Title: Does the inclusion of distillers dried grains with soluble (DDGS) in the diet of grow-finish pigs affect their susceptibility to and colonization with *Salmonella enterica*? – **NPB #11-159** **REVISED**

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Industry Summary:

As an alternative to counteract the increased feed costs, dried distillers grains with solubles (DDGS) have been increasingly included in pig diets. Much research has been conducted recently to evaluate growth performance and carcass characteristics associated with feeding DDGS to pigs. However, little is known about the potential effect of DDGS on the the susceptibility to infection or colonization with pathogens. Therefore, two experiments were conducted to determine if inclusion of DDGS in the diet of grow-finish pigs affects their susceptibility to or the intestinal levels and shedding of *Salmonella*. In experiment 1, 36 pigs (12 pigs/treatment) were assigned to 3 treatments: Control diet with no corn DDGS, diet with 20% corn DDGS, or diet with 40% corn DDGS. After an adaptation period of 2 weeks, each pig was inoculated with *Salmonella* and euthanized after 6 hours to determine their susceptibility to the challenge. In experiment 2, 40 pigs (20 pigs/treatment) were assigned to 2 treatments: Control diet with no corn DDGS or diet with 30% corn DDGS. After 2 weeks, each pig was inoculated with *Salmonella*; individual fecal samples were collected during 5 weeks, and pigs were euthanized at 3 and 5 weeks post-challenge to determine intestinal colonization. In experiment 1, no differences among treatments were observed on the susceptibility to *Salmonella* infection. In experiment 2, most pigs shed *Salmonella* at one of the fecal samplings during the study period, with control pigs having a higher cumulative shedding frequency than pigs receiving the diet with 30% DDGS. There was no difference between treatments regarding the average *Salmonella* fecal shedding level. Also, no difference between treatments was found on the frequency or levels of *Salmonella* in intestinal samples collected at 3 or 5 weeks post-challenge. In conclusion, dietary inclusion of corn DDGS does not alter the susceptibility to or colonization with *Salmonella* of grow-finishing pigs.

Keywords: swine, *Salmonella*, DDGS, food safety

Scientific Abstract:

As an alternative to counteract the increased feed costs, dried distillers grains with solubles (DDGS) have been increasingly included in pig diets. Much research has been conducted recently to evaluate growth performance and carcass characteristics associated with feeding DDGS to pigs. However, little is known about the effect of DDGS on the intestinal microbiota, and on the susceptibility to infection or colonization with pathogens. Therefore, two experiments were conducted to determine if inclusion of corn DDGS in the diet of grow-finish pigs affects their susceptibility to or the intestinal levels and shedding of *Salmonella*. In experiment 1, 36 pigs (12 pigs/treatment) were assigned to 3 treatments: Control diet with no corn DDGS, diet with 20% corn DDGS, or diet with 40% corn DDGS. After an adaptation period of 2 weeks, each pig was inoculated with *Salmonella* Typhimurium (10^4 cfu) and euthanized after 6 hours to determine their susceptibility to the challenge. In experiment 2, 40 pigs (20 pigs/treatment) were assigned to 2 treatments: Control diet with no corn DDGS or diet with 30% corn DDGS. After 2 weeks, each pig was inoculated with *Salmonella* Typhimurium (10^4 cfu); individual fecal samples were collected during 5 weeks, and pigs were euthanized at 3 and 5 weeks post-challenge to determine intestinal colonization. In experiment 1, no differences among treatments were observed on the susceptibility to *Salmonella* infection. In experiment 2, most pigs shed *Salmonella* at one of the fecal samplings during the study period, with control pigs having a significantly higher cumulative shedding frequency ($P<0.05$) than pigs receiving the diet with 30% DDGS (80% versus 50%). The overall average *Salmonella* shedding level was 2.2 $\log_{10}$ cfu/g of feces, with no difference between treatments ($P>0.10$). Also, no difference between treatments was found on the frequency or levels of *Salmonella* in intestinal samples collected at 3 or 5 weeks post-challenge. In conclusion, dietary inclusion of corn DDGS does not alter the susceptibility to or colonization with *Salmonella* of grow-finishing pigs.

Introduction:

Numerous ethanol production plants have been built over the last several years. As a consequence, the demand for corn for ethanol production has tremendously increased contributing to a marked increase in corn prices, which has lead to higher feed costs and reduced profits for pork producers. However, the expanded production of ethanol from corn has resulted in large amounts of distillers dried grains with solubles (DDGS) being available. As an alternative to counteract the increased feed costs, DDGS have been increasingly included in pig diets. According to Stein and Shurson (2009), DGGS can be included in diets fed to growing pigs in all phases of production, beginning at 2 to 3 weeks post-weaning, in increasing concentrations of up to 30%, and lactating and gestating sows can be fed diets containing up to 30 and 50%, respectively, without negatively affecting production performance. However, as feed costs continue to increase, producers have, in some occasions, been forced to adopt even higher inclusion levels of DDGS in the diets to maintain profitability.

Much research has been conducted recently to evaluate nutrient concentration and digestibility, growth performance, and carcass characteristics associated with feeding DDGS to pigs (Stein and Shurson, 2009). However, very little (based on very few published studies) is known about the effects of DDGS on the intestinal microbiota, as well as on its potential effects on the susceptibility to infection and colonization with pathogens (Whitney et al., 2006a,b,c; Yang et al., 2010b). By far, most of the research available on the effects of DDGS on the intestinal microbiota and pathogens has been conducted in beef cattle, particularly focusing on *Escherichia coli* O157:H7 (Jacob et al., 2008a, 2008b, 2009, 2010; Yang et al., 2010a, Wells et al., 2009; Edrington et al., 2010, Sharma et al., 2010).

Pork contamination occurs in the abattoir, along the slaughter and processing line. Incoming pigs carrying *Salmonella* in their intestinal tract increase the risk of contamination of carcasses and pork products. When a contaminated intestinal tract is lacerated, the whole carcass as well as neighboring carcasses are exposed. Therefore, it is critical to understand the dynamic of *Salmonella* infections in swine herds supplying the abattoirs (as well as other factors affecting it). In order to be able to develop effective intervention measures, it is essential that risk factors for the occurrence of *Salmonella* infections in swine herds be identified. By identifying and quantifying the effects of risk factors, interventions can be developed and applied to reduce *Salmonella* infection and carriage in the pigs at the herd level, which will reduce the contamination pressure at the abattoir.

The role of feed as a potential source of *Salmonella* is well established and reviewed (Crump et al., 2002; Davies et al., 2004). Because of that, and also because of its logical direct relation with the pigs' intestinal microbial ecology, several studies have investigated different aspects related to feed and feed management as potential risk factors for *Salmonella* infections in pigs. However, almost no research has been conducted on alternative feed ingredients. Feed constitutes the nutritional substrate for both the pigs and their gastrointestinal microbial ecosystem. Changes in feed composition may affect a variety of physiologic and metabolic processes within the gastrointestinal tract, including microbial populations. Due to the fact that DDGS contain 3-times more dietary fiber (mainly insoluble dietary fiber or NDF) than corn (Stein and Shurson, 2009; Urriola and Stein, 2010), the inclusion of DDGS in a diet increases the concentration of dietary fiber. Therefore, it is reasonable to hypothesize that differences in the digestibility of nutrients as well as in the passage rate of the digesta, and in the fermentation process in the large intestine will occur, which in turn will affect the microbiota present in the gastrointestinal tract of the pigs.

Therefore, based on the issues presented in this brief review, answers to the following two critical questions are critically needed: Does the inclusion of DDGS in the diet of grow-finish pigs increase their susceptibility to *Salmonella*? Does the inclusion of DDGS in the diet of grow-finish pigs increase intestinal levels and shedding of *Salmonella*?

Objectives:

The objectives of the experiments components of this project consist of determining if the inclusion of dried distillers grains with soluble (DDGS) in the diets affects:

1) the susceptibility of grow-finish pigs to Salmonella enterica (Experiment I);

2) the frequency and levels of Salmonella enterica shedding in grow-finish pigs (Experiment II); and

3) the levels of Salmonella enterica colonizing the intestinal tract of grow-finish pigs (Experiments I and II).

Materials & Methods:

To achieve the proposed objectives, a systematic approach was adopted using two sequential experiments. The decision of conducting two separate experiments was based on reducing potential confounding, reducing the number of animals needed, and animal housing and logistics requirements. The original experimental design was based on a single experiment, which essentially combined both experiments presented here. However, the execution of such large experiment required additional animals to be assigned to each of the three treatments originally defined (Control, 20% DDGS, and 40% DDGS), additional samples and sampling points, additional labor to care for the additional animals, and additional housing space (which is not available in our BSL-2 facility). Therefore, two smaller and more targeted experiments were designed to avoid these limitations and issues, improve efficiency of results, and minimize cost. With the first experiment, it was possible to determine if pigs fed diets containing DDGS are more susceptible to *Salmonella* infection and colonization, based on presence and concentration of the pathogen in a short post-infection period of time along the intestinal tract, attached to the ileal wall, and translocating to mesenteric lymph nodes. Moreover, this experiment allowed determining if different dietary inclusion levels of DDGS (20% vs. 40%) affect the susceptibility to the infection. With the second experiment, it was possible to determine if pigs fed a diet containing DDGS (at the most common inclusion level currently used in commercial operations in the U.S., 30%) are more likely to sustain a longitudinal colonization and shedding status, based on the frequency and levels of *Salmonella* shedding, as well as on the levels of *Salmonella* present in the intestinal tract and associated lymph nodes.

Both experiments were reviewed and approved by the Purdue Animal Care and Use Committee (PACUC Protocol # 1201000584).

Experiment I

A total of 36 grow-finishing pigs were used in this experiment. This experiment was conducted in two replicates (or blocks). Each replicate included the following treatments:

1) Control diet with no DDGS included (N = 6 pigs/replicate; 12 pigs total)
2) Diet containing 20% DDGS (N = 6 pigs/replicate; 12 pigs total)
3) Diet containing 40% DDGS (N = 6 pigs/replicate; 12 pigs total)

Animals were selected from the Purdue University's swine herd, and blocked by body weight and ancestry across treatments. Pigs were housed in individual pens with *ad libitum* water and feed access. Treatments were randomly assigned to each pig, and after 2 weeks of receiving the treatments, each pig were inoculated intranasally with a low dose (10^4 cfu/pig) of *Salmonella enterica* serovar Typhimurium (resistant to nalidixic acid). Immediately after inoculation, individual fecal samples were collected directly from the rectum every 120 minutes (i.e., every 2 h) to determine starting of shedding. At 6 hours post-inoculation, all pigs were humanely euthanized for necropsy, and sample collection including; ileal contents and tissue, mesenteric lymph node, cecal contents, and rectal contents. All samples were processed for the isolation and enumeration of the challenge *Salmonella* strain, including sequential enrichment in Tetrathionate broth (1:10; incubated at 37°C for 24 h) and Rappaport-Vassiliadis broth (incubated at 42°C for 24 h), followed by isolation on XLT-4 agar (incubated at 37°C for 24 h). The Rappaport-Vassiliadis broth and the XLT-4 agar were added with nalidixic acid to select for the challenge *Salmonella* strain used. For the enumeration of *Salmonella*, samples were serially diluted (10-fold), and spread on XLT-4 agar containing nalidixic acid. After incubation at 37°C for 24 h, colonies were counted and recorded. All these procedures have been previously described by Rostagno et al.(2011).

Experiment II

A total of 40 grow-finishing pigs were used in this experiment. This experiment was conducted in two replicates (or blocks). Each replicate included the following treatments:

1) Control diet with no DDGS included (N = 10 pigs/replicate; 20 pigs total)
2) Diet containing 30% DDGS (N = 10 pigs/replicate; 20 pigs total)

Animals were selected from the Purdue University's swine herd, and blocked by body weight and ancestry across treatments. Pigs were housed in individual pens with *ad libitum* water and feed access. Treatments were randomly assigned to each pig, and after 2 weeks of receiving the treatments, each pig was inoculated intranasally with a low dose (10^4 cfu/pig) of *Salmonella enterica* serovar Typhimurium (resistant to nalidixic acid). Individual fecal samples were collected daily (directly from the rectum) post-inoculation to determine shedding frequency and levels. At 3 and 5 weeks post-inoculation (i.e., 21 d and 35d p.i.), pigs were humanely euthanized for necropsy, and sample collection including; ileal contents and tissue, mesenteric lymph node, cecal contents, and rectal contents. All samples were processed for the isolation and enumeration of the challenge *Salmonella* strain, including sequential enrichment in Tetrathionate broth (1:10; incubated at 37°C for 24 h) and Rappaport-Vassiliadis broth (incubated at 42°C for 24 h), followed by isolation on XLT-4 agar (incubated at 37°C for 24 h). The Rappaport-Vassiliadis broth and the XLT-4 agar were added with nalidixic acid to select for the challenge *Salmonella* strain used. For the enumeration of *Salmonella*, samples were serially diluted (10-fold), and spread on XLT-4 agar containing nalidixic acid. After incubation at 37°C for 24 h, colonies were counted and recorded. All these procedures have been previously described by Rostagno et al.(2011).

Data Analysis

In both experiments, the experimental unit was the pig (i.e., individual animal). Treatments were compared, based on the frequency of *Salmonella*-positive samples as well as on the levels (i.e., concentration of bacteria per gram of sample) of *Salmonella* in the samples collected at each time-point. Comparison of frequencies was performed using Chi-square analysis, whereas comparison of bacteria levels was performed using analysis of variance (ANOVA), using the JMP software (version 9.0.0, SAS Institute, 2010). Quantitative bacteria data (i.e., counts or levels) were log-transformed prior to being subjected to statistical analysis. Statistical inferences were based on $P < 0.05$.

Results:

Objective 1:

Pigs receiving diet with 40% DDGS inclusion were the first to start shedding *Salmonella* in their feces, within 2 hours post-challenge.

The cumulative *Salmonella* fecal shedding frequency at 6 hours post-challenge was significantly higher ($P<0.05$) for pigs receiving diet with 40% DDGS inclusion (41.7%) in comparison to pigs receiving the control diet without DDGS (0%). Pigs receiving diet with 20% DDGS inclusion had an intermediate cumulative *Salmonella* fecal shedding frequency at 6 hours post-challenge (25%), which was not significantly different than the pigs receiving the other treatments (Figure 1).

There was no difference between treatments, based on the frequency of individual samples collected at necropsy, performed 6 hours post-challenge. Moreover, the comparison of the proportions of pigs positive for *Salmonella*, based on the combined results of all the sample types collected (i.e., pigs with at least one positive necropsy sample) also did not reveal any significant difference between the treatments (90.9% for control pigs, 100% for 20% DDGS pigs, and 100% for 40% DDGS pigs; Figure 2).

Objective 2:

Pigs receiving control diet (i.e., no DDGS) had a significantly higher ($P<0.05$) cumulative *Salmonella* fecal shedding frequency than pigs receiving diet with 30% DDGS inclusion at 35 days post-challenge (80% versus 50%, respectively; Figure 3).

However, no statistical difference was observed between pigs receiving control diet (i.e., no DDGS) and pigs receiving diet with 30% DDGS inclusion, based on the comparison of any of the samples collected during necropsies, at either 21 or 35 days post-challenge (Figure 4).

Objective 3:

There was no significant difference among treatments (in both experiments) on the levels of *Salmonella* shed in feces. Overall, the average *Salmonella* fecal shedding level observed was 2.2 $\log_{10}$ cfu/g.

There was no significant difference among treatments (in both experiments) on the levels of *Salmonella* in necropsy samples. In general, *Salmonella*-positive samples collected at necropsy contained between 1.88 and 2.85 $\log_{10}$ cfu/g.

Discussion:

In a series of studies, Whitney et al.(2006a,b,c) evaluated the effect of dietary inclusion of DDGS on length, severity, and prevalence of lesions caused by *Lawsonia intracellularis* in growing pigs, and reported inconsistent results, based on relatively high challenge doses (10^8 - 10^9 organisms). Yang et al.(2010) reported that pigs fed DDGS from corn tended to have increased concentrations of lactobacilli and *Enterobacteriaceae* compared to pigs fed distillers grains from other sources. Unfortunately, a control diet (i.e., with no DDGS) was not included in their study, which limits inferences in comparison to traditional corn-soybean diets. By far, most of the research available on the effects of DDGS on the intestinal microbiota and pathogens has been conducted in beef cattle, particularly focusing on *Escherichia coli* O157:H7. However, as the very few previously mentioned studies focusing on swine, results have been inconsistent, with some studies suggesting an increased risk of E. coli O157:H7 occurrence in cattle receiving diets with DDGS, whereas others have reported no effects.

A recently published study did not reveal any effect of wheat distillers dried grains with solubles on the prevalence of *Salmonella* in weaned pigs (Thomson et al., 2012). To the best of our knowledge, this is the only study other than our own study reported here focusing on the effects of DDGS on intestinal microbial populations, and *Salmonella* in particular, in swine. However, major differences should be pointed out, as Thomson et al. (2012) applied much higher *Salmonella* challenge dose, a very different challenge model, and wheat-based DDGS. Nevertheless, results were relatively similar. In general, both studies suggest that the dietary inclusion of DDGS does not markedly affect the susceptibility or colonization of pigs with *Salmonella*. Therefore, pork producers can continue to take advantage of the availability of this by-product of ethanol production to counteract the increased feed costs without a major concern on any potential pork safety risk due to *Salmonella* contaminations.

In conclusion, this study shows that dietary inclusion of DDGS does not alter the susceptibility to or colonization with *Salmonella* of grow-finishing pigs. However, if high dietary inclusion levels are to be used (i.e., >30%), further studies may be required to better define some effects observed in this study, such as a higher cumulative *Salmonella* fecal shedding within a short period of time post-infection, which may have implications for the pre-slaughter lairage practice.

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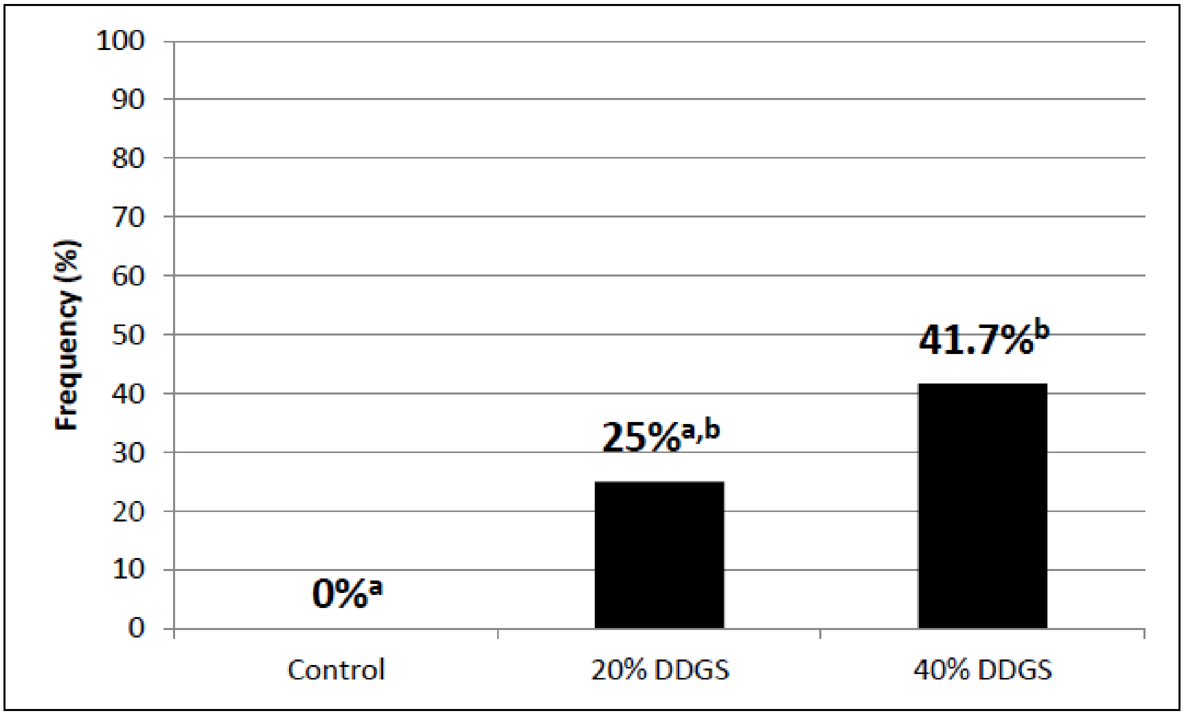
Figure 1. Cumulative *Salmonella* fecal shedding frequency at 6 hours post-infection.



Different superscript letters (a,b) indicate statistically significant difference (P<0.05).

Figure 2. Frequency of pigs with at least one sample collected at necropsy positive for *Salmonella*, 6 hours post-infection.

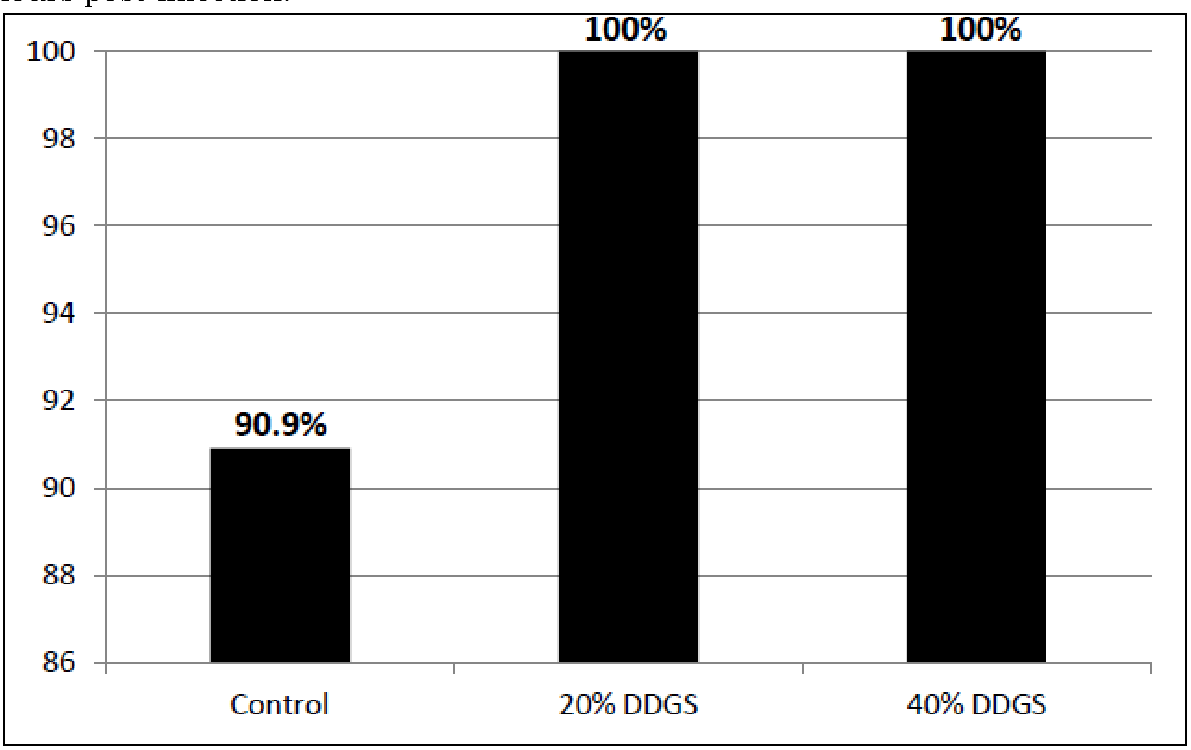
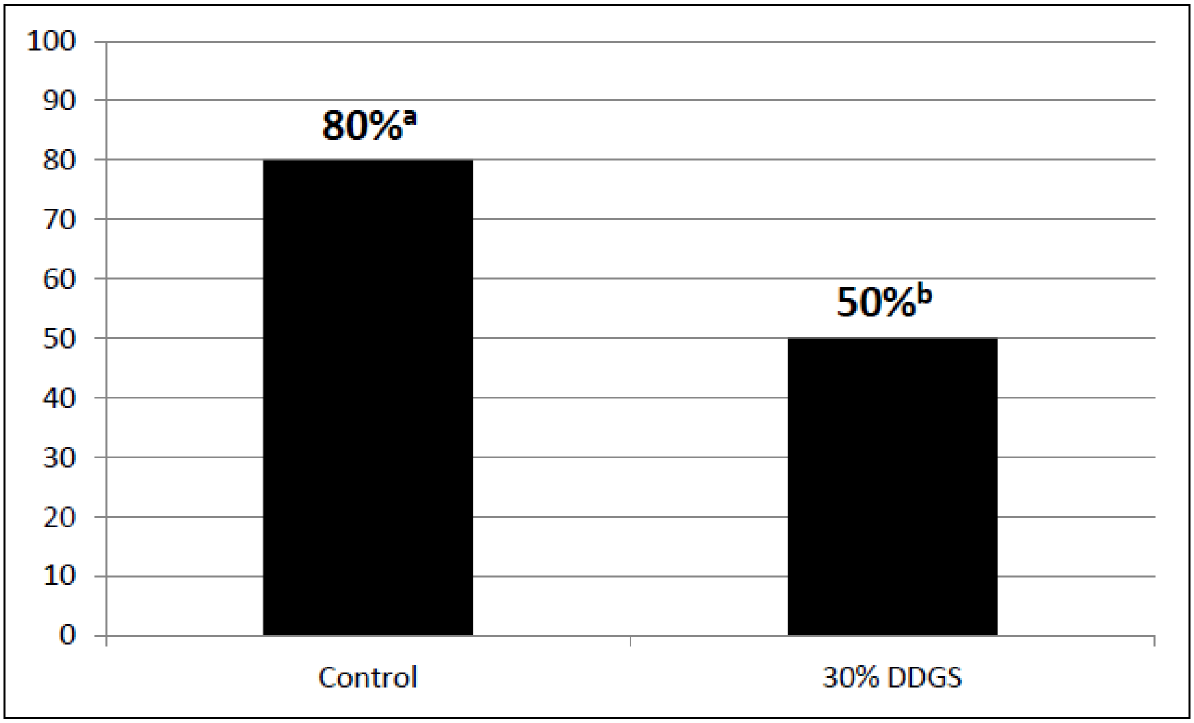


Figure 3. Cumulative *Salmonella* fecal shedding frequency at 35 days (5 weeks) post-infection.



Different superscript letters (a,b) indicate statistically significant difference ($P<0.05$).

Figure 4. Frequency of pigs with at least one sample collected at necropsy positive for *Salmonella*, 21 (3 weeks) and 35 (5 weeks) days post-infection.