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Effect of wheat bran on the health and performance of weaned pigs challenged with *Escherichia coli* K88^{+} ☆

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ABSTRACT

The leading cause of post-weaning diarrhoea in pigs is *Escherichia coli*. Previous studies showed that inclusion of wheat bran (WB) in the diet of weaned pigs decreased the number of pathogenic *E. coli* in the faeces and reduced the incidence of post-weaning diarrhoea. It is not clear whether it is the WB alone that improves gut health, or whether it is the particle size of the WB that is important. In this experiment we used an *E. coli* K88^{+} challenge model to test the importance of supplementing WB and particle size of the WB. A total of 36 individually-housed piglets (17 ± 0.77 d) were assigned randomly to one of four experimental groups. Treatments were: (1) a negative control diet (NC) based on corn, wheat, barley and soybean meal; (2) NC + 4% coarsely milled WB (WBc, 1088 μm); (3) NC + 4% finely milled WB (WBf, 445 μm); and (4) a positive control diet (PC) consisting of the NC diet supplemented with a commercial feed grade antibiotic mix. At 26 d of age, pigs were experimentally infected with 6.2×10^{9} cfu/mL of *E. coli* K88^{+}. Body weight, feed intake, and diarrhoea were monitored. Pigs were euthanized 7 d after infection. Ileal digesta and mucosa were taken for *E. coli* enumeration and for determination of SCFA and indices for richness and diversity of microbiota. There were no significant differences in ADG, ADFI, and G:F ratio attributable to dietary treatment. Inclusion of WB, either fine or coarse, significantly ($P<0.05$) decreased *E. coli* numbers in the ileal digesta. The use of WBc had an additional benefit because the *E. coli* K88^{+} numbers were significantly lower ($P<0.05$) and the SCFA in ileal digesta was higher ($P<0.05$) compared to WBf. We conclude that both WB per se, and the particle size of WB have an effect on gut health in weaned pigs.

1. Introduction

Post-weaning diarrhoea is a multifactorial disease provoked sometimes by certain strains of *Escherichia coli* and its expression is influenced by diet (Hampson, 1994). Some authors have reported that inclusion of fermentable carbohydrates in weaner pig diets may decrease post-weaning collibacilosis (PWC) by promoting proliferation of commensal microbiota and by decreasing protein fermentation in the digestive tract (Awati et al., 2006). In a recent experiment, we observed that inclusion of wheat bran (WB) in the diet of piglets from week 1 to 2 after weaning decreased the pathogenic *E. coli* numbers in the colon reducing the incidence of post-weaning diarrhoea (Molist et al., 2009). However it was not clear whether WB decreased PWD by modulating the microbial activity in the small intestine or through changes on the physico-chemical properties of digesta, for which the particle size is likely playing an important role. The aim of the present study was to investigate the effects of WB inclusion and particle size of WB on the microbial composition in the digesta and intestinal mucosa of newly weaned pigs challenged with enterotoxigenic *E. coli* K88^{+} (ETEC).

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2. Materials and methods

2.1. Animals and diets

The experimental protocol was reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (1993). A total of 36 Genesus ([Yorkshire × Landrace]♀ × Duroc♂) piglets weaned at 17 ± 1 d were obtained from the University of Manitoba's Glenlea Swine Research Unit. The pigs were weighed, individually-housed and randomly assigned to 1 of 4 experimental diets: (1) a negative control diet (NC) based on corn (32%), wheat (20%), barley (17%) and soybean meal (14%); (2) NC + 4% coarsely milled WB (WBc, 1088 μm); (3) NC + 4% finely milled WB (WBf, 445 μm); and (4) a positive control diet (PC) consisting of the NC diet supplemented with a commercial feed grade antibiotic mix (ASP-250: Chlortetracycline, Pencillin G, Sulfamethazine; Alpharma Inc., Fort Lee, NJ). All experimental diets were formulated to meet the NRC (1998) nutrient requirements for piglets weighing 7 to 12 kg (DE, 3400 kcal/kg; CP, 20.9%; Lys, 1.2%). The animals were housed in a Biohazard Level 2 animal facility that restricted access to unauthorized personal, and all individuals using the facility were trained in procedures related to biohazard containment. Animals had unlimited access to feed and water throughout the 2-wk study period, with the room temperature set at 29 ± 1 °C.

2.2. Experimental procedures and sampling

Animals received the experimental diets from day 1 to day 16 after weaning. On day 9, body weight (BW) and feed intake (FI) were recorded and faecal samples were taken for determination of *E. coli* population and microbial activity. After that, pigs received 6 mL (2.2×10^{10} cfu/mL) of a freshly prepared *E. coli* $K88^+$ inoculum following the procedure described by Bhandari et al. (2008). The severity of diarrhoea was assessed using the faecal consistency scoring method of Marquardt et al. (1999). On day 16 after weaning, BW and FI were recorded and animals were euthanized with an intravenous injection of sodium pentobarbitone (50 mg/kg BW). Piglets were bled, and the abdomen was immediately opened to sample ileal digesta and tissue. Segments of the ileum were placed in sterile containers before transportation to the laboratory for microbial analysis. Ileal digesta were divided into two subsamples of about 1 g that were immediately frozen at −80 °C for VFA and lactic acid determination and for the terminal restriction fragment length polymorphism (T-RFLP) analysis.

2.3. Analytical procedures

Dietary DM was determined by the standard AOAC (1996) method. Crude protein was quantified by a Leco NS 2000 Nitrogen Analyzer (Leco Corporation, St Joseph, MI). Gross energy was measured with a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Faecal samples were taken before the experimental infection (day 9) were weighed, diluted and plated on chromogenic *E. coli* media (BBL Levine Eosin Methylene Blue Agar; BD Company, Sparks, USA). Samples from the ileal tissue were also taken in day 16 for microbial assay. A blunt knife was used to scrape the mucosa down to the connective tissue, and the mucosa was then weighed and diluted 10-fold with anaerobic dilution and plated as described previously (Krause et al., 1995). Briefly, 10 μL droplets of medium were pipetted onto chromogenic *E. coli* media without antibiotic to count the bacterial *E. coli* population and with 0.5 μg/mL of levofloxacin (Fluka, Buchs, Germany) to determine the *E. coli* $K88^+$ serotype adhesion. Dilutions from 10^{-1} to 10^{-9} were plated, allowed to dry before inversion, and incubated at 39 °C for 24 h. The VFA and lactic acid determination were done by gas chromatography as described by Erwin et al. (1961). The extraction of DNA from ileal digesta, as well as the T-RFLP procedure and data analyses were done following the procedure described by Bhandari et al. (2008).

2.4. Statistical analyses

Data were subjected to ANOVA, with dietary treatment as the classification factor, using the GLM procedure (SAS Inc., 1999). Animal was considered as the experimental unit ($n = 9$). For performance data, initial BW was used as a covariate. The alpha level used for the determination of significance for all the analysis was 0.05 and trends (alpha<0.10) were also reported.

3. Results and discussion

3.1. Piglet performance

Growth performance was not affected by dietary treatments. The average daily feed intake (ADFI) was 231 g and the average daily gain (ADG) was 130 g for the 0 to 16 d period after weaning. The average final BW among treatments was 7.1 kg. These results agree well with those reported by Bhandari et al. (2008) and Wellock et al. (2007) who did not find performance differences within *E. coli* challenged pigs. However, we should remark that the number of animals and experimental conditions were not adequate to obtain clear conclusions from the performance of the animals.

3.2. Faecal score and microbiological analysis

The effect of WB on the *E. coli* population in the faeces before the experimental infection is shown in Table 1. No significant differences among treatments were found in the *E. coli* population before the challenge. Data related to the *E. coli* determination in the ileal mucosa, the *E. coli* $K88^+$ serotype count in the ileal mucosa, and the faecal scores (FS) after the *E. coli* K88 challenge are shown in Table 2. Irrespective of the particle size, supplementation of 4% WB in the diet of weaner pigs significantly reduced ($P<0.05$) *E. coli* population in the ileal mucosa compared with that from pigs fed the NC diet. Furthermore, inclusion of WBc significantly decreased ($P<0.05$) *E. coli* $K88^+$ adhesion to the ileal mucosa compared with that from pigs fed the NC diet. At the same time, FS was lower for piglets fed the WBc and PC diets than those fed a NC diet at 48 h ($P<0.05$) and