Poultry enteric inflammation model with dextran sodium sulfate mediated chemical induction and feed restriction in broilers

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ABSTRACT Gut inflammation is a cardinal event occurring in various gastrointestinal diseases regardless of etiology. A potential mechanism of action for antibiotic growth promoters and probiotics is alleviation or attenuation of such inflammation. In vivo inflammation models and markers to quantify changes in inflammation, such as paracellular leakage and tight junction function, are necessary tools in the search for methods to reduce enteric inflammation. Dextran sodium sulfate (DSS) and feed restriction (FRS), and fluorescein isothiocyanate dextran (FITC-d; 3 to 5 kDa) marker were evaluated for induction and assessment of enteric inflammation in broilers. Three independent experiments were conducted where birds received an inflammation inducer treatment and an oral gavage of FITC-d (2.2 mg/bird) 2.5 h before killing on d 4, followed by measurement of serum FITC-d levels and release of FITC-d from different regions of gastrointestinal tract (GIT) to evaluate tight junction function. Experiment 1 tested control (CON) and DSS; Experiments 2 and 3 evaluated CON, DSS, and FRS. In all experiments DSS, as well as FRS in Experiments 2 and 3, showed higher (P < 0.05) leakage of FITC-d into serum than CON, but FRS was not different from DSS. The amount of FITC-d retained in duodenal and cecal tissue was affected (P < 0.05) by FRS in Experiments 2 and 3, and DSS affected FITC-d retention in duodenum only, suggesting differences in gut passage or absorption/adsorption. In conclusion, DSS oral gavage and FRS could induce leaky gut, with changes in serum FITC-d and migration of FITC-d from GIT.

Key words: dextran sodium sulfate, FITC-dextran, gut leakage, feed withdrawal, inflammation

INTRODUCTION

In addition to numerous physiological functions including absorption of dietary nutrients, the gastrointestinal tract (GIT) plays a key role as a barrier against enteric pathogens, thus integrity and repair are vital for protection of animals against disease. The mucous layer, tight junctions between epithelial cells, and gut-associated lymphoid tissue help to maintain a homeostasis among dietary antigens, enteric pathogens, and beneficial microorganisms (van der Hulst et al., 1998). Disruption of gut health and its barrier could result in malabsorption of nutrients and translocation of a greater amount of enteric bacteria to various internal organs which may lead to diseases and reduced growth performance. Increasing grain prices, concerns over antibiotic growth promoters (AGP), and concerns for diminished animal well-being have driven a need for new sustainable disease management practices and improved feed efficiency for the poultry industry.

Although the mechanism by which AGP result in modulations of microbial presence, absence, and abundance patterns within the gut are in doubt, the effects of inclusion of AGP are clear (Butaye et al., 2003). Modulations of the gut microflora by AGP include suppression of bacterial pathogens, reduction of nutrient breakdown by the intestinal microflora, increased production of vitamins and other nutrients, and reduced production of ammonia (Butaye et al., 2003) and AGP have a profound impact on growth rate and feed efficiency, possibly through effects on stabilization of the microbial populations (Gunal et al., 2006). Importantly, these AGP are known to reduce intestinal disease frequency and severity, reduce mortality, while reducing feed usage and improving rate of gain in monogastric animals, including poultry (Dibner and Richards, 2005).

In the case of poultry, it has been proposed that one benefit of AGP is functional control of enteric inflammation (Niewold, 2007). Additionally, Niewold (2007) also pointed out that many popular AGP are drug classes that accumulate in phagocytes with known...
attenuation of the innate inflammatory response. This hypothesis is consistent with the observation that the intestinal walls of AGP-fed animals are thinner, which could be attributed to reduced influx and accumulation of inflammatory cells (Jukes et al., 1956). However, models of enteric inflammation are not currently well-developed in poultry, and hinder the search for both a complete understanding of the role of AGP in production, and for alternative additives that can provide the same, or better functions.

Murine models are extensively used to simulate human inflammatory bowel diseases, such as ulcerative colitis and Crohn’s disease. The most common rodent models include genetic engineering, spontaneous colitis, inducible colitis, and adoptive transfer (Hibi et al., 2002). Among these, dextran sodium sulfate (DSS), a heparin-like polysaccharide, induction of inflammatory bowel disease is the most common (Laroui et al., 2012). The major advantage of a DSS-induced gut inflammation model is, that by changing the concentration, there is more control over induction, onset, duration, and severity of inflammation produced (Perše and Cerar, 2012). Usually, murine models use 3 to 5% DSS (w:v) in drinking water and clinical signs are observed within 4 to 7 d (Johansson et al., 2010). However, the effect of DSS can vary in wild-type animals based on genetic susceptibility (species, strain, substrain, gender, and so on), type of DSS (molecular weight, manufacturer, and batch), and housing conditions, as well as intestinal microbial status (Perše and Cerar, 2012). Typical clinical signs produced by oral administration of DSS in murine models are loss of bw and bloody feces along with histological lesions such as shortening and loss of crypts and infiltration of inflammatory cells (Yan et al., 2009). An outstanding study conducted by Laroui et al. (2012) which explored the mechanism of action of DSS revealed that DSS formed nano-lipocomplexes with medium chain length fatty acids which helped it to disrupt tight junctions at murine colon. Yan et al. (2009) demonstrated an increase in paracellular leakage associated with DSS treatment by measuring the serum levels of fluorescein isothiocyanate dextran (FITC-d) 4 h after FITC-d oral gavage. Furthermore, Johansson et al. (2010) reported that DSS could produce rapid changes in inner colon mucus, which could make it more permeable to bacteria. These findings suggest that induction of gut inflammation through DSS and measurement of gut leakage using FITC-d could be an effective method to study gut health.

Recently, studies conducted in our laboratory were aimed at adapting the DSS murine gut inflammation model to poultry. We tested the DSS/FITC-d method for induction and measurement of gut leakage in broiler chickens. DSS produced clinical signs, and gross as well as histological lesions in poultry, similar to those of murine models (Menconi et al., 2015). Initial studies showed that chickens are more sensitive to DSS when compared to rodents, with dosages of 1.5 and 3% in drinking water causing severe morbidity and mortality (Menconi et al., 2015), whereas 2 to 5% DSS administered continuously for 4 to 9 d is commonly used to induce acute colitis in murine models (Perše and Cerar, 2012). Morphometric and histologic changes included shortened villi, reduced epithelial cell height, and increased goblet cell density in the duodenum and ileum of 0.75% DSS in drinking water treatment. In these studies, ceca were severely damaged and morphometric analysis was not conducted. However, clinical signs and lesions were not consistent with drinking water administration of DSS, but variations could be reduced by providing DSS via acute dose oral gavage administration (Kuttappan et al., 2014).

Furthermore, feed restriction is a common industry practice for control of bw in large breeder stock, and has been shown to decrease intestinal wall strength and increase circulating corticosterone levels (Bilgili, 1988; Mench, 1991). The present study was intended to evaluate how oral gavage of DSS and feed restriction in broiler chicks affected FITC-d leakage into circulation, measured as serum levels, and its distribution in GIT tissues.

**MATERIALS AND METHODS**

**Experimental Design**

In a preliminary experiment conducted in our laboratory, 2 doses oral gavage of 0.45 g DSS/mL/bird produced less mortality and more consistent results in 3- to 4-day-old birds when compared to 3 d 1 or 1.25% drinking water DSS administration, which resulted in highly variable levels of inflammation, presumably related to inconsistent drinking patterns in young birds (data not published). Also, for detection of inflammation related to DSS treatment, previous unpublished data from our laboratory has suggested that 2.5 h after oral gavage with FITC-d (2.2 mg/bird; molecular weight 3,000 to 5,000; Sigma–Aldrich Co., St. Louis, MO) is the optimal blood collection time point for broilers. Based on this preliminary data, 3 independent experiments were performed, each with slight modifications. For all experiments, day of hatch broilers chicks were grown to 3 d age on a broiler starter ration that met or exceeded National Research Council requirements (NRC, 1994). All animal handling procedures were in compliance with the Institutional Animal Care and Use Committee regulations at the University of Arkansas.

**FITC-d Fluorescence in Blood**

For detection of FITC-d in serum, blood was kept at room temperature for 3 h to allow clotting, and centrifuged (1,000×g for 15 min) to separate serum from red blood cells. Fluorescence levels of diluted serum (1:1 in PBS) were measured at excitation wavelength of 485 nm and emission wavelength of 528 nm (Synergy HT, multi-mode microplate reader, BioTek).
Instruments, Inc., VT), and FITC-d concentration/milliliter serum was calculated based on a standard curve.

**Tissue Levels of FITC-d**

To measure FITC-d released from intestinal tissue, 2.5 cm sections of GIT were collected, cleaned by flushing with Hanks buffered salt solution, weighed, and placed in tubes containing 10 mL Hanks buffer containing glutamine (0.3 g/L) and antimicrobial agents (Penicillin 100 U/mL; Streptomycin 0.01 mg/mL; Amphotericin B 0.25 μg/mL). Samples were collected from the descending duodenum, ileum from immediately proximal to the Meckel’s diverticulum, and a single entire cecum for tissue sampling. The tubes were incubated at 42°C and 100 μL buffer from each tube were sampled at time points described below. FITC-d levels of buffer in the tubes were determined by fluorescence measurement as described above for serum, with the final concentration reported as micrograms/gram ileal tissue.

Ascending duodenum samples from DSS and control (CON) chickens were collected for cryostat sectioning to view dispersion of FITC-d throughout intestinal sections. Fresh tissue samples were embedded in optimal cutting temperature compound (Tissue Tek, Sakura Finetek) and frozen at −80°C until slide sections were cut. To prepare slides, tissue sections were cut at 10 μm with a cryostat (CM1850, Leica, Germany), and mounted onto glass microscope slides and stored in dark conditions. Slides were observed on a Zeiss Imager.M2 (Zeiss, Germany) with a 20× Plan-APOCHROMAT 20×/0.8 objective. Tissue dispersion of FITC-d observed through Filter Set 38 1031 to 346 with an excitation of BP 470/40, beamsplitter of FT 495, and emission spectrum of BP 525/50. Images were captured with an Axio Cam MRm camera (Zeiss, Germany).

**Experiment 1**

For Experiment 1, 24 day-of-hatch broiler chicks were conminated in a single wire floor cage until 3 d, at which point they were randomly assigned to either control full-fed or DSS full-fed groups, with 12 chicks/group. The DSS birds were orally gavaged with 1 mL DSS (0.45 g/bird/d; molecular weight 40,000; Alfa Aesar, Ward Hill, MA) for 2 consecutive days. Birds in both groups were provided feed and water ad libitum throughout the study. Two and a half h after the second dose of DSS, an oral gavage of FITC-d was administered to all chicks. All birds were terminated by CO₂ inhalation 2.5 h after FITC-d oral gavage, at which time blood and ileum samples were collected. Ileal samples were incubated in Hanks buffer for 16 h, and samples collected at 1 and 16 h for fluorescence measurement.

**Statistical Analysis**

Data were analyzed using ANOVA (SAS 9.3, SAS Institute Inc., Cary, NC), and means were separated with Duncan’s significant difference test with $P < 0.05$ considered as significant. Individual birds were considered as the experimental unit for the entire analysis. Serum FITC-d data were occasionally showing extreme values which created high variations in the data affecting the statistical analysis. Thus, for serum FITC-d levels, data were truncated by removing outliers when they were not in the range of mean ± 2 SD. Tissue samples from birds with outlier serum were removed from the experiment, and number removed from each group (removed/total) are listed in tables and figures (Ghosh and Vogt, 2012).
RESULTS AND DISCUSSION

In all experiments, serum from DSS-treated chicks contained higher ($P < 0.05$) levels of FITC-d when compared to the respective CON groups (Tables 1 and 2). This finding is in accordance with a number of studies conducted in murine models which reported an increase in serum FITC-d as a result of oral DSS treatment (Shah et al., 2007; Brandl et al., 2009; Yan et al., 2009). DSS is reported to have an effect on gut epithelium by disrupting tight junctions and by increasing mucosal permeability (Yan et al., 2009; Johansson et al., 2010; Larouxi et al., 2012). As a result, FITC-d administered orally passes into blood through the compromised gut wall and can be recovered in serum. In addition, FRS for 29 to 34 h in 3- to 4-day-old birds resulted in higher FITC-d serum levels when compared to CON group in Experiments 2 and 3 (Table 2). Similarly, starvation in mice resulted in reduced gut epithelial cell proliferation and increased apoptosis (Chappell et al., 2003). Studies conducted in human patients found that parenteral nutrition and enteric starvation could result in decreased concentration of glutamine which may lead to morphologic changes and increased intestinal permeability (van der Hulst et al., 1993, 1994). Presumably, FRS in chickens may have resulted in damage to gut epithelium resulting in increased permeability. However, further studies are needed to understand the exact tissue changes in GIT associated with FRS in poultry.

Ex vivo evaluation of GIT permeability has been used as a method to determine gut leakage in animal models. In most of these studies, either an everted or noneverted intestinal section and a marker dye, like FITC-d or phenol red in buffer, and the rate of passage of dye across intestinal walls was estimated to determine gut leakage (Wilson and Wiseman, 1954; Nakamaru et al., 1998; Lambert et al., 2002; van der Meer et al., 2012). However, this method is time-consuming and results are often highly variable. Specially designed apparatus, such as Ussing chambers, are required to perform some permeability assays, which are slow and often lead to a low number of replications in experimental results (Sanders et al., 1995; Dixit et al., 2012). To test a simpler method, intestinal sections were collected after oral gavage with FITC-d, flushed to remove fecal contents, and placed into buffer solution. The intent was to determine levels of FITC-d released from tissue to evaluate whether the residual FITC-d that did not pass into blood was associated with gut leakage. Hanks buffer with added antimicrobial mixture (to reduce microbial degradation) and glutamine was used to extend the in vitro viability of these sections (van der Hulst et al., 1993, 1994). Only ileum was tested in Experiment 1, and although FITC-d levels in buffer from DSS treated samples were numerically higher after 1 h incubation, the value was not statistically different from CON, and DSS FITC-d levels at 16 h actually dropped to below that of CON. Results from the Experiment 2 assay showed that differences in FITC-d levels from tissue of FRS treated birds can be appreciated at least from 2 h incubation (Figure 1). Differences between control and FRS duodenal sections were lost by 8 h, but continued for ileal and cecal samples throughout the entire 16-h incubation period, with peak separation occurring at 8 h. Increased FITC-d recovery in buffer from tissue for DSS samples was noted only from duodenal tissue at 2 and 4 h, which may be due to decreased intestinal content passage related to cecal atrophy, which has been previously described (Menconi et al., 2015). As

<table>
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<tr>
<th>Table 1. Fluorescein isothiocyanate-dextran (FITC-d) levels in serum and release into Hank’s buffer from ileum sections that were collected from broiler chickens, Experiment 1.</th>
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<tr>
<td>Sodium concentration (μg/mL)</td>
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<td>CON</td>
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<tr>
<td>DSS</td>
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a,bSignificant ($P < 0.05$) difference within each column.
1Serum and tissue were collected 2.5 h after FITC-d administration.
Data were truncated to remove values ±2 SD from the group mean (1 removed from DSS: 1/23 total).
3CON = Control; DSS = Dextran sodium sulfate by oral gavage (0.45 g/bird, 2 doses).

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<tr>
<th>Table 2. Serum fluorescein isothiocyanate dextran (FITC-d) levels (micrograms per milliliter) of control (CON), dextran sodium sulfate (DSS), and feed restriction (FRS) from chicks in Experiments 2 and 3.</th>
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<tr>
<td>Sodium concentration (μg/mL)</td>
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<td>CON</td>
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<td>Experiment 2</td>
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<td>Experiment 3</td>
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a,bSignificant ($P < 0.05$) difference within each row.
Data were truncated to remove outliers ±2 SD from the group mean (1, 2, and 1 removed from CON, DSS, and FRS, respectively; 4/45 total in Experiment 2).
Serum was collected 2.5 h after FITC-d administration.
0.45 mg/bird/d, 2 doses.
All chicks were subjected to 34 h FRS total.
incubation continued, differences became less apparent (Figure 1) which could be due to photo-bleaching of FITC-d or from increased microbial damage to the tissue as incubation continued.

At 2 h incubation of tissue in Experiment 3, FITC-d recovery from buffer in FRS samples from duodenum and cecum were significantly higher than control tissue (Table 3). In DSS-treated birds, duodenum was the only tissue that released more FITC-d into Hank’s buffer at 2 h, and ileum from both DSS and FRS treated groups were no different than CON, likely due to high variability within each treatment group. After 6 h incubation, the same trend continued. As noted in Experiment 2, low FITC-d values from ceca in DSS-treated samples was likely due to atrophy of the tissue that decreased passage of FITC-d into the cecum. Plausibly, tissue damage caused by DSS or FRS could have resulted in such an increased tissue retention/release of FITC-d in these birds. Cryostat duodenum tissue sections observed under fluorescence microscopy showed paracellular dispersion of FITC-d consistent with decreased tight junction barrier function (Figure 2b), whereas control tissue showed less diffuse dispersion of FITC-d (Figure 2a).

In the case of FRS, present results showed consistently higher FITC-d release from all regions of GIT tested. Even though toxic effects of DSS have been noted throughout the GIT of chickens, lesions are more severe in ceca (Menconi et al., 2015). Variations in severity of DSS effects throughout the GIT likely explain the wide range of FITC-d recovered in Hanks buffer from different intestinal sections. Interestingly, DSS treatment resulted in lower ($P < 0.05$) FITC-d retention and release from ceca samples when compared to the respective controls at 2 h postincubation in Experiments 1 and 2. One possibility of such a difference is that DSS causes severe lesions on the cecal wall, which may results in a reduced ability to retain FITC-d in cecal tissue of DSS-treated birds. As mentioned above, another reason could be due to atrophy and irritation in ceca might have resulted in bypass of FITC-d followed by excretion without entering cecal sacs. Rath et al. (1998) reported that FITC could bind to granules present in chicken heterophils. So, infiltration of inflammatory cells at the site of gut inflammation due to DSS or FRS may have contributed to retention of FITC-d at different regions of the GIT tract. Clearly, paracellular retention of FITC-d occurs in tissue (Figure 2), but the mechanism by which this occurs is not yet understood, and future studies are warranted.

In conclusion, DSS-mediated chemically induced gut inflammation, as practiced in rodent models, can be replicated in broiler chickens, especially with an acute dose by oral gavage. Furthermore, these studies show that serum FITC-d levels can be a useful marker in poultry gut leakage and barrier function models. Both FRS and DSS resulted in increased FITC-d serum and may be equal models for induction of enteric inflammation. Finally, the FITC-d retained and released into Hank’s buffer from duodenum and ceca may also be a useful tool to evaluate changes in GIT, which needs further exploration to explain mechanisms of retention.
Table 3. Fluorescein isothiocyanate-dextran (FITC-d) released for different gastrointestinal tract (GIT) tissue section into Hank's buffer of GIT from control (CON), dextran sodium sulfate (DSS), and feed restriction (FRS) birds after 2 and 6 h postincubation (Experiment 3).

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<tr>
<th></th>
<th>2 h postincubation</th>
<th>6 h postincubation</th>
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<tr>
<td></td>
<td>Duodenum</td>
<td>Ileum</td>
</tr>
<tr>
<td>CON</td>
<td>2.63b ± 0.31</td>
<td>17.15 ± 4.11</td>
</tr>
<tr>
<td>DSS</td>
<td>7.00a ± 0.93</td>
<td>28.26 ± 6.50</td>
</tr>
<tr>
<td>FRS</td>
<td>8.89a ± 1.02</td>
<td>37.23 ± 14.33</td>
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a,bSignificant (P < 0.05) difference within each column.
1Tissues were collected 2.5 h after FITC-d administration.
2DSS dose was 0.45 mg/bird/d, and each chick received 2 doses.
3All chicks were subjected to 29 h total FRS.
∗Data was truncated to remove outliers ±2 SD from the group mean; 1 and 1 removed from DSS, and FRS, respectively, 2/38 total.

Figure 2. Fluorescence microscope image of duodenum from control (A) and dextran sodium sulfate (DDS) (0.45 g/bird/d, 2 doses; B) after gavage with fluorescein isothiocyanate-dextran (FITC-d). Arrows indicate infiltration of FITC-d into paracellular space between cells on the mucosal surface. M = mucosa, SM = submucosa, S = serosa.

REFERENCES


