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The Effects of Cellulose, Pectin and Starch on Standardized Ileal and Apparent Total Tract Amino Acid Digestibilities and Bacterial Contribution of Amino Acids in Feces of Growing Pigs

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ABSTRACT : Eight ileally cannulated pigs (BW 35.9±0.9 kg) were randomly allotted according to a 4×3 Latin square design to determine the effects of cellulose, pectin and starch on standardized ileal digestibility (SID) and apparent total tract digestibility (ATTD) of crude protein (CP) and amino acids (AA) as well as on the bacterial AA contribution in feces. The pigs were fed the control diet (20.2% CP, % dry matter (DM)) or one of the three experimental diets in which 25% of the control diet was substituted by cellulose, starch or pectin. Due to this substitution, dietary CP levels were lower in the cellulose (15.5% CP, % DM), pectin (15.4% CP, % DM) and starch diet (15.2% CP, % DM). Following a 15-d adaptation period, feces were collected for 5 d and ileal digesta for a total of 24 h. Starch increased SID of CP, while cellulose and pectin had no significant effect on the digestibility of CP. Overall, starch supplementation resulted in higher ($p<0.05$) SID values of histidine, isoleucine, threonine, alanine, aspartic acid, cysteine, glycine and serine compared with cellulose, while pectin decreased ($p<0.05$) SID of valine and proline compared with the starch and control diet. Both cellulose and pectin reduced ($p<0.05$) the ATTD of CP and AA, while starch decreased ($p<0.05$) ATTD of phenylalanine, alanine, proline and serine compared with the control. With regard to bacterial AA composition of the fecal mixed bacterial mass (MBM), cellulose supplementation increased ($p<0.05$) its content of N and almost all AA, except for valine, while pectin caused higher contents of arginine, histidine and proline compared with the control ($p<0.05$). The bacterial contribution of arginine in feces was higher ($p<0.05$) in the cellulose treatment, while pectin reduced ($p<0.05$) the bacterial contribution of leucine, alanine, glutamic acid and proline in feces compared with the control. In conclusion, the effects of cellulose, starch and pectin on SID were rather small. Bacterial activity in the large intestine can only explain the reduced ATTD values for arginine in the cellulose treatment, but not for the other AA in the cellulose and pectin treatments, suggesting higher endogenous losses of these AA in the large intestine. (**Key Words :** Amino Acids, Bacteria, Carbohydrates, Standardized Ileal Digestibility, Dietary Fiber, Pig)

INTRODUCTION

It is well accepted that alternative feed ingredients, also referred to as by-products, may have economical advantages in diet formulation for pigs. In general, these by-products which are mainly produced from cereal grains contain a broad range of dietary fiber components (Souffrant, 2001), such as cellulose and pectin. Dietary fiber has been described as an anti-nutrient as it has been associated with depressed nutrient digestibility and energy utilization (Wang et al., 2004; Yin et al., 2004). In particular, reductions in apparent ileal digestibilities (AID) of crude protein (CP) and amino acids (AA) have been ascribed to the presence of fiber in diets for pigs, but the results obtained are equivocal. Some authors observed a significant reduction in AID of CP and AA (Mosenthin et al., 1994), while others found hardly any effect (Wang et al., 2006). The main concern with using AID values in diet formulation for pigs is that AID values increase in a non-linear manner with the dietary CP and AA levels (Stein et al., 2007). By using standardized ileal digestibilities (SID) of CP and AA, which are corrected for basal ileal endogenous losses of CP and AA ($IAAL_B$), this disadvantage of AID values does not exist any more (Stein et al., 2007). However, information on the effect of different purified sources of fiber on SID values is still lacking. In addition, dietary fiber may influence the density and composition of the microbiota in the gastrointestinal tract of pigs, particularly in the large intestine, as the fermentation

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Table 1. Formulation of the control diet, as-fed basis

Ingredient (%)	Control diet
Corn	57.3
Soybean meal	14.0
Cornstarch	10.0
Sugar beet pulp	5.0
Potato protein	5.0
Dried egg white	3.0
Soy oil	2.0
Limestone	1.2
Vitamin-mineral premix[1]	1.0
Dextrose	1.0
DL-methionine	0.1
L-tryptophan	0.1
TiO_2	0.3

[1] Vitamin-mineral premix (BASU-Mineralfutter GmbH, Bad Sulza, Germany) provided per kg of diet: vitamin A, 4,000 IU; vitamin D_3, 500 IU; vitamin E, 15 IU; menadione, 150 μg; thiamine, 1.7 mg; riboflavin, 2.5 mg; pyridoxin, 3 mg; cobalamin, 18 μg; pantothenic acid, 10 mg, niacin, 15 mg; folic acid, 0.25 mg; biotin, 20 μg; choline chloride, 500 mg; Ca, 1.5 g; Na, 1 g; Mg, 500 mg; Zn, 100 mg; Fe, 100 mg; Mn, 20 mg; Cu, 6 mg; Co, 75 μg; I; 200 μg; Se, 300 μg.

of complex fibers mainly occurs in this gut segment (Bach Knudsen and Jørgensen, 2001; Wang et al., 2002). Therefore, changes in bacterial AA composition and excretion at the fecal level can be expected.

The objectives of the current study were to determine in growing pigs the effects of cellulose and pectin as purified fiber sources in comparison to cornstarch on SID of CP and AA. Secondly, the effect of these carbohydrates both on apparent total tract digestibility (ATTD) of CP and AA and bacterial AA composition and excretion in feces was studied.

MATERIALS AND METHODS

The experiment was conducted at the Institute of Animal Nutrition of the University of Hohenheim (Stuttgart, Germany). The experimental proposal, surgical procedures, and procedures for care and treatment of the pigs were reviewed and approved by the German Ethical Commission of Animal Welfare of the Provincial Government of Baden-Wuerttemberg. Care of the animals used in this experiment was in accordance with the guidelines established by the German Regulation for Care and Treatments of Animals (Lorz and Metzger, 1999).

Animals

Management of pigs and preparation of the experimental diets were previously described in detail (Metzler et al., 2007). Briefly, eight barrows (German Landrace×Piétrain) were fitted with a simple T-cannula at the distal ileum with an average BW of 35.9±0.9 kg according to the surgical procedures described by Li et al. (1993). The cannulas were prepared from polyvinylidenfluorid (internal diameter of the barrel 21 mm; wings 80 mm long and 21 mm wide; diameter of the retaining ring 60 mm; thread on the outside of the barrel, retaining ring and cap). The animals were housed individually in stainless steel metabolism crates (0.80 m×1.50 m) being able to freely move around and having visual contact with each other. Each metabolism crate was equipped with a low-pressure drinking nipple, which allowed free access to demineralized water. The room temperature was kept at 22±2°C. During the experiment, the skin around the cannula was cleaned with lukewarm water several times daily, dried and provided with a skin protecting paste (Stomahesive Paste, Convatec, Princeton, USA). Additionally, foamed material was put between the retaining ring and the skin to absorb leaking digesta in order to prevent erythrema. The pigs were allowed a recuperation period of 10 d. During this period, the feed allowance of a 20% CP (as-fed basis) grower diet was gradually increased, starting with 100 g/d the day after surgery until 1,000 g/d were consumed.

Experimental diets

The control diet was based on corn and soybean meal, and was ground to pass a sieve of 3.0 mm (Table 1). The control diet was formulated to meet or to exceed nutrient requirements for pigs of approximately 50 kg according to DLG (1991) recommendations. The daily P supply was adjusted at a level of 3 g/kg diet so that the apparent P absorption is not affected by intestinal homeostasis (Zimmermann et al., 2002). Titanium dioxide (TiO_2) was included as digestibility marker. The pigs received the control diet or one of the three experimental diets in which 25% of the control diet was either substituted by lignocellulose (Jeluxyl WEHO 500S, Jelu, Rosenberg, Germany), cornstarch (Sabamuehle GmbH, Nuernberg, Germany) or apple-pectin (Apple pectin Classic AU 202, Herbstreith & Fox KG, Neuenbuerg, Germany), in the following referred to as cellulose, starch and pectin treatment, respectively. Cellulose, pectin and starch contained 92.1, 91.1 and 87.9% DM and 1.5, 1.1 and 0.4% CP, (% DM), respectively. The CP and AA contents both of the control and experimental diets followed DLG (1991) recommendations for growing pigs. The carbohydrates were added separately to the control diet and mixed thereafter before being fed. The diets were fed as slurry by adding demineralized water (starch diet, 1:1 w/w; cellulose diet, 1:3 w/w; pectin diet, 1:2 w/w).

Experimental procedure

The pigs were randomly allotted to the experimental diets according to a duplicated 4×3 Latin square design. The diets were fed at a rate of approximately 2.1 times the maintenance requirement for metabolic energy (i.e. 106 kcal/kg $BW^{0.75}$) which corresponds to 1,000, 1,200 and 1,400 g/d (as-fed basis) in experimental period I, II and III,

respectively. The animals were fed twice daily, equal amounts each meal, at 0700 and 1900 h. Each experimental period comprised 22 d including an adaptation period of 15 d. To adapt the pigs to the experimental diets, the inclusion level of the supplemental carbohydrates was gradually increased from 5 to 25% within the first 8 d of the adaptation period. Total collection of feces was initiated at 0700 h on d 16, and ended on d 21 at 0700 h of each experimental period. Feces were collected using 3 L PE-bags and a silicon ring, which was attached to the anus region by means of skin adhesive (Medical Adhesive, Hollister, Libertyville, Illinois, USA) and sticking plaster. The bags were changed anytime feces were voided. Feces were stored at -32°C until freeze-dried. Ileal digesta were collected for a total of 24 h in two 12-h intervals: from 0700 to 1900 h on d 21, and from 1900 on d 22 to 0700 h the following day. The collection procedure was adapted from Li et al. (1993) using plastic tubings attached to the barrel of the cannula by elastic bands. The tubings contained 7 ml of 2.5 M formic acid to minimize further microbial activity. They were changed at least every 30 min or when the tubings were filled with digesta. The ileal digesta was stored at -32°C until freeze-dried. Feed intake was recorded daily. Body weight was measured at surgery and at the end of the experiment. Pigs which lost their hard cannulas during the experiment were fitted with soft cannulas prepared from a plastisol solution (Techniplast, FH & Sons Mfg., Ltd., Rexdale, ON, Canada) according to procedures adapted from Li et al. (1993).

Isolation of fecal mixed bacterial mass

After the conclusion of the experiment, fecal samples were thawed, and one aliquot was freeze-dried whereas the second aliquot was used to isolate fecal mixed bacterial mass (MBM). The isolation of the MBM was adapted from procedures described for the isolation of rumen bacteria (Yang et al., 2001). Initially, 500 g of the homogenized fecal sample were suspended in 1,000 ml of a solution containing 0.9% saline and thoroughly stirred in a Waring mixer (Waring Products Division, New Hartford, CT) for 2 min at high speed before being filtered through a double layer of cheese cloth. This procedure was repeated once. Afterwards, the MBM was isolated via differential centrifugation steps. The filtrate was centrifuged at 800×g for 30 min at 4°C (Megafuge 2.0R, Heraeus, Hanau, Germany). The supernatant was immediately transferred into a beaker to avoid renewed mixing of liquid and solid phase, and stored on ice until the start of the next centrifugation step. The second centrifugation step was performed at 27,000×g for 40 min at 4°C (Sorvall RC-5B Refrigerated Superspeed Centrifuge, DuPont Instruments, Bad Nauheim, Germany). The residue was transferred into plastic bottles by means of deionized water and stored at -32°C until being freeze-dried. After freeze-drying and prior to analyses of DM, nitrogen (N), and AA, the MBM was ground to pass through a 0.5 mm mesh screen using a lab ball mill (Mixer Mill MM2000; Retsch, Haan, Germany). Due to small sample size of MBM, contents of methionine and cysteine were not determined.

Chemical analyses

Samples of diets, carbohydrates and freeze-dried ileal digesta and feces were ground to pass a mesh screen of 0.5 mm. Thereafter, they were homogenized per animal prior to analyses. Analyses for DM, CP (N×6.25) and NDF in the control, cellulose, starch and pectin treatment as well as in ileal digesta and fecal samples were performed according to Naumann and Bassler (1997). The content of TiO_2 in the control diet as well as in fecal and ileal digesta samples was measured by means of Inductively Coupled Plasma Spectrometer (ICP-OES; JY 24, Jobin Yvon GmbH, Grasbrunn, Germany). Amino acids including diaminopimelic acid (DAP) in the control diet, ileal digesta and feces samples were determined according to Naumann and Bassler (1997). The amino acid composition of the hydrolyzates was determined with an automatic amino acid analyzer (Amino acid analyzer LC 3000, Eppendorf Biotronic, Hamburg, Germany).

Calculation and Statistical analysis

Apparent ileal (AID) and apparent total tract digestibility (ATTD) of CP and AA (%) was calculated using TiO_2 as digestibility marker according to equation (1):

$$AD_{CP\ or\ AA}\ (\%) = 100-((I_D \times D_I)/(I_I \times D_D)) \times 100\% \quad (1)$$

where $AD_{CP\ or\ AA}$ is the apparent digestibility of CP or AA in ileal digesta or feces (%), respectively; I_D is the marker concentration in the assay diet (g/kg of DM); D_I is the concentration of CP or AA in ileal digesta or feces (g/kg DM); I_I is the marker concentration in ileal digesta or feces (g/kg of DM); and D_D is the concentration of CP or AA in the assay diet (g/kg DM).

Standardized digestibility of CP and AA in ileal digesta (%) was calculated as described by Stein et al. (2007) according to equation (2):

$$SID_{CP\ or\ AA}\ (\%) = AID_{CP\ or\ AA}\ (\%) + (IAAL_B/D_D) \times 100\% \quad (2)$$

where $SID_{CP\ or\ AA}$ is the standardized digestibility of CP or AA in ileal digesta (%), $AID_{CP\ or\ AA}$ is the apparent digestibility of CP or AA in ileal digesta (%), and $IAAL_B$ are the basal ileal endogenous CP and AA losses (g/kg of

Table 2. Partial proximate nutrient and amino acid composition (% DM) and energy content (MJ ME/kg DM) of the control diet

	Control diet
DM	89.3
CP	20.2
NDF	12.3
ME[x]	15.7
Amino acids	
Indispensable	
Arginine (Arg)	1.09
Histidine (His)	0.51
Isoleucine (Ile)	0.80
Leucine (Leu)	2.02
Lysine (Lys)	1.09
Methionine (Met)	0.50
Phenylalanine (Phe)	1.06
Threonine (Thr)	0.89
Valine (Val)	0.99
Dispensable	
Alanine (Ala)	1.20
Aspartic acid (Asp)	1.95
Cysteine (Cys)	0.37
Glutamic acid (Glu)	3.27
Glycine (Gly)	0.84
Proline (Pro)	1.19
Serine (Ser)	1.11

[x] Calculated according to DLG (1991).

dry matter intake). Values for $IAAL_B$ were obtained from a literature survey (Jansman et al., 2002).

The bacterial contributions of N and AA in feces (%) were calculated as described by Caine et al. (1999) from the contents of DAP and N or AA in the MBM and feces using equation (3):

$$(DAP_{feces}/DAP_{MBM})\times(N_{MBM}/N_{feces})\times100\% \quad (3)$$
or
$$(DAP_{feces}/DAP_{MBM})\times(AA_{MBM}/AA_{feces})\times100\%$$

The data of this study were analyzed according to an incomplete (4 treatments×3 periods) duplicated Latin Square using the mixed procedure (PROC MIXED) of SAS (2001). For the determination of any influential observation on the model, the Cook's distance (Cook's D) test was used as measure. Any observation having a Cook's D greater than 0.5 was considered as influential and hence deleted from further analysis. The model included the effect of animal within the square, period and treatment. Animal and period were considered as random effects assuming a first order autoregressive variance-covariance structure (AR (1)). Degrees of freedom were approximated using Kenward-Rogers method (ddfm = kr). Significance level was set at $p\leq0.05$ and trends are discussed at $0.05<p<0.1$.

RESULTS

The animals recovered well from surgery. The average BW was 39.7±1.3 kg and 62.9±4.4 kg at the start and conclusion of the experiment, respectively. In total, three animals were removed from the experiment. One animal was removed due to loss of the cannula in the second

Table 3. Standardized ileal digestibility coefficients (%) of CP and AA in pigs fed the experimental diets[x]

Item (%)	Treatment				p-value	Trend ($p<0.1$)[y]
	Control	Cellulose	Pectin	Starch		
CP	75.1±2.58[ab]	69.8±1.14[a]	76.2±5.19[ab]	84.1±3.17[b]	0.08	-
Amino acids						
Indispensable						
Arg	82.7±2.32	82.9±2.53	81.9±4.63	88.5±2.46	0.63	-
His	77.0±2.32[ab]	74.9±1.21[a]	76.2±4.65[ab]	83.4±2.82[b]	0.24	-
Ile	75.2±2.51[ab]	74.6±1.53[a]	75.9±5.04[ab]	84.6±3.03[b]	0.32	-
Leu	78.5±2.12	77.8±1.71	76.8±4.23	84.6±2.46	0.49	2 vs. 4
Lys	75.7±2.31	74.2±1.81	76.3±4.61	81.6±2.69	0.47	2 vs. 4
Met	85.3±1.79	86.2±2.10	78.4±3.20	87.9±2.04	0.22	3 vs. 4
Phe	77.9±2.24	77.0±1.97	77.3±4.47	84.9±2.55	0.49	2 vs. 4
Thr	71.2±2.93[ab]	65.2±1.51[a]	72.5±5.89[ab]	80.4±3.58[b]	0.15	-
Val	75.4±4.22[a]	71.3±4.90[ab]	58.3±6.06[b]	79.7±4.80[a]	0.07	-
Dispensable						
Ala	73.0±2.65[ab]	70.3±1.33[a]	71.6±5.33[ab]	80.2±3.24[b]	0.21	-
Asp	74.5±2.36[ab]	71.3±1.19[a]	74.3±4.74[ab]	82.1±2.89[b]	0.17	-
Cys	73.4±3.29[ab]	63.5±3.87[a]	60.4±5.91[ab]	77.8±3.77[b]	0.11	1 vs. 3; 3 vs. 4
Glu	79.9±2.37	78.3±2.35	76.7±4.71	86.1±2.60	0.51	-
Gly	71.3±4.49[ab]	68.5±1.93[a]	73.5±9.03[ab]	81.1±5.53[b]	0.16	-
Pro	85.6±3.03[a]	80.9±3.49[ab]	68.6±4.92[b]	90.2±3.22[a]	0.06	-
Ser	76.4±2.27[ab]	72.3±0.98[a]	77.7±4.56[ab]	85.4±2.79[b]	0.08	-

[x] Values represent LS means±SEM. [y] 1 = Control, 2 = Cellulose treatment, 3 = Pectin treatment, 4 = Starch treatment.
[a, b, c] LS means in the same row with different letters differ at $p<0.05$.

Table 4. Apparent total tract digestibility coefficients (%) of CP and AA in pigs fed the experimental diets[x]

Item (%)	Treatment				p-value	Trend (p<0.1)[y]
	Control	Cellulose	Pectin	Starch		
CP	87.0±0.51[a]	74.9±0.59[b]	76.3±0.79[b]	88.2±0.55[a]	<0.01	-
Amino acids						
Indispensable						
Arg	91.8±0.70[a]	81.1±0.79[b]	78.7±0.99[b]	90.4±0.77[a]	<0.01	-
His	91.1±0.70[a]	77.6±0.78[b]	76.8±0.98[b]	89.2±0.76[a]	<0.01	-
Ile	87.4±0.89[a]	69.5±0.61[b]	65.6±1.70[b]	83.3±1.07[a]	<0.01	1 vs. 4; 2 vs. 3
Leu	90.0 ± 0.50[a]	76.5±0.56[b]	75.0±0.70[b]	88.4±0.55[a]	<0.01	1 vs. 4
Lys	87.0±1.84[a]	68.3±2.38[b]	69.5±2.97[b]	85.1±2.13[a]	0.01	-
Met	89.4±0.77[a]	76.5±0.79[b]	73.3±1.16[b]	87.4±0.82[a]	<0.01	2 vs. 3
Phe	88.9±0.48[a]	74.2±0.39[b]	71.7±0.90[b]	86.2±0.58[c]	<0.01	-
Thr	85.0±0.86[a]	64.1±1.03[b]	65.6±1.29[b]	82.3±0.92[a]	<0.01	-
Val	85.8±1.25[a]	68.6±1.39[b]	69.0±1.73[b]	84.1±1.36[a]	<0.01	-
Dispensable						
Ala	86.2±0.77[a]	65.6±0.93[b]	64.3±1.23[b]	82.7±0.84[c]	<0.01	-
Asp	88.3±0.92[a]	81.1±0.79[b]	78.7±0.99[b]	90.4±0.77[a]	<0.01	-
Cys	89.2±1.03[a]	73.3±1.24[b]	72.7±1.54[b]	87.2±1.11[a]	<0.01	-
Glu	91.8± 0.44[a]	78.5±0.45[b]	76.1±0.77[b]	89.7±0.51[a]	<0.01	-
Gly	85.1±1.11[a]	65.8±1.40[b]	66.6±1.68[b]	81.3±1.17[a]	<0.01	1 vs. 4
Pro	91.6±0.39[a]	78.2±0.36[b]	77.0±0.71[b]	89.7±0.45[c]	0.03	-
Ser	89.2±0.50[a]	73.9±0.57[b]	74.3±0.78[b]	87.1±0.54[c]	<0.01	-

[x] Values represent LS means±SEM. [y] 1 = Control, 2 = Cellulose treatment, 3 = Pectin treatment, 4 = Starch treatment.
[a, b, c] LS means in the same row with different letters differ at p<0.05.

experimental period, while two other pigs were excluded from the experiment in the third experimental period due to feed refusal and peritonitis, resulting in total in 6, 5, 5, and 4 observations for the control, cellulose, pectin and starch treatment, respectively. The analyzed chemical composition of the control diet is presented in Table 2. Due to the substitution of 25% of the control diet with cellulose, pectin or starch, the dietary CP contents were 20.2, 15.5, 15.4 and 15.2% (% DM) for the control, cellulose, pectin and starch treatment, respectively.

Standardized ileal digestibility of CP and AA

Standardized ileal digestibility coefficients of CP and AA are presented in Table 3. Starch inclusion increased SID values between 2.6 and 9.8 percentage units as compared with the control diet. These differences failed, however, to be significant. Moreover, there were no differences in SID values between the cellulose and control treatment. Pectin inclusion decreased (p<0.05) the SID of Val and Pro by 17 percentage units and tended (p<0.1) to decrease the SID of Cys by 13 percentage units compared with the control diet. Cellulose supplementation decreased SID values for CP, His, Ile, Thr, Ala, Asp, Cys, Gly and Ser (p<0.05), and there was a trend towards lower (p<0.1) SID values for Leu, Lys and Phe compared with the starch treatment. Moreover, the comparison of the starch with pectin treatment revealed for most AA up to 22 percentage units lower SID values in the pectin treatment. These differences were significant (p<0.05) for Val, and Pro and there was a trend (p<0.1) for Met and Cys. There were no significant differences in SID coefficients of CP and AA between the cellulose and pectin treatment.

Apparent total tract digestibility of CP and AA

Apparent total tract digestibility values of CP and AA are shown in Table 4. The substitution of the control diet with pectin, cellulose and starch reduced the digestibility of CP (p<0.01) and AA (p<0.03). Moreover, there was an animal effect for ATTD of CP (p<0.01) and for ATTD of most AA (p≤0.05), except for Lys, Met, Thr and Gly. Overall, the ATTD of CP and all indispensable and dispensable AA were reduced (p<0.05) in pigs fed the diets supplemented with cellulose or pectin, by approximately 10 to 22 percentage units compared with control pigs. Starch supplementation decreased (p<0.05) the ATTD of Phe, Ala, Pro and Ser and tended (p<0.1) to reduce the values of Ile, Leu and Gly compared with the control diet. However, these ATTD values were consistently higher (p<0.05) than those obtained for the cellulose and pectin treatment. Moreover, the ATTD values of the indispensable AA Ile and Met tended (p<0.1) to be lower in pigs of the pectin compared with the cellulose treatment.

Bacterial amino acids in MBM

Bacterial N and AA contents in the MBM were affected (p<0.05) by dietary carbohydrate substitution (Table 5). Cellulose increased (p<0.05) the content of N and of all AA, except for Val, in the MBM compared with the control and

Table 5. Bacterial AA composition (g/kg DM) in the fecal mixed bacterial mass of pigs fed the experimental diets[x]

Item (g/kg DM)	Treatment				p-value	Trend (p<0.1)[y]
	Control	Cellulose	Pectin	Starch		
N	53.7±1.69[a]	62.3±1.99[b]	59.3±2.63[ab]	50.7±1.82[a]	0.04	-
DAP	2.2±0.33	2.0±0.32	1.8±0.41	2.3±0.31	0.85	-
Amino acids						
Indispensible						
Arg	12.4±0.59[a]	16.3±0.57[b]	14.4±0.73[b]	11.9±0.55[a]	<0.01	1 vs. 4; 2 vs. 3
His	5.4±0.29[a]	6.8±0.28[b]	6.4±0.36[bc]	5.4±0.27[ac]	0.02	3 vs. 4
Ile	15.5±0.78[a]	18.6±0.76[b]	16.4±0.98[ab]	14.4±0.73[a]	0.04	-
Leu	25.1±1.22[a]	29.7±1.18[b]	26.7±1.52[ab]	23.4±1.14[a]	0.04	-
Lys	20.0±1.14[ac]	24.8±1.09[b]	22.9±1.41[a]	18.1±1.06[c]	0.02	-
Phe	16.1±0.63[a]	18.9±0.61[b]	17.0±0.79[ab]	14.9±0.59[a]	0.02	3 vs. 4
Thr	17.9±0.67[a]	21.4±0.65[b]	18.8±0.84[ab]	16.2±0.63[a]	<0.01	2 vs. 3
Val	18.0±0.93	21.8±0.89	19.4±1.15	16.7±0.86	0.03	-
Dispensible						
Ala	22.9±1.15[a]	27.1±1.11[b]	24.4±1.44[a]	21.0±1.08[a]	0.04	-
Asp	31.9±1.75[a]	38.8±1.69[bc]	36.2±2.18[ac]	30.4±1.63[a]	0.04	3 vs. 4
Glu	36.2±1.86[ac]	43.3±1.79[b]	42.1±2.31[a]	33.7±1.73[c]	0.02	1 vs. 3
Gly	16.0±0.65[a]	18.9±0.62[b]	17.5±0.80[ab]	15.2±0.60[a]	0.02	3 vs. 4
Pro	11.3±0.38[a]	13.4±0.37[b]	13.4±0.47[b]	10.9±0.36[a]	<0.01	-
Ser	15.8±0.49[ac]	18.9±0.48[b]	16.8±0.62[c]	14.6±0.46[a]	0.04	-

[x] Values represent LS means±SEM. [y] 1 = Control, 2 = Cellulose treatment, 3 = Pectin treatment, 4 = Starch treatment.
[a, b, c] LS means in the same row with different letters differ at p<0.05.

starch treatment. Similarly, pectin increased (p<0.05) the contents of Arg, His and Pro and tended (p<0.1) to enhance the content of Glu in the MBM, compared with control treatment. Moreover, the inclusion of pectin in the diet stimulated the incorporation of Arg, Lys, Glu, Pro and Ser (p<0.05) in the MBM and tended (p<0.1) to increase His, Phe, Asp and Gly contents in the MBM in comparison with pigs fed the diet supplemented with starch. On the other hand, the dietary inclusion of starch did not change the AA composition of the MBM in comparison with the control treatment, except for Arg whose content tended (p<0.1) to be lower than in control pigs.

The contribution of bacterial N to total N in feces was similar in all treatments ranging from 62 to 67%, while the contribution of bacterial AA to total AA recoveries in feces ranged from 49.4 (Leu, pectin treatment) to 89.5% (Arg, cellulose treatment) for the indispensable AA, and from 43.4 (Pro, pectin treatment) to 78.4% (Asp, cellulose treatment) for the dispensable AA (Table 6). Overall, the bacterial contribution of Arg to its total fecal recovery was significantly higher and that of Lys tended (p<0.1) to be higher in the cellulose compared with the control treatment. Pectin supplementation, in turn, decreased (p<0.05) the bacterial contribution of Leu, Ala, Glu, and Pro in feces and tended (p<0.1) to lower the bacterial contribution of Arg, His, Phe and Ser to their total fecal recovery compared with the control treatment. Except for Ile and Val, bacterial contribution of AA to total AA in feces was lower in pigs fed the diet supplemented with pectin rather than cellulose. The bacterial contribution of Arg, Lys, and Gly to their total fecal recovery was lower (p<0.05) and tended (p<0.1) to be lower for Phe and Asp in the starch compared with the cellulose treatment. In contrast, there was no difference between pigs fed either the starch or the diet supplemented with pectin except for the bacterial contribution of Pro in feces which tended (p<0.1) to be lower in the pectin than in the starch treatment.

DISCUSSION

Results of studies in which the effect of dietary inclusion of cellulose and pectin on AID of CP and AA was determined are equivocal. For example, Mosenthin et al. (1994) reported a considerable decrease in AID of CP and AA due to the dietary inclusion of 7.5% pectin. However, in another study no or only minor effects of pectin or cellulose, supplemented at levels of 5 or 10% to the control diet on AID of CP and AA could be observed (Sauer et al., 1991).

Part of the discrepancies between different studies may be attributed to the ingredient composition of the assay diet used as control. For example, Den Hartog et al. (1988), similar to the present study, used a control diet based on cereal grains, and the supplemental carbohydrates were included at the expense of a proportion of the control diet. The authors reported only minor reductions in AID of AA which is general agreement with the results reported herein. Substitution of the control diet by 25% pectin reduced SID of only a few AA while cellulose inclusion had even no effect on the SID of CP and all AA. In contrast, in the study by Mosenthin et al. (1994), pectin was included in a

Table 6. Contribution of bacterial N and AA to total N and AA in feces (%) of pigs fed the experimental diets[x]

Item (%)	Treatment				p-value	Trend ($p<0.1$)[y]
	Control	Cellulose	Pectin	Starch		
N	67.0±5.27	64.9±5.08	61.7±6.56	60.9±4.92	0.84	-
Amino acids						
Indispensible						
Arg	70.7±4.42[a]	89.5±4.40[b]	58.8±5.24[a]	66.5±4.42[a]	<0.01	1 vs. 3
His	63.3±4.34[ab]	67.5±4.32[a]	51.2±5.14[b]	58.1±4.34[ab]	0.14	1 vs. 3
Ile	70.8±4.16	81.1±4.14	68.2±4.93	59.1±4.16	0.03	-
Leu	64.8±4.61[a]	69.6±4.59[a]	49.4±5.46[b]	61.3±4.61[ab]	0.08	-
Lys	69.4±3.80[ab]	79.5±3.79[a]	64.4±4.51[b]	65.7±3.80[b]	0.08	1 vs. 2
Phe	67.0±4.23[ab]	75.0±4.21[a]	54.6±5.02[b]	62.9±4.23[ab]	0.06	1 vs. 3; 2 vs. 4
Thr	65.8±4.73[ab]	71.5±4.71[a]	57.3±5.37[b]	62.4±4.73[ab]	0.17	-
Val	68.1±4.25	77.2±4.23	57.7±5.03	64.8±4.25	0.07	-
Dispensible						
Ala	67.1±3.88[a]	72.0±3.86[a]	53.5±4.60[b]	62.6±3.88[ab]	0.05	-
Asp	70.1±4.39[ab]	78.4±4.37[a]	60.2±5.20[b]	66.5±4.39[ab]	0.10	2 vs. 4
Glu	66.2±4.23[a]	67.6±4.21[a]	51.3±5.01[b]	62.0±4.23[ab]	0.11	-
Gly	62.7±4.11[ab]	72.0±4.10[b]	56.3±4.88[a]	58.6±4.11[a]	0.10	-
Pro	56.6±4.04[a]	57.5±4.02[a]	43.4±4.78[b]	54.4±4.04[ab]	0.15	3 vs. 4
Ser	66.9±3.92[ab]	71.2±3.90[a]	55.2±4.80[b]	61.0±3.92[ab]	0.15	1 vs. 3

[x] Values represent LS means±SEM. [y] 1 = Control, 2 = Cellulose treatment, 3 = Pectin treatment, 4 = Starch treatment.
[a, b, c] LS means in the same row with different letters differ at $p<0.05$.

purified assay diet at the expense of 7.5% of cornstarch which reduced AID of AA up to 29 percentage units. Thus, it may be assumed, that supplemental pectin or cellulose may have a variable effect on the SID of AA, depending on diet composition in general and, more specifically, on the composition of the carbohydrate fraction of the assay diet.

There is evidence that already small changes in the physico-chemical properties among purified fibers may affect nutrient digestibility, but also intestinal morphology and the bacterial ecosystem differently. For example, McDonald et al. (2001) observed variations in intestinal morphology, daily growth rate and bacterial cell counts between weaned pigs fed diets either supplemented with a low or high viscous carboxymethylcellulose. It has to be mentioned, however, that in most studies, specifications of the fiber sources are missing. In the present study, lignocellulose with a crude fiber content of >65% (specification of the producer, Jelu) and apple-pectin with a methylation degree of 68-72% (specification of the producer, Herbstreith and Fox) were used. Mosenthin et al. (1994), in turn, used citrus pectin, while Den Hartog et al. (1988) added apple-pectin and carboxymethylcellulose as source of pectin and cellulose, respectively, to the assay diet.

A reduction in SID values for CP and AA may result from a lower rate of digestion and absorption of dietary protein and (or) from an increase in specific ileal endogenous CP and AA losses (Stein et al., 2007). These losses are induced by diet characteristics such as content and type of fiber or antinutritional factors (Stein et al., 2007). De Lange et al. (1989) reported considerably higher ileal endogenous losses of Gly and Pro in pigs when either 4% pectin or 7% purified cellulose were included in a nitrogen-free control diet at the expense of cornstarch, which is in support of lower SID of Pro and Gly for the pectin and cellulose as compared with the starch treatment in the present study. Gly and Pro have a high contribution to ileal endogenous protein losses of pigs (De Lange et al., 1989). Moreover, in rats, a higher secretion of glycine-conjugated bile acids as well as lower absorption of bile acids has been observed when 10% citrus pectin was included in the diet (Ide et al., 1990). Thus, the lower SID of Met, Val, Cys and Pro in the pectin treatment compared with the starch treatment may, at least in part, be attributed to an increase in specific ileal endogenous CP and AA losses. Pectin, for example, has been shown to stimulate the secretion of endogenous protein into the small intestine (Souffrant, 1991). Furthermore, it has been associated with an increase in digesta viscosity (Souffrant, 2001), reduced interactions between substrates and digestive enzymes (Bedford and Schulze, 1998); thereby reducing the rate of nutrient absorption in the small intestine (Bach Knudsen, 2001). Therefore, fiber sources, such as pectin or cellulose may reduce the digestion and absorption of exogenous and endogenous proteins in the small intestine resulting in lower SID values for CP and AA.

In contrast to the SID values, ATTD of CP and AA were consistently reduced by cellulose and pectin supplementation. The disappearance of protein and AA in the large intestine and the synthesis of bacterial protein and AA may reflect the catabolic and anabolic properties of the

microbiota in the large intestine as previously reported (Mosenthin and Rademacher, 2003). Due to their complex structure, it is assumed that pectin and cellulose are mainly fermented in the large intestine (Bach Knudsen and Jørgensen, 2001; Wang et al., 2002), thereby stimulating bacterial protein synthesis. As more energy in the form of cellulose and pectin becomes available to the microbes in the large intestine, it can be expected that relatively more nitrogen is incorporated into bacterial protein, which is subsequently voided in feces (Sauer et al., 1991; Mosenthin et al., 1994). Hence, the decrease in ATTD of CP and AA in pigs fed diets supplemented with cellulose and pectin may be ascribed, at least in part, to an increase in the amount of bacterial AA voided in feces. Although the contents of N and almost all AA in the fecal MBM was increased due to cellulose supplementation, only the bacterial contribution of Arg and Lys to their total recovery in feces was higher in pigs fed the diet with cellulose compared with control pigs. In addition, although the bacterial contents of Arg, His, Glu and Pro in the MBM of the pectin treatment were higher compared with the control, the bacterial contribution of Arg, His, Leu, Phe, Ala, Glu, Pro and Ser to total fecal recovery of these AA was even reduced when pectin was included in the diet. Hence, higher bacterial N and AA concentrations in the MBM do not necessarily result in higher bacterial proportions of these AA to total AA in feces as assumed in previous studies (Sauer et al., 1991; Wang et al., 2006). Overall, the bacterial N contribution in feces of approximately 64% was not affected by treatment in the present study, and is in agreement with previous studies in which 50 to 90% of the total N in feces was of bacterial origin (Sauer et al., 1991; Mosenthin et al., 1994). However, data on bacterial contribution of N and AA in feces may be confounded by the fact that the concentration of DAP in different bacterial species may differ. In particular, some gram-positive bacteria are completely devoid of DAP (Dufva et al., 1982). Some of the main cellulolytic bacteria in the large intestine of pigs, such as *Clostridium herbivorans* and *Ruminococcus flavefaciens* (Varel and Yen, 1997), belong to gram-positive bacteria. Moreover, Metzler et al. (2007a) reported distinctly lower DAP concentrations in feces of pigs fed 25% of cellulose compared with control pigs, while the total bacterial cell counts in feces, determined by amplification of 16S ribosomal DNA using quantitative realtime PCR, tended to increase in pigs fed with cellulose rather than the control diet (Metzler et al., 2007b). Therefore, some caution should be applied when referring to bacterial N and AA contributions in feces based on DAP measurements.

Evidence has been presented that mucin production and release are stimulated by volatile fatty acids (Sakata and Setoyama, 1995). Particularly pectin inclusion has shown to increase the volatile fatty acid concentration in feces (Metzler et al., 2006), so that it is possible that enhanced mucin production in the large intestine of pigs fed the diet supplemented with pectin may have caused higher endogenous losses of AA, thereby contributing to the reduction in ATTD of CP and AA. Moreover, insoluble fiber has shown to increase mucin production and epithelial cell turnover in the colon of growing pigs (Jin et al., 1994) which may partly explain the lower ATTD in the cellulose treatment. In addition, Wang et al. (2006) speculated that the adsorption of AA and peptides of dietary, endogenous or bacterial origin by dietary fiber may withhold them from absorption, thereby reducing the ATTD of CP and AA.

In conclusion, dietary inclusion of 25% cellulose, pectin and starch had only moderate effects on the SID of CP and most AA. Significantly lower SID of Val and Pro were observed when pectin was added to the diet compared with the control, whereas the dietary inclusion of starch resulted in higher SID values of CP and most AA compared with the cellulose treatment and in higher SID of Met, Val, Cys and Pro compared with the pectin treatment. In contrast, the ATTD of CP and all AA was reduced in the cellulose and pectin treatment compared with the control and starch treatment. Although the bacterial contents of AA increased in the cellulose treatment and for some AA also in the pectin treatment, only the bacterial contribution of Arg and Lys to their total recovery in feces was increased in the cellulose treatment, while the bacterial contribution of Arg, His, Leu, Phe, Ala, Glu, Pro and Ser in feces of the pectin treatment was even reduced. In contrast, the bacterial contribution of the remaining AA to total AA in feces was not significantly affected by dietary fiber. Thus, other factors, such as higher mucin secretion in the large intestine, may have been responsible for lower ATTD values of CP and AA.

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