Histopathological and morphometric changes induced by a dextran sodium sulfate (DSS) model in broilers

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ABSTRACT Oral administration of dextran sodium sulfate (DSS) is commonly used as an inducer of enteric inflammation in rodents. However, there is a dearth of knowledge regarding appropriate dosage, timing, or age responses in broilers for this potential inducer of inflammation without necrosis. Two experiments were conducted in day-of-hatch chicks to analyze clinical parameters and enteric histological changes induced by DSS when administered via drinking water (DW). In both experiments, birds were distributed into non-treated control or varying concentrations of DSS in DW. For both experiments, only 0.75% DSS in DW was histologically evaluated. In Experiment 1, chicks received DSS from day 3 to 11, and at 3, 6, and 8 d of treatment, chicks were weighed, and sections of the duodenum, ileum, and ceca were formalin fixed. The addition of 0.75% DSS caused depression, anemia, and watery bloody diarrhea, plus significantly (P < 0.05) decreased BW gain at all times. Shortened ileal villi at 6 d and duodenal villi at 8 d of treatment, reduced duodenal and ileal epithelial cell height at 3, 6, and 8 d, and increased duodenal goblet cell density at 6 and 8 d were observed in response to DSS administration (P < 0.05). In Experiment 2, birds received DSS from days 10 to 16 and were sampled at 3 and 6 d of treatment. Similar changes were found in ceca of treated birds. There was no significant change in the duodenal villus height and goblet cell density by 6 d of treatment, suggesting that 6 d of 0.75% DSS in DW was not sufficient for the reproduction of duodenal symptoms in these older birds. However, there was a significant decrease in ileal villus height and decreased ileal epithelial cell height at 3 and 6 d of treatment, as well as a significant decrease in BW compared to the control group. These findings indicate that DW administration of 0.75% DSS caused generalized mild and non-necrotic enteritis in broilers and that this compound may be useful for enteric inflammation modeling in poultry.

Key words: broiler, dextran sodium sulfate, enteric inflammation

INTRODUCTION

In addition to its role in endocrine and paracrine hormones production, nutrient permeability, water and electrolyte exchange, and digestion, intestinal mucosa is an important barrier for protecting animals against both commensal and pathogenic microorganisms and other insults (Kitajima et al., 1999, 2000; Sansonetti, 2004). The intestinal first line of defense is composed of the mucus layer and epithelium (Fasina et al., 2010). It has been proposed that intestinal epithelial cell defenses are essential to prevent inflammation, for example, by offering protection against microbial pathogens and oxidative stresses (Kawada et al., 2007). If the intestinal barrier is damaged and becomes nonselectively permeable, the submucosa and deeper layers will be subjected to continuous exposure to antigenic molecules from food and microorganisms, causing inflammatory conditions (Quinteiro-Filho et al., 2012).

Intestinal disorders in commercial broilers are important factors that lead to productivity losses, mortality, and further meat contamination (Lee et al., 2010). The development of an enteric inflammation model is important to further address the pathogenesis of intestinal inflammation as well as to test the efficacy of treatments designed to increase enteric integrity. Furthermore, a compelling proposed effect of antibiotic growth promoters in poultry is the reduction of low-level chronic inflammation (Niewold, 2007), and with pressures to remove such additives from poultry, robust high-throughput models other than performance trials may aid in the search for performance- and health-enhancing feed additives. Improved enteric health, beyond the prevention of infectious diseases, may improve the nutrient absorption,
performance parameters, and overall well-being of poultry flocks.

Dextran sodium sulfate (DSS) is a sulfated polysaccharide with a variable molecular weight (from 5 kDa to up to 1,400 kDa) that has been described as causing an acute chemical toxicity in intestinal epithelial cells (Yazbeck et al., 2012; Perse and Cerar, 2012). Moreover, DSS is an anticoagulant that has shown corrosive properties on the epithelia of the colon in experimental models with rodents, and this damage is likely to be related to common changes in colitis such as BW loss, consistency of feces, and presence of blood in the feces (Elsheikh et al., 2012).

Oral administration of DSS is commonly employed as an inducer of enteric inflammation in rodent models (Okayasu et al., 1990; Kitajima et al., 1999, 2000; Elsheikh et al., 2012). Furthermore, this induced colitis has been widely used as a model in studies of the pathogenesis and treatment of human inflammatory bowel disease (Kawada et al., 2007; Yan et al., 2009; Elsheikh et al., 2012). Nevertheless, a difference in susceptibility to DSS-induced intestinal inflammation among species has been reported (Kitajima et al., 2000). To our knowledge, no studies exist regarding DSS as a model of intestinal inflammation in chickens. Consequently, there is a dearth of knowledge regarding appropriate dosage, timing, or age responses, and extent of inflammation in chickens for this potential inducer of inflammation without necrosis. Therefore, the objective of these studies was to evaluate clinical parameters and enteric histological changes induced by DSS in broilers, with the aim of characterizing histological changes in the duodenum, ileum, and ceca in order to determine its potential effectiveness as an inducer of enteric inflammation.

**MATERIAL AND METHODS**

**Experimental Animals**

Day-of-hatch broilers were obtained from a local hatchery, placed in brooder batteries, and provided heat to maintain an age-appropriate temperature. Chicks were provided *ad libitum* access to water and a balanced unmedicated corn-soybean diet meeting the poultry nutrition requirements recommended by the National Research Council (1994). All animal handling procedures were in compliance with the Institutional Animal Care and Use Committee at the University of Arkansas.

**Dextran Sulfate Sodium Administration**

Two experiments were conducted to assess intestinal injury and inflammation caused by DSS in broilers. In both experiments, day-of-hatch chicks were randomly distributed into groups: control nontreated and DSS (LOT: H27Z029, Alfa Aesar, Ward Hill, MA) treated. DSS-treated groups were provided drinking water (DW) supplemented with DSS (MW 40 kDa), at levels and durations described subsequently, *ad libitum*. Control animals received normal DW *ad libitum*.

**Experiment 1**

A total of 80 day-of-hatch broiler chicks were randomly divided into 4 groups (n = 20 chickens): control nontreated, or DSS-treated at concentrations of 0.75% (wt/vol), 1.5% (wt/vol), or 3.0% (wt/vol) via DW starting at day 3. BW was recorded at day of hatch and days 3, 6, 9, and 11. At days 6, 9, and 11 (3, 6, and 8 d of DSS treatment, respectively), 5 birds from each treatment were humanely killed by CO$_2$ inhalation, and sections of duodenum, ileum, and cecum were collected for histological analysis.

**Experiment 2**

A total of 84 day-of-hatch broiler chicks were randomly divided into 3 groups (n = 28 chickens). The groups were distributed as control nontreated or DSS-treated at concentrations of 0.75% (wt/vol) or 1.5% (wt/vol) via DW starting at day 10. BW was recorded at days 10, 13, and 16. At days 13 and 16 (3 and 6 d of DSS treatment, respectively), 10 birds from each treatment were humanely killed by CO$_2$ inhalation, and sections of duodenum, ileum, and ceca were collected for histological analysis.

**Histology and Morphometric Analysis of Intestine**

Intestinal sections were standardized: for duodenum, a 0.5-cm section was collected from the middle of the descending duodenum; for ileum, a 0.5-cm section was collected from the mid-ileum at Meckel’s diverticulum; and a 0.5-cm section was collected from the middle of one ceca. Cecal sections were straightened on a flat piece of paper and fixed with mucosa facing up. Duodenal, ileal, and cecal sections were fixed in 10% neutral buffered formalin and embedded in paraffin, sectioned (5 mm thick), set on a glass slide, and stained with hematoxylin and eosin (H&E) or Periodic Acid–Schiff (PAS), then examined by light microscopy. Photomicrographs of random selected fields of each intestinal sample were acquired using a microscope equipped with a Leica DFC450C camera and Leica v.3.8.0 software (Leica Application Suite) and used for morphometric analysis. ImageJ 1.47v software (Rasband, 1997–2012) was used for the morphometric analysis of villus height (H&E), epithelial cell height (H&E), and goblet cell density (PAS). For villus height of the duodenum and ileum, under a magnification of 4×, an average of 12 villi per bird were measured, with a total of 5 birds per group per sampling day in Experiment 1 and an average of 7 villi per bird were measured, with a total of 10 birds per group per sampling day in Experiment 2. Villus height was measured from the tip of the villus...
to the top of the muscularis mucosa and reported in millimeters.

For epithelial cell height of the duodenum and ileum, under a magnification of 10×, 3 to 5 selected fields were photographed and 10 epithelial cells were measured per field in both Experiment 1 (5 birds/group) and Experiment 2 (10 birds/group). Epithelial cell height was measured from the base (middle of the villus) to the tip of the cell and reported in micrometers.

Goblet cell count and surface area measurement of the duodenum and ileum were measured under a magnification of 20×. Five selected fields per bird were photographed, with a total of 5 birds/group in Experiment 1, and 4 selected fields per bird were taken, with a total of 10 birds/group in Experiment 2. Goblet cell density was measured as number of PAS positive goblet cells/mm² of epithelial area, as described by Fasina et al. (2010).

**Data and Statistical Analysis**

BW gain from these studies was calculated using the initial BW at the beginning of DSS treatment (day of hatch in Experiment 1 and day 10 of age in Experiment 2) as a covariate and subjected to ANOVA using the GLM procedure of SAS, with significance reported at \( P < 0.05 \); means were further separated using Duncan’s multiple range test (SAS Institute, 2002). The mean values of each measured morphometric parameter for each chicken were entered as raw data in the database and were subjected to ANOVA (SAS Institute, 2002) and to mean separation using Duncan’s multiple range test at a 5% level of significance.

**RESULTS AND DISCUSSION**

In addition to characterizing morphometric and histologic lesions induced in broilers by DW administration of DSS, multiple levels of inclusion were tested to determine the dose that would optimally induce enteric pathologies without severe distress. Higher doses of DSS in the DW of chicks in both experiments – 3.0% and 1.5% in Experiment 1 and 1.5% in Experiment 2 – resulted in adverse clinical signs, such as morbidity, lethargy, severe bloody diarrhea, and malaise, and were terminated early without sample collections. Furthermore, unreported experiments testing 0.062, 0.125, 0.25, and 0.5% DSS in DW did not result in significant differences in BW gain between control and all treatment groups, suggesting that doses lower than 0.75% did not effectively cause clinical signs of disease (data not shown). The apparent tight dose range of DSS in chickens was not consistent with that of rodents, typically ranging from 1 to 5% depending on the desired severity of disease (Okayasu et al., 1990; Kitajima et al., 1999; Elsheikh et al., 2012), and is indicative of an increased susceptibility to its toxic effects. Oehlers et al. (2013) did, however, report a similar tight dose range with DSS when administered to zebrafish, suggesting that toxic effects vary with species of animal.

The addition of 0.75% DSS in DW caused depression, anemia, and watery and occasionally bloody diarrhea as well as significant (\( P < 0.05 \)) BW loss measured after 3, 6, and 8 d of treatment in Experiment 1 and continued to segregate throughout the study. Significant (\( P < 0.05 \)) weight loss in Experiment 2 was noted after 6 d of treatment (Table 1). Chickens receiving DSS in the DW developed histologically apparent inflammation in the duodenum, ileum, and ceca; however, the intestinal inflammation had different severities depending on the segment. Severe damage was found in ceca. Ceca have been reported as the most permeable segment of gastrointestinal tract (GIT), and, thus, perhaps are more susceptible to external substances and bacterial byproducts (Kitajima et al., 2000). Held in the ceca, molecules in solution, as well as solid particles, can be acted on by cecal secretions, bacteria, fungi, and other microorganisms, and fluid has time to be absorbed (Clench, 1999).

Moreover, subjective histological evaluation of the ceca from nontreated control chickens revealed a well-defined crypt length and no edema in mucosa and submucosa (Figure 1A). In contrast, ceca sections from DSS-treated chickens, from both Experiments 1 and 2, showed loss of epithelial cell and crypt architecture, submucosal edema with inflammatory cell infiltration, and hemorrhage in lamina propria at all time points (Figure 1B), which correlated with the gross lesions of atrophy and blood-filled ceca observed during necropsy. These lesions and alterations were similar to what has been described from colons of DSS-treated rodents. In general, the most common histopathological findings described in the colon of rodents treated with DSS were crypt and epithelial cell injury, granulocytes, and mononuclear immune cell infiltration, edema, and ulceration (Kawada et al., 2007). According to Yan et al. (2009), mice treated with 3.5% DSS in DW for different time periods showed BW loss, blood in the

<table>
<thead>
<tr>
<th>Table 1. Effect of 0.75% DSS in DW on BW gain from Experiments 1 and 2.</th>
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<tr>
<td>BW gain (g) – Experiment 1</td>
</tr>
<tr>
<td>3 d (6d)**</td>
</tr>
<tr>
<td>6 d (9d)</td>
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<tr>
<td>8 d (11d)</td>
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<tr>
<td>Control 45.13 ± 2.75a</td>
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<tr>
<td>45.20 ± 3.59a</td>
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<tr>
<td>50.60 ± 9.22a</td>
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<tr>
<td>DSS 0.75% 36.86 ± 2.93b</td>
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<td>20.33 ± 5.95b</td>
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<td>19.75 ± 3.28b</td>
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<td>BW gain (g) – Experiment 2</td>
</tr>
<tr>
<td>3 d (13d)</td>
</tr>
<tr>
<td>6 d (16d)</td>
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<tr>
<td>Control 92.9 ± 3.43a</td>
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<tr>
<td>130.8 ± 7.94a</td>
</tr>
<tr>
<td>DSS 0.75% 93.5 ± 2.99b</td>
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<tr>
<td>68.9 ± 6.30b</td>
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Values expressed as mean ± standard error. In Experiment 1, \( n = 20 \) at 3 and 6 d of treatment, and \( n = 15 \) at 8 d of treatment. In Experiment 2, \( n = 28 \) at 3 d of treatment, and \( n = 18 \) at 6 d of treatment.

**Means with different superscripts within the same column, per experiment, differ significantly (\( P < 0.05 \)).

**Initial BW was recorded on the day of DSS DW treatment initiation.

**Days of treatment with DSS in DW; numbers in parentheses indicate age of chicks.
Table 2. Effect of 0.75% DSS in DW on duodenal epithelial cell height, villus height, goblet cell density (3, 6, and 8 d of treatment) from Experiment 1. DSS treatment was initiated at 3 d of age.

<table>
<thead>
<tr>
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<th>3 d (6d)*</th>
<th>6 d (9d)</th>
<th>8 d (11d)</th>
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<tbody>
<tr>
<td>Duodenal epithelial cell height (μm)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>98.95 ± 6.13°</td>
<td>110.54 ± 4.85°</td>
<td>97.13 ± 4.26°</td>
</tr>
<tr>
<td>DSS 0.75%</td>
<td>83.97 ± 1.62b</td>
<td>76.54 ± 5.03b</td>
<td>77.01 ± 1.70b</td>
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<tr>
<td>Duodenal villus height (mm)</td>
<td></td>
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<tr>
<td>Control</td>
<td>1.25 ± 0.05a</td>
<td>4.89 ± 0.15a</td>
<td>5.25 ± 0.13a</td>
</tr>
<tr>
<td>DSS 0.75%</td>
<td>1.10 ± 0.10b</td>
<td>4.24 ± 0.33b</td>
<td>4.57 ± 0.08b</td>
</tr>
<tr>
<td>Duodenal goblet cell density (number of goblet cells/mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>230.79 ± 35.5a</td>
<td>212.22 ± 16.4b</td>
<td>214.75 ± 13.5b</td>
</tr>
<tr>
<td>DSS 0.75%</td>
<td>239.79 ± 24.6a</td>
<td>307.56 ± 20.1a</td>
<td>350.52 ± 9.3a</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard error, n = 5 per group per sampling day.

*Days of treatment with DSS in DW; numbers in parentheses indicate age of chicks.

Means with different superscripts within the same column, per element, differ significantly (P < 0.05).

EFFECT OF DEXTRAN SODIUM SULFATE IN BROILERS

In another study, Kitajima et al. (1999) described crypt loss and separation of the crypt base from the muscularis mucosa as the first findings observed after 2 to 3 d of DSS (5% in the DW) treatment in mice. They also reported that after 4 to 5 d of DSS treatment, crypt loss became more severe, and the lamina propria and submucosa had more infiltration of inflammatory cells as compared to control nontreated rodents, suggesting that DSS had an inhibitory effect on cell proliferation, since crypt cells actively proliferate in the intestinal mucosa (Kitajima et al., 1999). Rose et al. (2012) evaluated histopathological changes in the colon of wild-type mice after oral treatment with 1 or 3% DSS and showed significant loss of crypts and edema of the lamina propria in mice treated with 1% DSS for 7 d. They additionally noted mucosal and submucosal lymphocyte, neutrophil, and macrophage infiltration and erosions in mice treated with 3% DSS for 7 d. After 7 d of DSS removal and recovery time, the colon of the mice showed a complete recovery in the group treated with 1% DSS, but no recovery in the group treated with 3% DSS because of the extensive colon injury (Rose et al., 2012). Additionally, Yazbeck et al. (2011) showed that administration of 2% DSS for 6 d in mice resulted in a colon inflammation that extended to the small intestine, suggesting that the small intestine can be also damaged.

In the present study, histological morphometric analysis of the duodenum showed a significant shortening (P < 0.05) of the epithelial cell height at 3, 6, and 8 d of administration and villus height at day 8 of chickens treated with DSS beginning on day 3 of age, though villus height was not significantly affected until 8 d of treatment (Table 2). A significant (P < 0.05) increase in duodenal goblet cell density at days 6 and 8 was also observed in DSS-treated broilers (Table 2). Intestinal goblet cells, located in the villi, are responsible for producing secretory mucins (Fasina et al., 2010). It has been proposed that mucus secretion increases in response to commensal and pathogenic bacteria, and thus the determination of goblet cell numbers could be used to estimate mucin production (Fasina et al., 2010).

According to Fasina et al. (2010), infection with Salmonella Typhimurium in chickens increased the density of goblet cells relative to surface area of villi. The authors suggested that by the release of bioactive factors or activation of immune cells, microorganisms can affect goblet cells and mucus layer (Fasina et al., 2010). A change in mucin production, which is important in epithelial protection, directly affects the epithelial barrier function (Dharmani et al., 2011). The enumeration of filled, empty, or releasing mucin goblet cells in the crypts of DSS-treated rats revealed alterations in the number and morphology of mucin-secreting activity of goblet cells. Interestingly, when DSS-treated groups were compared to the control, a decrease in the number

Figure 1. Micrographs of cecal tissues showing differences in histologic parameters between control nontreated (A) and DSS treated (B). HE. Scale bar = 200 μm. Panel B shows loss of epithelial cells and crypt architecture, submucosal edema, and inflammatory cell infiltration and hemorrhage in lamina propria.
of filled goblet cells and, at the same time, an increase in the number of empty and releasing mucin goblet cells were shown (Dharmani et al., 2011).

Histological morphometric analysis of the ileum also showed a significant shortening ($P < 0.05$) of epithelial cell height at all 3 sampling points in Experiment 1 and villus height only at 6 d of treatment in chickens treated with 0.75% DSS (Table 3). No changes in goblet cell density was observed in the ileum of DSS-treated chickens from Experiment 1 (Table 3). Minor changes in the small intestine (ileum) morphology of DSS-treated rats has been described in rodents, with increases in ileal crypt cell proliferation, but sometimes no effects on villus height (Geier et al., 2009; Yazbeck et al., 2011).

In Experiment 2, there was no significant change in the duodenal villus height and goblet cell density, which suggests that 6 d of treatment is not enough to cause histologically significant changes in older broilers and that age or gastrointestinal development may play a role in the toxic effects of DSS (Table 4). This change in susceptibility to the toxic effects of DSS could be due to gastrointestinal developments since it is well known that the GIT undergoes rapid growth and development during the first weeks of life. However, there was a significant decrease ($P < 0.05$) in duodenal epithelial cell height after 6 d of treatment with 0.75% DSS in DW (Table 4), which was temporally associated with the significant ($P < 0.05$) BW loss noted in Table 1. Contrary to Experiment 1, DSS affected the ileum of older chickens in Experiment 2, with a significant shortening ($P < 0.05$) of the epithelial cell height and villus height at both sampling points (Table 5). According to Beal et al. (2006), the main signs of an intestinal immune response are villus atrophy and hyperplasia of enterocytes in the crypts. Ileal goblet cell density significantly increased ($P < 0.05$) at treatment day 3 but was not sustained to day 6 (Table 5). As described by Yazbeck et al. (2011) in rodents, the effect of DSS in the small intestine could be due to direct damage or a secondary and compensatory response to harm in the colon.

As a mechanism of action, DSS has been primarily reported to cause mucosal epithelial cell damage with inflammation due to cytotoxic effects, dysfunction of lamina propria immune cells, changes in the intestinal microbiota (Kitajima et al., 1999; Elsheikh et al., 2012), and production of free radicals due to the increase in macrophages and neutrophils (Yazbeck et al., 2011). By causing damage to the epithelial cells, DSS can provoke an increase in the mucosal permeability, allowing bacterial subproducts to permeate the mucosa and induce an inflammatory response (Kitajima et al., 1999). Additionally, it has been reported that DSS can cause intestinal mucus loss, allowing bacteria and other antigens to contact and damage epithelial cells (Morgan et al., 2013).
After 7 d of DSS treatment in DW, mice that received 5 kDa molecular weight DSS showed inflammation in the ceca and upper colon; those that received 40 kDa showed more severe inflammation compared to 5 kDa, which was located in the lower colon. However, mice that received 500 kDa DSS showed no colitis, which could be explained as a lack of passage of the molecule through the mucosal membrane (Kitajima et al., 2000). A higher sulfur content in high-molecular-weight DSS, such as 40 kDa, was suggested as the main cause of a more severe inflammation (Kitajima et al., 2000).

Histopathological analysis of the ceca, as well as morphometric analysis of the duodenum and ileum, suggested that 0.75% DSS, administered orally in DW, caused generalized enteritis in broiler chickens, consequently causing BW loss, diarrhea, and intestinal bleeding. Though the toxic dose range for chickens is small, thereby limiting the flexibility of DSS as a model for causing different severities of intestinal inflammation, an inclusion rate of 0.75% appears to affect BW, cause gross lesions, and significantly affect microscopic architecture and structure in a manner consistent with rodent models commonly used to study inflammatory diseases. DSS-induced colitis is commonly used as an animal model of intestinal bowel disease, and these findings indicated that this compound may be useful for enteric inflammation modeling in poultry and forstudying methods to ameliorate intestinal inflammation.

REFERENCES


