, et al., 2006). Therefore, increased availability of these compounds during conditions of stress could provide a benefit to the host. At the end of the trial, cumulative feed conversion ratio was significantly ($P<0.05$) improved in broilers fed any of the three sources of sulfate compared to control. These results are comparable to those seen by Ruiz-Feria et al. (2011), where broilers on a diet with SBS finished with lower FCR compared to a control diet. Chadwick et al., (2020) indicated that broilers fed SBS at 0.5% under coccidiosis challenge showed significantly ($P<0.05$) lower cumulative FCR compared to control.

We hypothesize that SBS may be ameliorating the negative effects associated with coccidia in the gastrointestinal tract by maintaining a higher level of gut integrity compared to Control. Broilers fed a control diet seemed to experience the highest loss in performance during the last week of the trial, where the ($P<0.05$) differences in feed conversion vs sulfate-fed broilers further developed. KBS-fed broilers had consistently numerically lower cumulative feed consumption compared to Control. Feed consumption data showed no significant differences among the treatments during the experimental period (0 to 18 d).

No consistent trend was observed on intestinal pH from a dietary effect. Intestinal pH data 7-18 days is summarized on Table 6. No significant differences were observed at day 7. By the second week of the experimental period, a dietary effect was observed with broilers fed the encapsulated form of SBS showing lower ($P<0.05$) jejunum pH compared

to all other dietary treatments. Both KBS and SBS had significantly lower ileum pH compared to Control.

At day 18, KBS-fed birds had higher ($P<0.05$) duodenum pH but lower ($P<0.05$) jejunum pH compared to SBS. In addition, SBS-fed birds finished with a significantly higher ileum pH compared to Control and SBS encapsulated treatments. A low pH in the gastrointestinal tract may improve the solubility and absorption of mineral salts (Guinotte et al., 1995). Future research should include crop and gizzard pH measurements. According to Jiménez-Moreno et al. (2009) a lower gizzard pH could increase pepsin activity and mineral absorption.

In regards to feed pH, there was a reduction in pH noticed among the different diets. A reduction of 0.70 points in pH was observed between KBS, SBS and the other two dietary treatments. No statistical analysis was possible due to each sample was obtained from only one replicate batch. Further feed pH analysis with replication was conducted in experiment two.

Experiment II

A summary of all the results from experiment two are shown in Table 7. Experiment two showed significant ($P<0.001$) differences in mineral content from SBS diets. Increasing SBS in the diet reduced ($P < 0.001$) pH and significantly increased sodium and sulfur content ($P < 0.001$) of the diet. During analysis of the coefficient of variation (CV) of sulfur, there was a diet × particle size interaction ($P = 0.029$) noted.

The feed industry standard for many years has been a % CV of less than 10 using a single source tracer such as salt or trace mineral (Clarke, 2007). This resulted from an

increased CV in the poultry diet containing 10 lb./ton SBS compared to all other treatments. Even though this difference was detected, all treatments had an acceptable (< 10% CV) CV for sulfur content.

Correct poultry feeding requires a homogenous mixture of macro and micro ingredients to ensure all formulated nutrients are effectively supplied to meet the bird's nutrional requirements. There was no evidence of difference for sodium CV with increasing concentrations of SBS in the diet.

Based on CV of sulfur and sodium, it is concluded that SBS can be adequately mixed in the diet when added with micro ingredients using a predetermined mixed time.

Conclusions and applications

1. Increasing SBS in the diet reduces ($P < 0.001$) pH and increases sodium and sulfur content ($P < 0.001$) of the diet.
2. All treatments had an acceptable (< 10% CV) CV for sulfur content and it was concluded that SBS can be adequately mixed in the diet when added with micro ingredients.
3. SBS and Encapsulated SBS does not appear to modulate intestinal pH and similar performance advantages (higher body weights, improved feed conversion) can be expected when using animal feed grade sodium bisulfate or potassium bisulfate vs Control.
4. The results of this experiment provide practical information for the poultry industry as it relates to nutritional strategies that could be further studied as means to maintain performance under coccidiosis vaccine and offer flexibility in feed formulation.

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Table 1 Starter diets and calculated nutrient composition experiment I

	Control 0-18 d	[1]SBS En 0-18 d	[2]SBS 0-18 d	[3]KBS 0-18 d
Ingredients, %				
Corn, yellow grain	58.58	58.50	58.50	58.50
Soybean meal, dehulled	34.77	34.77	34.77	34.77
Monocalcium Phosphate	1.51	1.51	1.51	1.51
Limestone	1.48	1.48	1.48	1.48
Sodium chloride	0.44	0.36	0.26	0.44
Mineral mix**	0.05	0.05	0.05	0.05
DL Methionine	0.28	0.28	0.28	0.28
Lysine	0.17	0.17	0.17	0.17
Vitamin Premix*	0.25	0.25	0.25	0.25
Fat A/V blend	2.45	2.48	2.48	2.48
SBS Encapsulated	--	0.15	--	--
SBS	--	--	0.30	--
KBS	--	--	--	0.30
Total	100	100	100	100
Calculated and (Analyzed) Nutrient Composition				
CP, %	21.0 (19)	21.0 (19)	21.0 (20)	21.0 (19)
ME, kcal/kg	3025	3022	3022	3022
Crude Fat, %	5.0	5.0	5.0	5.0
Lysine HCl, %	1.30	1.30	1.30	1.30
Methionine, %	0.61	0.61	0.61	0.61
TSAA, %	0.97	0.97	0.97	0.97
Tryptophan	0.27	0.27	0.27	0.27
Threonine	0.82	0.82	0.82	0.82
Arginine	1.45	1.45	1.45	1.45
Valine, %	1.00	1.00	1.00	1.00
Calcium, %	0.90	0.90	0.90	0.90
Available Phosphorous, %	0.45	0.45	0.45	0.45
Sodium	0.20 (.23)	0.20 (.19)	0.20 (.18)	0.20 (.23)
Chloride, %	0.36	0.26	0.24	0.36
Sulfur, %	(.24)	(.27)	(.32)	(.32)

*Vitamin premix added at this rate yields 11,023 IU vitamin A, 2,800 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls. **Trace mineral premix added at this rate yields 149.6 mg manganese, 55.0 mg zinc, 26.4 mg iron, 4.4 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contain less than 1% mineral oil. Dietary treatments were as follow: [1]Animal feed grade sodium bisulfate encapsulated at 0.15% inclusion. [2]Animal feed grade sodium bisulfate not encapsulated at 0.30% inclusion. [3]Animal feed grade potassium bisulfate not encapsulated at 0.30% inclusion.

Table 2 Experimental treatments experiment I

Treatment	*Group	Dietary inclusion SBS per ton of feed	No. of birds
T1	Control	0 %	72
T2	SBS Encapsulated	0.15 %	72
T3	SBS not encapsulated	0.30 %	72
T4	KBS not encapsulated	0.30 %	72

*Animal feed grade sodium bisulfate (SBS); Potassium bisulfate (KBS).

Table 3 Diets and calculated nutrient composition experiment II

	*Poultry diet (1,000 µm)			*Swine diet (700 µm)		
	0 lb/ton SBS	5 lb/ton SBS	10 lb/ton SBS	0 lb/ton SBS	5 lb/ton SBS	10 lb/ton SBS
Corn,	56.50	56.25	56.00	70.19	69.94	69.69
Soybean meal	35.37	35.37	35.37	24.35	24.35	24.35
Fat	4.83	4.83	4.83	3	3	3
Dicalcium phosphate	1.63	1.63	1.63	0.5	0.5	0.5
Calcium carbonate	0.85	0.85	0.85	0.875	0.875	0.875
Sodium chloride	0.39	0.39	0.39	0.35	0.35	0.35
DL- methionine	0.26	0.26	0.26	0.045	0.045	0.045
Trace mineral	0.08	0.08	0.08	0.15	0.15	0.15
Vitamin premix	0.05	0.05	0.05	0.15	0.15	0.15
L - lysine	0.03	0.03	0.03	0.29	0.29	0.29
L-threonine 98.5	0.01	0.01	0.01	0.07	0.07	0.07
Phytase	-	-	-	0.03	0.03	0.03
Sodium bisulfate	0.00	0.25	0.50	0	0.25	0.5
Total	100	100	100	100	100	100

*Particle size of treatments is representative of commercial poultry and swine diets

Table 4 Experimental treatments experiment II

*Treatment	Particle Size (microns)	Inclusion SBS per ton of feed
A	700	0
B	700	0.25 %
C	700	0.50 %
D	1000	0
E	1000	0.25 %
F	1000	0.50 %

*Each treatment was replicated three times
Particle size of treatments is representative of commercial poultry and swine diets

Table 5 Effect of dietary supplementation of SBS on performance of 18-day-old broilers (g)

Average Body weights (g)

Age (days)	Control	SBS En[1]	SBS[2]	KBS[3]	SEM	P*
7	175^{b}	190^{ab}	202^{a}	186^{ab}	5.48	0.0113
14	528	537	540	533	8.23	0.7508
18	767^{C}	886^{A}	849^{AB}	829^{B}	17.57	0.0001
Feed Consumption (g/bird)						
0 –7	180	184	189	177	1.36	0.3453
0–14	566	561	566	533	3.22	0.4246
0–18	541	550	529	532	17.25	0.3542
Feed Conversion (feed: gain)						
0–7	0.8088	0.7737	0.7816	0.8193	0.0213	0.5331
0–14	1.1717^{A}	1.1825^{A}	1.1750^{A}	1.0967^{B}	0.0233	0.0451
0–18	1.2817^{A}	1.2525^{AB}	1.2275^{BC}	1.1983^{C}	0.0189	0.0343

Each value represents the mean value for each group.
[A, B] Means within a row lacking a common superscript differ significantly (P<0.001)
[a, b] Means within a row lacking a common superscript differ significantly (P<0.05).
*Main effect P-value
Dietary treatments were as follows:
[1]Animal feed grade sodium bisulfate encapsulated at 0.15% inclusion
[2]Animal feed grade sodium bisulfate not encapsulated at 0.30% inclusion
[3]Animal feed grade potassium bisulfate not encapsulated at 0.30% inclusion
Each value represents the mean value for each group

Table 6 The effect of dietary supplementation of SBS on intestinal pH on days 7, 14, and 18

Age (days) **Tissue	Control	SBS En[1]	SBS[2]	KBS[3]	SEM	P*
D - 7 d	6.27	6.21	6.29	6.29	0.048	0.6241
D – 14 d	6.15	6.23	6.24	6.25	0.028	0.0700
D – 18 d	6.25	6.26	6.18	6.29	0.018	0.6241
J - 7 d	6.16	6.06	6.12	6.16	0.046	0.3856
J – 14 d	6.10	5.99	6.13	6.12	0.053	0.2221
J – 18 d	6.11	6.14	6.14	6.08	0.019	0.6241
I – 7 d	6.53	6.70	6.56	6.79	0.108	0.2975
I – 14 d	6.40	6.25	6.14	6.18	0.095	0.2654
I – 18 d	6.30	6.31	6.49	6.38	0.048	0.5241

*Main effect P-value
** D= duodenum, J= jejunum, I= ileum
Each value represents the mean value for each group
Feed pH: Control: 5.83, [1]5.83, [2]5.23, [3]5.10

Table 7 Effect of Sodium Bisulfate (SBS) inclusion rate and diet type on mix uniformity of SBS as measured by sodium and sulfur[1]

									Probability, $P <$		
	Swine Diet			Poultry Diet					SBS		
SBS, lb./ton	0	5	10	0	5	10	SEM	Interac	Diet	Linear	Quadratic
pH	6.15	5.95	5.68	6.1	5.9	5.8	0.03	0.128	0.4	0.001	0.404
Ca, %	0.81	0.81	0.80	1.00	1.01	1.03	0.021	0.607	0.001	0.761	0.867
P, %	0.41	0.40	0.41	0.62	0.65	0.66	0.008	0.122	0.001	0.038	0.715
K, %	0.74	0.73	0.74	0.92	0.93	0.93	0.016	0.854	0.001	0.822	0.805
Mg, %	0.17	0.17	0.17	0.21	0.21	0.23	0.013	0.578	0.001	0.362	0.663
Zn ppm	141.2	137.2	142.1	113.9	120.8	130.3	7.77	0.604	0.013	0.289	0.674
Mn ppm	53.4	50.67	53.33	50.53	54.77	52.87	1.651	0.143	0.853	0.506	0.900
Cu ppm	24.12	24.76	24.21	20.49	20.41	22.12	1.330	0.696	0.009	0.531	0.899
S, %	0.18	0.23	0.29	0.24	0.30	0.35	0.003	0.494	0.001	0.001	0.544
Na, %	0.17	0.21	0.25	0.18	0.23	0.29	0.012	0.644	0.073	0.001	0.756
Mo, %	0.70	0.72	0.79	1.49	1.36	1.42	0.065	0.383	0.001	0.926	0.286
Coefficient of Variation, %											
Ca	7.2	6.2	6.2	5.1	5.5	8.4	1.36	0.303	0.845	0.415	0.474
P	5.7	4.6	3.3	3.6	4.2	4.8	0.69	0.068	0.554	0.470	0.938
K	4.3	4.4	3.4	3.6	4.5	6.3	0.65	0.045	0.174	0.183	0.912
Mg	5.0	5.1	4.3	4.8	4.2	7.1	1.02	0.208	0.530	0.478	0.478
Zn	16.5	25.8	18.9	15.7	18.0	20.0	2.58	0.236	0.255	0.221	0.091
Mn	12.8	16.3	15.3	14.3	12.3	14.3	2.52	0.571	0.576	0.615	0.950
Cu	17.5	26.3	20.5	22.8	23.5	18.9	3.15	0.406	0.915	0.887	0.093
S	4.8	4.7	3.6	4.3	3.7	6.2	0.61	0.029	0.451	0.547	0.315
Na	7.8	9.5	6.4	10.0	8.8	11.2	1.43	0.204	0.102	0.968	0.788
Mo	16.6	23.4	15.4	37.9	11.9	10.2	10.95	0.318	0.865	0.211	0.805

[1]Each of the six treatments were mixed in three separate batches to provide three replications per treatment. As the mixer was discharged, 10 stream line samples were collected in equally space time intervals for calculation of the mean and coefficient of variation or each replicate.

3 DIETARY SUPPLEMENTATION OF 25-HYDROXYCHOLECALCIFEROL AND ITS IMPACT ON PERFORMANCE, INTESTINAL MORPHOLOGY AND VITAMIN D STATUS IN BROILERS SUBJECTED TO COCCIDIOSIS VACCINE

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Summary

The objective of this study was to compare the effect of dietary D_3 and 25-OH-D_3 on performance, intestinal integrity, and vitamin-D status of broilers subjected to a 2x recommended dose coccidiosis vaccine. Broiler chicks were placed in floor pens for a 21-day-trial-period. Experimental dietary treatments were formulated with a mineral-vitamin-premix devoid of D_3 and supplemented with dietary 25-OH-D_3 and/or vitamin-D_3 from a commercially available D_3 source (CS). Birds were randomly assigned to a vaccinated vs not-vaccinated group for each of the four dietary treatments and arranged in a completely randomized block design. Twenty-five-male Cobb500 broilers per replicate, six pen replicates per treatment were used, resulting in a 4x2 factorial arrangement. The dietary treatments consisted of (Control) 2750 IU/Kg vitamin-D supplemented by D_3, 2750 IU/Kg vitamin-D supplemented by 25-OH-D_3, 2750 IU/Kg vitamin-D supplemented 50:50 by D3 and 25-OH-D_3, lastly 1375 IU/Kg vitamin D by 25-OH-D_3. Feed consumption (FC), body weights (BW), and feed conversion (FCR) were calculated at d-7, 14, and 21. Serum concentration of 25-OH-D_3, tibia breaking strength (TBS), and lesion scores were measured on day 10, and 21. There was a difference ($P<0.05$) in BW, FCR, and TBS between one or more 25-OH-D_3 dietary treatments and Control. Cumulative FCR was improved ($P<0.05$) in 25-OH-D_3 treatments compared to Control. TBS at d-10, and 21 was greater ($P<0.05$) between 25-OH-D_3 treatments compared to Control. Overall, the results indicate dietary 25-OH-D_3 positively impacts TBS, 25-OH-D_3 in serum, and performance, with more noticeable differences in coccidiosis vaccinated groups.

Key words: coccidiosis, broiler, 25-OH-D_3, vitamin-D, bone

Description of the problem

In commercial poultry production, balancing cost and performance is an everyday challenge that requires an understanding of the interrelated nature of many production factors. Nutrition plays a critical role in maintaining production and economic feasibility. There are many strategies and additives aimed at maximizing performance, and maintaining animal health and welfare while keeping costs within acceptable margins. There are many changes that have transpired in the poultry industry in regards to the use of antibiotics, which has resulted in major impacts on gut health, specifically with regards to coccidiosis and necrotic enteritis. Coccidiosis is a major concern within the poultry industry for its detrimental effects in poultry health and substantial economic losses that surpass $3 billion dollars worldwide (Anon, 2013). Coccidiosis is caused by intestinal infection of coccidia protozoan parasites, which are commonly found in broiler grow out houses (Merck Veterinary Manual, 2016). Currently, poultry nutritionists are concerned about the potential effect of coccidiosis vaccine on vitamin D status and how to better implement nutritional strategies in order to increase the safety margin to guarantee maximum genetic potential. Malabsorption syndrome is a common observation in birds challenged with coccidiosis, (Allen and Fetterer, 2002). Previous research indicates the use of 25-hydroxycholecalciferol (25-OH-D_3) as replacement or partial replacement of cholecalciferol (D_3) in broiler diets can result in significant differences in performance and bone mineralization. However, in order to better comprehend the benefits and limitations of vitamin D metabolites as a nutritional strategy especially in antibiotic free production, future research should aim to evaluate these nutritional strategies at different

dietary concentrations and in the presence of a coccidiosis challenge. It has been reported that vitamin D may have a role in modulating the morphological and functional development of intestinal villus mucosa (Shinki et al., 1991).

Commercial broilers often are exposed to stressful conditions and field challenges that can generate a malabsorption syndrome, and as a result have an impaired absorption or liver hydroxylation of vitamin D_3. The rapid growth of broilers makes the first part of the broiler grow out cycle fundamentally important. In our lab, the ability of broilers to absorb vitamin D can be hindered depending on sources and vaccine challenge. As enteric health in commercial broilers decreases, the risk for a loss in performance increases. Nutritionists have to be aware of field issues that can hamper the ability of the flock to utilize all the nutrients carefully formulated in a diet.

The use of dietary 25-OH-D_3 has been shown to be more effective than D_3 in improving overall performance of broilers (Yarger et al., 1995) and as replacement or in addition to vitamin D_3 is effective in promoting performance, enhancing bone mineralization (Leyva-Jimenez, 2019), and modifying avian immunity (V.G. Gómez, et. al. 2013). Because D_3 is not considered biologically active until it undergoes a series of hydroxylation reactions, it becomes important to understand how to better utilize the commercially available D_3 metabolites. This is particularly important under antibiotic-free production, where conditions of malabsorption can be seen at higher incidence because of the common problems associated with coccidiosis. Measuring vitamin D absorption by determining the serum concentration of 25-hydroxycholecalciferol, an active metabolite

of vitamin D, could provide us an indication of an improved status on birds challenged with coccidiosis vaccine.

Therefore, the objective of this study was to compare the effect of D_3 and 25-OH-D_3 on performance, intestinal integrity, and vitamin D status of broilers subjected to a 2x recommended dose coccidiosis vaccine.

Materials and methods

All animal handling and care practices were in accordance with an approved animal use protocol by the Institutional Animal Care and Use Committee of Texas A&M University. Birds were allocated in an environmentally controlled rearing facility located at Texas A&M University Poultry Research Center on wood shavings. 1,200 Cobb-500 one -day-old male broiler chicks were obtained from Cobb-Vantress hatchery (Timpson, Texas). All birds were weighed individually and grouped together by weight so that birds could be allocated to each pen with similar initial body weights and variance (±5 g). Separate rooms were used for vaccinated vs unvaccinated birds – two rooms per group, four rooms total. At day of placement, male Cobb 500 birds received a 2X recommended dose of live coccidiosis vaccine via oral gavage. Twenty-five randomized birds were placed into each pen (1,200 birds / 2 vaccine groups = 600/ 4 dietary treatments = 150/6 replicates = 25 birds/each pen). Six pen replicates per group were used. Birds were randomly assigned to a vaccinated or not vaccinated group for each of the four dietary treatments. Replicates were arranged in a completely randomized block design in a 4 (diets) x 2 (vaccinated vs not vaccinated) x 2 rooms factorial arrangement. Pen average was the unit of measurement for all performance variables. Individual birds were the unit of measure for gut integrity,

vitamin D status, tibia breaking strength, and bone ash. A basal corn-soy broiler starter diet was formulated using a customized mineral-vitamin premix devoid of D_3 and supplemented with 25-OH-D_3 by Bio-D™ 500 (Bio-D) from Huevepharma (Peachtree, GA) at the different levels indicated in table 1. Commercial type diets were formulated at a concentration of 2750 IU vitamin D / Kg rather than the 200 IU concentration recommended by NRC. The diets were formulated at these levels in order to better capture a response that could be practical and of relevance to the industry. The negative control groups received 2750 IU vitamin D/ Kg from a commercially available D_3 source, AHI, (Nacogdoches, Texas). The positive control groups received 2750 IU vitamin D /Kg supplemented by 25-OH-D_3 from Bio-D. Chemical analysis of the feed was conducted prior to the feeding of each diet. Nutrient composition of each diet was confirmed by the Feed and Water Environmental Lab UGA (Athens, Ga). Concentration of vitamin D in the feed was chemically analyzed at Heartland Assays Laboratories (Ames, Iowa) via liquid chromatography mass-spectrometry (LC/MS). Feed and watering were available *ad libitum* during all experimental periods. No antibiotics or coccidiostats were used during the study. Birds were monitored daily with regard to general flock condition, temperature, lighting, water, feed, and any unanticipated events for the rearing facility. At day of placement, birds were vaccinated via oral gavage with Huvepharma ADVENT®-Vaccine containing a mixture of viable sporulated oocysts from *E. acervulina, E. maxima and E. tenella* at 2X recommended dose. In previous work conducted at our lab, we have seen the use of 2X recommended dose ADVENT vaccine via oral gavage has resulted in significant differences in performance and gut integrity when compared to control birds.

Performance

Feed consumption and body weight were recorded on day 0, 7, 14 and 21 to calculate weight gain, feed: gain ratio, feed: weight ratio, productivity index, and mortality. The experimental birds were observed daily for any clinical signs and mortality. Mortality and body weight of dead birds were recorded daily.

Vitamin D status (ELISA)

At day 10 and 21 of the trial, three birds per pen were selected and used to collect blood samples, drawn from the jugular vein. Blood samples were placed in a micro centrifuge tube and then centrifuged for 15 min. at 3,000 rpm. The sample was used to evaluate vitamin D status using a commercial ELISA 25-OH-Vitamin D Kit Eagle Biosciences® (Nashua, NH). The serum was stored at -80 °C until further analysis (Leyva et al., 2019).

Bone mineralization

At day 10 and 21 of the trial, three birds per pen were euthanized by CO_2. The same three birds euthanized per pen on each sampling day were used for all parameters measured. Both tibias from the same three birds per pen were removed. Right tibiae bones were defatted in 4 L of petroleum ether for 48-h and then dried in a forced draft oven at 105 °C until constant weight. Subsequently, the dried bones were ashed 24 hours at 650 °C. Percent bone ash was calculated based on starting dry bone weight and remaining ash. The left tibiae were cleaned from any adhering tissue and used to assay breaking strength using a Texture Technologies TA.XT Plus® Texture Analyzer (Hamilton, MA) with a 50 kg load cell, a crosshead speed of 100 mm/min with the tibia supported on a 3 point standing ring and a 3.0 cm constant span (Leyva-Jimenez, 2019).

Intestinal morphology

At day 10 and 21 of the trial, jejunum and ileum samples were flushed and fixed in 10% formalin from the three selected birds per pen. The jejunum samples were taken in the middle point between the bile ducts and the Meckel's diverticulum. The ileum samples were taken 8 cm proximal to the ileocecal junction. Samples were processed and villus height and crypt depth were measured from 5 complete villi and villus-associated crypts for each sample (Feng et al., 2007).

Lesion scores

At day 10 and 21 of the trial the upper and middle small intestine and ceca of the three selected birds per pen were scored for coccidial lesions. Lesions were scored from 0 – 4 as descried by Johnson and Reid (1970). Where 0 score represents no gross lesions and 4 indicates severe damage to the intestine.

Data analysis

Significance was accepted at $P \leq 0.05$. Arcsine transformation were conducted for all % variables for analysis. Collected data was analyzed as two way- ANOVA using the GLM Procedure of SPSS for a completely randomized design where treatment diets were used as blocks in the model. If significance, means were separated by Duncan's Multiple Range Test. No room effects were observed in any of the parameters measured. Room arrangement was not included in the final statistical analysis**.**

Results and discussion

The results herein indicate that the use of dietary 25-OH-D_3 metabolite can provide an improved safety margin under a coccidiosis vaccine challenge than birds fed D3 alone.

Furthermore, birds fed a control diet using cholecalciferol showed significantly lower live performance after the first and second coccidiosis cycles compared to birds fed a diet supplemented by 25-OH-D_3.

Vitamin D deficiency has been reported to depress the cellular immune responses in young broiler chicks (Aslam et al., 1998). In the literature, vitamin D metabolites have been reported to decrease turkey osteomyelitis (Huff et al., 2002). Multiple reports in the literature identify serum 25-OH-D_3 concentration as an effective indicator of the vitamin D status of a broiler. The half-life of 25-OH-D_3 is close to three weeks making it ideal to test vitamin D status. During the present trial, significant differences were observed in serum 25-OH-D_3 concentration at day 10 and 21. Prior to the beginning of the study, we hypothesized that birds fed the control diet would be negatively impacted by the coccidiosis vaccine challenge and the malabsorption conditions would hindered its vitamin D status ultimately impacting performance as well. On day 10, significantly ($P<0.001$) lower 25-OH-D_3 serum concentration were observed in birds fed the control diet compared to all other dietary treatments. Results obtained in previous experiments conducted by our lab have allowed us an opportunity to observe differences in activation of cell-mediated immune response between the period following a primary immunization and secondary immunization which can affect live performance (Suarez et al., 2018).

There was an overall dietary effect ($P<0.001$) on serum 25-OH-D_3 concentration (Table 3). Birds fed the diet containing the 25-OH-D_3 supplement alone had significantly greater concentrations of serum 25-OH-D_3 compared to birds fed any other treatment. There was no evidence of difference on serum 25-OH-D_3 concentration due to a vaccine

effect at day 10. On day 21, an interaction between diet and vaccine was observed where vaccinated birds that were fed a control diet showed ($P<0.001$) the lowest serum 25-OH-D_3 concentration compared to all other treatments. Birds fed the diet with 2750 IU vitamin D3/Kg supplemented by the metabolite 25-OH-D_3 had the highest serum 25-OH-D_3 concentration compared to all other dietary treatments in both vaccinated and not vaccinated birds. Yarger et al., 1995 reported serum 25-OH-D_3 concentrations increase more rapidly in birds fed 25-OH-D_3 than in those fed vitamin D3 alone. Remarkably, birds fed the diet lower in vitamin D (1375 IU/Kg) but formulated with the metabolite 25-OH-D_3 showed significantly better vitamin D status compared to Control and no significant differences with birds fed a diet containing 50:50 split in vitamin D sources. On day 21, a similar trend was observed in addition to an interaction between dietary and vaccine effect. Birds fed the control diet had significantly ($P<0.001$) lower serum 25-OH-D_3 concentration compared to all other dietary treatments regardless of a vaccine effect.

Vaccinated birds that were fed the control diet finished with significantly ($P<0.001$) lower vitamin D status than any other group. The biggest significant difference observed in serum 25-OH-D_3 concentration was in birds fed the control diet where non-vaccinated birds had significantly higher concentration than those that received the vaccine. Numerically, the difference between the lowest concentration of 25-OH-D_3 and the highest serum concentration reported was shown in vaccinated birds. Birds fed the control diet finished with (98 ng/mL vs the highest concentration reported 191 ng/mL). Birds that were fed a diet containing 2750 IU vitamin D /Kg using the 25-OH-D_3 dietary supplement had significantly ($P<0.001$) better vitamin D status than control birds

regardless of vaccination. This indicates that the vitamin D status of birds is not compromised when fed a 25-OH-D_3 dietary supplement under a coccidiosis vaccine challenge. Furthermore, results indicate that under coccidiosis vaccine stress a dietary supplementation of 1375 IU vitamin D/ Kg results in significantly better vitamin D status than in a control diet with D_3 alone. Control birds that did not receive the coccidiosis vaccine challenge showed significantly higher vitamin D status compared to the vaccinated control birds. These results reinforced the hypothesis that a diet formulated with 25-OH-D_3 provides a better safety margin for vitamin D supplementation compared to feeding D_3 alone under coccidiosis vaccine challenge.

Table 11 summarizes tibia breaking strength data collected at day 10 and 21. No significant differences ($P>0.05$) were observed on day 10. By the end of the trial at day 21, there was a vaccine and dietary main effect ($P<0.001$). Birds that received the vaccine showed significantly lower tibia breaking strength compared to those not vaccinated. Birds fed the control diet had the lowest tibia breaking strength compared to all dietary treatments in both vaccinated and not vaccinated groups. All birds fed diets containing 25-OH-D_3 supplementation had significantly greater tibia breaking strength that those fed a control diet but no significant differences existed among them. Vitamin D supplementation has been shown to be closely related to a decreased incidence of bone disorders in great part due to its role in the absorption of calcium and phosphorus, and proper bone mineralization (Garcia et al, 2013). Reports in the literature suggest that dietary 25-OH-D_3 may be effective in reducing incidence of tibia dyschondroplasia (Atencio et al. 2005; Yarger et al., 1995). Greater severity of rickets in chickens suffering

from coccidiosis has been reported in the literature as well. Birds fed a diet formulated using dietary 25-OH-D_3 at any of the two concentrations used during this trial showed significantly ($P<0.001$) better TBS than control birds. Birds that did not receive the coccidiosis vaccine showed significantly higher TBS compared to vaccinated birds. Similar results were observed by Leyva et al., 2019.

Bone ash percent data shows significant ($P<0.001$) differences observed at day 10 and 21. A vaccine effect at day 10 shows birds that received the vaccine had significantly lower bone ash % than those that were not vaccinated. Birds fed the control diet consistently showed significantly lower bone ash % compared to any other treatment throughout the trial. A dietary effect was observed at day 10 and 21. Control birds had significantly ($P<0.001$) lower bone mineralization compared to all other dietary treatments. Birds fed a diet containing 25-OH-D_3 had significantly higher bone ash percent compared to birds fed a control diet. Both bone mineralization parameters indicate that 25-OH-D_3 results in improved tibia breaking strength and bone ash percent compared to a diet with D_3 alone under coccidiosis vaccine challenge. Similar results are reported in the literature (Yarger et al., 1995; Leyva et al., 2019).

Results of mean body weights are summarized in Table 12. There were differences ($P<0.001$) between vaccinated and non-vaccinated birds during the second and third week of the trial with vaccinated birds having lower body weight. This is consistent with multiple reports from the literature, (Peek, 2011, Chapman, et al., 2013; Ahmad, et al., 2016), and correlate with first and second cocci cycling also reported in the literature. No significant differences were observed between dietary treatments but birds fed diets

containing the 25-OH-D_3 metabolite had numerically higher body weights at the end of the trial. Feed consumption data is summarized on Table 5. No significant differences ($P>0.05$) between treatments were observed. Feed consumption was not significantly impacted by a vaccine or dietary effect throughout the study. Multiple reports have found no difference in feed consumption from feeding different vitamin D_3 sources (Yarger et al., 1995; Leyva et al., 2019). All dietary treatments finished with very similar cumulative feed consumption.

Feed conversion ratio data shows a vaccine and dietary effect that was observed throughout the trial. Cumulative feed conversion was significantly higher ($P<0.001$) in birds fed the control diet. It has been reported the use of live vaccines can be associated with decreased performance due to the induced of sub-clinical infection after vaccination (Lillehoj and Trout, 1993). The biggest difference in feed conversion was observed between birds fed the control diet and those fed the lower concentration of vitamin D_3 (1375 IU). There was a difference of 7 points by the end of the trial (1.205 vs 1.137, respectively). By the end of the trial, feed conversion was significantly higher in vaccinated birds compared to not vaccinated groups with a difference of 4 points by the end of the trial (1.18 vs 1.14, respectively). A dietary effect was observed during the third week with birds that were fed the control diet showing significantly higher feed conversion ratio compared to all other treatments. Vaccinated birds consistently showed higher feed conversion ratios throughout the trial. During the 21-day study, vaccinated birds showed decreased live performance compared to unvaccinated groups. Throughout the study, feed conversion ratio was significantly ($P<0.001$) higher in vaccinated groups compared to

unvaccinated groups. These results are consisted with (Cantor and Bacon, 1978; McNutt and Haussler, 1973; Yarger et al., 1995). Interestingly, birds fed the diet containing the lowest concentration of vitamin D (1375 IU vitamin D/Kg) showed similar performance to those fed a diet with higher concentrations of vitamin D and significantly ($P<0.001$) better feed conversion ratio than those birds fed the control diet.

Table 13 contains the intestinal morphology data. A vaccine and dietary effect were observed only at day 21. Shortening of the villi and increased crypt depth has been reported during a coccidia challenge (Fernando and McCraw, 1973). Birds that did not receive the vaccine showed significantly ($P<0.001$) higher villi height in the duodenum compared to vaccinated birds. However, at day 21, vaccinated birds showed significantly higher villi height in the jejunum section. A dietary effect at day 21 showed birds fed the control diet had significantly ($P<0.05$) higher villi height in the jejunum section compared to those fed the diet formulated at 2750 IU vitamin D with 25-OH-D_3. No further dietary effects on villi height were observed throughout the trial. Crypt depth was measured at day 10 and 21. Only a vaccine effect was observed during the trial. At day 10, vaccinated birds consistently showed significantly ($P<0.001$) greater crypt depth in all sections of the small intestine. Duodenum crypt depth was significantly greater in vaccinated birds and both jejunum and ileum finished with numerically greater crypt depth. For villi height: crypt depth ratio results only a vaccine effect was observed. Duodenum villi height: crypt depth ratio in birds that receive the vaccine was significantly ($P<0.001$) lower at days 10 and 21 compared to not vaccinated birds. The same effect was observed in the jejunum at day 10. Vaccinated birds consistently showed numerically lower villi height: crypt depth

ratio throughout the study. Villi height and crypt depth measurement results did not allow for the establishment of any direct correlation from a dietary effect suggesting better intestinal absorption from 25-OH-D_3 dietary inclusion. Vaccinated birds consistently showed significantly ($P<0.001$) poorer gut morphology including lower villi height and greater crypt depth. Chou and coworkers (2009) reported that supplemental 25-OH-D_3 positively modulated small intestine morphology but under no coccidiosis challenge. Lesion scores were significantly different ($p<0.05$) between vaccinated and not vaccinated groups, indicative of a good vaccine challenge.

Conclusion and applications

1. The live coccidiosis vaccine challenge presented to birds at day of placement resulted in significant impacts to performance and vitamin D status of birds fed a diet absent of dietary 25-OH-D3 compared to control (cholecalciferol).

2. Reduced intestinal integrity associated with the coccidiosis challenge was observed during the trial, which resulted in significantly lower bone mineralization, and serum 25-OH-D3 levels in birds fed a control diet.

3. A combination of dietary D3 and 25-OH-D3 equally formulated to provide 2750 IU vitamin D/ Kg was more effective than D3 alone in maintaining bone mineralization and improving serum 25-OH-D3 levels in broilers subjected to a live coccidiosis vaccine challenge.

4. The results of the present study suggest the use of dietary 25-OH-D3 compared to inclusion of D3 alone provides a significantly improved safety margin for performance and bone mineralization.

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Table 8 Experimental treatments

TRT[1]	Diet (IU's of Vitamin D_3)/Kg	Bio-D 500™ g/metric ton feed[2]
T1	NVac CON D_3 at 2750 IU	D_3 supplement + 0g/ MT Bio-D
T2	NVac 25-OH-D_3 at 2750 IU	No D_3 supplement + 988g/ MT Bio-D
T3	NVac D3-1375 IU + 25-OH-D_3 at 1375 IU	D_3 supplement + 494 g/MT Bio-D
T4	NVac 25-OH-D_3 at 1375 IU	No D_3 supplement + 494 g/MT Bio-D
T5	CON D_3 at 2750 IU	D_3 supplement + 0g/MT Bio-D
T6	25-OH-D_3 at 2750 IU	No D_3 supplement + 988g/MT Bio-D
T7	D3-1375 IU + 25-OH-D_3 at 1375 IU	D_3 supplement + 494 g/MT Bio-D
T8	25-OH-D_3 at 1375 IU	No D_3 supplement + 494 g/MT Bio-D

[1]T1-T4: Non-vaccinated.
[2]At inclusion of 494 g Bio-D / m ton → 34.5 µg/Kg 25-OH-D_3 →1375 IU/Kg of feed. On day 1 of the study, birds were vaccinated with Huevepharma ADVENT®-Vaccine (Peachtree City, GA) containing a mixture of viable sporulated oocysts from *E. acervulina, E. maxima and E. tenella* at 2X recommended dose.

Table 9 Diets and calculated nutrient composition

Starter 0-21 days	(-) Control D_3 2750 IU/kg	25-OH-D_3 2750 IU/kg	50:50 1375 IU/kg each	25-OH-D at 1375 IU/kg
Ingredients, %				
Corn, yellow grain	58.59	58.59	58.59	58.61
Soybean meal, dehulled solvent	34.77	34.77	34.77	34.77
Monocalcium Phosphate	1.51	1.51	1.51	1.51
Limestone	1.48	1.48	1.48	1.48
Sodium chloride	0.45	0.45	0.45	0.45
Customized vitamin-mineral	0.24	0.24	0.24	0.24
DL Methionine	0.28	0.28	0.28	0.28
Lysine	0.17	0.17	0.17	0.17
Fat A/V blend	2.45	2.45	2.45	2.45
Bio-D™ 500	--	0.06	0.03	0.03
D_3 (Animal Science)	0.06	--	0.03	--
Total	100	100	100	100
Calculated and (Analyzed)				
CP, %	22.0 (21.0)	22.0 (21.8)	22.0 (21.3)	22.0 (21.0)
ME, kcal/kg	3025	3025	3025	3025
Calculated D_3 µg/Kg: *25-OH-D3 µg/Kg*	69: *<5*	<5: *69*	35: *35*	<5: *35*
Analyzed D_3 µg/Kg: *25-OH-D3 µg/Kg*	79: *<5*	<5: *72*	49: *42*	10: *34*
Crude Fat, %	5.0	5.0	5.0	5.0
Lysine HCl, %	1.30	1.30	1.30	1.30
Methionine, %	0.61	0.61	0.61	0.61
TSAA, %	0.97	0.97	0.97	0.97
Tryptophan	0.27	0.27	0.27	0.27
Threonine	0.82	0.82	0.82	0.82
Arginine	1.45	1.45	1.45	1.45
Valine, %	1.00	1.00	1.00	1.00
Calcium, %	0.91(0.94)	0.91(0.92)	0.91(0.94)	0.91(0.99)
Available (Phosphorous), %	0.45 (0.71)	0.45 (0.71)	0.45 (0.73)	0.45 (0.72)
Sodium	0.20	0.20	0.20	0.20
Chloride, %	0.36	0.36	0.36	0.36

[1]Vitamin premix added at this rate yields 11,023 IU vitamin A, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. A customized mineral-vitamin premix devoid of D_3 was utilized. [2]Trace mineral premix added at this rate yields 149.6 mg manganese, 55.0 mg zinc, 26.4 mg iron, 4.4 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. NRC (1994) Cholecalciferol requirement for starter diet (200 IU/kg feed or 5 µg/kg of feed). 1 IU D_3 = 0.025 µg cholecalciferol. Cholecalciferol was supplemented as D_3–30, Animal Science Products Inc., Nacogdoches, TX and 25-hydroxycholecalciferol was supplemented as Bio-D, Huvepharma Inc., Peachtree, GA. 494 g Bio-D/MT → 34.5 µg/Kg 25-OH-D_3 →1375 IU vitamin D/ Kg of feed.

Table 10 Effect of dietary supplementation of vitamin D_3 and 25-hydroxycholecalciferol on serum 25-hydroxycholecalciferol concentration of 21-day-old broilers (ng/mL)

	Vaccinated			Diet				
Age (days)	No	Yes	SEM	(-) Control D_3 2750 IU/kg	25-OH-D_3 2750 IU/kg	1375 IU/kg each	25-OH-D_3 1375 IU/kg	SEM
10[1]	178	181	5.7	118^{C}	222^{A}	197^{B}	180^{B}	8.0
21[2]								
	Not vaccinated			121^{Z}	192^{X}	167^{Y}	160^{Y}	5.8
	Vaccinated			98^{Z}	191^{X}	185^{X}	156^{Y}	5.8

[A,B,C] Within day 10 diet treatments, means lacking a common superscript differ significantly (P<0.001). Each value represents the mean value for each group.
[X,Y,Z] Within day 21 means, means lacking a common superscript differ significantly (P<0.001)
[1] Day 10 Vaccine main effect P = 0.7318, Diet main effect P < 0.0001.
[2] Day 21 Vaccine*Diet interaction P = 0.0053.

Table 11 Effect of dietary supplementation of vitamin D_3 and 25-hydroxycholecalciferol on tibia breaking strength (Kg of force) and bone ash (%)

	Vaccinated			Diet				
Age (days)	No	Yes	SEM	(-) Control D_3 2750 IU/kg	25-OH-D_3 2750 IU/kg	1375 IU/kg each	25-OH-D_3 1375 IU/kg	SEM
10[1]	7.76	7.86	1.61	7.56	8.04	8.09	7.55	2.27
21[2]	32.48[A]	27.31[B]	6.68	27.15[B]	30.83[A]	30.78[A]	30.82[A]	9.45
Bone ash (%)								
10[3]	49.7[A]	47.9[B]	0.34	46.9[B]	50.0[A]	49.0[A]	49.3[A]	0.48
21[4]	50.1	50.5	0.17	49.5[C]	50.9[A]	50.7[A]	50.1[B]	0.25

[A, B] Within vaccine or diet treatments, means within a row lacking a common superscript differ significantly ($P<0.001$). Each value represents the mean value for each group.
[1] Day 10 Vaccine main effect P = 0.6483, Diet main effect P = 0.1672
[2] Day 21 Vaccine main effect P < 0.0001, Diet main effect P = 0.0126
[3] Day 10 Vaccine main effect P = 0.0003, Diet main effect P < 0.0001
[4] Day 21 Vaccine main effect P = 0.0813, Diet main effect P = 0.0006

Table 12 Effect of dietary supplementation of vitamin D_3 and 25-hydroxycholecalciferol on performance of 21-day-old broilers

Average Body weights (g)

	Vaccinated				Diets					
Age (days)	No	Yes	SEM	P*	D1	D2	D3	D 4	SEM	P*
7	175	179	1.6	0.1607	177	176	178	177	2.2	0.9102
14	492^{A}	453^{B}	4.7	0.0001	480	463	481	467	6.7	0.1727
21	953^{A}	888^{B}	13.7	0.0019	886	927	932	939	19.3	0.2323
Average Body weight gain										
0 –21	911^{A}	847^{B}	13.1	0.0019	844	885	890	897	19.3	0.2324
Feed Consumption (g/bird)										
0 –7	128	126	1.6	0.4056	129	125	127	127	2.3	0.5708
7 -14	382	375	5.0	0.3222	390	378	383	365	7.1	0.1017
14-21	696	702	19.6	0.8417	699	691	702	704	27.7	0.9874
Feed Conversion (feed: gain)										
0–7	0.95^{A}	0.92^{B}	0.01	0.0038	0.95	0.92	0.92	0.94	0.01	0.2665
7–14	0.99^{B}	1.09^{A}	0.01	0.0001	1.05	1.06	1.03	1.02	0.02	0.3942
0–14	0.98^{B}	1.03^{A}	0.01	0.0001	1.02	1.01	0.99	0.99	0.01	0.3293
14–21	1.44^{B}	1.58^{A}	0.03	0.0070	1.67^{A}	1.44^{B}	1.50^{B}	1.43^{B}	0.05	0.0033
0-21	1.14^{B}	1.18^{A}	0.01	0.0043	1.21^{A}	1.14^{B}	1.15^{B}	1.137^{B}	0.02	0.0029

D1: Control D_3 at 2750 IU/kg; D2: 25-OH-D_3 at 2750 IU/kg;
D3: 1375 IU/kg each; D4: 25-OH-D_3 at 1375 IU/kg
$^{A, B}$ Within vaccine or diet treatments, means within a row lacking a common superscript differ significantly ($P<0.001$). * Main effect P value.
Each value represents the mean value for each group.

Table 13 Effect of dietary supplementation of vitamin D_3 and 25-hydroxycholecalciferol on intestinal morphology in broilers

	Vaccinated				Diet					
Age (days)	No	Yes	SEM	P**	D1	D2	D3	D4	SEM	P**
Villi height (μm) day 10 and 21										
D - 10 d	1920	1927	33	0.8738	1989	1870	1882	1954	48	0.2252
D – 21 d	2547^{A}	2381^{B}	45	0.0019	2401	2471	2514	2472	66	0.6404
J – 10 d	842	852	21	0.7420	861	795	843	889	29	0.1536
J – 21 d	1268^{B}	1444^{A}	32	0.0002	1425^{a}	1250^{b}	1358^{ab}	1392^{a}	45	0.0424
I – 10 d	659	689	15	0.1490	652	670	682	694	21	0.5185
I – 21 d	930	990	24	0.0839	964	960	968	948	34	0.9803
Crypt depth (μm) day 10 and 21										
D - 10 d	144^{B}	171^{A}	5	0.0003	156	158	152	165	7	0.6239
D - 21 d	195^{B}	238^{A}	7	0.0001	210	205	224	229	9	0.2577
J – 10 d	115^{B}	126 ^{A}	4	0.0248	114	121	125	122	5	0.4092
J – 21 d	147	161	6	0.1086	158	152	153	152	9	0.9555
I – 10 d	104^{B}	118^{A}	4	0.0111	107	105	113	118	5	0.2898
I – 21 d	145	147	4	0.7116	144	144	148	147	6	0.9446
Villi height: Crypt Depth Ratio (μm) day 10 and 21										
D – 10 d	14.0^{A}	11.5^{B}	0.42	0.0001	13.4	12.5	12.9	12.3	0.60	0.5532
D - 21 d	13.5^{A}	10.6^{B}	0.46	0.0001	12.0	12.7	11.9	11.5	0.64	0.6030
J – 10 d	6.3^{A}	5.1^{B}	0.27	0.0047	5.8	5.4	5.8	5.8	0.38	0.8529
J – 21 d	6.8	6.4	0.24	0.2741	7.0	6.7	6.3	6.4	0.33	0.3809
I – 10 d	6.5	6.1	0.20	0.1498	6.3	6.6	6.2	6.1	0.28	0.5746
I – 21 d	6.6	6.8	0.20	0.4714	6.8	6.7	6.7	6.7	0.28	0.9925

* D= duodenum, J= jejunum, I= ileum.
** Main effect P value.
$^{A, B}$ Means within a row lacking a common superscript differ significantly (P<0.001).
$^{a, b}$ Means within a row lacking a common superscript differ significantly (P<0.05).
Each value represents the mean value for each group. Villi height and crypt depth were measured from five well-oriented villi and villi-associated crypts for each sample.

4 EFFECT OF ANIMAL FEED GRADE SODIUM BISULFATE SUPPLEMENTATION ON PERFORMANCE, INTESTINAL MORPHOLOGY AND VITAMIN D STATUS OF BROILERS SUBJECTED TO A COCCIDIOSIS VACCINE CHALLENGE

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Summary

This study analyzed the effect of sodium bisulfate (SBS) supplementation on performance, intestinal morphology, and vitamin D3 status of broilers subjected to a 2X dose coccidiosis vaccine. Coccidiosis causes detrimental effects to poultry health and substantial economic losses to the poultry industry worldwide. At day of placement, 800 one-day-old male Cobb500 broiler chicks were randomly assigned to a vaccinated or not vaccinated group for each of the two dietary treatments, control or SBS. Broilers were placed in floor pens for a 21-day trial period. SBS was supplemented at 0.4% at the expense of sodium chloride. Replicates were arranged in a completely randomized block design with twenty-five broilers per replicate and eight replicates per treatment, a 2x2 factorial arrangement. Body weights (BW), feed consumption (FC), and mortality-corrected feed conversion (FCR) were calculated at day 7, 14, and 21. Serum concentration of 25-hydroxycholecalciferol (25-OH-D3), villi and crypt measurements from small intestine, and tibia ash (TBA) were measured on day 10, and 21 along with lesion scores. Lesion scores were significantly higher ($P<0.05$) for vaccinated groups compared to not vaccinated groups, suggestive of a good vaccine challenge. At day 21 there was a significant improvement ($P<0.05$) in 25-OH-D3 serum concentration, BW, FCR, and 10 d TBA from SBS compared to Control. In conclusion, the data suggests that SBS can positively impact cumulative FCR, 25-OH-D3 serum concentration, and TBA in broilers.
Key words: coccidiosis, broiler, sodium bisulfate, bone, vitamin-D

Description of the problem

In the poultry industry, many changes have transpired in regards to the use of antibiotics. In commercial broiler production, it has resulted in major impacts on gut health, specifically with regards to coccidiosis and necrotic enteritis. Coccidiosis is a major concern within the poultry industry for its detrimental effects in poultry health and substantial economic losses that surpass $3 billion dollars worldwide (Anon, 2013). Coccidiosis is caused by intestinal infection of coccidia protozoan parasites, which are commonly found in broiler grow out houses (Merck Veterinary Manual, 2016). Major focus is put in the first stages of the growth curve of a broiler in order to supply the nutrients that can support the fast growth curve. The industry has looked at several strategies to maintain flock health and performance without the use of antibiotics by incorporating and restudying strategies such as coccidiosis vaccination, probiotics, prebiotics, essential oils and acidifiers. There is great concern in the industry that the shift that has happened in commercial production in regards to the absence of antibiotics will increase coccidiosis problems as well as most enteric related issues (Cervantes, 2015). As enteric health in commercial broilers decreases, the risk for a loss in performance increases.

Nutritionists have to be aware of field issues that can hinder the ability of the flock to utilize all the nutrients carefully formulated in a diet. Multiple strategies are currently being studied in order to combat problems associated with antibiotic free production. Intestinal health has received major focus as it is necessary to maintain production standards and maintain animal health. The current study focused on the use of animal feed

grade sodium bisulfate (SBS). Multiple studies have reported positive effects on broiler performance from dietary supplementation of SBS (Ruiz-Feria et al., 2011; Kassem et al., 2012; Chadwick et al., 2020). This compound of acidic nature has shown positive effects on poultry health and grow-out conditions due to its ability to effectively shift excreta pH to retain ammonia in order to improve air and litter quality, while having various effects on bacteria found in the environment (Pope and Cherry, 2000). It is currently used in pet food as an effective feed acidifier. It is worth mentioning that most of the studies with SBS reported in the literature were conducted in the absence of a coccidiosis vaccine challenge.

A common problem associated with coccidiosis is the poor performance that often results from a compromised intestinal integrity and reduced nutrient absorption. Our lab has observed that vitamin D status can be contingent on the vitamin D source and vaccine challenge in part because D_3 is not considered biologically active until it undergoes a series of hydroxylation reactions in the kidney and liver. Yarger et al, (1995) compared the effect of feeding two different sources of vitamin D at different concentration levels and found a significant dose response with serum 25-hydroxycholecalciferol vitamin D_3 (25-OH-D_3) concentrations increasing more rapidly in birds fed 25-OH-D_3 than in birds fed cholecalciferol alone. Prior to the beginning of this study, we hypothesized that a nutritional strategy that could help ameliorate the intestinal challenges related to coccidiosis could have a positive effect on vitamin D absorption, which has been associated with performance, enhancing bone mineralization, reducing incidence of bone abnormalities, improving intestinal morphology (Leyva-Jimenez, 2019), and modulating

avian immunity (V.G. Gómez, et. al. 2013). Measuring vitamin D absorption by looking at the serum concentration of 25-OH-D_3, an active metabolite of vitamin D could provide an indication of an improved vitamin D status on broilers challenged with coccidiosis vaccine.

It is well known that commercial broilers often are exposed to stressful conditions, pathogens, and field challenges that can generate an impaired absorption of nutrients. The nutritional status of a bird can determine the level and efficacy of its response to stress, for example coccidiosis (Yun, et al., 2000). Animal feed grade sodium bisulfate may promote performance of broilers under coccidiosis vaccine challenge by supporting intestinal integrity and function. Improved intestinal integrity with the feeding of SBS under conditions of enteric stress can result in higher absorption and we hypothesize that it may be reflected on an improved vitamin D status.

Therefore, this study aimed to analyze the effect of dietary supplementation of animal feed grade sodium bisulfate on performance, intestinal morphology, and vitamin D_3 status of broilers subjected to a 2X dose coccidiosis vaccine challenge.

Materials and methods

At day 0, broilers were allocated in an environmentally controlled rearing facility located at Texas A&M University Poultry Research Center on wood shavings. Twenty-five randomized male Cobb 500- broilers were placed into each pen. Eight replicates per group in a completely randomized block design were used (25 * 32 = 800) a 2x2 factorial arrangement. Separate rooms were used for vaccinated vs unvaccinated broilers – two rooms per group, 4 rooms total. All broilers were weighed individually and grouped together so that broilers could be allocated to each pen with nearly identical initial body weights and variance (±5 g). Pen average was the unit of measurement for all performance variables. Individual broilers were the unit of measure for gut morphology, vitamin D status, tibia breaking strength, and bone ash.

All animal handling and care practices were in accordance with an approved animal use protocol by the Texas A&M institutional animal care and use committee (IACUC).

A basal corn-soy broiler starter diet was formulated. Rather than the 200 IU/Kg Vit D concentration recommended by NRC (1994), experimental diets were formulated at a concentration of 2750 IU/Kg of Vit D_3 using a commercially available D_3 source (AHI, Nacogdoches, Texas). A customized mineral-vitamin premix devoid of D_3 was used. The diets were formulated at 2750 IU to be congruent with commercial type diets and in order to better capture a response that could be practical and of importance to the industry. The positive control groups received 0.4% of animal feed grade sodium bisulfate (SBS; Jones-Hamilton Co; Walbridge, Ohio) at the expense of sodium chloride. All diets had the same

formulated concentration of sodium. Chemical analysis of the feed was conducted prior to the feeding of each diet. Nutrient composition of each diet was confirmed by the Feed and Water Environmental Lab UGA (Athens, Ga) via near infrared spectroscopy. Concentration of vitamin D in the feed was analyzed at Heartland Assays Laboratories (Ames, Iowa) via liquid chromatography mass-spectrometry (LC/MS). Feed and watering method was *ad libitum* during all experimental periods. No antibiotics or coccidiostats were used during the study. Broilers were monitored daily with regard to general flock condition, temperature, lighting, water, feed, and any unanticipated events for the rearing facility. On day 1 of the study, broilers in one half of the pens were vaccinated via oral gavage with Huvepharma ADVENT®-Vaccine containing a mixture of viable sporulated oocysts from *Eimeria acervulina, Eimeria maxima and Eimeria tenella* at 2X recommended dose.

Performance

Feed consumption and body weight were recorded on day 0, 7, 14 and 21 to calculate weight gain, feed:gain ratio, feed:weight ratio, productivity index, and mortality. The experimental broilers were observed daily for any clinical signs and mortality. Mortality and body weight of dead broilers were recorded daily.

Vitamin D status

At day, 10 and 21 three broilers per pen were randomly selected, individually weighed and used to collect blood samples, drawn from the jugular vein. The same three broilers were euthanized by CO_2 and were used for all parameters measured in order to study potential correlations between factors. Blood samples were placed in a micro centrifuge

tube and then centrifuged for 15 min. at 3,000 rpm. The serum was stored at -80 °C until further analysis. Vitamin D status was evaluated by analyzing the stored serum samples using a commercial ELISA 25-OH-Vitamin D kit via manufacturer's protocol (Eagle Biosciences, Nashua, NH).

Lesion scores

At day-10 and 21 of the trial, the three euthanized broilers per pen were scored for coccidial lesions in the upper and middle small intestine and ceca. Lesions were scored from 0 – 4 as described by Johnson and Reid (1970). Where 0 score represents no gross lesions and 4 indicates severe damage to the intestine.

Intestinal morphology

At day 10, and 21 the three euthanized broilers per pen were utilized for intestinal morphology assessment. Jejunum and ileum samples were obtained, flushed and fixed in 10% formalin. Jejunum samples were taken in the middle point between the bile ducts and the Meckel's diverticulum. The ileum samples were taken 8 cm proximal to the ileocecal junction. Samples were processed and villus height and crypt depth were measured from 5 complete villi and villus-associated crypts for each sample (Feng et al., 2007).

Bone mineralization

At day-10 and 21 of the trial, both tibias from the three euthanized broilers per pen were removed. Right tibiae bones were defatted in 4 L of petroleum ether for 48-h and then dried in a forced draft oven at 105°C until samples were of constant weight. Finally, the dried bones were ashed 24 hrs. at 650°C. Percent bone ash was calculated based on starting dry bone weight and remaining ash. The left tibiae were cleaned from any adhering tissue

and used to assay breaking strength using a Texture Technologies TA. XT Plus® Texture Analyzer (Hamilton, MA) with a 50 kg load cell, a crosshead speed of 100 mm/min with the tibia supported on a 3-point standing ring and a 3.0 cm constant span (Leyva-Jimenez, 2019).

Statistical analysis

No room effects were observed in any of the parameters measured. Room arrangement was not included in the final statistical analysis**.** Data were analyzed as two way- ANOVA using the GLM Procedure of SPSS (SPSS Inc., Chicago, IL) for a completely randomized design where treatment diets were used as blocks in the model. If significance, means were separated by Duncan's Multiple Range Test. Significance was reported at $P \leq 0.05$.

Results and discussion

Performance

Results of all performance parameters are summarized in Table 16. There were significant differences ($P<0.05$) at day 21 between dietary treatments with SBS-fed broilers having higher body weights. A vaccine effect was observed at day 14 with vaccinated broilers having significantly lower (6%) body weights. Throughout the study, vaccinated broilers had numerically lower body weights than non-vaccinated broilers. Unvaccinated broilers were kept isolated from vaccinated broilers and the absence of lesion scores show that there was in fact an absence of a coccidia challenge to those groups. Lesion scores were significantly higher ($P<0.05$) for vaccinated groups compared to non-vaccinated groups, suggestive of a good vaccine challenge.

The presence of lesions in the small intestine confirms effective delivery of the coccidiosis vaccine and provides further evidence of the digestive and immune challenge to the broilers. Lower body weights at day 14 are most likely the result of vaccinated broilers being expose to the first coccidia cycling, which allowed for differences to be more noticeable. There are several reports that show broilers receiving a vaccine challenge having lower body weights shortly after immunization and as a normal immune response to the challenge (Merck Manual, 2016).

A dietary effect was observed at the end of the trial with SBS fed broilers finishing with mean body weights significantly ($P<0.001$) higher than Control broilers. Several studies in the past have shown a coccidiosis vaccine effect on body weights (Peek, 2011, Chapman, et al. 2013, Ahmad, et al. 2016). A dietary effect was more noticeable in the concluding part of the study, which suggests that the difference became apparent as coccidia-induced gut damage increased as the oocyst populations likely peaked. Feed consumption data showed no significant differences among the treatments during the experimental period (0 to 21 d). SBS-fed broilers had numerically lower cumulative feed consumption compared to Control. Overall, vaccinated broilers showed numerically higher feed consumption throughout the trial compared to non-vaccinated broilers.

In addition, no significant dietary effect was observed through the study. These results are consistent with research data (Suarez et al., 2018) between vaccine-challenged broilers vs not vaccinated. Feed consumption often does not appear to be significantly different due to the constant feed availability and proper environmental conditions presented in a trial. In previous studies conducted at our lab studying antigen specific

immune response following Newcastle Disease Virus immunization there were no significant differences in cumulative feed consumption (Suarez et al., 2018).

Cumulative feed conversion ratio (0-21d) was significantly lower ($P<0.001$) for SBS-fed broilers, and at the end of the trial a difference of 0.11 existed. The greatest development in feed:gain was observed during the last week of the trial (14-21d). Vaccinated broilers had significantly ($P<0.001$) higher feed conversion ratio by the end of the second week and finished numerically higher than Control broilers by day 21, which is consistent with published data. A dietary effect was noticed during the first and third week. SBS fed broilers had significantly ($P<0.001$) lower feed conversion ratios. Sodium bisulfate is composed of sodium, hydrogen, and sulfate ions. All these ions play key roles in gut function such as acid–base balance, electrolyte homeostasis, and in the case of sodium absorption of sugars and fluids. (Gennari, and Weise 2008). Sulfate serves a key component for the maintenance of tissues (Hooge, et al., 1999; Ahmad, et al., 2006). Therefore, increased availability of these compounds during conditions of stress could provide a benefit to the host. At the end of the trial, cumulative feed conversion ratio was significantly ($P<0.001$) improved in SBS fed broilers. These results are comparable to those seen by Ruiz-Feria et al. (2011), where broilers on a diet with SBS finished with lower FCR compared to a control diet. SBS fed broilers finished with a feed conversion ratio significantly ($P< 0.001$) lower than those of vaccinated broilers.

This difference suggests that under vaccine challenge broilers fed SBS can maintain a significantly improved performance. However, as a result of feed utilization rather than an increased feed consumption.

Chadwick, and coworkers (2020) indicated that broilers fed SBS at 0.5% under coccidiosis challenge resulted in significantly ($P<0.05$) lower cumulative FCR compared to control. We hypothesize that SBS may be ameliorating the negative effects associated with coccidia in the gastrointestinal tract by maintaining a higher level of gut integrity compared to Control. Broilers fed a control diet seemed to experience the highest loss in performance during the last week of the trial, where the significant ($P<0.001$) differences in feed conversion vs SBS further developed. Coccidiosis can generate its most detrimental impact to the gastrointestinal tract close to day 21 of age depending on litter conditions and other factors (Merck Manual, 2016). I

In addition, results obtained in previous experiments have allowed us an opportunity to observe differences in activation of cell-mediated immune response between the period following a primary immunization and secondary immunization which can affect live performance differently contingent on the age at which the challenge is presented (Suarez et al. 2019).

Intestinal morphology

Table 17 summarizes villi height taken on days 10 and 21. Samples taken on day 10 showed a significant difference ($P<0.001$) in the duodenum with broilers fed a control diet having higher villi height. Vaccinated broilers showed a numerically higher villi height at day 10. No significant differences were observed in the jejunum at day 10. A vaccine and dietary effect were observed in the jejunum at day 21. Broilers fed a control diet and those vaccinated showed significantly higher ($P<0.05$) villi height in the jejunum compared to SBS-fed birds and not vaccinated broilers.

In addition, at day 10 in the ileum section vaccinated broilers had significantly ($P<0.05$) higher villi height compared to non-vaccinated broilers. No other dietary effect or vaccine effect of significance was observed at day 21. Vaccinated broilers consistently showed numerically higher villi height in all sections of the small intestine compared to non-vaccinated broilers except in the duodenum at day 21. This may be indicative of compensatory hypertrophy, which has been reported in other coccidiosis models (Fernando and McCraw, 1973). These differences did not show any trend during the trial or correlated with performance nor lesion scores. It is likely that the difference in villi height was related to an exposure to coccidia earlier in the life of vaccinated broilers, which allowed for an earlier immune response in vaccinated broilers compared to not vaccinated broilers. Suarez, ad coworkers (2018) reported significant differences in antigen specific immune response as a result of maternally-derived antibodies and different immunization protocols. Chou, and coworkers (2009) showed that it is possible that coccidiosis vaccine can cause an increased variability in intestinal response of chickens.

Table 18 summarizes results of crypt depth from days 10 and 21. Significant differences ($P<0.001$) at day 10 in the duodenum and ileum were observed with vaccinated broilers having higher crypt depths compared to non-vaccinated broilers Vaccinated broilers consistently had higher crypt depth in all sections of the small intestine during the study. Broilers fed the control diet had significantly ($P<0.001$) higher crypt depth in duodenum at day 10 and ileum at day 21 compared to SBS-fed broilers. At day 21, duodenum of vaccinated broilers had significantly higher crypt depth compared to non-

vaccinated broilers. Increased crypt depth in order to compensate for epithelial cell loss has been reported during a coccidia challenge (Fernando and McCraw, 1973).

Therefore, a lower crypt depth can be interpreted as less damage happening in the gut or maintenance of gut integrity due to that in the intestinal mucosa, the renewal development that occurs continually aids the proliferating cells in the mucosal crypts differentiate predominantly to enterocytes and be released into the lumen from the villus tip after migration (Zuni et al., 2010). A compromised gut integrity would then lead to increased cell turnover along the crypt/villus axis, leading to immature enterocytes and as a result poor nutrient absorption. Chadwick, and coworkers (2020) reported significantly ($P<0.05$) lower crypt depth in broilers fed SBS at 0.5% under 10X coccidiosis-vaccine challenge compared to Control. Throughout the study, SBS fed broilers had lower crypt depth and by day 21 it was significantly ($P<0.001$) lower compared to control.

Villi height to crypt depth ratio data is summarized on Table 19. At day 10, vaccinated broilers had significantly lower ($P<0.001$) ratios in all sections of the small intestine. SBS-fed broilers had a significantly higher ($P<0.001$) ratio in the jejunum at day 10 and finished with numerically higher ratios in all other sections throughout the study except at day 21 in the jejunum. At day 10, the three sections of the small intestine of vaccinated broilers were significantly lower compared to those unvaccinated. The jejunum both at day 10 and 21 had lower ratios compared to the duodenum and ileum. A dietary effect was noticeable with SBS fed broilers on average having higher villi height to crypt depth ratios and at day 10 showing significantly ($P<0.001$) higher ratio compared to control. In the literature, villi height to crypt depth ratio is described as a parameter to

measure gut integrity and nutrient absorption potential. A higher villi height to crypt depth ratio indicates an improved intestinal nutrient absorption capacity and their measurements are significant indicators of intestinal integrity (Shirani et al., 2019).

Vitamin D status

Results from serum 25-OH-D_3 concentration at days 10 and 21 are summarized on table 20. It is well accepted that an accurate representation of vitamin D status can be measured by looking at 25-OH-D_3 in circulation which has a half-life close to three weeks (Yarger et al., 1995). SBS-fed broilers had significantly higher serum 25-OH-D_3 concentration ($P<0.001$) at day 21 and numerically higher concentration ($P<0.07$) compared to Control at day 10. Vaccinated broilers showed numerically lower concentrations at days 10 and 21. The lowest concentration was seen at day 21 in vaccinated broilers and broilers fed the control diet. SBS-fed broilers showed at day 10 the highest concentration reported of 25-OH-D_3 during the trial.

Vitamin D deficiency has been reported to depress the cellular immune responses in young broiler chicks (Aslam et al., 1998). In the literature, vitamin D metabolites have been reported to decrease turkey osteomyelitis (Huff et al., 2002). It is well accepted that 25-OH-D_3 bypasses the requirement for liver hydroxylation, it experiences tight regulation from the renal alpha-hydroxylase, which allows for less side effects in absorption in comparison to l,25-$(OH)_2D_3$ (Yarger et al. 1995).

An objective of this trial was to determine if animal feed grade sodium bisulfate could ameliorate the negative effects commonly associated with coccidiosis.

Malabsorption syndrome is a common observation in broilers challenged with coccidiosis (Allen and Fetterer, 2002).

One parameter affected by coccidiosis, that has been described in the literature and we have seen in our lab, is vitamin D status. We hypothesized that a nutritional strategy that could help ameliorate the negative effects of coccidiosis by maintaining gut integrity could result in different vitamin D concentrations in serum when compared to a control diet. Both SBS and control diets were formulated to provide 2750 IU/Kg vitamin D using the same vitamin source. Commercial type diets follow this inclusion level instead of the 200 IU recommended by the NRC.

A dietary effect was detected at day 21 with control broilers showing significantly ($P<0.05$) lower 25-OH-D_3 concentration in serum compared to SBS fed broilers. An initial thought was given to the possibility that the improved performance observed in SBS fed broilers could be the result of improved vitamin D status compared to control as it is well studied that higher 25-OH-D_3 concentration in serum correlate with improved overall performance (Yarger et al. 1995).

Bone mineralization

Tibia breaking strength (TBS) results showed no significant ($P>0.05$) differences among the treatments. Numerically higher TBS was observed in SBS-fed broilers at day 10 and 21. Vaccinated broilers showed lower TBS at day 10 and 21.

Bone ash percentage results from sampling days 10 and 21 are reported in table 21. At day 10, a significant ($P<0.001$) difference between vaccinated and not vaccinated broilers was reported. Non-vaccinated broilers had higher % bone ash. At day 10, SBS-

fed broilers showed a significantly ($P<0.001$) higher bone ash % compared to broilers fed a control diet. At day 21, no significant differences were observed between the treatments. Bone mineralization is an important parameter to measure and under commercial conditions it can allow for detection of leg problems which can ultimately affect animal health and profit margins. Bone ash is a good indicator of bone mineralization and relative bioavailability of P in poultry (Shastak et al., 2012). Low bone ash percent may reflect a broilers' need for Ca and P and may reflect an overall lower level of homeostasis (Künzel, et al. 2019).

Vaccinated broilers finished with lower bone ash and at day 10 they had significantly ($P<0.001$) lower bone ash compared to unvaccinated broilers. Vitamin D status results observed during the trial agree with the reported bone mineralization results.

Conclusions and applications

1. No interaction between vaccine and dietary effect were observed consistently during the study. In non-challenged birds, animal feed grade sodium bisulfate appeared to generate improved broiler performance. SBS fed birds showed significantly improved feed conversion ratio and increased body weights.

2. No consistent effect was reported on tibia breaking strength. Bone ash percentage was significantly improved at day 10 from a dietary effect vs control, which can be of value during the early development of chicks.

3. SBS fed broilers maintained 25-OH-D_3 concentration in serum and had significantly higher concentration by day 21. This can help provide a higher safety margin from a diet formulation standpoint.

4. The main mechanism of action of SBS remains to be elucidated and further research is necessary to more accurately describe its effect as a nutritional strategy.

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Table 14 Diets and calculated nutrient composition

	Starter - Control 0-21 d	Starter - SBS 0-21 d
Ingredients, %		
Corn, yellow grain	58.58	58.35
Soybean meal 46%, dehulled solvent	34.77	34.77
Monocalcium Phosphate	1.51	1.51
Limestone	1.48	1.48
Sodium chloride	0.44	0.26
Mineral mix[1]	0.05	0.05
DL Methionine	0.28	0.28
Lysine, HCl	0.17	0.17
Vitamin Premix[2]	0.25	0.25
Fat A/V blend	2.45	2.48
SBS	--	0.40
Total	100	100
Calculated and (Analyzed) Nutrient Composition		
CP, %	22.0 (21.8)	22.0 (20.6)
ME, kcal/kg	3025	3020
Calculated D_3 µg/Kg	69	69
Analyzed D_3 µg/Kg	79	78
Crude Fat, %	5.0	5.0
Lysine, %	1.30	1.30
Methionine, %	0.61	0.61
TSAA, %	0.97	0.97
Tryptophan	0.27	0.27
Threonine	0.82	0.82
Arginine	1.45	1.45
Valine, %	1.00	1.00
Calcium, %	0.90 (0.94)	0.90 (0.99)
Available Phosphorous, %	0.45 (0.37)	0.45 (0.36)
Sodium	0.20 (.22)	0.20 (0.19)
Chloride, %	0.36	0.24
Sulfur	(0.07)	(0.15)

[1]Vitamin premix yields 11,023 IU vitamin A, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. Experimental diets formulated at 2750 IU of Vit D from a commercially available D_3 source, AHI, (Nacogdoches, TX) and using a customized mineral-vitamin premix devoid of D_3. [2]Trace mineral premix yields 149.6 mg manganese, 55.0 mg zinc, 26.4 mg iron, 4.4 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix has less than 1% mineral oil. Animal feed grade sodium bisulfate (SBS; Jones-Hamilton Co; Walbridge, OH) at 0.4% added at the expense of sodium chloride.

Table 15 Experimental treatments

Treatment	Dietary inclusion of SBS (%)	Coccidial Vaccinated Birds
T1	D_3 supplement + 0% SBS	NO
T2	D_3 supplement + 0.4% SBS	NO
T3	D_3 supplement + 0% SBS	YES
T4	D_3 supplement + 0.4% SBS	YES

8 replicates per treatment. 2x2 factorial arrangement. Separate rooms were used for vaccinated vs unvaccinated broilers – two rooms per group, 4 rooms total. On day 1 of the study, birds were vaccinated with Huevepharma ADVENT®-Vaccine (Peachtree City, GA) containing a mixture of viable sporulated oocysts from E. acervulina, E. maxima and E. tenella at 2X dose.
1 IU of D_3 =0.025 µg cholecalciferol. All diets were formulated at 2750 IU vit D/ Kg using a commercially available D_3 source, AHI, (Nacogdoches, Texas) and using a customized mineral-vitamin premix devoid of D_3. Animal feed grade sodium bisulfate obtained from Jones-Hamilton (Walbridge, OH) was added at the expense of salt.

Table 16 Effect of dietary supplementation of SBS on performance of 21-day-old broilers (g)

Average Body weights (g)								
	Vaccination				Diets			
Age (days)	No	Yes	SEM	P*	Control	SBS	SEM	P*
7	180	179	2.15	0.799	177	178	2.15	0.125
14	488^{A}	459^{B}	5.91	0.002	479	481	5.91	0.134
21	923	912	14.31	0.579	886^{B}	949^{A}	14.31	0.006
Feed Consumption (g/bird)								
0 –7	128	125	2.46	0.376	129	125	2.46	0.243
7–14	382	390	8.19	0.477	390	382	4.41	0.344
14–21	675	716	21.8	0.200	699	691	21.85	0.790
Feed Conversion (feed: gain)								
0–7	0.933	0.905	0.012	0.104	0.950^{A}	0.888^{B}	0.012	0.001
7–14	1.011^{A}	1.085^{B}	0.019	0.001	1.050	1.070	0.019	0.460
0–14	0.982^{B}	1.042^{A}	0.012	0.002	1.018	1.006	0.012	0.505
14–21	1.501	1.534	0.042	0.589	1.673^{A}	1.362^{B}	0.042	<.0001
0–21	1.148	1.172	0.012	0.177	1.214^{A}	1.104^{B}	0.012	<0.001

^{A,B} Within vaccine or diet treatments, means within a row lacking a common superscript differ significantly (P<0.01). Each value represents the mean value for each group.
*Main effect P-value

Table 17 The effect of dietary supplementation of SBS on villi height (µm) at days 10 & 21

	Vaccine				Diet			
Age (days)	No	Yes	SEM	P*	Control	SBS	SEM	P*
**Tissue								
D - 10 d	1924	1949	36.0	0.618	2005^{A}	1868^{B}	36.0	0.009
D – 21 d	2461	2511	55.84	0.536	2430	2542	55.84	0.159
J - 10 d	851	876	16.4	0.277	875	852	16.4	0.319
J – 21 d	1288^{b}	1397^{a}	33.56	0.025	1416^{A}	1269^{B}	33.56	0.003
I – 10 d	639^{b}	679^{a}	12.49	0.026	657	661	12.49	0.851
I – 21 d	931	952	27.22	0.576	974	909	27.22	0.097

$^{A, B}$ Within vaccination or diet treatments, means within a row lacking a common superscript differ significantly (P<0.01).
$^{a, b}$ Means within a row lacking a common superscript differ significantly (P<0.05).
*Main effect P-value
** D= duodenum, J= jejunum, I= ileum
Each value represents the mean value for each group.

Table 18 The effect of dietary supplementation of SBS on crypt depth (µm) at days 10 & 21

	Vaccine				Diet			
Age (days)	No	Yes	SEM	P*	Control	SBS	SEM	P*
**Tissue								
D - 10 d	126^{B}	165^{A}	4.87	<0.0001	155^{A}	136^{B}	4.87	0.008
D – 21 d	188^{B}	225^{A}	7.79	0.001	207	206	7.79	0.863
J – 10 d	108	117	4.45	0.183	115	109	4.45	0.366
J – 21 d	153	157	6.18	0.586	155	155	6.18	0.979
I – 10 d	95^{B}	116^{A}	4.33	0.001	105	105	4.33	0.907
I – 21 d	135	139	3.83	0.392	145^{A}	129^{B}	3.83	0.005

$^{A, B}$ Means within a row lacking a common superscript differ significantly (P<0.01)
Each value represents the mean value for each group.
*Main effect P-value
** D= duodenum, J= jejunum, I= ileum

Table 19 Effect of dietary SBS on villi height: crypt depth ratio at days 10 & 21

	Vaccine				Diet			
Age (days)	No	Yes	SEM	P*	Control	SBS	SEM	P*
**Tissue								
D - 10 d	15.9^{A}	12.2^{B}	0.490	<0.0001	13.6	14.4	0.490	0.239
D – 21 d	14.1^{A}	11.3^{B}	0.607	0.002	12.5	12.8	0.607	0.735
J – 10 d	7.1^{A}	5.5^{B}	0.258	<0.0001	5.9^{B}	6.7^{A}	0.258	<0.0001
J – 21 d	7.2	6.4	0.355	0.116	7.2	6.3	0.355	0.095
I – 10 d	6.9^{A}	6.2^{B}	0.192	0.011	6.5	6.6	0.192	0.684
I – 21 d	7.0	7.0	0.232	0.879	6.8	7.2	0.232	0.229

$^{A, B}$ Means within a row lacking a common superscript differ significantly (P<0.01)
Each value represents the mean value for each group.
*Main effect P-value
** D= duodenum, J= jejunum, I= ileum.

Table 20 Response to dietary supplementation of SBS on serum 25-OH-D_3 concentration (ng/mL)

	Vaccine				Diet			
Age	No	Yes	SEM	P*	Control	SBS	SEM	P*
10 d	130	123	6.69	0.492	118	135	6.69	0.071
21 d	121	114	5.33	0.366	110[b]	125[a]	5.33	0.044

[a, b] Means within a row lacking a common superscript differ significantly ($P<0.05$). Each value represents the mean value for each group.
*Main effect P-value

Table 21 Response to dietary supplementation of SBS on bone ash (%)

	Vaccine				Diet			
Age	No	Yes	SEM	P*	Control	SBS	SEM	P*
10 d	48.9[A]	47.4[B]	0.393	0.012	46.9[B]	49.5[A]	0.393	<0.0001
21 d	49.3	50.0	0.219	0.169	49.5	50.0	0.219	0.135

[A, B] Within vaccination or diet treatments, means within a row lacking a common superscript differ significantly (P<0.001)
Each value represents the mean value for each group.
*Main effect P-value

5 CONCLUSIONS

In conclusion, the results from this series of studies further documents the performance advantage previously reported in the literature with regards to use of animal feed grade sodium bisulfate. Its main mechanism of action remains to be elucidated and further research is necessary to more accurately describe its effect as a nutritional strategy. The results herein reported do serve as a robust scientific foundation to expand research with this compound. SBS does not appear to modulate intestinal pH. Similar performance advantages (higher body weights, improved feed conversion) can be expected when using animal feed grade sodium bisulfate or potassium bisulfate vs Control. Increasing SBS in the diet reduces ($P < 0.001$) pH and increases sodium and sulfur diet content ($P < 0.001$).

A vigorous coccidiosis vaccine challenge was developed where a 2X recommended dose of live coccidiosis vaccine at day of placement significantly impacted performance, vitamin D and intestinal morphology. Detrimental enteric conditions associated with onset of the coccidiosis challenge was observed during the trial, which resulted in significantly lower bone mineralization, and serum 25-OH-D_3 concentration in birds fed a control diet. The results of the present study suggest the use of dietary 25-OH-D_3 compared to inclusion of D_3 alone provides a significantly improved safety margin for performance and bone mineralization. Furthermore, it is suggested that dietary 25-OH-D_3 compared to D_3 can be used as a nutritional strategy to surpass common challenges associated with the use of live coccidiosis vaccine.