Effect of wheat distillers dried grains with solubles or sugar beet pulp on prevalence of *Salmonella enterica* Typhimurium in weaned pigs

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Effect of wheat distillers dried grains with solubles or sugar beet pulp on prevalence of *Salmonella enterica* Typhimurium in weaned pigs[1]

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ABSTRACT: *Salmonella enterica* Typhimurium (ST) is of concern in the swine industry with relevance for animal health and consumer safety. Nutritional strategies might help to reduce ST infection and transmission. This study examined the potential of wheat (*Triticum aestivum*) distillers dried grains with solubles (DDGS) and sugar beet (*Beta vulgaris*) pulp (SBP) to alter intestinal microbial communities and ST shedding using a Trojan model. Weaned pigs (n = 105; 28.5 ± 3.5 d of age) were separated into 3 treatment groups (7 pigs/pen) and fed a wheat-based control diet or the control diet formulated with 15% wheat DDGS or 6% SBP inclusion. Following 12 d of diet adaptation, 2 pigs/pen were inoculated with 2 x 10^9 cfu ST, resistant to novobiocin and nalidixic acid. Fecal swabs were taken from infected pigs and pen-mates (contact pigs) for 9 d following challenge, enriched in nutrient broth for 24 h, and plated on selective media to determine prevalence of ST. The ranges of prevalence of ST in feces were from 90 to 100% in challenged pigs and 74 to 78% in contact pigs. No influence of treatment on rectal temperature and prevalence of ST in contact pigs were observed. Fifteen contact pigs were euthanized per treatment group on 9 and 10 d postchallenge to enumerate in intestinal contents (ileum, cecum, and proximal colon), *Lactobacillus* spp., Enterobacteriaceae, and *Clostridium* clusters I, VI, and XVIa by quantitative PCR (qPCR) and to determine ST prevalence by selective culture. No significant effects of diet were observed with respect to ST prevalence in feces, ileum, cecum, colon, and lymph nodes of contact pigs. Compared with the control diet, DGGS and SBP diets showed a trend towards increased ($P < 0.1$) number of *Lactobacillus* species in the cecum and colon. Although both wheat DGGS and SBP tended to increase the *Lactobacillus* spp. neither of the feed ingredients affected ST prevalence.

Key words: challenge model, distillers dried grains with solubles, *Salmonella* Typhimurium, sugar beet pulp

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INTRODUCTION

Salmonella is a common zoopathogen found in the feed and food chain worldwide (Callaway et al., 2008) and a major contributor to foodborne illness. Contamination of pork products at processing and environmental contamination are potential sources of *Salmonella* infection in humans. The significance of *Salmonella* as a public health concern has lead to a focus on pre- and postharvest *Salmonella* control programs in North America. As a result, a number of feed additives and physicochemical characteristics of feed have been investigated for contribution to preharvest control strategies in swine (Burkey et al., 2004; Pieper et al., 2012). Several mechanisms may be effective for control of *Salmonella* colonization in vivo. For example, mannan oligosaccharides may interfere with type 1 fimbriae-mediated attachment of *Salmonella* to enterocytes limiting epithelial invasion and colonization persistence (Althouse et al., 2003; Burkey et al., 2004). Alternatively, commensal microbiota including microbial-origin short chain fatty acids modulated by fiber source may exclude *Salmonella* via direct antimicrobial effects or down regulate genes required for *Salmonella* translocation (Gantois et al., 2006).

This study was conducted to evaluate two industrial by-products, namely sugar beet pulp (**SBP**) and wheat-based distillers dried grains with solubles (**DDGS**), and their influence on *Salmonella* colonization and transmission in pigs. We hypothesized that microbial

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fermentation of fiber components in both ingredients and mannan oligosaccharides present in yeast cell walls found in DGGS would modify the intestinal environment reducing favorability for *Salmonella* colonization.

MATERIALS AND METHODS

One hundred five (Duroc × Landrace) piglets (8.4 ± 0.2 kg BW) were assigned to 15 pens with 7 pigs each, balanced for BW, and kept in a level 2 biocontainment facility. Pigs in each pen were assigned to 1 of 3 experimental diets including a control diet containing (g/kg on as-fed basis) wheat (57), soybean (*Glycine max*) meal (**SBM**; 20), soy protein concentrate (4), and fish meal (4) and similar diets in which SBM was replaced with wheat DDGS or SBP at 15 and 6%, respectively. All diets met nutrient requirements and were offered ad libitum. A Trojan challenge model was used as previously described (Pieper et al., 2012) using *Salmonella enterica* serotype Typhimurium (**ST**) var. Copenhagen selected for novobiocin and nalidixic acid resistance. After a 12 d adaptation period, two Trojan pigs were randomly selected from each pen, moved to a separate pen, and orally challenged with 10^9 cfu ST at each of 0800 and 1400 h. Pigs were returned to their original pens 1 h after the second challenge. Nonchallenged pen-mates were designated "Contact" pigs. Rectal temperature and fecal scores (1 to 3 scale; higher score for wetter feces) were recorded and anal fecal swabs were collected from all pigs at 2-d intervals following the challenge. Ten days following challenge, 3 Contact pigs were euthanized 5 h after the morning meal provided after an 8-h overnight fast. Mesenteric lymph nodes (**MLN**) and digesta from ileum, cecum, and colon was collected and processed as described (Pieper et al., 2012). Briefly, ST prevalence was determined by 24 h enrichment of sample in selenite broth (Naladixic acid and Novabiocin) followed by culture on brilliant green agar. Copy number of members of major microbial groups was determined based on 16s ribosomal RNA gene using quantitative PCR (**qPCR**). *Salmonella* prevalence was compared using Fisher's exact test (SAS Institute, Inc. Cary, NC). All other data were analyzed using one-way ANOVA (SAS). Differences were considered significant at $P < 0.05$. Experiments were conducted in accordance with University of Saskatchewan Animal Use Protocol number 20110036.

RESULTS AND DISCUSSION

In the current study, a Trojan challenge model (Callaway et al., 2006) was used to more closely approximate *Salmonella* exposure in commercial herd settings. Direct challenge of Trojan pigs revealed near constant shedding of ST (Table 1) whereas the prevalence (74–78%) of ST in feces of Contact pigs was lower consistent with previous findings (Pieper et al., 2012). Although transient inappetence, fever, and diarrhea have been reported following ST challenge in the pig (Burkey et al., 2004), no obvious clinical signs were observed in the current study with exception of a mean increase in rectal temperature of 0.4 ± 0.1, 0.6 ± 0.1, and 0.5 ± 0.1°C in control, DGGS, and SBP pigs respectively, which was not affected by treatment. Inclusion of SBP or wheat DGGS did not affect the prevalence of ST in fecal swabs collected throughout the postinfection period (Table 1) nor in intestinal contents (Table 2) or MLN (15, 14, and 12 of 15 pigs positive for ST in control, DGGS, and SBP, respectively) collected on 10 d postchallenge.

Interestingly, despite the markedly different carbohydrate profile of these two feed ingredients, the profile of major bacterial taxonomic groups was very similar. A trend ($P < 0.1$) towards increased number of *Lactobacillus* species in both cecum and colon contents was observed in SBP and to a lesser extend pigs fed DGGS compared with the wheat-based control diet. Sugar beet pulp supported fewer potentially putrefactive *Clostridium* cluster 1 ($P < 0.05$) bacteria in the colon but otherwise neither feed ingredient appeared to sufficient alter microbial colonization to alter *Salmonella* prevalence. Although we have confirmed *Salmonella* agglutination to yeast species present in wheat DGGS (data not shown), the affinity or inclusion level of yeast cell components may be insufficient to cause agglutination and interference with enterocyte attachment in vivo. However, no reduction in ST shedding was reported on inclusion of mannan oligosaccharide, a major yeast cell wall component, in pigs (Burkey et al., 2004), which is in good agreement with the current findings.

We conclude that neither SBP nor DDGS offer improvement in control of ST shedding and transmission

Table 1. Number of Trojan and Contact pigs with fecal swabs positive for *Salmonella*. Pigs were fed a control wheat-based diet or a similar diet containing 15% wheat distillers dried grains with solubles (DGGS) or 6% sugar beet pulp (SBP)

Diet	Number (n) of positive animals[1]				
	Days postchallenge				
	2	4	6	8	Total shedding days
Trojan pigs					
Control (n = 10)	10	9	9	9	37
DDGS (n = 10)	9	10	10	9	38
SBP (n = 10)	10	10	10	9	39
Contact pigs					
Control (n = 25)	22	20	18	16	74
DDGS (n = 25)	20	20	19	18	78
SBP (n = 25)	22	19	19	18	78

[1]Data are from a total of 5 pens per diet representing 2 Trojan pigs (directly challenged) and 5 Contact pigs (pen-mates) per pen. Values not different by Fisher's exact test.

Table 2. Abundance (log 16s ribosomal RNA gene copy number/g contents) of major bacterial taxonomic groups and prevalence of *Salmonella* in intestinal contents in Contact pigs (3/pen, n = 15) fed a control wheat-based diet or a similar diet containing 15% wheat distillers dried grains with solubles (DDGS) or 6% sugar beet pulp (SBP)

	Bacteria number (log_{10} gene copy number/g contents)						*Salmonella* prevalence
	Total bacteria	*Lactobacillus* spp.	Enterobacteria	Cl.[1] cluster I	Cl. cluster IV	Cl. cluster XIVa	No. positive
Ileum							
Control	10.4	10.4	8.8	10.0	7.9	8.6	13
DDGS	10.6	10.4	8.8	10.4	7.8	8.2	11
SBP	10.7	10.4	8.6	10.2	8.0	8.8	12
P value	0.24	0.90	0.73	0.32	0.51	0.08	0.89
SEM	0.09	0.08	0.11	0.10	0.09	0.10	na[2]
Cecum							
Control	10.9	10.4	8.9	9.9	10.4	10.9	15
DDGS	10.9	10.5	8.7	10.0	10.2	10.9	15
SBP	10.8	10.6	8.7	9.6	10.1	11.0	12
P value	0.62	0.09	0.60	0.12	0.22	0.69	0.09
SEM	0.05	0.07	0.12	0.07	0.07	0.05	na
Colon							
Control	10.8	10.4	8.9	9.8	10.3	10.9	14
DDGS	10.6	10.5	8.7	9.8	10.2	10.8	14
SBP	10.9	10.6	8.6	9.4	10.3	11.0	10
P value	0.36	0.06	0.64	0.05	0.97	0.18	0.17
SEM	0.04	0.09	0.11	0.08	0.03	0.04	na

[1]Cl. = *Clostridium*.

[2]na = not applicable.

in weaned pigs.

LITERATURE CITED

Althouse, C., S. Patterson, P. Fedorka-Cray, and R. E. Isaacson. 2003. Type 1 fimbriae of *Salmonella enterica* serovar Typhimurium bind to enterocytes and contribute to colonization of swine in vivo. Infect. Immun. 71:6446–6452.

Burkey, T. E., S. S. Dritz, J. C. Nietfeld, B. J. Johnson, and J. E. Minton. 2004. Effect of dietary mannanoligosaccharide and sodium chlorate on the growth performance, acute-phase response, and bacterial shedding of weaned pigs challenged with *Salmonella enterica* serotype Typhimurium. J. Anim. Sci. 82:397–404.

Callaway, T. R., T. S. Edrington, J. A. Byrd, R. C. Anderson, R. B. Harvey, K. J. Genovese, J. L. McReynolds, and D.J. Nisbet. 2008. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. J. Anim. Sci. 86 (E. Suppl.):E163–E172.

Callaway, T. R., J. L. Morrow, T. S. Edrington, K. J. Genovese, S. Dow, J. Carroll, J. W. Dailey, R. B. Harvey, T. L. Poole, R. C. Anderson, and D. J. Nisbet. 2006. Social stress increases fecal shedding of *Salmonella* Typhimurium by early weaned piglets. Curr. Issues Intest. Microbiol. 7:65–72.

Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, I. Hautefort, A. Thompson, J. C. Hinton, and F. Van Immerseel. 2006. Butyrate specifically down-regulates *Salmonella* pathogenicity island 1 gene expression. Appl. Environ. Microbiol. 72:946–949.

Pieper, R., J. Bindelle, G. Malik, J. Marshall, B. G. Rossnagel, P. Leterme, and A. G. Van Kessel. 2012. Influence of different carbohydrate composition in barley varieties on *Salmonella* Typhimurium var. Copenhagen colonisation in a "Trojan" challenge model in pigs. Arch. Anim. Nutr. 66:163–179.