Influence of dietary fiber on luminal environment and morphology in the small and large intestine of sows

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ABSTRACT: In this study, the effect of feeding different types and amounts of dietary fiber (DF) on luminal environment and morphology in the small and large intestine of sows was studied. Three diets, a low-fiber diet (LF) and 2 high-fiber diets (high fiber 1, HF1, and high fiber 2, HF2) were used. Diet LF (DF, 17%; soluble DF 4.6%) was based on wheat and barley, whereas the 2 high-fiber diets (HF1: DF, 43%; soluble DF, 11.0%; and HF2: DF, 45%; soluble DF, 7.6%) were based on wheat and barley supplemented with different coproducts from the vegetable food and agroindustry (HF1 and HF2: sugar beet pulp, potato pulp, and pectin residue; HF2: brewers spent grain, seed residue, and pea hull). The diets were fed for a 4-wk period to 12 sows (4 receiving each diet). Thereafter, the sows were killed 4 h postfeeding, and digesta and tissue samples were collected from various parts of the small and large intestine. The carbohydrates in the LF diet were well digested in the small intestine, resulting in less digesta in all segments of the intestinal tract. The fermentation of nonstarch polysaccharides in the large intestine was affected by the chemical composition and physicochemical properties. The digesta from pigs fed the LF diet provided low levels of fermentable carbohydrates that were depleted in proximal colon, whereas for pigs fed the 2 high-DF diets, the digesta was depleted of fermentable carbohydrates at more distal locations of the colon. The consequence was an increased retention time, greater DM percentage, decreased amount of material, and a decreased tissue weight after feeding the LF diet compared with the HF diets. The concentration of short-chain fatty acids was consistent with the fermentability of carbohydrates in the large intestine, but there was no effect of the dietary composition on the molar short-chain fatty acid proportions. It was further shown that feeding the diet providing the greatest amount of fermentable carbohydrates (diet HF1, which was high in soluble DF) resulted in significant morphological changes in the colon compared with the LF diet.

Key words: coproduct, carbohydrate, digestibility, luminal environment, morphology, sow

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INTRODUCTION

Dietary carbohydrates are a diverse group of compounds, which differ considerably in chemical and structural composition and physicochemical properties. Dietary carbohydrates can be classified as sugars, oligosaccharides, and 2 broad classes of polysaccharides: starch and nonstarch polysaccharides (NSP). Nonstarch polysaccharides along with lignin are the main constituents of the plant cell walls and are defined as dietary fiber (DF; Theander et al., 1994; Bach Knudsen, 1997). It is well established that the different carbohydrates have different fates and functional properties in the gastrointestinal tract. Most of the starch is enzymatically digested to glucose in the small intestine and absorbed at this site together with free sugars. Nonstarch polysaccharides are fermented by bacteria mostly in the large intestine to produce short-chain fatty acids (SCFA), which serve as nutrients for the epithelium and as oxidative fuel for body tissues (Bach Knudsen et al., 2001; Bach Knudsen, 2005). The fraction of NSP along with lignin not fermented in the large intestine will maintain their physical properties, hold water, and increase the bulk of digesta materi-
als (Bach Knudsen et al., 2001), which together with the nutrients released as SCFA may influence the morphology of the large intestine (Friedel and Levine, 1992). The crypts are the principal site of cell proliferation in the intestinal mucosa, and it has been hypothesized that intake of high-fiber diets increases the rate of turnover of intestinal mucosal cells in growing pigs (Jin et al., 1994; Brunsgaard, 1998).

In an accompanying paper (Serena et al., 2008), we have studied the digestion of carbohydrates and energy utilization in sows fed diets with contrasting levels and physiochemical properties of dietary fiber. The cereals wheat and barley of a low-fiber (LF) diet were substituted with coproducts characterized to be high in either soluble DF (sugar beet pulp, potato pulp, or pectin residue) or insoluble DF (obtained by substitution of approximately two-thirds of the former coproducts with pea hull, seed residues, and brewers spent grain), and the digestion of carbohydrates and energy utilization was studied. It was evident that the DF level affected the ileal flow of nutrients, in particular carbohydrates that increased from 190 g/d when feeding the LF diet to 538 to 539 g/d when feeding the 2 high-fiber (HF) diets.

We hypothesized that the contrasting properties of DF would result in different fermentation patterns of NSP and physical properties, thereby inducing changes in

### Table 1. Ingredients and chemical composition of the low-fiber (LF), high-fiber 1 (HF1), and high-fiber 2 (HF2) diets

| Item                              | LF    | HF1   | HF2   |
|-----------------------------------|-------|-------|-------|
| Ingredient, % as-fed basis        |       |       |       |
| Barley                            | 42.0  | 14.5  | 14.5  |
| Wheat                             | 42.0  | 14.5  | 14.5  |
| Sugar beet pulp                   | —     | 14.0  | 5.0   |
| Pectin residue                    | —     | 14.0  | 5.0   |
| Potato pulp                       | —     | 14.0  | 5.0   |
| Seed residue                      | —     | —     | 13.5  |
| Pea hulls                         | —     | —     | 13.5  |
| Brewers spent grain               | —     | —     | 13.5  |
| Soy oil                           | 5.0   | 5.0   | 5.0   |
| Soybean meal, toasted             | 7.5   | 21.6  | 8.4   |
| Vitamin and mineral premix\(^1\) | 0.2   | 0.2   | 0.2   |
| Monocalcium phosphate             | 2.2   | 0.9   | 0.9   |
| Calcium carbonate                 | 0.6   | 1.0   | 0.5   |
| NaCl                              | 0.3   | 0.3   | 0.3   |
| Chromic oxide                     | 0.2   | 0.2   | 0.2   |
| Chemical composition, % of DM     |       |       |       |
| Ash                               | 5.3   | 5.9   | 6.8   |
| CP                                | 13.6  | 18.0  | 16.5  |
| Fat                               | 8.5   | 8.6   | 9.7   |
| Total carbohydrates               | 68.9  | 62.4  | 65.4  |
| Sugars                            | 2.1   | 2.9   | 1.9   |
| Fructan                           | 0.9   | 0.5   | 0.6   |
| Starch                            | 51.9  | 21.8  | 25.3  |
| Total NSP\(^2\)                   | 14.0 (4.6) | 37.2 (11.2) | 37.6 (7.6) |
| Cellulose                         | 3.1   | 15.5  | 16.8  |
| NCP\(^3\)                        | 10.9  | 21.7  | 20.9  |
| Klason lignin                     | 3.3   | 5.9   | 7.5   |
| Dietary fiber                     | 17.3  | 43.1  | 45.1  |
| Cr\(_2\)O\(_3\)                   | 0.23  | 0.22  | 0.22  |
| GE, MJ/kg                         | 17.5  | 18.5  | 18.0  |
| Essential amino acids, g/kg of DM |       |       |       |
| Lysine                            | 6.46  | 10.2  | 7.96  |
| Methionine + cystine              | 5.31  | 5.80  | 5.31  |
| Threonine                         | 5.10  | 7.01  | 6.06  |
| Viscosity, mPa.s\(^4\)            | 0.89  | 1.64  | 1.16  |
| Swelling, mL/g of DM              | 3.81  | 6.53  | 5.72  |
| Water-binding capacity, g/g of DM | 1.32  | 3.43  | 3.10  |

\(^1\)Provided the following per kilogram of final diet: 8,800 IU of vitamin A as retinol acetate, 1,000 IU of vitamin D as cholecalciferol, 60 mg of all \(\alpha\)-tocopherol acetate, 2.2 mg of menadione, 2.2 mg of thiamine, 5.5 mg of riboflavin, 3.3 mg of pyridoxine, 16.5 mg of \(\beta\)-pantothenic acid, 22 mg of niacin, 1.65 mg of folic acid, 220 \(\mu\)g of biotin, 22 \(\mu\)g of cyanocobalamin, 60 mg of butylated hydroxytoluene, 100 mg of \(\text{Fe as FeSO}_4\cdot 7\text{H}_2\text{O}\), 150 mg of \(\text{Zn as ZnO}\), 28 mg of Mn as MnO, 20 mg of Cu as CuSO\(_4\)·5H\(_2\)O, 304 \(\mu\)g of I as KI, and 300 \(\mu\)g of Se as Na\(_2\)SeO\(_3\).

\(^2\)NSP = nonstarch polysaccharides; values in parentheses are soluble NSP.

\(^3\)NCP = noncellulosic polysaccharides.

\(^4\)mPa.s = millipascal seconds.
the morphological characteristics of the gastrointestinal tract.

The aim of this particular study was to investigate the effect of the 3 particular diets on the luminal environment and the morphological characteristics in the small and large intestine of sows.

MATERIALS AND METHODS

Experimental Design

Experiments complied with the guidelines of The Danish Animal Experiments Inspectorate, Ministry of Justice, Copenhagen, Denmark, with respect to animal experimentation and care of the animals under study. Twelve nonpregnant sows with an initial average BW = 232 ± 26 kg were selected after weaning of their first or second litter (Danish Landrace × Yorkshire; Faculty of Agricultural Sciences Swineherd, Foulum, Denmark). The sows were fed 3 experimental diets, a LF control diet and 2 generic HF diets: high fiber 1 (HF1) or high fiber 2 (HF2), with different proportions between soluble and insoluble DF. The LF diet was based on wheat and barley, and the 2 HF diets were supplemented with different coproducts (residues from the industrial production: potato pulp, KMC, Kartoffelmelcentralen Amba, Brande, Denmark; sugar beet pulp, Danisco Sugar A/S, Assens, Denmark; pectin residue, CP Kelco ApS, Lille Skensved, Denmark; brewers spent grain, Carlsberg A/S, Fredericia delivered by Agro-Korn A/S, Videbæk, Denmark; pea hull, Prodana Seeds A/S, Odense, Denmark; and seed residues, DLF Trifolium A/S, Roskilde, Denmark; Table 1). Wet co-products (sugar beet pulp, potato pulp, pectin residue, and brewers spent grain) were all dried to DM content above 87.5%. The diets were formulated to contain different types and levels of DF. The LF diet contained 15% DF, and the 2 HF diets contained approximately 40% DF. The HF1 diet had a high content of soluble DF provided from sugar beet pulp, potato pulp, and pectin residue, and HF2 had a high content of insoluble DF obtained by substitution with the coproducts pea hull, seed residue, and brewers spent grain. The diets were formulated to meet the Danish minimum recommendations for essential macro- and micronutrients (Jørgensen and Tybirk, 2005). The diets were milled to pass through a 2-mm screen, and chromic oxide (2.0 g/kg of feed) was added as a solid-phase marker.

The sows were fed once daily (at 0700 h) with 2,000 g as fed to standardize the intake of DF. After a 4-wk period, the sows were slaughtered at 4 h postfeeding. The gastrointestinal tract was quickly removed, and digesta were collected from the stomach, 3 equal lengths of the small intestine (Si1, Si2, and Si3), the cecum, and 4 parts of equal length of the colon (Co1, Co2, Co3, and Co4). The content and tissues (not washed) were weighed and the length of the small intestine and colon measured. The pH was measured in all segments. Tissue samples for morphological characterizations were taken in duplicate from the mid small intestine (Si50), the cecum, and the mid colon (Co50). The samples were immediately transferred to either 10% neutral-buffered formaldehyde or Clark’s fluid [25% glacial acetic acid (Merck 63, Darmstadt, Germany) in absolute alcohol].

Analytical Methods

Diets were analyzed in triplicate for physicochemical properties (water-binding capacity, swelling, and viscosity), as described by Serena (2005).

All chemical analyses were performed in duplicate. Chromic oxide, lactic acid (LA), and SCFA were measured in wet material; all other analysis was done in

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Table 2. Weight of content and of tissue in the gut and the length of the small and large intestine at 4 h after feeding low-fiber (LF), high-fiber 1 (HF1), or high-fiber 2 (HF2) diets

| Item                                      | LF   | HF1  | HF2  | SEM  |
|-------------------------------------------|------|------|------|------|
| Weight of content (wet material), kg       |      |      |      |      |
| Stomach                                   | 4.3b | 6.1a | 5.6a | 0.30 |
| Small intestine                           | 1.5b | 2.4a | 2.1a | 0.16 |
| Cecum                                     | 1.0a | 1.1ab| 0.6b | 0.13 |
| Colon                                     | 4.1b | 6.4ab| 8.0a | 0.61 |
| Total                                     | 10.9b| 16a  | 16a  | 0.82 |
| Tissue weight, kg                         |      |      |      |      |
| Stomach                                   | 1.2  | 1.5  | 1.4  | 0.04 |
| Small intestine                           | 2.5  | 2.5  | 2.6  | 0.07 |
| Cecum                                     | 0.5  | 0.4  | 0.3  | 0.02 |
| Colon                                     | 2.0b | 2.9a | 2.6ab| 0.14 |
| Total                                     | 6    | 7.3  | 6.9  | 0.24 |
| Length of the gut, m                      |      |      |      |      |
| Small intestine                           | 15.7 | 17.2 | 17.0 | 0.54 |
| Large intestine                           | 5.9  | 5.1  | 5.9  | 0.20 |

Within a row, means without a common superscript are different (P < 0.05).

Mean values of 4 sows per diet.
freeze-dried materials. Diets and gut material were analyzed for DM by drying to a constant weight at 103°C, ash according to the AOAC (1990), and for nitrogen by the Kjeldahl method using Kjeltech 1035 tecator (Foss Tecator AB, Höganäs, Sweden; AOAC, 1990). Fat was determined by extraction with diethyl ether after acid hydrolysis and analyzed as described by Stoldt (1952) and chromic oxide by the method of Schürch et al. (1950). Starch was analyzed by an enzymatic-colorimetric method according to Bach Knudsen (1997) and sugars (glucose, fructose, and sucrose) and fructan by the enzymatic-calorimetric method of Larsson and Bengtsson (1983). Total NSP and their constituent sugars were determined by gas-liquid chromatography for neutral sugars and by a colorimetric method for uronic acids using a modification of the methods described by Theander and Åman (1979) and Englyst et al. (1982), as described by Bach Knudsen (1997).

Content of cellulose was calculated as

\[
\text{cellulose} = \text{NSP}_{\text{glucose}} (12 \text{ mol/L } \text{H}_2\text{SO}_4) - \text{NSP}_{\text{glucose}} (2 \text{ mol/L } \text{H}_2\text{SO}_4) \]

Total noncellulosic polysaccharides were calculated as

\[
\text{total noncellulosic polysaccharides} = \text{rhamnose} + \text{fucose} + \text{arabinose} + \text{xylose} + \text{mannose} + \text{galactose} + \text{glucose} + \text{uronic acids};
\]

Soluble noncellulosic polysaccharides were calculated as

\[
\text{soluble noncellulosic polysaccharides} = \text{total noncellulosic polysaccharides} - \text{insoluble noncellulosic polysaccharides};
\]

and DF was calculated as

\[
\text{DF} = \text{total noncellulosic polysaccharides} + \text{cellulose} + \text{lignin}.
\]

Klason lignin was measured gravimetrically as the residue resistant to hydrolysis by 12 mol/L of H\text{SO}_4 (Theander and Åman, 1979). The gut content was analyzed for total LA and SCFA by GC, as described in detail by Jensen et al. (1995).

Tissue samples for morphological and histological analysis were placed for 24 h in 10% neutral-buffered formaldehyde, cleaned of remaining digesta using deionized water, and dehydrated and infiltrated with paraffin wax. Three slides were prepared from each sample, and each slide contained a minimum of 4 sections that were 4-µm thick and were made at least 50-µm apart.

The slides were processed for carbohydrate histochemistry using either the periodic acid-Schiff's (which stains neutral mucins) reaction or the Alcian blue reaction at either pH 2.5 (which stains carboxylated or sulfated types of acidic mucins) or pH 1.0 (which stains sulfomucins; Kiernan, 1990). Carbohydrate histochemistry on the periodic acid-Schiff's- and Alcian blue-stained samples was evaluated as described previously by Brunsgaard (1997).

The slides processed for neutral mucins were further used to determine the area, the height, and the density of the intestinal villi and the crypts and the thickness of the muscularis externa using an image analyses system (Quantimet 500MC, Leica, Cambridge, UK; Hedemann et al., 2006). All measures were done using a light microscope at 10× magnification.

Samples for mitotic counts were stained with the Feulgen reaction, and the mitotic counts in the crypts were performed as described by Goodlad (1994).

Calculations and Statistical Analysis

The content of polysaccharide residues was calculated as anhydro sugars, and all apparent digestibilities were calculated relative to the Cr$_2$O$_3$ concentration:

The digestibility was calculated as

\[
\text{digestibility of X (\% of intake)} = \left(1 - \frac{\text{Cr}_2\text{O}_3(\text{material}) \times X_{(\text{diet})}}{\text{Cr}_2\text{O}_3(\text{diet}) \times X_{(\text{material})}}\right) \times 100,
\]

where $X$ = the concentration of specific nutrients in the diet and the digesta. When calculating starch digestibility at the ileum, it is generally assumed that free glucose in ileum digesta derives from starch.

Mean transit time in the segments was calculated as:

\[
\text{mean transit time (h)} = \frac{\text{Cr}_2\text{O}_3(\text{GI}) \times 24}{\text{Cr}_2\text{O}_3(\text{day})}.
\]

where Cr$_2$O$_3$(GI) = the amount of Cr$_2$O$_3$ in the segment of gastrointestinal tract and Cr$_2$O$_3$(day) = the daily intake of Cr$_2$O$_3$.

The correlation between pH and SCFA concentrations in the gut segments was done using PROC CORR (SAS Inst. Inc., Cary, NC).

Comparison of treatment effects (i.e., diets) in a given intestinal segment was accomplished by a simple ANOVA based on the model

\[
X_i = \mu + \alpha_i + \varepsilon_i,
\]

where $X_i$ = the dependent variable; $\mu$ = the mean of the variable; $\alpha_i$ = treatment (diet LF, HF1, and HF2); and $\varepsilon_i$ accounts for unexplained variation. The GLM procedure in SAS with a level of significance of $P < 0.05$ was used in this case.
Investigation of the effect of diets over a range of intestinal segments was carried out using the following general model:

\[ X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \mu_k + \varepsilon_{ijk}, \]

where \( X_{ijk} \) = the dependent variable; \( \mu \) = the mean of the variable, \( \alpha_i \) = the effect of diets (LF, HF1, and HF2); \( \beta_j \) = the effect of segment (cecum and Co1 to 4); \( \gamma_{ij} \) = the interaction between diet and segment; and \( k \) = an individual sow. The variance component, \( \mu_k \sim N(0, \tau^2) \), accounts for the repeated measurements made on the same individual, thereby rendering these observations correlated. The error term, \( \varepsilon_{ijk} \sim N(0, \sigma^2) \), represents unexplained variation. The variance interaction between segment and diet was described as random by using \( \gamma_{ij} \sim N(0, \sigma^2) \) [Type = arh (1)]. The MIXED procedure in SAS with a level of significance of \( P < 0.05 \) was used in this case.

### RESULTS

In our diet formulation, proper adjustments were done to ensure a sufficient supply of amino acids, but unexpectedly, HF1 had a greater protein content (% of DM) and diet HF2 a greater fat content than planned (Table 1). The content of carbohydrates was greatest in the LF diet with 69% compared with 62 to 65% in diets HF1 and HF2. The amount of starch was greatest in diet LF (52%) compared with the 2 HF diets (22 to 25%). The amount of cellulose was high in the HF diets (16 to 17%) and decreased in LF (31%). As expected, the 2 HF diets were similar in total NSP but varied in the amount of soluble and insoluble NSP. The percentage of soluble NSP out of total NSP was 30% for HF1 and 20% for HF2.

Feeding the HF diets resulted in significantly greater amounts of wet material in the small intestine, whereas only pigs fed the HF2 diet had a greater \( (P < 0.05) \) amount of wet material in the colon compared with the LF-fed pigs (Table 2). Tissue weights of the stomach, small intestine, and cecum were not affected by diet, whereas the colon of HF1-fed pigs was heavier \( (P < 0.05) \) compared with LF-fed pigs (Table 2).

The digestibility of carbohydrates varied among diets (Table 3). The digestibility of starch was high with all diets but with some difference among the diets in terms of the site where maximum digestibility was reached. Practically complete digestion was reached in Co1 when feeding diet LF, in Co3 when feeding diet HF1, and for diet HF2, approximately 3% of ingested

### Table 3. Digestibility (% of intake) of starch, nonstarch, cellulose, and noncellulosic polysaccharides along segments of the gastrointestinal tract in sows fed low-fiber (LF), high-fiber 1 (HF1), or high-fiber 2 (HF2) diets

| Segment | n  | LF  | HF1 | HF2 | LF  | HF1 | HF2 | LF  | HF1 | HF2 | LF  | HF1 | HF2 |
|---------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ileum   | 6  | 96a | 92b | 93b | 41a | 23b | 26b | 11  | 6   | 4   | 50a | 34b | 40b |
| Cecum   | 4  | 98a | 90b | 89b | 10  | 14  | 12  | -57 | -59 | -23 | -57 | -59 | -23 |
| Co1     | 4  | 99b | 93b | 93b | 47b | 63a | 42a | 11  | 49b | 24b | 58b | 71b | 54b |
| Co2     | 4  | 100b| 98b | 93b | 49b | 74a | 55b | 10  | 65b | 43b | 61b | 80b | 62b |
| Co3     | 4  | 100b| 99b | 96b | 46b | 76a | 61b | 10  | 67b | 53b | 61b | 82b | 65b |
| Co4     | 4  | 100b| 99b | 97b | 47b | 79a | 64b | 7b  | 72a | 57b | 59b | 84b | 69b |
| Feces   | 6  | 100b| 100b| 97b | 48b | 78a | 67b | 9b  | 72a | 64b | 60b | 82b | 69b |
| SEM     |    | 0.45| 6.93| 3.12| 2.18|      |      |      |      |      |      |      |      |

**P-value**

- Diet(D) \(<0.001\)
- Segment(S) \(<0.001\)
- D × S \(<0.001\)

*Within a row and nutrient component, means without a common superscript are different \((P < 0.05)\).*

1 Data from ileum and feces are mean values from another study (Serena, 2005) with 6 T-cannulated sows in a repeated 3 × 3 Latin square design. These data are therefore not included in statistical analysis for repeated measurement.

2 Co1 to Co4 = segment 1 to 4 of the colon.

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Figure 1. Dry matter (%) in segments of the gastrointestinal tract of sows fed low-fiber (LF), high-fiber 1 (HF1), or high-fiber 2 (HF2) diets. □ LF; ○ HF1; ○ HF2. Ce = cecum; Co1 to Co4 = segment 1 to 4 of the colon. Mean values for 4 sows per diet. Vertical bars are SD.
starch was not digested after passage of the total tract. The digestibility of NSP in cecum was decreased compared with the ileum for all diets. The digestibility of NSP increased along the colon but reached a plateau at different sites; for diet LF, it was at Co1/Co2; with diet HF1, it was at Co2/Co3; and with diet HF2, it was at Co3/Co4. The greatest total tract digestibility of NSP was shown for diet HF1, followed by diet HF2, and the least for diet LF. The digestibility of cellulose and NCP generally followed that of NSP with the exception that the difference among diets was greater for cellulose than for NCP.

The DM content increased along the distal parts of the gastrointestinal tract from 10 to 11% in Si3 to 28 and 33% in Co4 for HF diets (HF1 and HF2) and diet LF, respectively (Figure 1). Significant differences in DM content among diets were seen at all sampling points along the large intestine. From cecum and until Co2, there were differences in DM content among all 3 diets. In Co3 and Co4, the DM content was greater in sows fed LF than in sows fed the HF diets.

For the entire large intestine, digesta was retained 13 h longer when feeding diet LF (75 h) than was the case when feeding the 2 HF diets (62 h; Figure 2).

The concentration (mmol/kg of material) of organic acids is shown in Table 4. Lactic acid was only detected in ileal digesta, whereas the branched-chain fatty acids (BCFA) only were present in the large intestine. The concentration of SCFA in most segments of the large intestine was significantly decreased in LF-fed pigs compared with pigs fed the HF diets. The greatest concentration of SCFA varied along the large intestine, with the greatest values detected in Co1.

The pH in digesta increased from 6.2 in cecum to 6.7 in Co4 but was not affected by the dietary composition (Table 4). A negative correlation between the SCFA concentration and pH was shown across all diets in the different segment of the gut (r = 0.78; P < 0.001).

The variation in the SCFA profile of cecal and colonic content among diets was approximately as follows: acetate, 65 ± 6.2%; propionate, 21 ± 3.1%; butyrate, 11 ± 4.0%; and BCFA, 3 ± 1.8% (Table 5).

The morphology of the small intestine and the cecum was not affected by diet composition (Table 6). However, when feeding diet HF1, crypt depth in Co50 was significantly greater, and crypt area in Co50 had a tendency to be greater, compared with LF. The mucin characteristics of villi and crypts were not affected by diet and are presented as mean values for all 3 diets (Table 7). The staining area of neutral mucins was greater in all segments than the staining area of acidic or sulfo mucins.

**DISCUSSION**

Our study with ileal-cannulated sows clearly documented that types and levels of DF had a profound effect on the amount of nutrients, in particular carbohydrates, potentially available for fermentation in the large intestine and the physicochemical properties of digesta. In the present study, it was the intention to investigate to what extent these changes would affect the luminal environment and the morphology in the small and large intestine of pigs.

To track changes in the apparent digestibility of carbohydrates from ileum and along the large intestine, we used chromic oxide, which is a widely used solid-phase marker for this purpose (Warner, 1981; Mroz et al., 1996). Although the digestibility of starch and NSP progressed as expected in all segments of the colon, the digestibility of starch (diets HF1 and HF2) and NSP (all diets) was decreased in the cecum compared with the ileum presumably because the sudden movement of digesta in connection with the slaughtering process (the consequence is underestimation of the digestibility in the cecum but not in the colon).

The digestibility of starch from the LF diet was generally greater compared with the HF diets in spite of the fact that the bulk of starch in all diets was derived from cereals. Starch in cereals has an open structure, which enables an easy access for the salivary and pancreatic α-amylases in the small intestine (Englyst et al., 1992). The explanation for why the digestibility of starch is lower at ileum and at all sites of the large intestine and the physicochemical properties of digesta. In the present study, it was the intention to investigate to what extent these changes would affect the luminal environment and the morphology in the small and large intestine of pigs.

Solubility and lignification are 2 important parameters influencing the fermentability of NSP (Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1993; Glitsø et al., 1998): solubility by enhancing the surface area available for bacterial enzymes and lignification by cementing together cell wall polysaccharides and hindering the accessibility of bacterial enzymes to cell wall polysaccharides (Bach Knudsen and...
In agreement with these observations, the digestibility of NSP was greatest for diet HF1, intermediate for diet HF2, and least for diet LF. It should be noted that the degradation profile of the NSP along the large intestine reached a plateau at different sites, which to a large extent reflected the amount and solubility of NSP that reached the large intestine and the type of NSP degraded.

Fermentation in the large intestine produces SCFA as the major energy-containing end product, whereas LA is primarily formed in the stomach and small intestine (Argenzio and Southworth, 1975). The concentrations of LA and SCFA in the distal small intestine were similar to studies with growing pigs (Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1993), as were the concentration of SCFA throughout the various segments of the large intestine (Argenzio and Southworth, 1975; Bach Knudsen et al., 1993). In the present study, the greater concentration of SCFA in Co1 and Co2 is consistent with the observation that in growing pigs the cecum and proximal colon are the sites with the greatest saccharolytic activity (Bach Knudsen et al., 1991). This is also the case in the present study, which demonstrated greater concentration of SCFA in Co1 and Co2 than in other segments of the large intestine. Branched-chain fatty acids are primarily formed in regions with low saccharolytic activity (Macfarlane and

Table 4. Organic acids along segments of the gastrointestinal tract in sows fed low-fiber (LF), high-fiber 1 (HF1), or high-fiber 2 (HF2) diets and pH along segments of the gastrointestinal tract (mean of diets)¹

| Segment² | Total organic acids | Lactate | Short-chain fatty acids | Branched-chain fatty acids |
|----------|---------------------|---------|------------------------|---------------------------|
|          | LF | HF1 | HF2 | LF | HF1 | HF2 | LF | HF1 | HF2 | LF | HF1 | HF2 | pH |
| Si3      | 43 | 39  | 33  | 21³ | 8³  | 7³  | 22 | 31  | 27 | ND³ | ND | ND | —   |
| Cescum   | 112 | 152 | 123 | ND | ND | ND | 109³ | 151³ | 121³ | 2.9³ | 1.5³ | 1.4³ | 6.02 |
| Co1      | 83³ | 146³ | 115³ | ND | ND | ND | 80³ | 143³ | 123³ | 3.4³ | 3.2³ | 2.4³ | 6.24 |
| Co2      | 65³ | 104³ | 85³ | ND | ND | ND | 62³ | 101³ | 82³ | 2.8³ | 3.7³ | 2.6³ | 6.62 |
| Co4      | 62³ | 92³ | 82³ | ND | ND | ND | 60³ | 87³ | 78³ | 2.8³ | 4.4³ | 3.1³ | 6.74 |
| SEM      | 4.4 | 0.7 | 4.7 | 0.15 | <0.001 | 0.04 | 0.015 | <0.001 | 0.04 | 0.015 | <0.001 | 0.04 |

P-value

Diet (D) <0.001 | <0.001 | <0.001 | <0.001 | 0.33
Segment (S) <0.001 | <0.001 | <0.001 | <0.001 | 0.03
D × S <0.001 | <0.001 | <0.001 | 0.03

²Within a row and nutrient component, means without a common superscript are different (P < 0.05).
¹Mean values for 4 sows per diet.
²Si3 = the last third of the small intestine; Co1 to Co4 = segment 1 to 4 of the colon.
³ND = nondetected.

Hansen, 1991; Cherbut et al., 1991; Glitsø et al., 1998). In agreement with these observations, the digestibility of NSP was greatest for diet HF1, intermediate for diet HF2, and least for diet LF. It should be noted that the degradation profile of the NSP along the large intestine reached a plateau at different sites, which to a large extent reflected the amount and solubility of NSP that reached the large intestine and the type of NSP degraded.

Fermentation in the large intestine produces SCFA as the major energy-containing end product, whereas LA is primarily formed in the stomach and small intestine (Argenzio and Southworth, 1975). The concentrations of LA and SCFA in the distal small intestine were similar to studies with growing pigs (Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1993), as were the concentration of SCFA throughout the various segments of the large intestine (Argenzio and Southworth, 1975; Bach Knudsen et al., 1993). In the present study, the greater concentration of SCFA in Co1 and Co2 is consistent with the observation that in growing pigs the cecum and proximal colon are the sites with the greatest saccharolytic activity (Bach Knudsen et al., 1991). This is also the case in the present study, which demonstrated greater concentration of SCFA in Co1 and Co2 than in other segments of the large intestine. Branched-chain fatty acids are primarily formed in regions with low saccharolytic activity (Macfarlane and

Table 5. Molar distribution (%) of short-chain fatty acids along segments of the gastrointestinal tract in sows fed low-fiber (LF), high-fiber 1 (HF1), or high-fiber 2 (HF2) diets³

| Segments² | Acetate | Propionate | Butyrate | Branched-chain fatty acids |
|-----------|---------|------------|----------|---------------------------|
|           | LF | HF1 | HF2 | LF | HF1 | HF2 | LF | HF1 | HF2 | LF | HF1 | HF2 |
| Ileum     | 92³ | 97³ | 93³ | 0  | 0  | 1 | 8.3³ | 2.6³ | 6.2³ | 0  | 0  | 0  |
| Cescum    | 69³ | 76³ | 76³ | 20³ | 17³ | 16³ | 8.3 | 6.3 | 6.8 | 2.4³ | 0.0³ | 0.4³ |
| Co1       | 63³ | 69³ | 69³ | 22³ | 18³ | 19³ | 11.5 | 10.1 | 10.3 | 2.7³ | 1.0³ | 1.1³ |
| Co2       | 61 | 60 | 60 | 23³ | 20³ | 22³ | 12.5³ | 17.1³ | 16.3³ | 4.3³ | 2.3³ | 2.1³ |
| Co3       | 62 | 61 | 59 | 24³ | 20³ | 25³ | 10.7³ | 15.4³ | 13.3³ | 4.7³ | 3.8³ | 3.2³ |
| Co4       | 66³ | 67³ | 61³ | 22³ | 21³ | 25³ | 8.9 | 8.9 | 11.6 | 4.8 | 5.0 | 4.0 |
| Feces     | 71³ | 74³ | 65³ | 18³ | 16³ | 21³ | 7.9³ | 6.9³ | 11.1³ | 5.2³ | 5.6³ | 4.7³ |
| SEM       | 1.5 | 1.0 | 0.51 | 0.23 |<0.001 | <0.001 | <0.001 | <0.001 |<0.001 |<0.001 |<0.001 |<0.001 |

P-value

Diet (D) <0.001 |<0.001 |<0.001 |<0.001 |<0.001
Segment (S) <0.001 |<0.001 |<0.001 |<0.001 |<0.001
D × S <0.001 |<0.001 |<0.001 |<0.001 |<0.001

²Within a row and nutrient component, means without a common superscript are different (P < 0.05).
³Mean values for 4 sows per diet.
⁴Co1 to Co4 = segment 1 to 4 of the colon. Data from ileum and feces are the mean values from another study (Serena, 2005) with 6 T-cannulated sows in a repeated 3 × 3 Latin square design were used. These data are therefore not included in statistical analysis for repeated measurements.
Cummings, 1991), and generally, there is a greater overall concentration when feeding a diet providing low amounts of fermentable carbohydrates (Macfarlane et al., 1992; Bach Knudsen et al., 1993; Macfarlane and Macfarlane, 2003). Accordingly, BCFA were present in cecum and Co1-Co2 in a greater proportion when feeding diet LF compared with the HF diets. The explanation is likely due to a shortage of fermentable carbohydrates leading to a shift to protein fermentation when feeding LF compared with the HF diets.

Studies showing the effect of SCFA concentration on pH in the intestinal tract are in disagreement with each other (Argenzio and Southworth, 1975; Fleming et al., 1985; Partanen and Mroz, 1999). We showed no significant difference in the pH in colonic material among the diets, although a greater concentration of SCFA in segment Co2 to Co4 was observed when feeding the HF diets compared with LF. We observed a general decrease in pH with an increased SCFA concentration throughout all segments of the large intestine.

The DM percentage and the amount of digesta retained throughout the different segments of the colon are in good agreement with the fermentation pattern of NSP from the different diets in the large intestine. Thus, the decreased DM percentage when feeding diet HF1 is a combined effect of high fermentability of NSP and a high microbial biomass formation, which makes the colonic material moister. At sites with the most significant degradation of NSP, Co1, and Co2, we showed a difference between DM percentage in colonic mate-

### Table 6. Morphology of segments of the gastrointestinal tract in sows fed low-fiber (LF), high-fiber 1 (HF1), or high-fiber 2 (HF2) diets

| Item                  | LF   | HF1  | HF2  | SEM  | P-value |
|-----------------------|------|------|------|------|---------|
| Mid small intestine   |      |      |      |      |         |
| Villi height, µm      | 443  | 444  | 493  | 23   | 0.70    |
| Villi area, µm²       | 45,195 | 45,191 | 49,750 | 2,019 | 0.68    |
| Villi density, n/mm   | 7.9  | 7.5  | 7.7  | 0.1  | 0.60    |
| Crypt depth, µm       | 213  | 243  | 232  | 14.0 | 0.70    |
| Crypt area, µm²       | 6,079 | 7,497 | 6,681 | 522  | 0.58    |
| Crypt density, n/mm   | 21.8 | 20.2 | 19.8 | 0.3  | 0.21    |
| Mitose, n/crypt       | 3.8  | 4.9  | 4.2  | 0.3  | 0.49    |
| Muscle, µm             | 395  | 619  | 390  | 59   | 0.22    |
| Cecum                 |      |      |      |      |         |
| Crypt depth, µm       | 441  | 505  | 407  | 22   | 0.18    |
| Crypt area, µm²       | 23,626 | 25,869 | 20,852 | 1,242 | 0.28    |
| Crypt density, n/mm   | 13   | 11.6 | 12.3 | 0.2  | 0.14    |
| Mitose, n/crypt       | 2.4  | 3.5  | 2.9  | 0.2  | 0.10    |
| Muscle, µm             | 718  | 874  | 590  | 65   | 0.28    |
| Mid colon             |      |      |      |      |         |
| Crypt depth, µm       | 330² | 492² | 389² | 25   | 0.01    |
| Crypt area, µm²       | 15,751² | 23,201² | 19,812² | 1,338³ | 0.06    |
| Crypt density, n/mm   | 15.5 | 15.4 | 14.0 | 0.3  | 0.50    |
| Mitose, n/crypt       | 1.2  | 1.6  | 1.0  | 0.1  | 0.13    |
| Muscle, µm             | 772  | 513  | 420  | 76   | 0.16    |

¹Means in a row without a common superscript are different (P < 0.05).
²Mean values for 4 sows per diet.

### Table 7. Staining area of mucins on the villi of the small intestine and in the crypts of the small intestine, cecum, and colon of sows

| Item                  | Neutral | Acidic | Sulfo | Staining area, % of total area |
|-----------------------|---------|--------|-------|-------------------------------|
|                      | Mean    | SEM    | Mean  | SEM  | Mean    | SEM    | Mean    | SEM    | Neutral | Acidic | Sulfo |
| Mid small intestine   |         |        |       |      |         |        |         |        |         |        |       |
| Villi                 | 3,249   | 354    | 2,200 | 116  | 2,494   | 134    | 7       | 5      | 6       |
| Crypt                 | 1,455   | 94     | 1,165 | 83   | 314     | 36     | 22      | 17     | 5       |
| Cecum                 | 7,010   | 665    | 4,548 | 376  | 4,435   | 347    | 30      | 20     | 19      |
| Mid colon             | 8,427   | 546    | 5,822 | 484  | 7,295   | 609    | 44      | 30     | 37      |

¹Data are mean and SEM from all animals and all diets (n = 12).
²Neutral mucins (periodic acid-Schiff’s staining), acidic mucins (Alcian blue reaction at 2.5 pH), and sulfo mucins (Alcian blue reaction at 1.0 pH).
rial after feeding diet HF1 and HF2, but the difference disappeared as the digesta moved more distal in the colon (Co3 and Co-4). In contrast, diet HF2 (high in insoluble DF) acted as a fecal-bulking agent that holds water in the unfermented DF. This is also reflected in the amount of material in the colon being almost 2-fold greater when feeding diet HF2 compared with feeding LF diet. The amount of material in the stomach and small intestine was greater when feeding HF1 (not significantly different from HF2) in response to the greater water-binding capacity and swelling of this diet before degradation in the intestinal tract.

The total accumulated mean transit time of the large intestine was 13 h longer when feeding diet LF compared with the 2 HF diets. This is a reflection of the greater flow of digesta from the small to the large intestine when feeding HF diets (Serena, 2005). Transit time also depends on the length of the entire gut (Jørgensen et al., 1996), especially the length of the cecum-colon. However, our results did not indicate that the large intestinal length was affected by HF diets.

To our knowledge, it is the first time that morphological characteristics of intestinal tissue have been studied on sows, and most comparative data are from growing-finishing pigs (Brunsgaard, 1997, 1998; Hedeemann et al., 2005). In the present study, the weight of stomach, small intestine, and cecum did not vary significantly among diets as observed by Brunsgaard (1998). We showed a significantly greater weight of colon when feeding HF1 compared with LF, and these results are in agreement with studies by Jørgensen et al. (1996) and Eastwood and Brydon (1985). In contrast, Jin et al. (1994) were not able to show differences between feeding a high or low DF diet with respect to the weight of the large intestine in growing pigs. The greater tissue weight in the colon when feeding HF1 compared with LF is not caused by increased length but may be due to the greater supply of energy to the colonic epithelium when feeding HF1 as demonstrated by the greater absorption of SCFA to the portal vein Serena (2005).

With regards to the gut morphology, we showed a greater crypt depth in the colon when feeding the highly fermentable diet (HF1) compared with diet LF. This result was also shown by Jin et al. (1994) in growing-finishing pigs consuming a HF compared with a low DF diet (P < 0.1). However, in the present study, there was no difference between diet HF2 and diet LF, suggesting that the effect of DF on gut morphology was more ascribed to the type rather than the level of DF.

Previous research reported that DF increases the rate of turnover of intestinal mucosal cells in growing pigs (Jin et al., 1994; Brunsgaard, 1998). However, in the present study with sows, there was no difference in mitotic counts among dietary treatments, and the absolute level was only 10 to 17% of that of growing pigs. These data together with the greater number of crypts especially in the small intestine of sows compared with growing pigs (Brunsgaard, 1997) suggest a more mature gut of sows relative to growing pigs.

A 1.5 to 3.0 times thicker muscularis externa in sows relative to growing pigs (Brunsgaard, 1998) could be caused by the greater feed intake and a more coarse feed structure.

In our study, there were no differences among diets in mucins or in the crypts of the small intestine, cecum, and colon. This is in contrast to other studies in which high-DF diets compared with low-DF diets increased the number of mucin-secreting goblet cells in rats (Enns et al., 1994; Sakata, 1997), resulting in a possible increase in protection of the gut (Brownlee et al., 2003). A study by Shimotoyodome et al. (2001) showed that soluble DF increased fecal secretion, epithelial production, thickness of the mucus layer, and amount of luminal mucus. The reason why we did not observe any effect of the level or type of DF on the above-mentioned parameters could be due to the age of the animals and that feeding sows high-DF diets for 4 wk is not sufficient to change these mucin characteristics in the gut.

It is assumed that acidic and sulfo mucins can protect the gut against pathogenic bacteria (Belley et al., 1999; Deplancke and Gaskins, 2001). In sows, we detected a decreased area of acidic and sulfo mucins in the colon compared with the colon of growing pigs (Hedemann et al., 2002). The reason could be that the microflora utilize mucin as energy (Forstner, 1978; Deplancke and Gaskins, 2001) and that the gut in sows is protected by a significant number of stable microflora (Guarner and Malagelada, 2003). The area of mucins on the villi is 2 to 5 times greater than that observed for growing pigs (Hedemann et al., 2005). Increased mucin-staining area in growing pigs has been associated to increased binding of Salmonella to ileal tissue. Whether this is the case in sows as well needs to be further investigated.

Although we believe that the observed differences in morphology of growing pigs compared with sows is a consequence of aging, it is also important to consider that during the lactation period sows have consumed in the order of 7 to 8 kg of feed per day. The differences introduced by the diets in the study period in this perspective may be relative small.

In conclusion, our results show that the LF diet was well digested in the small intestine and resulted in substantially less materials in all segments of the gastrointestinal tract. In the large intestine, sows fed LF were depleted of fermentable carbohydrates in the mid colon, whereas for the 2 high-DF diets, the colon contained more digesta, had a decreased DM content, and was depleted of fermentable carbohydrates at more distal locations. The various polysaccharides of NSP were broken down at different sites of the large intestine. The diets affected concentration of SCFA in the large intestine but had only a minor effect on SCFA proportions. The morphology of the intestinal tract of sows was different from that of growing pigs, and feeding sows a diet containing a high amount of soluble DF resulted in significantly morphological changes in the colon compared with the low DF diet.
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