Impact of feeding fermentable proteins and carbohydrates on growth performance, gut health and gastrointestinal function of newly weaned pigs

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Jeaurond, E. A., Rademacher, M., Pluske, J. R., Zhu, C.H. and de Lange, C. F. M. 2008. **Impact of feeding fermentable proteins and carbohydrates on growth performance, gut health and gastrointestinal function of newly weaned pigs**. Can. J. Anim. Sci. **88**: 271–281. Feeding fermentable carbohydrates (FC) to weanling pigs may reduce the negative impact of proteolytic fermentation on gastrointestinal health and function. A total of 144 newly weaned pigs [6.23 kg body weight (BW); six pens per treatment; six pigs per pen) were used to determine the interactive effects of feeding additional fermentable protein (FP) and FC on growth performance, gastrointestinal function and intestinal health. Dietary treatments, based on a 2×2 factorial arrangement, were: (1) basal diet (control); (2) control + 10% poultry meal (PM) as FP source; (3) control + 5% beet pulp (BP) as FC source; and (4) control + 10% PM and 5% BP. Diets were formulated to be similar in digestible energy (DE) and digestible amino acid contents. In general, no interactive effects of FC and FP were observed ($P > 0.10$). During the 3-wk post-weaning period, feeding FP reduced average daily gain (ADG) (242 vs. 269 g d^{-1}; $P < 0.05$), while FC increased ADG (269 vs. 243 g d^{-1}; $P < 0.05$). Overall, feed intake did not differ between treatments ($P > 0.10$). On days 14 and 28 post-weaning, *Clostridia* spp. counts in colon contents, counts of white cells and segmented neutrophils in blood were lowered ($P < 0.05$) by feeding FC. Blood urea nitrogen was increased by feeding FP (9.5 vs. 6.5 mg dL^{-1}; $P < 0.05$), while ammonia concentration in colon contents was lowered by FC (154 vs. 193 μg mL^{-1}) ($P = 0.06$). Among biogenic amines, levels of tyramine (140 vs. 304 nmol g^{-1} DM) and spermidine (174 vs. 219 nmol g^{-1} DM) in colon contents were lowered ($P < 0.05$) by FC. Acetic, proprionic and butyric acid contents in colon contents were increased by feeding FC, while valeric and caproic acid content decreased by feeding FP ($P < 0.05$). Feeding FC and FP had no effect ($P > 0.10$) on colon histology, pH, fecal consistency score and organ weights. Results suggest that FP and FC have independent effects on newly weaned pigs, while effects appear partly related to changes in gut microbiota.

Key words: Enteric fermentation, fiber, gastrointestinal function, protein, pigs

Jeaurond, E. A., Rademacher, M., Pluske, J. R., Zhu, C.H. et de Lange, C. F. M. 2008. **Les protéines et les glucides fermentables et leur impact sur la croissance, la santé intestinale et le fonctionnement du système digestif des porcelets**. Can. J. Anim. Sci. **88**: 271–281. Donner des glucides fermentables (GF) aux porcelets sevrés peut atténuer l'incidence négative de la fermentation protéolytique sur la santé et le fonctionnement du tube digestif. Les auteurs ont recouru à 144 porcelets fraîchement sevrés (6,23 kg de poids corporel; 6 enclos par traitement; 6 sujets par enclos) pour préciser les interactions entre les protéines fermentables (PF) et les GF sur la croissance, le fonctionnement du système digestif et la santé intestinale. Les rations, dispensées selon un arrangement factoriel en 2×2, étaient les suivantes : (1) ration de base (témoin); (2) témoin + 10 % de farine de volaille comme source de PF; (3) témoin + 5 % de pulpe de betterave en tant que source de GF; (4) témoin + 10 % de farine de volaille et 5% de pulpe de betterave. Les rations étaient formulées pour contenir la même quantité d'énergie digestible et d'acides aminés digestibles. Dans l'ensemble, il n'y a pas d'interaction entre les PF et les GF ($P > 0{,}10$). Au cours des trois semaines qui ont suivi le sevrage, les PF ont diminué le gain quotidien moyen (242 c. 269 g par jour; $P < 0{,}05$), alors que les GF l'ont augmenté (269 c. 243 g par jour; $P < 0{,}05$). La prise alimentaire ne varie pas avec le traitement ($P > 0{,}10$). Le 14e et le 28e jour après le sevrage, le nombre de Clostridium sp. dans le contenu du côlon et celui de leucocytes et de neutrophiles cloisonnés dans le sang étaient plus faibles ($P < 0{,}05$) chez les sujets recevant des GF. Les PF augmentent la concentration d'urée dans le sang (9,5 c. 6,5 mg par dL; $P < 0{,}05$), mais les GF réduisent celle d'ammoniaque dans le côlon (154 c. 193 μg par mL; $P = 0{,}06$). Parmi les amines biogènes, les GF réduisent ($P < 0{,}05$) la concentration de tyramine (140 c. 304 nmol par gramme de matière sèche) et de spermidine (174 c. 219 nmol par gramme de matière sèche) dans le côlon. Les GF augmentent toutefois celle des acides acétiques, propionique

Abbreviations: **ADFI**, average daily feed intake; **ADG**, average daily gain; **BP**, sugar beet pulp; **BUN**, blood plasma urea nitrogen; **CFU**, colony-forming units; **DE**, digestible energy; **FC**, fermentable carbohydrates; **FP**, fermentable protein; **LAB**, lactic acid-producing bacteria; **PM**, poultry meal; **VFA**, volatile fatty acids; **WBC**, white blood cell

et butyrique dans le côlon alors que les PF diminuent celle des acides valérique et caproïque ($P < 0,05$). Les GF et les PF n'ont aucune incidence ($P > 0,10$) sur l'histologie du côlon, son pH, la consistance des fèces et le poids des organes. Les résultats laissent croire que les PF et les GF agissent indépendamment sur les porcelets nouvellement sevrés, et que leurs effets découlent en partie d'une modification de la microflore intestinale.

Mots clés: Fermentation entérique, fibres, fonctionnement du système digestif, protéines, porcs

For the formulation of pig diets, feed ingredients are routinely characterized based on digestible nutrient content, while little consideration is given to the impact of indigestible nutrients. The dietary content of undigested protein may unintentionally be increased by the inclusion of protein sources such as peas and poultry meal, and provide a substrate for microbial fermentation [de Lange et al. 2003; Centraal Veevoeder Bureau (CVB) 2004]. Proteolysis is the first step in the utilization of protein by bacteria. Subsequent deamination and decarboxylation of amino acids limits their availability to the host and yields several putrefactive compounds including ammonia, amines, branched fatty acids, indoles, phenols and sulfur-containing compounds (Swanson et al. 2002). These compounds can have toxic effects on pigs and can influence function and diversity of the gut microbiota (Gaskins 2003).

It is well documented that young pigs are faced with many changes and challenges at weaning (Pluske et al. 1997). These changes, particularly in environment and diet, increase the pigs' susceptibility to harmful micro-organism and enteric diseases, which are associated with decreases in feed intake and growth performance (van Beers-Schreurs et al. 1998). This susceptibility to disease is largely a result of the delicate balance between commensal and pathogenic bacteria in the gut of newly weaned pigs (Williams et al. 2001).

The inclusion of carbohydrates with prebiotic properties in pig diets to stimulate establishment of beneficial enteric micro-organisms may limit the proliferation of enteric pathogens and decrease formation of toxic fermentation products (Williams et al. 2001). When the availability of carbohydrates for microbial fermentation is limiting, undigested protein can be deaminated, used as an energy source, and yield potentially harmful compounds. On the other hand, microbes can utilize ammonia and amines to synthesize microbial protein, provided that sufficient fermentable carbohydrates are available (Ravindran et al. 1999). This would suggest there is an optimum balance between fermentable protein (FP) and fermentable carbohydrates (FC) in pig diets.

It was hypothesized that feeding FC is a means to reduce the negative impact of proteolytic fermentation on newly weaned pigs. The objective of this study was to determine the impact of feeding FP from poultry meal (PM) and FC from sugar beet pulp (BP) on the growth performance, health, proteolytic fermentation and gut microbiota of newly weaned pigs.

MATERIALS AND METHODS

Animals and Diets

This study was conducted at the Arkell Research Station of the University of Guelph and approved by the local Animal Care Committee. A total of 144 newly weaned Yorkshire pigs, with an average body weight of 6.23 ($\pm$0.53 kg) and 16 to 19 d of age, were selected from 38 litters. There were two equal and subsequent blocks of 12 pens of six piglets, with six pens per dietary treatment (Namkung et al. 2004). Based on a 2×2 factorial design, pens of pigs were exposed to one of the following four dietary treatments: (1) basal diet (control); (2) control + 10% PM (treatment FP); (3) control + 5% BP (treatment FC); and (4) control + 10% PM and 5% BP (treatment FP + FC) (Table 1). The pigs were fed ad libitum and had free access to water. The diets were formulated to have similar digestible energy (DE) and standardized ileal digestible lysine contents, while

Table 1. Ingredient composition (%) of experimental diets

Dietary treatment[z]	Control	FP	FC	FP + FC
Corn	47.00	43.49	42.17	38.71
Soybean meal	8.00	8.00	8.00	8.00
Fat (animal/vegetable) blend	2.00	2.00	2.00	2.00
Wheat	20.00	20.00	20.00	20.00
Whey[y]	10.00	10.00	10.00	10.00
Casein	10.00	4.80	10.00	4.80
Poultry meal[x]	–	10.00	–	10.00
Sugar beet pulp, dried[w]	–	–	5.00	5.00
Lysine·HCl	–	0.16	–	0.14
Methionine	0.05	0.10	0.05	0.05
Threonine	0.05	0.10	0.05	0.05
Titanium dioxide	0.10	0.10	0.10	0.10
Limestone	1.10	0.40	0.95	0.30
Dicalcium phosphate	1.10	0.25	1.13	0.25
Salt	0.10	0.10	0.10	0.10
Premix[v]	0.50	0.50	0.50	0.50

[z] Dietary treatments represent basal diet (control), basal diet with 10% poultry meal (source of fermentable protein; FP), basal diet with 5% sugar beet pulp (source of fermentable carbohydrate; FC) and basal diet with 10% poultry meal and 5% sugar beet pulp (FP + FC).
[y] Whey supplied by Pastells, Cambridge, ON, Canada.
[x] Poultry meal supplied by Rothsay, Dundas, ON, Canada.
[w] Sugar beet pulp, dried, supplied by ADM, Woodstock, ON, Canada.
[v] Mineral and vitamins premix supplied the following per kg of feed: Cu, 15 mg (from $CuSO_4 5H_2O$); Zn, 104 mg (from ZnO); Fe, 100 mg (from $FeSO_4 1H_2O$); Mn, 19 mg (from MnO); I, 0.3 mg (from $Ca[IO_3]_2$); Se 0.3 mg (from Na_2SeO_3); vitamin A, 10 000 IU; vitamin D, 1000 IU; vitamin E, 40 IU; vitamin K, 2.5 mg; choline, 570 mg; pantothenic acid, 16 mg; riboflavin, 5 mg; folic acid, 2 mg; niacin, 25 mg; thiamine, 1.6 mg; vitamin B6, 1.8 mg; biotin, 0.20 mg; vitamin B_{12}, 0.025 mg.

ensuring that lysine was the first limiting amino acid, but to differ in contents of FP and FC (Table 2). Estimates of nutrient content and digestibility values for the feed ingredients were derived from CVB (2004), and FP was calculated as the difference between ileal and fecal digestible crude protein (CP) contents in the feed ingredients. Estimated contents of FC in the diets varied between 5.94 and 8.51%. Diets were formulated to achieve rather extreme diet FP and FC levels to explore interactive effects between FP and FC (Williams et al. 2001; de Lange et al. 2003), and without compromising pig performance as a result of feeding too much BP (Schiavon et al. 2004).

Table 2. Calculated and analyzed content of the main nutrients in the experimental diets (%)[z]

Dietary treatment[y]	Control	FP	FC	FP+FC
Calculated content				
Digestible energy (MJ)	14.86	14.79	14.74	14.66
CP (%)[x]	20.15	21.87	20.17	21.77
Crude fat (%)	4.24	5.32	4.09	5.18
Apparent ileal digestible CP (%)	17.49	17.53	17.26	17.37
Apparent fecal digestible CP (%)	18.04	18.82	17.92	18.73
Fermentable CP (%)[w]	0.55	1.29	0.67	1.36
Fermentable carbohydrates (%)[v]	6.14	5.94	8.51	8.31
Lysine (%)	1.27	1.33	1.28	1.34
Methionine (%)	0.51	0.53	0.51	0.47
Methionine plus cysteine (%)	0.78	0.83	0.77	0.77
Threonine (%)	0.88	0.93	0.89	0.89
Tryptophan (%)	0.26	0.25	0.27	0.25
Standardized ileal digestible lysine (%)	1.18	1.18	1.18	1.17
Calcium (%)	0.81	0.81	0.80	0.80
Phosphorus (%)	0.65	0.65	0.65	0.65
Sodium (%)	0.20	0.22	0.21	0.23
Potassium (%)	0.61	0.65	0.63	0.67
Chloride (%)	0.25	0.33	0.25	0.33
Analysed content (%)				
CP	19.7	21.7	20.2	21.4
Starch	38.0	33.7	32.8	32.3
NDF	7.3	7.9	9.5	11.5
ADF	2.9	3.0	3.4	3.7
Ash	4.7	4.2	4.6	4.7
Crude fat	3.5	4.4	3.8	4.5
Lysine	1.14	1.29	1.20	1.30
Methionine	0.47	0.51	0.50	0.44
Methionine plus cysteine	0.71	0.80	0.75	0.73
Threonine	0.82	0.93	0.88	0.87
Calcium	1.09	0.75	0.78	0.90
Phosphorus	0.70	0.61	0.60	0.65
Sodium	0.32	0.25	0.28	0.26
Potassium	0.63	0.68	0.63	0.71

[z]Values were calculated based on nutrient contents and digestibility values according to Centraal Veevoeder Bureau (2004).
[y]See Table 1.
[x]CP, crude protein.
[w]Calculated as: apparent fecal digestible CP content – apparent ileal digestible CP content (%).
[v]Calculated from content and apparent fecal digestibility of remaining organic material (organic matter minus the sum of crude protein, crude fat, starch and sugars) in each of the ingredients.

Data and Sample Collection

Per-pen feed usage and BW of individual pigs was monitored at weekly intervals for 4 wk post-weaning. Feces were scored weekly for consistency by visual inspection on a per-pen basis and based on at least three randomly chosen fresh droppings per pen (scale of 1 to 5; loose to solid). The person conducting the feces consistency scoring was not aware of the assignments of treatments to pens. Fresh fecal samples were collected aseptically by rectal palpation from three randomly chosen pigs per pen at bi-weekly intervals, pooled per pen and stored at -20°C for subsequent analyses. For each of the diets, a representative sample was obtained as well, and stored at -20°C for nutrient analyses.

The median pig in each pen, based on BW, was sacrificed on days 14 and 28 post-weaning with an intravenous injection of 2.0 ml of Euthanasol (pentobarbital 340 mg mL^{-1}) (Schering-Plough, Pointe-Claire, QC) for sampling of digesta and intestinal tissue. The pH in fresh cecum and the colon contents was determined using an accumet probe AP63 (Fisher Scientific Company, Ottawa, ON). Digesta were sampled from the mid-colon and frozen immediately to characterize microbiota and nutrient contents. The liver, spleen and cecum were weighed and mid-colon tissue samples were taken for visual inspection of lesions. Digesta samples were obtained from the terminal ileum, to determine nutrient digestibility. In this procedure, a 1-m segment of the ileum just prior to ileo-cecal valve was isolated; its contents were then gently removed and stored frozen at -20°C.

Blood samples were collected from pigs that were sacrificed at days 14 and 28 post-weaning via orbital-sinus puncture to determine white blood cell (WBC) counts and blood plasma urea nitrogen (BUN) levels. Blood was collected in evacuated tubes containing EDTA for the measurement of WBC counts. Blood collected in non-heparinized evacuated tubes was centrifuged at $1200 \times g$ for 10 min to separate the plasma for measurement of BUN. Blood plasma was stored at -20°C for further analyses.

Sample Processing and Chemical Analyses

Frozen feces and digesta samples were freeze-dried and ground using a grinder (Thomas-Wiley Mill, Philadelphia, PA) through a 1-mm screen. Diets, and freeze-dried samples of ileal digesta and feces, were analyzed for analytical dry matter and titanium oxide contents according to the Association of Official Analytical Chemists (AOAC 1995) methods. Diets were analyzed for contents of ash, fat, starch (enzymatic method) according to AOAC (1995) methods and at Agri-Food laboratories (Guelph, ON). Contents of volatile fatty acids (VFA) in digesta were measured in thawed samples of mid-colon digesta using gas chromatography according to Zahn et al. (1997). Briefly, the standards and samples were run isothermally at 90°C for 18 min with the detector set at 300°C and injector set at 200°C

on a Varian GC 3400 with a Supelco Nucol column (15 mm, 0.53 mm i.d., 0.50-mm coating) and connected to a Varian Star Data Acquisition Workstation (Version 5.3;Varian Canada Inc. Mississauga, ON). Ammonia concentration was determined using the phenol-hypochlorite colorimetric method (Weatherburn 1967), while biogenic amines were determined using high performance liquid chromatography (University of Guelph, Laboratory Services Division, Guelph, ON) according to Salazar et al. (2000).

Feed, and freeze-dried samples of ileal digesta and feces were analyzed for crude protein contents using an induction furnace and thermal conductivity Nitrogen Gas Analyzer (LECO FP-428, LECO Corporation, 3000 Lakeview Ave., St Joseph, MI). The gross energy contents of diets and freeze-dried feces were determined using an IKA 5000 bomb calorimeter (IKA, Staufen, Germany). Diets and ileal digesta samples were analyzed for amino acid content according to Llames and Fontaine (1994) and in the laboratory of Degussa AG (Hanau, Germany).

Total WBC were determined and differentiated into different cell types according to Odink et al. (1990). Blood urea nitrogen (BUN) content was measured colorimetrically using a BUN diagnostic kit from Teco diagnostics (Anaheim, CA).

Assessment of Microbiota

Culturing of bacteria was performed with an emphasis on intestinal anaerobes: *Clostridia* spp., total coliforms and lactic acid-producing bacteria (LAB) (primarily *Bifidobacteria* spp. and *Lactobacillus* spp.). Colon contents were serially diluted (10^{-1} to 10^{-8}) with sterile PBS and cultured as described by Namkung et al. (2004). Coliforms were grown aerobically on a MacConkey agar (Oxoid Ltd., Hampshire, UK) and the colony-forming units (CFU) were counted after 24 h inoculation at 37°C. LAB were grown anaerobically on a MRS agar (Oxoid Ltd.) at 37°C for 48 h and *Clostridia* spp. was grown on Blood agar (Oxoid Ltd.) for 48 h and at 37°C.

Calculations and Statistical Analyses

Apparent ileal and apparent fecal nutrient digestibility values were derived according to Nyachoti et al. (1997) and using titanium dioxide as an indigestible marker. Growth performance data were calculated as described by Namkung et al. (2004).

The data were analyzed statistically using the mixed model procedure of SAS software (SAS Institute, Inc. 1999) to assess effects of dietary treatments. The experimental design was a 2×2 factorial arrangement in a randomized block design, where pen was the experimental unit for growth performance data and nutrient digestibility. For all other measures, pig was the experimental unit. The statistical model included the effects of pen ($n = 6$) or pig ($n = 12$) as random effects; block ($n = 2$), dietary treatment ($n = 4$; main effects of FP and FC and the FP $\times$ FC interaction) were considered fixed effects. When appropriate time of sampling ($n = 2$; 2 and 4 wk post-weaning) and interactions between the time of sampling and dietary treatments effects were considered as fixed effects. Pig BW at weaning was used as a co-variable where applicable. For analyses of growth performance and blood parameters data, the repeated measurements procedure of SAS was used as well; the Corrected Akaike Information Criterion was used to identify the covariance structure that fitted best the variances for weekly measurements and covariance between measurements across time periods. A probability of $P < 0.05$ was accepted as significant and differences between treatments with $P < 0.10$ were identified as trends.

RESULTS

All pigs seemed in good health and no signs of abnormalities in behavior were observed during this study. For reasons not related to treatments, seven pigs were removed from the trial. Because the dietary treatment had no effect on colon histology and fecal consistency score ($P > 0.10$), these results are not presented.

Analyzed contents of CP, lysine, methionine, methionine plus cysteine, threonine, calcium, phosphorus and sodium in diets were consistent across treatments and similar to calculated values (Table 2), indicating that the diets were prepared properly. For most amino acids, and in all diets, analyzed contents were slightly lower than anticipated, while this discrepancy appeared largest for the control diet (Table 2). Analyzed ash contents (Table 2) suggest that minerals supplied by PM were accounted for largely be reducing the contents of limestone and dicalcium phosphate in the PM-containing diets (Table 2). As anticipated, the inclusion of PM in the diets increased the analyzed crude fat contents by approximately 1 percentage unit, while the inclusion of BP in the diets increased the NDF content by 2.2 to 3.6 percentage units, reflecting the fat and NDF contents in these feed ingredients (CVB 2004).

Fecal DM digestibility showed an interactive effect ($P < 0.05$) of FC and FP; it was higher for the FP treatment than for the control treatment ($P < 0.05$), but when PM was including in the BP-containing diet (treatment FP + FC vs. treatment FC) it did not influence fecal DM digestibility (Table 3). Treatment effects on fecal energy digestibility tended to be similar to those for DM digestibility (Table 3; $P = 0.06$). The determined DE content was higher in the FP diet than in the control diet ($P < 0.05$) and intermediate in FC and FP + FC diets. Apparent ileal amino acid and fecal CP digestibility did not differ among the treatments ($P > 0.10$). However, there was a clear trend ($P = 0.07$) for PM containing diets (treatments FP and FP + FC) to contain more FP than diets with no PM (treatments control and FC) (Table 3).

Table 3. Determined apparent fecal digestibility (%) of dry matter, energy and crude protein (CP), diet DE content (MJ kg^{-1} DM), ileal digestibility (%) of CP and selected amino acids, and fermentable CP content (g kg^{-1}) in diets that vary in content of fermentable carbohydrates (FC) and fermentable protein (FP)[zy]

						P values[w]		
Dietary treatment	Control	FP	FC	FP+FC	SE[x]	FP	FC	FP × FC
Fecal dry matter digestibility (%)	83.7*b*	85.7*a*	85.1*ab*	84.5*ab*	0.58	0.214	0.804	0.026
Fecal energy digestibility (%)	85.8	87.4	86.7	86.1	0.58	0.377	0.780	0.063
Diet DE content (MJ kg^{-1} DM)	16.5*b*	17.3*a*	16.8*ab*	17.0*ab*	0.11	<0.001	0.735	0.011
Fecal CP digestibility (%)	78.5	80.4	80.5	79.0	1.06	0.784	0.831	0.129
Ileal dry matter digestibility (%)	65.4	64.2	71.3	68.1	5.30	0.688	0.363	0.855
Ileal CP digestibility (%)	67.4	56.5	66.5	64.0	5.70	0.220	0.999	0.185
Diet fermentable CP content (g kg^{-1}DMI)[x]	2.11	5.00	2.40	3.38	1.00	0.070	0.993	0.139
Apparent ileal digestibility (%) of selected amino acids								
Lysine	73.0	61.9	66.9	66.7	6.20	0.388	0.967	0.336
Methionine	81.3	71.3	76.9	72.7	4.10	0.085	0.700	0.474
Cysteine	48.1	39.2	53.1	52.6	9.10	0.604	0.310	0.642
Threonine	63.1	53.8	60.7	59.9	6.50	0.433	0.771	0.510
Diet ileal digestible lysine content (g kg^{-1}DM)	9.35	8.97	9.02	9.74	0.72	0.119	0.774	0.991

[z]See Table 1; values represent least square means.
[y]Standard error for treatment means based on $n = 6$.
[w]Probability of main effects of FP and FC, as well as their interaction (FP × FC).
[x]Calculated as Diet CP content × (fecal CP digestibility – ileal CP digestibility)/100.
a, *b* Means within a row followed by different letters differ ($P < 0.05$).

No interactive effects of feeding additional FP and FC on growth performance were observed ($P > 0.10$; Table 4). However, the response to dietary treatments differed between weeks ($P < 0.05$) and, therefore, weekly as well as overall growth performance is presented (Table 4). During the first week post-weaning, feeding additional FC did not influence ADG, average daily feed intake (ADFI) and gain:feed ($P > 0.10$). However, feeding additional FP tended to reduce average daily gain (ADG) ($P = 0.09$). During the second week post-weaning, feeding additional FP tended to decrease ADG ($P = 0.07$), while feeding additional FC tended to increase ADG ($P = 0.09$). During the third week post-weaning feeding additional FC increased ADG ($P < 0.05$). Including additional FP in the diet reduced ADG ($P < 0.05$) between days 1 and 21 post-weaning, while including additional FC in the diet increased ADG ($P < 0.05$) and tended to increase feed efficiency ($P = 0.09$) over that period. Over the entire 4-wk post-weaning period, feeding additional FP reduced ($P < 0.06$) ADG and ADFI, whereas dietary treatments did not influence gain:feed ($P > 0.10$).

There were no interactive effects ($P > 0.10$) of feeding additional FP and FC on organ weights, digesta pH, blood parameters and ammonia concentration in colon contents (Tables 5 and 6). Moreover, no interactive effects ($P > 0.10$) between dietary treatments and sampling time (2 vs. 4 wk post-weaning) were observed, and data were pooled across the two sampling times. Organ weights were not affected by dietary treatments ($P > 0.10$; Table 5). The pH of digesta from the cecum was not influenced by dietary treatment ($P > 0.10$). The counts of WBC and segmented neutrophils in blood plasma were lowered ($P < 0.05$) by the inclusion of additional FC in the diet (Table 5). Blood urea nitrogen was increased ($P < 0.01$) with the inclusion of additional FP, while ammonia concentration tended to be lowered ($P = 0.06$) by the inclusion of additional FC in the diet (Table 6).

The conventional culturing methods showed no interactive effects of feeding additional FC and FP on bacterial counts in colon contents ($P > 0.10$; Table 7). The counts of *Clostridia* spp. were lower in pigs fed additional FC ($P = 0.02$), and tended to increase ($P = 0.08$) by feeding additional FP (Table 7). Yet, no dietary treatment effects on counts of coliforms or LAB were found in this study ($P > 0.10$). The ratio of LAB:coliforms was greater than 100:1 across all treatments.

In regard to amine concentrations in colon contents (Table 8), an interactive effect of feeding additional FC and FP was observed for spermine; its concentration was higher for treatment FC than for the other treatments (32.2 vs. 18.7, 20.5 and 20.0 nmol g^{-1} DM SE 3.4; for FC vs. control, FP and FP+FC, respectively). However, the biogenic amine concentrations in mid-colon content were quite variable within pigs as reflected by the high standard error (Table 8). Feeding additional FC resulted in a decrease in tyramine ($P < 0.01$) and spermidine ($P = 0.03$) concentrations. Yet, the sum of concentrations of the six biogenic amines that were measured did not differ between dietary treatments ($P > 0.10$).

Table 4. Average daily gain (g d^{-1}), average daily feed intake (g d^{-1}) and feed efficiency (gain:feed, g d^{-1}) during the first 4 wk post-weaning of pigs that were fed diets that vary in content of fermentable carbohydrates (FC) and fermentable protein (FP)[z]

						P-values[x]		
Dietary treatment	Control	FP	FC	FP+FC	SE[y]	FP	FC	FP × FC
Average daily gain								
Week 1	129	106	116	101	11.0	0.09	0.41	0.76
Week 2	237	196	247	235	13.5	0.07	0.09	0.31
Week 3	410	377	471	440	20.5	0.13	0.01	0.99
Week 4	627	594	629	607	25.2	0.26	0.78	0.74
Week 1–3	259	226	279	258	10.8	0.02	0.02	0.58
Week 1–4	357	321	378	340	10.6	<0.01	0.08	0.94
Average daily feed intake								
Week 1	160	183	154	147	19.0	0.67	0.27	0.43
Week 2	301	274	332	315	8.8	0.03	<0.01	0.56
Week 3	647	545	616	588	47.2	0.18	0.89	0.47
Week 4	881	813	908	875	34.5	0.20	0.26	0.64
Week 1–3	369	368	334	350	17.3	0.13	0.67	0.62
Week 1–4	497	454	503	481	16.0	0.06	0.33	0.52
Gain:feed								
Week 1	0.809	0.662	0.742	0.679	0.07	0.14	0.72	0.55
Week 2	0.785	0.717	0.743	0.743	0.03	0.29	0.82	0.30
Week 3	0.649	0.713	0.783	0.750	0.05	0.78	0.14	0.39
Week 4	0.715	0.731	0.699	0.702	0.03	0.79	0.55	0.85
Week 1–3	0.704	0.686	0.758	0.738	0.03	0.52	0.09	0.96
Week 1–4	0.721	0.710	0.752	0.708	0.02	0.25	0.52	0.47

[z]See Table 1; values represent least square means and are adjusted for initial body weight (covariable in the statistical model); responses to dietary treatments differ across weeks ($P<0.05$).
[y]Standard error mean for treatment means based on $n=6$ (six pens of six pigs) per treatment.
[x]Probability of main effects of FP, FC, as well as their interaction (FP × FC).

No interactive effects ($P>0.10$) of feeding additional FC and FP were observed for VFA concentrations in colon contents (Table 9). The acetic, proprionic and butyric acid concentrations were increased with feeding additional FC ($P<0.05$), while valeric and caproic acid concentrations were decreased with feeding additional FP ($P=0.05$).

DISCUSSION

Fermentable carbohydrates (oligosaccharides, non-starch polysaccharides, resistant starch) may decrease the concentration of putrefactive compounds that are generated during proteolytic fermentation by providing the microbiota an additional source of energy (Williams et al. 2001; Swanson et al. 2002). Although both PM and

Table 5. Organ weights, digesta pH and counts of total white blood cells (WBC) and specific white blood cell types in starter pigs that were fed diets that vary in content of fermentable carbohydrates (FC) and fermentable protein (FP)[z]

						P-values[y]		
Dietary treatment	Control	FP	FC	FP+FC	SE[y]	FP	FC	FP × FC
Liver wt (g)	413	419	419	424	19.4	0.677	0.704	0.963
Spleen wt (g)	21.96	22.93	23.51	23.52	1.72	0.699	0.399	0.696
Cecum wt (g)	35.26	37.70	37.84	35.40	3.98	1.000	0.962	0.394
Cecum pH	5.59	5.55	5.59	5.50	0.13	0.512	0.790	0.802
Colon pH	6.37	6.24	6.23	6.09	0.13	0.152	0.118	0.942
WBC (10^{+9} L^{-1})	21.86	19.87	17.13	16.87	1.78	0.391	0.005	0.500
Platelets (10^{+9} L^{-1})	701	772	734	675	71.83	0.905	0.539	0.212
Segmented neutrophils (10^{+9} L^{-1})	10.45	9.31	7.44	6.92	1.24	0.363	0.005	0.730
Lymphocytes (10^{+9} L^{-1})	10.21	9.16	8.15	8.77	1.25	0.820	0.186	0.352
Monocytes (10^{+9} L^{-1})	1.04	0.88	0.94	0.80	0.18	0.273	0.513	0.942

[z]See Table 1; means represent least square mean values for pigs sacrificed on day 14 and day 28, as there were no interactive effects of dietary treatment and sampling day.
[y]Standard error mean for treatments based on $n=6$.
[x]Probability of main effects of FP, FC, as well as their interaction (FP × FC).

Table 6. Blood urea nitrogen content (mg dL^{-1}) and ammonia concentration (μg mL^{-1}) in mid-colon (as is) in starter pigs that were fed diets that vary in content of fermentable carbohydrates (FC) and fermentable protein (FC)[z]

						P values[x]		
Dietary treatment	Control	FP	FC	FP+FC	SE[y]	FP	FC	FP × FC
Blood urea nitrogen	6.54	8.79	6.42	10.12	1.08	0.000	0.435	0.347
Ammonia	195	191	147	161	28.31	0.794	0.062	0.658

[z]See Table 1; means represent least square mean values for pigs sacrificed on day 14 and day 28, as there were no interactive effects of dietary treatment and sampling day.
[y]Standard error mean for treatments based on $n = 6$.
[x]Probability of main effects of FP, FC, as well as their interaction (FP × FC).

BP are routinely used in pig diets, their interactive effects on pig performance, health and digestive function have to our knowledge not been evaluated. Poultry meal has been used as an alternative and relatively inexpensive protein source in weanling pig diets with no change in growth performance when compared with other protein sources (e.g., Zier et al. 2004). Gebbink et al. (1999) showed no BW gain effects of 10% BP inclusion in pig diet, other than an increase in VFA content of feces, while Schiavon et al. (2004) found that the inclusion of 120 g kg^{-1} BP in the diet can improve the health status, based on the incidence of diarrhea and fecal excretion of coliforms and clostridia, with minor negative effects on growth performance of piglets between 21 and 64 d of age.

In the current study, the inclusion of PM as a source of FP in the diet resulted in a decrease in ADG, whereas the use of BP as a source of FC increased ADG in newly weaned pigs. Feed consumption was similar across treatments, eliminating any palatability concerns about the use of either PM or BP in weaner pig diets. The observed negative growth response to feeding FP may be related to available (ileal digestible) amino acid intake, metabolic cost of N excretion, changes in gut microbiota, immune system stimulation, or the production of toxic metabolites from proteolytic fermentation. The positive growth response to feeding FC may be related to gut fill, growth of visceral organs or enhanced digestive function. The absence of an interactive effect of feeding FP and FC on growth performance indicates that FP from PM and FC from BP have independent effects on newly weaned pigs.

In this study, neither apparent ileal nor apparent fecal digestibility of CP, DM and amino acids differed among treatments (Table 3). The latter may be attributed to the relatively large variability, in particular for the ileal digestibility values. Moreover, the observed apparent CP and amino acid digestibilities were somewhat lower than published values (CVB 2004; NRC 1998), which are established in heavier pigs with a more fully developed digestive capacity. However, including additional PM in the diet increased the dietary CP content and tended to increase the dietary FP content. Therefore, the experimental design allowed for an assessment of the impact of feeding FP on newly weaned piglets. During the first few weeks of the study, and when pig growth performance was most sensitive to dietary amino acid levels, pigs on the control diet achieved numerically the best growth performance (Table 4), even though the analyzed amino acid contents in the control diet were low relative to the other diets. In addition, and across diets, high analyzed amino acid contents were confounded with low ileal amino acid digestibility values, yielding similar ileal digestible lysine contents. It is thus unlikely that the observed differences in analyzed amino acid contents across the experimental diets were related

Table 7. Counts of different microbial types (log CFU mL^{-1}; as is) in digesta sampled from the mid-colon of starter pigs and that were fed diets that vary in content of fermentable carbohydrates (FC) and fermentable protein (FP) (mean values for pigs sacrificed 2 and 4 wk post-weaning)[z]

						P-values[x]		
Dietary treatment	Control	FP	FC	FP+FC	SE[y]	FP	FC	FP × FC
Total coliforms	6.36	6.63	6.51	6.53	0.25	0.58	0.92	0.64
Clostridia spp.	3.09	3.49	2.91	3.00	0.14	0.08	0.02	0.27
Lactic acid bacteria (LAB)	9.32	9.36	9.27	9.07	0.13	0.53	0.20	0.34
LAB:Total coliforms	2.94	2.73	2.69	2.54	0.26	0.49	0.41	0.91

[z]See Table 1; means represent least square mean values for pigs sacrificed on day 14 and day 28.
[y]Standard error mean for treatments based on $n = 6$.
[x]Probability of main effects of FP, FC, as well as their interaction (FP × FC).

Table 8. Contents of biogenic amines (nmol g^{-1} DM) in digesta sampled from the mid-colon of starter pigs and that were fed diets that vary in content of fermentable carbohydrates (FC) and fermentable protein (FP) (mean values for pigs sacrificed 2 and 4 wk post-weaning)[z]

						P-values[x]		
Dietary treatment	Control	FP	FC	FP+FC	SE[y]	FP	FC	FP × FC
Tyramine	351	249	139	141	52.9	0.35	<0.01	0.33
Histamine	145	181	146	138	23.7	0.56	0.38	0.36
Putrescine	357	560	443	463	87.9	0.21	0.95	0.30
Cadaverine	1119	1068	931	968	208	0.97	0.49	0.83
Spermidine	216	221	191	157	19.1	0.43	0.03	0.30
Spermine	18.7*b*	20.5*b*	32.2*a*	20.0*b*	3.4	0.13	0.06	0.04
Total amines[w]	2207	2300	1883	1886	338	0.89	0.28	0.90

[z]See Table 1; means represent least square mean values for pigs sacrificed on day 14 and day 28.
[y]Standard error mean for treatments based on $n = 6$.
[x]Probability of main effects of FP, FC, as well as their interaction (FP × FC).
[w]Total amines represents the sum of tyramine, histamine, putrescine, cadaverine, spermidine and spermine.
a, *b* Means followed by different letters differ ($P < 0.05$).

to treatment effect on the pigs' response. More specifically, diet DE and apparent ileal digestible amino acid contents, especially lysine, were either significantly or numerically higher in the PM-containing diets (Table 3). Apparently the nutritional value of PM was somewhat underestimated when the experimental diets were formulated. As a result, in the current study, the observed reductions in ADG in pigs fed PM-containing diets cannot be attributed to a lack of available nutrient intake and can be attributed largely to the increased supply of FP from PM.

In the current study, the impact of adding BP to the diet on diet FC content was not established clearly. Even though no diet effects on ileal and fecal DM digestibility were observed, numerically the ileal DM digestibility was higher when BP was included in the diet. This suggests that BP was digested or fermented prior to the terminal ileum, which may explain why no interaction between diet FC and FP content was observed. Schiavon et al. (2004) concluded that the lack of remarkable effects of BP on feed consumption and growth over time was mainly due to the considerable proportions of BP digested in the upper gut.

Some enteric diseases may be linked to enteric fermentation such as post-weaning *E. coli* diarrhea. Lack of treatment effects on diarrhea occurrence and an associated lack of change in colon morphology suggest that the gut environment was not altered after weaning to an extent to cause these changes in the current study. In contrast, in a study by Dong et al. (1996) lesions were observed in gut of pigs when dietary crude protein levels were increased up to 30%. The low level of anti-nutritional factors in the diet, due to the low dietary inclusion level of vegetable protein sources like soybean products, probably contributed to the low occurrence of diarrhea as well (Engle 1994). Visceral organ weights and digesta pH were also not influenced by dietary treatment, partly due to the high variability within treatment groups and small sample size. In other studies, Chiba et al. (1995) observed an increase in liver and

Table 9. Contents of volatile fatty acid (g kg^{-1}) in digesta sampled from the mid-colon of starter pigs and that were fed diets that vary in content of fermentable carbohydrates (FC) and fermentable protein (FP) (mean values for pigs sacrificed 2 and 4 wk post-weaning)[z]

						P-values[x]		
Dietary treatment	Control	FP	FC	FP+FC	SE[y]	FP	FC	FP × FC
Acetic acid	2.65	3.27	3.56	3.44	0.24	0.31	0.03	0.14
Proprionic acid	1.91	1.89	2.41	2.24	0.19	0.64	0.03	0.68
Iso-butyric	0.14	0.08	0.23	0.10	0.05	0.07	0.28	0.48
Butyric acid	1.28	1.33	1.54	1.56	0.12	0.79	0.05	0.89
Iso-valeric	0.28	0.20	0.36	0.20	0.06	0.05	0.53	0.48
Valeric acid	0.41	0.38	0.54	0.41	0.05	0.17	0.17	0.32
Caproic acid	0.16	0.07	0.32	0.07	0.08	0.05	0.37	0.34

[z]See Table 1; means represent least square mean values for pigs sacrificed on day 14 and day 28.
[y]Standard error mean for treatments based on $n = 6$.
[x]Probability of main effects of FP, FC, as well as their interaction (FP × FC).

kidney weights in pigs fed meat protein and feather meal diets. Swanson et al. (2002) also found no effect on fecal pH in dogs fed diets with added oligosaccharides, attributing the results to the high absorption rate of volatile fatty acids in the colon.

The inclusion of FC in the diet resulted in a decrease in blood plasma counts of WBC and segmented neutrophils (Table 5). Zhang et al. (2002) noted that 1 wk after weaning, WBC counts increased significantly and were associated with post-weaning stress in piglets. Because WBC and segmented neutrophils are a defense mechanism against pathogens, lower WBC counts and segmented neutrophils reflect lower exposure to pathogens or a reduced immune response in pigs fed BP. It must be noted that the average levels of WBC and segmented neutrophils were within the acceptable range for pigs fed additional FC, while they were slightly elevated for pigs fed additional FP.

Ammonia is a toxic waste product of amino acid deamination or urea hydrolysis by microbial urease (Visek 1984; Anderson et al. 1999). Ammonia is very volatile and absorbed quickly from the intestinal lumen, which may have influenced the ammonia levels in colon contents. Yet, a tendency for FC to decrease ammonia content in digesta from the colon was noted in the current study (Table 6). Also, in other studies, the inclusion of FC in the diet tended to decrease the ammonia concentration in colon contents (Smith and Macfarlane 1998; Flickinger et al. 2003). These observations are consistent with the suggestion that the amount of N incorporated into bacterial protein, increases with increasing dietary supply of FC (Morgan and Whittemore 1988).

Urea within the blood stream is synthesized primarily from absorbed ammonia or ammonia derived from deamination of amino acids (Zervas and Zijlstra 2002). As anticipated, in the current study, BUN was significantly increased in pigs fed additional FP, reflecting increased ammonia uptake from the hindgut and reduced nitrogen utilization efficiency. As a result, the energy cost of urea synthesis contributed to the observed reduction in growth performance of pigs fed additional FP (Birkett and de Lange 2001). Feeding additional FC may shift N excretion from urea in urine to bacterial protein in feces (Morgan and Whittemore 1988); however, based on fecal N excretion, we did not notice such shift in this study. The latter is consistent with Zervas and Zijlstra (2002), who observed no interactive effects between protein and fiber intake on N excretion or N retention.

Around the time of weaning, the pig's enteric microbiota are unstable and vulnerable to proliferation of pathogens. This unstable microbiota may be altered by manipulation of diet composition. Three categories of bacteria were selected to assess gut health. Coliforms were chosen as an indicator of toxins producing bacteria, *Clostridia* spp. are known to produce amines as well as toxins, and LAB are considered by some to promote development of a healthy gut (Allison and Macfarlane 1989; Gilliland 1990). The conventional bacterial culturing methods yielded no difference in counts of coliforms and LAB among dietary treatments. However, feeding additional FC significantly reduced the *Clostridia* spp. and feeding additional FP tended to increase *Clostridia* spp. counts. These results are in agreement with a study by Flickinger et al. (2003), where the fecal *Clostridium perfringens* counts in dogs were lowered by inclusion of additional carbohydrates in the diet, and with increased counts of *Clostridium perfringens* in the ceca of broilers with increasing dietary protein level (Elwinger et al. 1994). These findings suggest that restricting the intake of FP and increasing intake of FC is a means to reduce the proliferation of harmful *Clostridium* spp. in the hindgut of young pigs, and thus to enhance gut health and animal productivity. Konstantinov et al. (2003) reported that feeding FC increased bacterial diversity and promoted a more rapid stabilisation of the bacterial community. The characterization of diet effects on microbiota diversity should be explored further.

Volatile fatty acids, and butyric acid in particular, are the main sources of energy for colonocytes and are the key to a healthy and efficient digestive tract (Hallman et al. 1995; Swanson et al. 2002). Volatile fatty acids may also decrease digesta pH and have potent antimicrobial effects on many pathogenic species (Williams et al. 2001). In the current study, feeding additional FC increased the contents of acetic, propionic, and butyric acids in digesta sampled from the colon. The increased contents of VFA in colonic digesta may contribute to observed improvements in growth performance of pigs fed FC, by providing additional fermentable substrate.

Amines, such as putrescine, spermine and spermidine, are widespread in all organisms, yet high levels are toxic and facilitate cell death (Teti et al. 2002). Colonic bacteria such as *Clostridia* spp. produce amines, yet little is known about the physiological effects of the various microbially produced amines (Allison and Macfarlane 1989). Normally, amines are rapidly absorbed from the colon and are either detoxified by the gut mucosa or liver, or are excreted in the urine (Gaskins 2001). Increased amine production by the intestinal bacteria has been associated with diarrhea at weaning in pigs with high putrescine and cadaverine concentrations (Teti et al. 2002). In the current study, the decrease in tyramine and spermidine concentrations in colon contents as a result of feeding FC may be reflective of a shift from proteolytic fermentation towards a more saccharolytic fermentation. This is in agreement with the observed reduction in counts of *Clostridia* spp. with the inclusion of FC in the diet. The trend towards an increase in spermine concentration in colon contents with the addition of FC to the diet may contribute to improved pig growth performance (Allison and Macfarlane 1989).

CONCLUSIONS AND IMPLICATIONS

In conclusion, no interactive effects of feeding additional fermentable protein (FP) and fermentable carbohydrates (FC) to newly weaned pigs were found on growth performance or digestive function. The inclusion of 10% poultry meal as a source of FP in the diet resulted in a decrease in ADG, whereas the use of 5% BP as a source of FC increased ADG in newly weaned pigs. Feeding additional FC decreased the counts of WBC and segmented neutrophils. The inclusion of FC in the diet tended to decrease ammonia content in digesta from the colon, while BUN was significantly increased in pigs fed FP. Feeding FC significantly reduced the *Clostridia* spp. and feeding FP tended to increase *Clostridia* spp. counts in digesta sampled from the mid colon. Feeding FC increased the contents of acetic, proprionic, butyric acids in colon contents. The mechanism whereby feeding FP, or poultry meal, and FC, or BP influence pig growth performance require further exploration, but appear related to changes in gut microbiota, beneficial effects of volatile fatty acids, and the energy cost of urinary N excretion. The negative impact of feeding FP and the positive impact of feeding FC on starter pig performance should be considered when formulating pig diets, especially when restrictions are imposed on the use of antibiotics in pig diets to manipulate gut microbiota and animal productivity.

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