



## Dietary supplementation of viscous and fermentable non-starch polysaccharides (NSP) modulates microbial fermentation in pigs<sup>☆</sup>

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### ABSTRACT

Purified non-starch polysaccharides (NSP) affect intestinal nutrient flow and hence microbial fermentation in pigs. Therefore, we investigated the effect of NSP differing in viscosity and fermentability on colonic fermentation and bacterial populations in eight ileal-cannulated barrows (BW 30 kg) fed a cornstarch-casein based diet supplemented with 5% low fermentable, low viscous cellulose (CEL), low fermentable, high viscous carboxymethylcellulose (CMC), high fermentable, low viscous oat  $\beta$ -glucan (LG), or high fermentable, high viscous oat  $\beta$ -glucan (HG) in a double  $4 \times 4$  Latin square. Ileal DM flow was higher ( $P < 0.05$ ) for CEL, LG and HG than for CMC. Thus, more fermentable substrate entered the colon resulting in higher ( $P < 0.01$ ) SCFA levels in faeces with CEL, LG and HG. The LG raised ( $P < 0.05$ ) faecal molar proportion of butyrate 1.5 to 3 folds compared to CEL and CMC. LG also increased branched-chain fatty acids compared to CMC, indicating increased colonic fermentation of branched-chain AA that coincided with increased ( $P < 0.05$ ) flow of protein into the large intestine. In contrast, faecal counts of *C. perfringens* cluster were lower ( $P < 0.05$ ) for LG and CMC compared to CEL and HG. Less colonic fermentable substrate with CMC corresponded to lower faecal numbers of lactobacilli and higher numbers of *Bacteroides*-like and *Enterobacteriaceae* ( $P < 0.05$ ). In conclusion, effects on microbial fermentation were more linked to the chemical structure of individual NSP than to their shared physical properties, such as viscosity and fermentability.

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### 1. Introduction

Non-starch polysaccharides (NSP)-rich by-products of the food and bio-processing industry are increasingly included in diets for pigs. However, the NSP induced changes in digestion and absorption of nutrients in the small intestine influence the availability of nutrients for fermentation in the lower parts of the gastro-intestinal tract (GIT). For instance, soluble NSP such as oat  $\beta$ -glucans increase intestinal viscosity,

thereby reducing the ileal digestibility of nutrients and increasing the substrate for fermentation in the large intestine (Bach Knudsen et al., 2000). Furthermore, differences in the fermentability of NSP influence in which part of the GIT particular NSP are fermented. This knowledge allows the formulation of diets that stimulate fermentation in specific sections of the GIT (Williams et al., 2005). As bacterial populations differ in their substrate preference, the profile and total amount of short-chain fatty acids (SCFA) produced as well as the bacterial community can potentially be manipulated through different sources of NSP (Botham et al., 1998). Therefore, the aim of the present study was to investigate effects of four NSP differing in fermentability and viscosity on dry matter (DM) and protein flow into the large intestine, and on the fermentation profile and bacterial populations in the distal colon of grower pigs.

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## 2. Material and methods

### 2.1. Diet, animals and experimental procedures

A semi-purified basal diet consisting of cornstarch and casein was formulated to meet or to exceed the nutrient requirements for growing pigs according to NRC (1998). A total of 0.3% of titanium dioxide (TiO<sub>2</sub>) was added as digestibility marker. The basal diet was supplemented with 5% purified sources of NSP: low fermentable, low viscous cellulose (CEL; TIC Pretested Ticacel MCC FG-100; TIC GUMS, White Marsh, MD, USA); low fermentable, high viscous carboxymethylcellulose (CMC; TIC Pretested Ticacelose CMC 6000 F, TIC GUMS); high fermentable, low viscous oat  $\beta$ -glucan (LG; OatVantage, GTC Nutrition, Missoula, MT, USA); or high fermentable, high viscous oat  $\beta$ -glucan (HG; Viscofiber, Cevena By-products, Edmonton, AB, Canada). Cellulose, CMC, LG and HG were selected with respect to their *in vitro* viscosities that were 0.3, 285, 20 and 210 mPas for CEL, CMC, LG and HG, as determined in 0.5% solution using a rheometer (UDS 200, Paar Physica, Glenn, VA) at a shear rate of 12.9/s and 20 °C. The analysed digestible energy contents were 3.41, 3.41, 3.58 and 3.49 Mcal/kg for the CEL, CMC, LG and HG diets and the analysed CP contents were 14.83, 14.71, 16.27 and 15.67% for the CEL, CMC, LG and HG diets. Eight ileal-cannulated cross-bred barrows (Duroc  $\times$  Large White; BW 30  $\pm$  1.3 kg) were fed one of the experimental diets in a double 4  $\times$  4 Latin square. The pigs were allowed to consume the experimental diets at a rate of 3  $\times$  the maintenance requirement for energy (3  $\times$  110 kcal DE/kg BW<sup>0.75</sup>; NRC, 1998). Pigs were fed twice daily two equal meals at 0800 and 1600 as a mash and had free access to water. The four experimental periods consisted of a 10-d adaptation period followed by a 3-d collection of faeces and then a 4-d collection of ileal digesta daily from 08.00 to 20.00. All procedures in this study were approved by the Animal Care and Use Committee for Livestock at the University of Alberta.

### 2.2. Chemical and statistical analyses

Diets and freeze-dried ileal digesta and faeces were analysed for DM, TiO<sub>2</sub> and crude protein (CP) (AOAC, 1995). In fresh ileal digesta and faeces, SCFA were determined using gas chromatography. Bacterial groups were measured using quantitative PCR. Briefly, total DNA was isolated from intestinal samples using chloroform-phenol extraction. Group-specific primers for *Lactobacillus*, *Clostridium coccooides* cluster, *Clostridium perfringens* cluster, *Enterobacteriaceae* family (Wise and Siragusa, 2006) and *Bacteroides-Prevotella-Porphyrromonas* (Rinttilä et al., 2004) were used for quantitative PCR amplification in a Fast 7500 Realtime System (Applied Biosystems, Foster City, USA).

Data were statistically analysed using the PROC MIXED of SAS including effects of pig within square, period, and treatment. Pig and period were considered as random effects assuming a compound symmetry variance-covariance structure. Differences were considered as significant at  $P < 0.05$ .

## 3. Results

Supplementation of CEL, LG and HG raised ( $P < 0.01$ ) the ileal flow of DM and CP compared to CMC (Table 1). Moreover, CEL, LG and HG resulted ( $P = 0.01$ ) in a higher postileal disappearance of DM compared to CMC. Faecal total SCFA concentration was higher ( $P < 0.05$ ) with CEL, LG and HG than with CMC (Table 2). Molar ratio of acetate was higher ( $P < 0.05$ ) with CEL and CMC compared to LG and HG, while propionate ratio was increased ( $P < 0.05$ ) by CMC compared to the other NSP. Faecal proportions of butyrate and branched-chain fatty acids were higher ( $P < 0.05$ ) with LG compared to CMC and CEL. CMC reduced ( $P < 0.05$ ) faecal numbers of lactobacilli compared to CEL, but increased ( $P < 0.05$ ) numbers of *C. coccooides* cluster and *Bacteroides-Prevotella-Porphyrromonas* compared to CEL, LG and HG. Moreover, CMC increased ( $P < 0.05$ ) *Enterobacteriaceae* in faeces compared to LG and HG. Both CMC and LG caused lower numbers of *C. perfringens* cluster ( $P < 0.05$ ) compared to CEL and HG.

## 4. Discussion

Low fermentable, high viscous CMC reduced the flow of DM and CP into the large intestine, indicating an improved digestion in the upper GIT compared to the other NSP fractions. In contrast, likely due to faster transit and thus less exposure of the diet to the host's digestive enzymes (Low, 1982), low fermentable, low viscous CEL increased the flow of DM and CP into the large intestine. Similarly, both low and high viscous oat  $\beta$ -glucans enhanced the flow of fermentable substrate into the large intestine which was reflected in higher faecal SCFA concentration. The higher availability of fermentable substrate in the large intestine for the CEL, LG and HG diets stimulated fermentation in this segment of the gut as indicated by increased postileal DM disappearance and higher total SCFA concentrations for these diets compared to CMC. However, CEL is almost non-fermentable for intestinal microbes. Thus, fermentation in the large intestine must have mainly occurred from easily fermentable dietary nutrients contained in the ileal effluent. Starch content in digesta was estimated as the difference between DM, CP, ash and fibre, which was lowest in diets containing CMC (data not shown). Starch, protein and ash amounted to approximately 250 g/kg DM the ileal effluent

**Table 1**

Ileal flow of DM and CP into the large intestine and faecal flow of DM (g/kg DM intake) of pigs fed diets supplemented with cellulose (CEL), carboxymethylcellulose (CMC), low viscous oat  $\beta$ -glucan (LG) or high viscous oat  $\beta$ -glucan (HG).

Diets	Low fermentable		High fermentable		SEM	P-value
	Low viscous	High viscous	Low viscous	High viscous		
Ileal DM flow	308 <sup>a</sup>	165 <sup>b</sup>	242 <sup>c</sup>	277 <sup>ac</sup>	1.7	<0.001
Ileal CP flow	42 <sup>ab</sup>	26 <sup>c</sup>	39 <sup>b</sup>	47 <sup>a</sup>	2.0	<0.001
Postileal DM disappearance	129 <sup>a</sup>	23 <sup>b</sup>	102 <sup>a</sup>	126 <sup>a</sup>	20.3	0.010

<sup>abc</sup>LS means within a row with different superscripts are significantly different at  $P < 0.05$ .

**Table 2**

Effects of cellulose (CEL), carboxymethylcellulose (CMC), low viscous oat  $\beta$ -glucan (LG) and high viscous oat  $\beta$ -glucan (HG) on bacterial groups, concentration of total SCFA and molar proportions of SCFA in faeces of pigs.

Diet	Low fermentable		High fermentable		SEM	P-Value
	Low viscous	High viscous	Low viscous	High viscous		
	CEL	CMC	LG	HG		
<i>Bacterial groups (log<sub>10</sub> 16S rDNA gene copies/g fresh matter)</i>						
<i>Lactobacillus</i> spp.	6.9 <sup>a</sup>	5.6 <sup>b</sup>	6.2 <sup>ab</sup>	6.1 <sup>ab</sup>	0.41	0.202
<i>Clostridium perfringens</i> cluster	7.2 <sup>a</sup>	2.5 <sup>b</sup>	3.3 <sup>b</sup>	6.5 <sup>a</sup>	0.93	0.005
<i>Clostridium coccoides</i> cluster	7.7 <sup>b</sup>	8.7 <sup>a</sup>	6.4 <sup>c</sup>	7.1 <sup>bc</sup>	0.40	0.007
<i>Bacteroides-Prevotella-Porphyromonas</i>	8.9 <sup>b</sup>	10.5 <sup>a</sup>	8.0 <sup>c</sup>	8.4 <sup>bc</sup>	0.20	<0.001
<i>Enterobacteriaceae</i> family	9.0 <sup>ab</sup>	10.3 <sup>a</sup>	7.0 <sup>c</sup>	7.6 <sup>bc</sup>	0.58	0.004
Total SCFA ( $\mu$ mol/g fresh matter)	61 <sup>a</sup>	28 <sup>b</sup>	69 <sup>a</sup>	64 <sup>a</sup>	6.87	0.005
<i>Molar proportion (%)</i>						
Acetate	71 <sup>a</sup>	72 <sup>a</sup>	62 <sup>c</sup>	67 <sup>b</sup>	1.25	<0.001
Propionate	14 <sup>b</sup>	18 <sup>a</sup>	14 <sup>b</sup>	15 <sup>b</sup>	1.11	0.087
Butyrate	6 <sup>bc</sup>	3 <sup>c</sup>	10 <sup>a</sup>	7 <sup>ab</sup>	1.06	0.003
Branched-chain fatty acids	7 <sup>b</sup>	6 <sup>b</sup>	11 <sup>a</sup>	9 <sup>ab</sup>	1.15	0.039

<sup>abc</sup>LS means within a row with different superscripts are significantly different at  $P < 0.05$ .

with CEL. CEL and CMC stimulated acetate fermentation, whereas LG promoted butyrate fermentation compared to CEL and CMC. Stimulation of butyrate may be desirable due to its beneficial effects on intestinal cell proliferation (Kien, 2007). LG and HG increased proportions of branched-chain fatty acids, products of branched-chain amino acid fermentation which is coupled to the formation of ammonia, amines, and malodorous components. Because different bacterial species have different substrate preferences and the amount of fermentable substrate entering the large intestine varied, the four NSP promoted the growth of bacterial populations in the colon differently. Decreased flow of fermentable substrate into the large intestine together with the low fermentability of CMC was not only reflected in lower SCFA concentrations, but also in the decreased number of lactobacilli, while *Bacteroides-Prevotella-Porphyromonas* and *C. coccoides* cluster, which both include cellulolytic bacteria, were higher in faeces compared to other NSP. Further shifts in the colonic microbiota with CMC and LG compared to CEL and HG included decreased number of the *C. perfringens* cluster, which comprises most clostridial pathogens, and the lower number of *Enterobacteriaceae* with LG compared to CEL and CMC. Particularly, growth-stimulation of *Enterobacteriaceae* in the distal colon by viscous CMC (McDonald et al., 2001) was confirmed by this study, likely

reflecting changes in the mucus composition and production in the large intestine (Piel et al., 2005). Thus, changing the amount and nature of fermentable substrate entering the large intestine stimulates growth of potential pathogenic bacterial groups which may impair gut health.

In conclusion, dietary inclusion of CEL, CMC, LG and HG selectively stimulated fermentation and growth of bacterial groups in the large intestine. Effects on fermentation were linked to the chemical structure of individual NSP and their effect on nutrient flow rather than to shared physical properties, such as viscosity and fermentability.

### Conflict of interest

None of the authors have a conflict of interest related to the work in the manuscript.

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