A high dietary concentration of inulin is necessary to reduce the incidence of swine dysentery in pigs experimentally challenged with *Brachyspira hyodysenteriae*

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Abstract

A total of sixty surgically castrated male pigs (Large White × Landrace) weighing 31·2 (SD 4·3) kg were used in a randomised block experiment to examine the effect of added dietary inulin (0, 20, 40 and 80 g/kg) on the occurrence of swine dysentery (SD) and on fermentation characteristics in the large intestine after experimental challenge with the causative spirochaete *Brachyspira hyodysenteriae*. The pigs were allowed to adapt to the diets for 2 weeks before each pig was challenged orally four times with a broth culture containing *B. hyodysenteriae* on consecutive days. Increasing dietary levels of inulin linearly (P=0·001) reduced the risk of pigs developing SD; however, eight out of fifteen pigs fed the diet with 80 g/kg inulin still developed the disease. The pH values in the caecum (P=0·072) tended to decrease, and in the upper colon, the pH values did decrease (P=0·047) linearly with increasing inulin levels in the diets, most probably due to a linear increase in the concentration of total volatile fatty acids in the caecum (P=0·018), upper colon (P=0·01) and lower colon (P=0·013). In addition, there was a linear reduction in the proportion of the branched-chain fatty acids isobutyric acid and isovaleric acid in the caecum (P=0·015 and 0·026) and upper colon (P=0·011 and 0·013) with increasing levels of dietary inulin. In conclusion, the present study showed that a diet supplemented with a high level of inulin (80 g/kg) but not lower levels reduced the risk of pigs developing SD, possibly acting through a modification of the microbial fermentation patterns in the large intestine.

Key words: *Brachyspira hyodysenteriae*; Inulin; Pigs; Swine dysentery

Swine dysentery (SD) is a contagious mucohaemorrhagic diarrhoeal disease that mainly occurs in pigs in the grower/finisher phase. The essential causative agent of SD is the anaerobic intestinal spirochaete *Brachyspira hyodysenteriae*, and this pathogen acts in association with other anaerobic members of the large-intestinal microbiota to induce extensive inflammation and necrosis of the epithelial surface of the caecum and colon(1). It is known that the pigs’ diet can have a strong influence on colonisation by *B. hyodysenteriae* and on the occurrence of clinical signs of SD. Several studies have been undertaken to elucidate the effects of different types of carbohydrates on colonisation with *B. hyodysenteriae* and on the incidence of SD, but the results have been contradictory(2–5). Recently, a diet containing chicory root and sweet lupin was shown to offer protection against SD(6), and subsequently we demonstrated that feeding pigs 80 g/kg inulin but not lupin prevented SD following experimental challenge with *B. hyodysenteriae*(7). Inulin is a mildly sweet, white polysaccharide that is normally extracted from chicory root.

Physiologically, fructo-oligosaccharides such as inulin are classified as dietary fibre and are resistant to complete enzymatic degradation in the small intestine. Undigested fibre entering the caecum and colon functions as a substrate for fermentative processes and generates a higher luminal concentration of volatile fatty acids (VFA), which in turn can cause lower luminal pH values(8). Inulin is mainly fermented in the large intestine to VFA, lactate and gas by *Bifidobacteria* and *Lactobacilli* species(9). In addition, dietary inulin supplementation may regulate metabolic activity, decreasing the

Abbreviations: BCFA, branched-chain fatty acids; N–NH₃, NH₃ nitrogen; SD, swine dysentery; VFA, volatile fatty acids.

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protein:carbohydrate ratio in the hindgut. As a result, carbohydrate fermentation may suppress the formation of branched-chain fatty acids (BCFA) and \( \text{NH}_3 \) produced from protein fermentation\(^{(10)}\).

Dietary supplementation with inulin is expensive, and currently no information is available concerning the level of dietary inulin inclusion that is necessary to reduce the occurrence of SD and cause changes in the microbiota in the large intestine of pigs. Accordingly, the present study was designed to determine whether dietary inclusion of less than 80 g/kg inulin could prevent pigs that were experimentally challenged with \( B. \ hyodysenteriae \) from developing the disease. The hypothesis tested was that a diet supplemented with 80 g/kg inulin would decrease the risk of pigs developing SD and reduce protein fermentation in the hindgut.

**Materials and methods**

The present study was conducted with the approval of the Murdoch University Animal Ethics Committee (R2186-08). Animals were cared for according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes \( ^{(11)} \).

**Animals and housing**

A total of sixty surgically castrated commercial pigs (Large White \( \times \) Landrace) were obtained at weaning from a commercial specific-pathogen-free piggery known to be free of SD. At weaning, the pigs were housed in a group at Murdoch University and were offered the same commercially formulated diets without any feed additives or antimicrobial compounds until they reached a body weight of 31.2 (SD 4.28) kg. At this time, the pigs were allocated to one of the four experimental diets based on their body weight. The pigs were housed in a temperature-controlled animal house in three identical rooms. Each room had four pens in a square arrangement so that each pen was adjacent to two other pens. The pens were raised above the ground and had fully slatted plastic floors and wire mesh sides that allowed contact between the animals and passage of manure between the pens. In each room, there was one pen of five pigs per experimental diet (i.e. there were fifteen pigs per experimental diet). Each pen was equipped with a dry-feed single space feeder without water and two drinking bowls. Throughout the study, the pigs had \( \text{ad libitum} \) access to feed and water. Group housing was chosen to facilitate transmission of the pathogenic bacteria within and between groups\( ^{(4,7,12)} \). The pigs were allowed to adapt to the diets for 2 weeks before being challenged with \( B. \ hyodysenteriae \).

**Experimental design and diets**

The experimental design was a completely randomised block arrangement, with four dietary treatments differing in the amount of added dietary inulin (0, 20, 40 and 80 g/kg). The four diets were formulated, as shown in Table 1, to meet or exceed the nutrient requirements for pigs of this genotype, and all diets contained the same energy and protein (amino acids) contents. Inulin (Orafti\(^{\text{TM}}\); Orafti, Tienen, Belgium) was added to the diets at the expense of triticale and barley. The diets were produced in mash form using the same batch of raw materials and did not contain any antimicrobials.

**Challenge with Brachyspira hyodysenteriae and assessment of swine dysentery**

Australian \( B. \ hyodysenteriae \) strains WA1 and B/Q02 were obtained as frozen stocks from the culture collection at the Reference Centre for Intestinal Spirochetes, Murdoch University. They were thawed and grown in Kunkle's pre-reduced anaerobic broth containing 2% (v/v) fetal bovine serum and 1% (v/v) ethanolic cholesterol solution\( ^{(13)} \), and were incubated at 37°C on a rocking platform until early log-phase growth was achieved.

Each morning for four consecutive days, all pigs were challenged via a stomach tube with 100 ml broth culture containing approximately 10\(^{9} \) colony-forming units/ml of \( B. \ hyodysenteriae \), made up of equal numbers of each of the two spirochaete strains. At this time, the pigs had an average body weight of 41.1 (SD 4.47) kg.

The pigs were weighed weekly, and rectal swabs were taken from all pigs twice a week for spirochaete culture. Visual faecal consistency scoring (1, firm, well formed; 2, soft; 3, loose; 4, watery; 5, watery with mucus/blood) was conducted daily. Watery diarrhoea with mucus/blood was considered as indicating SD, and pigs showing these signs were removed for post-mortem examination within 48 h. All other pigs were removed for necropsy 42 d after the first day of challenge.

**Post-mortem**

Euthanasia was by captive bolt stunning followed by exsanguination. The gastrointestinal tract was removed immediately and divided into seven segments by ligatures: stomach, duodenum, jejunum, ileum, caecum, upper colon and lower colon. The presence, distribution and nature of gross lesions in the large intestine were recorded\( ^{(14)} \), and bacteriological swabs were taken from the wall of the caecum and proximal colon for spirochaetal culture. The luminal contents were then removed by gently squeezing the material from the gut segment. The empty segments and collected material were weighed, and representative samples were collected in sterile plastic tubes that were snap-frozen in liquid N\(_2\) within 10 min of euthanasia. Samples for DM and VFA examination were stored at \(-20^\circ\text{C}\) until analysis.

For determination of \( \text{NH}_3 \) nitrogen (N-N\(_{\text{NH}_3}\)), digesta samples were diluted 1:1 (w/v) with TCA (10%), mixed, snap-frozen in liquid N\(_2\) and stored at \(-80^\circ\text{C}\) until analysis. The pH values of the digesta were measured by inserting the electrode of a calibrated portable pH meter (Schindengen pH Boy-2; Schindengen Electric MFG, Tokyo, Japan) into the collected sample. The DM content of samples was measured using the AOAC method (930.15)\( ^{(15)} \).
Histological measurements

A ring-like cross-section of the ileum was collected and immediately fixed in 10 % neutral-buffered formalin. Measurements of villous height and crypt depth were conducted as described by Hansen et al. (7).

Bacteriological analysis

Bacteriological swabs taken from faeces, caecum and colon were streaked onto selective agar plates designed for isolation of Brachyspira species (16), consisting of Trypticase Soya agar (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) containing 5 % (v/v) defibrinated sheep blood, spectinomycin (400 {mg}/ml), and colistin and vancomycin (each 25 {mg}/ml) (Sigma, St Louis, MO, USA). The plates were incubated for 5–7 d at 37°C in a jar with an anaerobic environment generated using a GasPak Plus disposable H₂ + CO₂ generator envelope with a Pd catalyst (Becton Dickinson Microbiology Systems, Franklin Lakes, NJ, USA). The presence of low, flat, spreading growth of spirochaetes on the plate and any haemolysis around the growth were recorded. Spirochaetes were confirmed by selecting areas of suspected growth, resuspending in PBS and examining the suspension under a phase-contrast microscope at 400 × magnification. Spirochaetes were identified as B. hyodysenteriae on the basis of strong b-haemolysis, microscopic morphology and PCR results of an NADH oxidase gene for cell growth on the plates. The PCR primers and conditions have been described previously (17).

Analysis of feed, organic acids and ammonia nitrogen

The N content of the feed was determined with a N analyser (LECO FP-428; LECO Corporation, St Joseph, MI, USA) by a combustion method (American Organization of Analytical Chemists 990.03) (15). Crude protein was calculated by multiplying the N content by 6·25. Crude fat was measured using the AOAC Soxhlet method (960.39) (15).

The concentrations of organic acids (formic acid, VFA, lactic acid and succinic acid) in the ileal contents were analysed by the method described by Jensen et al. (18). The VFA concentrations in caecal and colon contents were determined as described by Heo et al. (19).

Concentrations of N-NH₃ were measured according to the method by Weatherburn (20). Briefly, the supernatant was
pigs fed 80 g/kg inulin were less likely to have faecal samples that were culture positive for B. hyodysenteriae during the experimental period, eight out of fifteen did develop the disease.

**DM, pH and ammonia nitrogen**

The DM content in the caecum decreased linearly ($P=0.007$) with increasing dietary inulin levels (Table 3). The opposite occurred in the lower colon, with the DM content increasing linearly ($P=0.007$) with increasing inulin levels. The DM content in the ileum and upper colon were not influenced by diet. The pH values in the caecum tended to decrease ($P=0.072$) and in the upper colon decreased ($P=0.047$) linearly with inulin levels in the diets. In the ileum and lower colon, the pH values were not influenced by diet. N-NH$_3$ concentrations in the caecum, upper colon and lower colon were unaffected by dietary inulin levels (Table 3).

**Organic acids in digesta**

Diet did not affect the total concentration of organic acids or the molar proportion of the organic acids in the ileum, except for a tendency towards a linear decrease in the proportion of propionic acid ($P=0.060$; Table 4). On the other hand, a linear increase in total VFA concentration was observed in the caecum ($P=0.018$), upper colon ($P=0.001$) and lower colon ($P=0.013$). In the caecum, increasing dietary inulin tended to linearly increase the molar proportion of propionic acid ($P=0.067$), whereas a linear reduction in the percentage of isobutyric acid ($P=0.015$) and isovaleric acid ($P=0.026$) was observed. In the upper colon, there was a linear decrease, or there tended to be a decrease, in the percentage of acetic acid ($P=0.065$), isobutyric acid ($P=0.011$) and isovaleric acid ($P=0.013$) with increasing levels of dietary inulin. In contrast, an increase or tendency towards a linear increase was found for propionic acid ($P=0.038$), butyric acid ($P=0.070$) and valeric acid ($P=0.007$). In the upper colon, there was a linear increase in the percentage of butyric acid ($P=0.026$), valeric acid ($P=0.005$) and caproic acid ($P=0.051$), but a tendency towards a linear decrease in the proportion of isobutyric acid ($P=0.056$).

Overall, total VFA concentrations and pH in digesta were negatively correlated in the ileum (Pearson’s $r = 0.29$;

### Table 2. Number of positive pigs and relative risk* of a pig being culture positive for *Brachyspira hyodysenteriae* or showing clinical signs of swine dysentery (fifteen pigs per dietary treatment)

<table>
<thead>
<tr>
<th>Inulin (g/kg)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>Inulin</th>
<th>Linear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs challenged</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigs with clinical swine dysentery</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative risk of clinical swine dysentery</td>
<td>1.9</td>
<td>1.8</td>
<td>1.6</td>
<td>1.0</td>
<td>0.022</td>
<td>0.001</td>
</tr>
<tr>
<td>Pigs shedding <em>B. hyodysenteriae</em> in faeces (culture)</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative risk of culture positive <em>B. hyodysenteriae</em> faeces</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
<td>1.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pigs with culture positive <em>B. hyodysenteriae</em> colon content at euthanasia</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative risk of culture positive <em>B. hyodysenteriae</em> colon content at euthanasia</td>
<td>1.5</td>
<td>1.5</td>
<td>1.3</td>
<td>1.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* The risk of an event in the group of interest compared with the reference group.
of a broth culture with approximately 10^8 colony-forming units/ml viable cells in the previous study (7). With this and 661 (SEM 43·4) g/d, respectively. Overall, pig performance regard, a higher level of infectious challenge may have

100 ml of a broth containing approximately 10^9 colony-

the pigs were challenged on four consecutive days with

of the broth used to challenge the pigs: in the present study,

B. hyodysenteriae

concentration of inulin in our previous study(7). This differ-

in 15 % of the pigs (three out of twenty pigs) receiving this

inulin developed the disease, whereas disease occurred only

in 0·58; P<0·001). The significant effects of dietary inclusion rates of inulin were not protective, demonstrating that a high concentration of dietary inulin is required for protection

Discussion

The model of SD used in the present study was effective, as all fifteen pigs fed the control diet developed disease. In this setting, 80 g/kg dietary inulin inhibited the development of SD, confirming our previous findings(7). However, lower concentrations of inulin were not protective, demonstrating that a high concentration of dietary inulin is required for protection if it is used as the sole intervention. In the present study, more than half of the pigs (eight out of fifteen pigs) fed 80 g/kg inulin developed the disease, whereas disease occurred only in 15 % of the pigs (three out of twenty pigs) receiving this concentration of inulin in our previous study(7). This difference might be explained by the amount and concentration of the broth used to challenge the pigs: in the present study, the pigs were challenged on four consecutive days with 100 ml of a broth containing approximately 10^9 colony-forming units/ml of B. hyodysenteriae compared with 80 ml of a broth culture with approximately 10^8 colony-forming units/ml viable cells in the previous study(7). With this regard, a higher level of infectious challenge may have overwhelmed the protection afforded by the inulin.

Nonetheless, the present findings are in accordance with other researchers who also found that fermentable dietary carbohydrates reduced the incidence of SD(6,21). In a field study, Bilic & Bilkie(21) observed that a diet with wheat shorts and maize starch reduced the incidence of SD but, as in the present study, total protection against SD was not achieved. In contrast, Thomsen et al.(6) found that an organic diet based on dried chicory roots and lupins completely protected pigs against SD after experimental challenge with B. hyodysenteriae. In our previous study, we demonstrated that pigs fed 80 g/kg inulin had a reduced risk of developing SD, while the onset of disease was delayed in pigs fed lupins(7); however, unlike Thomsen et al.(6), it was found that a small number of pigs fed the various ‘protective’ diets developed the disease. These discrepancies are most probably due to differences in virulence of the different strains of B. hyodysenteriae employed or differences in the experimental diets used.

In contrast, feeding fermentable carbohydrates from sugarbeet pulp, wheat shorts and potato starch failed to prevent the development of SD(2), while feeding diets that contained wheat, barley or oat groats resulted in an almost 100 % incidence of SD(12). This demonstrates that the amount and properties of the dietary carbohydrates are important considerations when formulating diets to control infections with B. hyodysenteriae.

The present findings seemingly contradict previous findings, where diets supplemented with soluble NSP and resistant starch were associated with the development of SD compared with diets based on cooked white rice and animal proteins(4,5,12). On the other hand, attempts to reproduce these results by Lindecrona et al.(5) and Kirkwood et al.(2) failed, which again could be due to the difference in the experimental designs such as differences in rice processing or virulence of the strains of B. hyodysenteriae(12).

Fermentation of inulin by the indigenous microbiota results in the production of VFA and gases(9), so consequently the
luminal pH values in the caecum and upper colon decreased with increasing levels of dietary inulin. Similarly, the concentration of VFA in the caecum, upper colon and lower colon increased when pigs were fed higher concentrations of inulin. Generally, dietary inclusion of inulin has shown contradictory results with respect to luminal pH values and VFA concentrations. Hansen et al. observed that feeding 80 g/kg inulin to pigs experimentally challenged with \textit{B. hyodysenteriae} had no influence on large-intestinal pH values and total VFA concentrations, whereas Halas et al. using weaner pigs and Loh et al. using grower pigs observed lower total VFA concentrations in the large intestine when diets were supplemented with inulin. Nonetheless, the negative correlations between digesta VFA concentration and luminal pH observed in the present study support the notion that undigested carbohydrate entering the large intestine functions as a substrate for fermentative processes which generates a higher concentration of VFA to cause a decrease in pH.

In the present study, feeding increasing levels of inulin significantly influenced the proportion of VFA in the luminal contents, which concurs with previous findings. According to Cummings & Macfarlane, dietary inulin mainly stimulates lactobacilli that produce lactic and acetic acids. However, no increase in acetic acid concentration was observed in the present study, and hence butyrate and valerate concentration should not be affected. However, Bindelle et al. reported that in human subjects, butyrate-producing bacteria can be net utilisers of acetate to the extent that the proportion of acetate can be the reciprocal of the concentration of butyrate due to bacterial cross-feeding. Indeed, Molbak et al. suggested that feeding inulin can cause an increase in lactate-producing bacteria, which in turn can stimulate lactate-utilising butyrate producers such as \textit{Megasphaera elsdenii}.

VFA produced by the intestinal microbiota are commonly divided into straight-chain fatty acids and BCFAs. Straight-chain fatty acids such as acetic, propionic and butyric acids are produced from carbohydrate fermentation, while BCFAs such as isobutyric and isovaleric acids are produced by fermentation of proteins. In addition, fermentation of protein in the
large intestine normally becomes more evident as carbohydrate availability becomes a limiting factor for microbial fermentation\(^\text{(18)}\). Collectively, this could explain the decline in BCFA in the caecum and colon with increasing levels of dietary inulin, as the preferential metabolism of inulin by carbohydrate-fermenting bacteria may have lessened the activity of the proteolytic bacteria. In agreement, Jensen et al.\(^\text{(18)}\) showed that the production of protein metabolites from microbial fermentation may be reduced by inclusion of NSP.

In addition to BCFA, protein fermentation may be accompanied by an increased production of \(\text{NH}_3\), indole, phenols, amines and \(S\)-containing compounds.\(^\text{(33)}\) In the present study, \(\text{N-NH}_3\) was measured as a marker of protein fermentation in the large intestine, which nonetheless was unaltered by dietary inulin levels. This lack of increased luminal \(\text{N-NH}_3\) concentrations in the large intestine could be a result of \(N\) assimilation by bacteria and/or growth of the caecal and colonic biomass possibly coupled with acidification of the large-intestinal contents, resulting in the conversion of ammonia to the less diffusible \(\text{NH}_4^+\) ion.\(^\text{(32)}\)

Collectively, the data from the present study suggest that dietary supplementation with inulin probably influenced bacterial populations in the gut, resulting in the observed changes in VFA levels and proportions. These changes may have affected the pathogenesis of SD, for example by inhibiting colonisation by the spirochaete. Different dietary effects on the expression of SD are most probably linked to diet-related changes in the intestinal microbiota. Different dietary effects on the expression of SD are most probably linked to diet-related changes in the intestinal microbiota. Different dietary effects on the expression of SD are most probably linked to diet-related changes in the intestinal microbiota.

**Conclusion**

The present study demonstrated that pigs fed 80 g/kg inulin, but not lower concentrations, have a reduced risk of developing clinical SD after experimental challenge with \(B.\) *hyodysenteriae*. These results confirm previous findings by our group.\(^\text{(27)}\) In addition, the present study underlines that these relative high and expensive dietary inclusion levels are necessary to induce changes in the intestinal fermentation characteristics. Diets supplemented with 80 g/kg inulin may protect pigs against developing SD by modifying the microbiota in the gastrointestinal tract.

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