Effects of sugar beet pulp on growth and health status of weaned piglets

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ABSTRACT

The effects on growth performance and on some health parameters due to the inclusion of sugar beet pulp in antibiotic-free diets for weaned piglets were evaluated on a commercial farm. A conventional diet (C) and one containing 120 g/kg of sugar beet pulp (SBP) were fed to 668 weaned piglets from 21 to 64±3 days of age. Every two weeks, live weight and feed consumption (FC) of the two groups were recorded by pens. Fecal samples were collected from 8 pairs of piglets for each treatment to evaluate the evolution over the time of the apparent digestibility of the nutrients, as well as the volatile fatty acids content and the microbial count in the feces. The dietary treatment did not affect FC. In comparison to C, SBP slightly reduced average daily gain over 36 to 49 (528 vs. 498 g/d; P<0.05) and 50 to 64±3 (677 vs. 631 g/d, respectively; P<0.01) days of age. Digestibility of NDF of diet C increased with age from 441 to 526 g/kg whereas that of diet SBP increased from 465 to 638 g/kg. The differences between diets became significant after 36 days of age (P<0.01). From 29 to 35 days of age higher contents of water (793 vs. 713 g/kg; P<0.01), acetic (322 vs. 206 µmol/g dm; P<0.01) propionic (108 vs. 81 µmol/kg dm; P<0.01) acids and lower counts for fecal-coliforms (6.9 vs. 8.2 $\log_{10}$/g; P<0.01), clostridia (1.3 vs. 2.3 $\log_{10}$/g; P<0.01) and *Staphylococcus* spp. (6.7 vs. 8.1 $\log_{10}$/g; P<0.01) were found in the feces of the SBP piglets compared to those of C. These differences progressively disappeared with time. Some piglets showed clinical signs of purulent arthritis and meningitis, but no signs of diarrhea were observed. The SBP group showed, with respect to C, a significantly lower number of piglet deaths caused by meningitis (15 vs. 30 ‰, respectively; χ^2, P<0.05), and a significantly lower number of piglets removed because lack of growth (33 vs. 76 ‰, respectively; χ^2, P<0.01). No clear evidence to explain this result was found, however it was concluded that the inclusion of 12% of sugar beet pulp in antibiotic-free diets can improve the health status of piglets with little effect on growth performance.

Key words: Sugar beet pulp, Fiber, Microbial flora, Health, Pigs.

RIASSUNTO
EFFETTO DELLE POLPE SECCHE DI BIETOLA SULL'ACCRESCIMENTO
E LO STATO DI SALUTE DI SUINETTI IN POST-SVEZZAMENTO

Il presente lavoro si è prefisso di valutare, in condizioni commerciali e su un elevato numero di soggetti, gli effetti dovuti all'inclusione di polpe secche di barbabietola in mangimi privi di antibiotici sulle prestazioni produttive e sullo stato di salute di suinetti in post-svezzamento. Sono stati utilizzati 668 suinetti svezzati a 21 giorni di vita, suddivisi in due gruppi omogenei per sesso e peso. I suinetti sono stati alimentati con mangimi di controllo (C) o con mangimi contenenti 120

g/kg di polpe secche di bietola non melassate (SBP), fino ad una età di 64±3 giorni. I rilievi, effettuati con cadenza bisettimanale, hanno riguardato il peso vivo e i consumi di mangime per box di allevamento. Ogni 2 settimane, da 8 coppie di suinetti per tesi, sono stati raccolti campioni di feci mediante la tecnica del grab sample. Le feci sono state analizzate per valutarne la composizione chimica, i contenuti di NDF e ADF, e di acidi grassi volatili e i valori di conta su piastra per diverse categorie microbiche. La digeribilità apparente è stata valutata utilizzando le ceneri acido insolubili come indicatore endogeno. Per ciascuna tesi si sono registrati il numero di suinetti morti o rimossi per mancato accrescimento. I consumi alimentari dei suinetti dei due trattamenti sono stati simili. Rispetto al controllo, la dieta SBP ha leggermente ridotto la velocità di crescita tra i 36 e i 49 (528 vs 498 g/d; P<0,05) e tra i 50 e 64 (677 vs 631 g/d; P<0,01) giorni di vita. Tra i 29 e i 64 giorni di vita la digeribilità dell'NDF della dieta C è aumentata da 441 a 526 g/kg, mentre quella della dieta SBP è aumentata da 465 al 638 g/kg. Le differenze tra i trattamenti sono risultate significative solo dopo i 36 giorni d'età. Le feci prodotte dai suinetti SBP nell'intervallo d'età compreso tra 29 e 35 giorni hanno evidenziato, rispetto al controllo, maggiori contenuti d'acqua (793 vs 713 g/kg; P<0,01), di acidi acetico (322 vs 206 μmol /g ss; P<0,01) e propionico (108 vs 81 μmol/kg ss; P<0,01) e minori valori di conta microbica per coliformi fecali (6,9 vs 8,2 $\log_{10}$/g; P<0,01), clostridi solfito-riduttori (1,3 vs. 2,3 $\log_{10}$/g; P<0,01) e Staphylococcus spp. (6,7 vs 8,1 $\log_{10}$/g; P<0,01). Le differenze non sono risultate significative nei successivi periodi di controllo, nonostante il contestuale aumento della digeribilità dei costituenti fibrosi apportati dalle polpe. La sola causa di mortalità osservata è stata una forma di meningite. Non si sono osservati casi di diarrea. Rispetto al controllo, con la dieta SBP si sono osservate minori percentuali di suinetti morti (30 vs 15‰, χ^2, P<0,05) e rimossi per mancata crescita (76 vs 33‰, χ^2, P<0,01). Anche se le relazioni di causa-effetto di queste differenze non sono state chiarite, i risultati suggeriscono che l'inclusione fino a 120 g/kg di polpe secche di barbabietola, in mangimi senza antibiotici può consentire sensibili miglioramenti dello stato di salute dei suinetti in post svezzamento con limitate riduzioni delle prestazioni di crescita.

Parole chiave: *Polpe secche di bietola, Fibra, Flora microbica, Salute, Suino.*

Introduction

Piglets weaned around 3 to 4 weeks of age are immature both for their potential to digest nutrients (lack of enzymes) and to ferment fiber (underdeveloped microflora), therefore the inclusion of high levels of fiber in the diet can lead to a reduction in feed intake, impaired growth performance and increasing risks of intestinal disease, such as diarrhea (Low, 1985). Nevertheless, some experiments (Longland *et al.*, 1994; Lizardo *et al.*, 1997) conducted on weaned piglets have clearly shown that the replacement of appreciable amounts of cereals by fermentable fiber ingredients, such as sugar beet pulp, led to negligible differences in growth performance. The use of fermentable fiber could be beneficial due to its low bulking effect and because it could stimulate a more rapid development of a non-pathogenic intestinal microflora with a large production of VFA (Williams *et al.*, 2001). These compounds are used as an energy source for the intestinal endothelial tissues, which protect the host from pathogenic microbes and toxins (Velazquez *et al.*, 1997; Piva *et al.*, 1996; 2002). The use of fermentable fiber to manipulate the intestinal environment becomes of interest in order to reduce the addition of antibiotics in piglet feed (Mosenthin *et al.*, 2001; Piva *et al.*, 2002). This experiment was designed to evaluate, on a large number of subjects, the effects of the replacement of cereals by sugar beet pulp in antibiotic-free diets on feed consumption, growth performance and health of 3 to 9 weeks old piglets. In the same context the variation over time of the apparent digestibility of nutrients, fecal concentration of VFA, viable count of some microbial groups, DNA alterations and inflammatory status of rectum mucosa were investigated.

Material and methods

Animals and diets

The trial was conducted on a commercial farm from March to June 2001. Four batches of piglets (White Cross breed) weaned at 21 days of age, for a total of 668 subjects, were transferred over time in four rooms, each containing 22 pens, in groups of 7-8 subjects. Piglets remained in the trial until they were sold. Piglets of two batches were sold at 62 days of age; the others were sold at 67 days of age. The rearing rooms were equipped with an automated system of heating and of ventilation. Environmental temperature ranged from about 23 to 26 °C. The pens in each room were divided in

Table 1. Feed and chemical composition and metabolizable energy (ME) content of the diets[1].

		Phase I starter diets		Phase II starter diets	
		C	SBP	C	SBP
Ingredients:					
Barley meal	g/kg	180	180	150	150
Barley popped grains	"	100	100	50	50
Wheat meal	"	110	70	160	130
Wheat middlings	"	30	-	70	-
Maize meal	"	203	150	178	150
Unmolassed sugar beet pulp	"	-	120	-	120
Soybean meal	"	150	150	180	190
Full fat soy	"	30	30	30	30
Dried whey	"	120	120	80	80
White fish meal	"	40	45	30	30
Poultry fat	"	-	-	15	15
Sugar-cane molasses	"	-	-	20	20
Salt	"	3.0	3.0	3.0	3.0
Calcium carbonate	"	9.4	6.8	9.3	6.8
Bicalcium phosphate	"	9	10	11	12
L-Lysine hydrochloride	"	2.5	2.2	1.8	1.36
DL-methionine	"	2.0	2.1	1.6	1.7
Threonine	"	1.4	1.2	1.0	0.9
Trace elements/vitamin mix[2]	"	9.7	9.7	9.3	9.3
Chemical composition:					
Dry matter	g/kg	890	890	890	890
Crude protein	g/kg DM	192	195	207	201
Lysine	"	14.8	14.8	14.0	14.0
Ether extract	"	55	54	65	63
NDF	"	133	185	140	186
ADF	"	47	73	48	72
SDF	"	18	30	16	30
AIA[3]	"	3.7	4.4	2.9	4.0
ME[4]	MJ/kg DM	14.5	14.0	14.9	14.6

[1] C, control diet; SBP, sugar beet pulp diet; NDF, neutral detergent fiber; ADF, acid detergent fiber; SDF, soluble dietary fiber;

[2] Trace elements /vitamin mix supplied (per kg of diet): zinc, 210 mg; copper, 150 mg; retinol, 6.0 mg; cholecalciferol, 75 µg; α-tocopherol, 40 mg; menadione, 5.0 mg; thiamin, 4.0 mg; riboflavin, 6.4 mg; pyridoxine, 4.0 mg; cyanocobalamin, 40 µg; ascorbic acid, 50 mg; D-pantothenic acid, 19.2 mg, niacin, 40.0 mg; biotin, 0.1 mg; folic acid, 0.8 mg and flavors.

[3] Mean values of 5 replications (standard deviation of mean values were 0.06, 0.17, 0.22 and 0.37 g/kg, respectively, in the same order for the 4 feeds shown in the table).

[4] ME, metabolizable energy estimated from the proportion of each ingredient in the diets and the tabulated ME values proposed by NRC (1998).

two groups, balanced for sex and body weight, and assigned to one of two antibiotic-free dietary treatments. From 21 to 35 days of age the control group received a phase I starter feed and for the following days a phase II starter feed. The diets of the other group were formulated from the previous ones by replacing parts of maize, wheat and wheat middlings with 120 g/kg of unmolassed sugar beet pulp (Table 1). The level of inclusion of sugar beet pulp was intentionally chosen to be similar to that used by others (Longland *et al.*, 1994; Lizardo *et al.*, 1997) who failed to find any significant effect on feed consumption and growth performance, but on a small number of pigs kept under experimental conditions of rearing. The proportion of barley in the feed for the two groups was kept constant to avoid interference due to different levels of β-glucans (Longland *et al.*, 1994). Some other small adjustments were made to keep the diets isonitrogenous. The feeds were supplemented with synthetic amino acids in order to supply at least 14.0 g/kg of dry matter of lysine and to meet the ratios of the other amino acids to lysine in the ideal protein. Samples of feeds were collected to be analyzed and the resulting chemical composition of diets is given in Table 1. Feed was distributed in pellet form and the piglets had unrestricted access to fresh drinking water from two nipple drinkers placed in each pen.

Records made on all piglets

The piglets were weighed by pen at 21, 35, 49 days of age, and at the end of the trial (62 or 67 days of age). Feed intake was monitored weekly by weighing the residue in the feeders. Previous observation made on the same farm indicated that the post weaning mortality of piglets was around 23 ‰, but medicated feeds were routinely used. Thus, in this trial the piglets were controlled every morning for clinical signs of disease, with emphasis on diarrhea. The care, the use and the health control of piglets were performed under veterinary assistance. Dead piglets and those showing a serious lack of growth (less than about 30% compared to the average daily gain of the other pigs of the same pen) were individually weighed and removed. Piglets showing a serious lack of growth were transferred to other separate pens and treat-

ed with antibiotics (Supramox). The proportions of piglets that died or were removed because of serious lack of growth were considered as indexes of illness (Black *et al.*, 1999).

Collection of biological materials

For each treatment, 8 pairs of piglets were randomly chosen from 8 pens and ear tagged. These subjects were used as donors of fecal samples and fragments of rectum mucosa.

For digestibility measurements, fecal grab samples were collected twice daily at the following weeks of age: 4th (22, 23, 26 and 27 days of age), 5th (29, 30, 33 and 34 days of age), 7th (41, 42, 43 and 44 days of age), 9th (55, 56, 57 and 58 days of age). Samples were immediately frozen and stored at –20 °C. However, the amounts of feces collected from the 22nd to the 27th day of age were not sufficient to perform the chemical analysis.

A second set of grab samples was collected from the same piglets at 31, 45 and 59 days of age to be analyzed for pH and VFA contents. The pH level was immediately measured with a portable pHmeter (Crison, Basic 20) on sub-samples of about 1 g diluted in 25 cc of distilled water. The remaining amounts were, for preservation, immediately diluted (10:1 ratio proportion) with a solution consisting of a 0.01 (w/w) solution of mercuric chloride and 0.05 (v/v) o-phosphoric acid in distilled water, according to the methodology described by Jouany (1982).

A third set of samples was collected at 25, 32, 46 and 60 days of age. The feces were put in aseptic bags, stored at 4 °C and immediately sent to the laboratory for the viable microbial count. During the same days, five fragments of mucosa were collected by endoscopy from the first rectal loop of each piglet and stored at –80 °C in liquid N to be analyzed for oxidative damage of DNA (DNA adducts) and myeloperoxidase activity (MPO).

Chemical and biological analysis

Fecal samples were thawed, weighed and pooled in composite samples to be representative of each pair of piglets and of the following periods: 29 to 35, 36 to 49, 50 to 64 days of age, homogenized and oven dried at 105 °C. Dried feces and air-dry diets were ground in a sample mill through

a 1-mm screen prior to analysis. Proximate composition was measured according to AOAC (2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as described by Van Soest *et al.* (1991). Hemicelluloses were calculated as differences between NDF and ADF. Soluble dietary fiber (SDF) content was determined according to the AOAC (2000) method n. 991.43 with a previous treatment of fecal samples with ether (Prosky *et al.*, 1988). To calculate the total tract apparent digestibility, feed and feces were also analyzed for their content in acid insoluble ash (AIA) as internal marker (Scipioni and Lambertini, 1981; Kavanagh *et al.*, 2001). Five grams of feed and fecal samples were ashed at 500 °C, treated with 50 ml of 2 N HCl, boiled, filtered and washed with distilled water up to pH 7, ashed again and weighed. Measurements were carried out on 5 replications for feed and in duplicate for feces.

The second set of fecal samples was thawed, centrifuged at 8000 g for 5 min, filtered, and the supernatant was analyzed for VFA by HPLC (Shimadzu Class VP, SCL 10ADVP, Kyoto, Japan) with an Aminex HPX-87H (30 cm x 7.8 mm) cation exchange column (Bio-rad, Hert, UK). The column temperature was maintained at 85 °C and the flow rate of the water mobile phase at 0.6 ml/min.

On the third set of fresh fecal samples the viable cell count for the following microbial categories was performed: total viable bacteria, assayed in plate count agar (Merck n.1.05463) after 48 h at 31 °C; *Enterobacteriaceae*, assayed in violet red bile lactose Agar (Oxoid CM107) after 24 h at 37 °C; fecal coli forms, assayed in MacConkey Agar (Merck n. 1.05465) after 24 h at 37 °C; *Escherichia* coli assayed in coli form agar (Merck n. 1.0426) after 24 h at 37 °C; sulphite-reducing clostridia in vegetative forms, assayed in SPS Agar (Merck n.1.10235) after 48 h at 37 °C, sulphite-reducing clostridia spores, assayed as above, by previously pasteurizing at 72 °C per 15 min; *Staphylococcus* spp. and *Micrococcus* spp. assayed in Baird-Parker Agar (Merck n. 1.05406), after 48 h at 37 °C per 48 h; *Lactobacillus* spp. assayed in MRS Agar (Merck n.1.10660) after 48 h at 37 °C, fecal *Enterococcus* spp., assayed in SB Agar (Oxoid CM 377) after 48 h at 37 °C and 44 h at 45 °C;

yeast and mold assayed in OGYA Agar (Oxoid CM 545) after 72 h at 20/22 °C.

Fragments of rectum mucosa were analyzed for their content in adducts of DNA. DNA was extracted, purified and analyzed according to Fraga *et al.* (1990). After enzymatic hydrolysis and filtration, the purified DNA was analyzed for its content of 8-OH-deoxyguanosine (8OHdG) and normal deoxyguanosine (dG). The two molecules were separated by HPLC (Shimadzu, Kyoto, Japan) using a 3µm Supelcosil LC-18-DB analytical column (7.5 cm x 4.6 mm, Supelco, Bellefonte, PA) equipped with 5 mm guard column cartridge (Supelguard TM LC-18-DB). 8OHdG was detected by using an electrochemical detector (ESA Coulochem II 5200 A, Bedform, MA) and dG by measuring the UV absorbance at 254 nm. The degree of DNA alteration was measured at the molecular ratio between 8OHdG and dG.

Other tissue fragments were analyzed for their myeloperoxidase activity (MPO), to measure the inflammatory status of the lower part of the intestine (Soderholm *et al.*, 2002). The fragments were homogenized in a separation buffer (EDTA-bromide, pH 6, Sigma Co, St. Louis, Missouri) and centrifuged. The supernatant was added to a solution of O-diasidin solution (Sigma Co, St. Louis, Missouri) and hydrogen peroxide in a phosphate buffer. The colorimetric reaction absorbance was measured by using a spectrophotometer. The MPO activity was expressed in unit per mg of fresh tissue. A unit of activity is the amount of enzyme required to convert 1 ml of hydrogen peroxide in water in 1 minute at 24 °C.

Calculations and statistical analysis

Apparent digestibility coefficients of the diets were computed as described by Scipioni and Lambertini (1981). Apparent digestibility of dry matter, NDF, hemicellulose and ADF of the test ingredient, the sugar beet pulp, was computed as (T-C)/S, where T is the computed amount (g) of nutrient component digested from SBP diet, C is the amount (g) of nutrient component digested from the non sugar beet pulp ingredients of the diet SBP assuming it had the same digestibility value of diet C, and S is the amount (g) of the nutrient compound derived from sugar beet pulp in the SBP diet (Longland *et al.*, 1994).

Data were analyzed with the GLM procedure of SAS (1988) according to the following split-plot model:

$$Y_{ijkl} = \mu + B_i + A_j + D_k + BA_{ij} + AD_{jk} + e_{ijkl}$$

where: Y_{ijkl} was the dependent variable; m was the overall mean; B_i was the batch effect; A_j was the age effect; D_k was the diet effect; BA_{ij} was the BxA interaction; AD_{jk} was the AxD interaction and e_{ijkl} was the residual error. The significance of the differences between diets within each week of age was tested by running orthogonal contrasts. The effects of other interactions were not considered since from a previous analysis they resulted not significantly different. The proportions of dead and removed piglets, with respect to the total number of heads reared for each treatment and age, were subjected to a χ^2 analysis to compare differences due to the diet, the age and their interaction (SAS, 1988).

Results

Piglets performance and health status

Growth performance and the number of dead and removed piglets during the trial are shown in Table 2. Some piglets showed, at gross and histo-pathological evaluation, clinical signs of purulent arthritis and meningitis, probably due to *Streptococcus suis* (Staats *et al.*, 1997). This occurred in particular during the first and the second week from weaning. The number of dead and removed piglets declined with age in both treatments. The total number of C piglets that died was about double of that observed for SBP (30 vs. 15 ‰, χ^2, P<0.05). Diet C was also associated with a significantly higher number, with respect to SBP of removed piglets (76 vs. 33 ‰, respectively; χ^2, P<0.01). About 80% of the removed piglets showed the same clinical signs of the dead piglets. When they were moved to separate pens and treated

Table 2. Piglets, dead and removed, growth performance and feed consumption for the control (C) and the sugar beet pulp (SBP) diets.

Items		Diet	Age, days			P values for main effects			SEM
			21 to 35	36 to 49	50 to 64	Diet	Age	Diet x Age	
Piglets in trial	n.	C	326	305	294				
		SBP	342	334	332				
Piglets dead[1]	‰	C	12	9	9	0.025	0.165	0.041	-
		SBP	9	3	3				
Piglets removed[1]	"	C	52	24	0	<0.001	<0.001	<0.001	-
		SBP	15	15	3				
Final live weight[2]	kg	C	8.2	15.6	26.9	0.335	<0.001	0.864	0.2
		SBP	8.1	15.1	25.6				
Daily live weight gain[2]	g/d	C	194	528[b]	677[B]	0.007	<0.001	0.074	8
		SBP	196	498[a]	631[A]				
Daily feed consumption[2]	"	C	253	687	1062	0.300	<0.001	0.800	13
		SBP	249	648	1036				
Feed conversion ratio[2]	kg/kg	C	1.30	1.30	1.57	0.811	<0.001	0.264	0.08
		SBP	1.27	1.30	1.64				

[a,b; A,B] *Mean values between two consecutive rows within a column with unlike superscript letters were significantly different from orthogonal contrast; with small letters: P<0.05; with capital letters: P<0.01.*

[1] *P values obtained by χ^2 analysis*

[2] *Each value is the mean of 44 observations (4 batches x 11 pens).*

with a therapeutic dose of Supramox their health conditions were re-established. No clinical signs of diarrhea or other pathologies were observed in the piglets of the two groups.

Feed consumption increased markedly with age (P<0.01), but it was not affected by the dietary treatment. There was no difference in average daily gain between the two dietary treatments from 21 to 35 days of age, but later the piglets receiving the C diets grew progressively faster with respect to those on SBP (528 *vs.* 498 g/d, P<0.05 from 36 to 49 d of age; 677 *vs.* 631 g/d, P<0.01 from 50 to 64 d of age). However, considering the whole period of growth the average daily gain of the SBP group was about only 6.4% lower than that of the C group. The dietary treatment did not affect the feed conversion ratio.

Fecal dry matter content and apparent digestibility coefficients

Piglets receiving the SBP diets produced feces with a significantly (P<0.01) higher moisture content as compared to the piglets of the control group, but only from 29 to 49 days of age (Table 3). The difference in the fecal moisture between treatments gradually disappeared with time and the interaction Diet x Age was significant (P<0.01). A significant correlation (r = +0.76) between fecal moisture and ADF was observed for the feces of the SBP group (Figure 1).

The partial replacement of cereal products with sugar beet pulp slightly lowered the apparent digestibility coefficients of dry matter, crude protein and crude fat (P<0.01) and markedly increased those of the various fibrous constituents

Table 3. Fecal content of moisture and AIA (g/kg DM) and apparent digestibility coefficients (g/kg) of piglets fed the control (C) and the sugar beet pulp (SBP) diets.

Items	Diet	Age, days			P value for main effects			SEM
		29 to 35	36 to 49	50 to 64	Diet	Age	Diet x Age	
Faecal content:								
Moisture	C	713[A]	751[A]	745	<0.01	<0.01	<0.01	4
	SBP	793[B]	791[B]	757				
AIA	C	17	165[A]	18[A]	<0.01	<0.01	0.02	1
	SBP	18	20[B]	23[B]				
Apparent digestibility:								
Dry matter	C	784[B]	822[B]	840	<0.01	<0.01	0.75	3
	SBP	760[A]	803[A]	824				
Crude protein	C	707[b]	763	822[b]	<0.01	<0.01	0.33	8
	SBP	654[a]	749	789[a]				
Crude fat	C	731[b]	728[b]	763[b]	<0.01	<0.01	0.24	11
	SBP	619[a]	694[a]	716[a]				
NDF	C	441	489[a]	526[A]	<0.01	<0.01	0.25	13
	SBP	465	553[b]	638[B]				
Hemicellulose	C	547	578[A]	601[A]	<0.01	<0.01	0.37	13
	SBP	583	643[B]	703[B]				
ADF	C	245	317[A]	382[A]	<0.01	<0.01	0.21	16
	SBP	286	412[B]	535[B]				

[a,b; A,B]*Mean values between two consecutive rows within a column with unlike superscript letters were significantly different from orthogonal contrasts; with small letters: P<0.05; with capital letters: P<0.01.*

Each value is the mean of 8 observations (1 obs. for 8 pens).

Figure 1.   Relationship between moisture and ADF fecal contents of piglets receiving the control diet (C, O; -------; $Y_C = 11.4X + 1242$; R^2 0.110) and the sugar beet pulp diet (SBP, •,———; $Y_{SBP} = 28.6X - 788.4$; R^2 0.583)
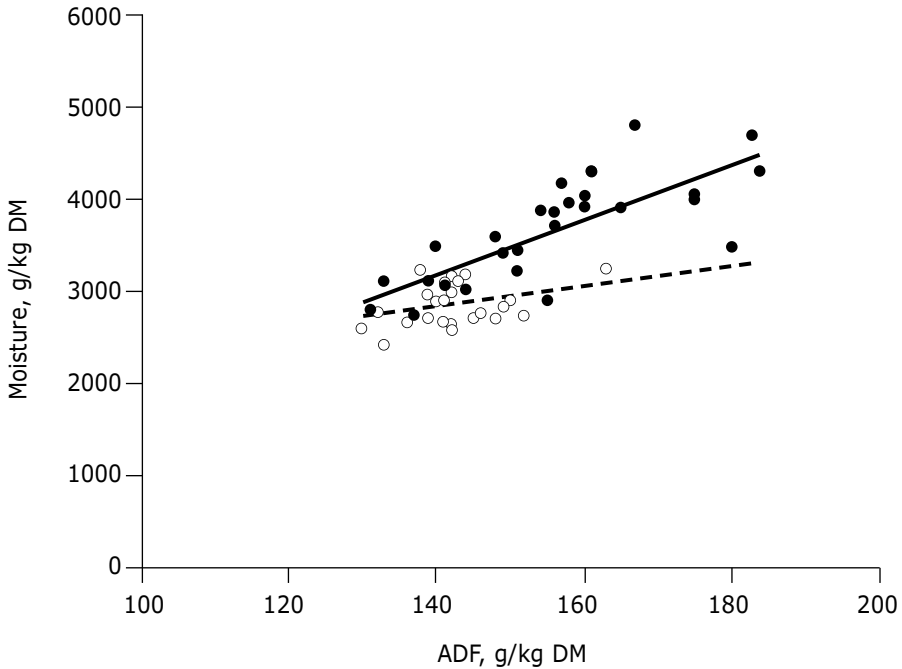
(P<0.01). The estimates of the digestible coefficients for the dry matter and the fibrous compounds of the test ingredient are given in Table 4. These coefficients were computed, according to the method of Longland *et al.* (1994), from the data given in Table 3. The ability of piglets to digest this ingredient markedly increased with age; the apparent digestibility coefficient of dry matter and

Table 4.   Digestive utilization of sugar beet pulp[1] in piglets (g/kg).

Items	Age, days		
	29 to 35	36 to 49	50 to 64
Dry matter	584	664	707
NDF	516	690	878
Hemicellulose	709	876	1069
ADF	332	516	703

[1] *Apparent digestibility of sugar beet pulp (AD) components was evaluated from data given in Table 3 as (T-C)/S; where T is the amount (g) of nutrient component digested from SBP diet, C is the amount (g) of nutrient component digested from the non sugar beet pulp ingredients of the diet SBP (assuming the same digestibility values of diet C), and S is the amount (g) of the nutrient compound derived from sugar beet pulp in the SBP diet (Longland et al., 1994).*

Table 5.   End products of fermentation (μmol/g DM) in faeces of piglets fed the control (C) and the sugar beat pulp (SBP) diets.

Items	Diet	Age, days			P value for main effects			SEM
		29 to 35	36 to 49	50 to 64	Diet	Age	Diet x Age	
Acetic acid	C	206[A]	184[A]	221	<0.01	0.01	0.03	8
	SBP	322[B]	258[B]	253				
Propionic acid	C	81[A]	77[a]	91	<0.01	0.01	0.18	3
	SBP	108[B]	88[b]	98				
Butyric acid	C	46	45	58	0.46	<0.01	0.65	2
	SBP	47	44	63				
Ethanol	C	23[a]	21[A]	16[a]	<0.01	<0.01	0.87	1
	SBP	19[b]	15[B]	11[b]				
Lactic acid	C	11[a]	9	6	0.07	<0.01	0.37	1
	SBP	8[b]	7	6				
pH	C	6.56	6.62	6.59	0.17	0.74	0.91	0.08
	SBP	6.49	6.60	6.51				

[a,b; A,B] *Mean values between two consecutive rows within a column with unlike superscript letters were significantly different from orthogonal contrasts; with small letters: P<0.05; with capital letters: P<0.01.*
Each value is the mean of 16 observations (2 obs. x 8 pens).

NDF from sugar beet pulp increased from 584 to 707 g/kg and from 516 to 878 g/kg, respectively.

End product of fermentation and microbes in the faeces

Fecal concentration of the various end products of fermentation is given in Table 5. The values are in the ranges observed by others (Stanogias and Pearce, 1985). The 29-35 days old piglets fed the SBP diet produced feces containing higher concentrations of acetic (P<0.01) and propionic acids (P<0.01), a similar concentration of butyric acid and a lower concentration of ethanol (P<0.05) and lactic acid (P<0.05), with respect to control. These differences became less pronounced with age and during the last period no significant differences due to the treatment were observed for these compounds, except for ethanol. For acetic acid the interaction DxA was significant (P<0.05).

Age also had significant effects on the viable cell count of all the various microbial groups (Table 6). The feces collected from the control group showed, with respect to SBP, significantly higher viable cell counts for sulphite-reducing clostridia spores (P<0.01), *Staphylococcus* spp. (P=0.02) and yeast and molds (P<0.01). Orthogonal contrasts also indicated that from 29 to 35 days of age the viable cell count for several microbial categories was significantly higher (P<0.01) for control with respect to SBP.

Parameters of health status of intestinal tissue

The DNA adducts in the rectum mucosa fragments and the index of DNA oxidation significantly decreased with age (P<0.01) (Table 7). No significant difference was observed between dietary treatments. However, the residual variability was high. Standard deviation of this measurement, within treatment, was higher for the control group compared to the SBP group (sd: 54.6 *vs.* 46.9 OHdG/10[5] dG). Similarly, there was no difference on the MPO activity due to the dietary treatment and also in this case the residual variability was high.

Table 6. Viable cell count for some microbial categories ($\log_{10}$/g) in fresh faeces of piglets fed the control (C) and the sugar beet pulp (SBP) diets.

Items	Diet	Age, days				P value for main effects			SEM
		21 to 28	29 to 35	36 to 49	50 to 64	Diet	Age	Diet x Age	
Microbial categories:									
Enterobacteriaceae	C	7.8	8.5[B]	8.0	6.4	0.31	<0.01	<0.01	0.3
	SBP	7.9	7.1[A]	8.1	6.9				
Coliforms	C	7.8	8.2[B]	7.9	6.0	0.16	<0.01	<0.01	0.3
	SBP	7.7	6.9[A]	7.7	6.5				
Escherichia coli	C	7.7	8.0[B]	7.6	6.0	0.75	<0.01	0.02	0.3
	SBP	7.4	7.1[A]	7.9	6.6				
Bacillaceae									
Clostridia (vegetative forms)	C	3.8	3.1	3.4	3.0	0.48	<0.01	0.92	0.3
	SBP	3.7	2.7	3.3	3.0				
Clostridia (spores)	C	3.2	2.3[B]	1.7	2.1[b]	<0.01	<0.01	0.04	0.3
	SBP	3.3	1.3[A]	1.3	1.5[a]				
Micrococcaceae									
Staphylococcus spp.	C	5.1	8.1[B]	8.4	9.2	0.02	<0.01	0.01	0.5
	SBP	5.5	6.7[A]	7.8	9.0				
Micrococcus spp.	C	2.9	1.0	1.1	1.4	0.83	<0.01	0.63	0.4
	SBP	2.8	1.0	1.5	1.3				
Lactobacillaceae									
Lactobacillus spp.	C	7.2	6.5	7.6	7.1	0.50	<0.01	0.76	0.3
	SBP	7.2	6.7	7.3	7.1				
Enterococcus spp.	C	5.1	2.4	2.6	2.3	0.70	<0.01	0.55	0.5
	SBP	5.0	3.1	2.4	2.3				
Yeast and moulds	C	5.6	7.7[B]	6.1[b]	4.6	<0.01	<0.01	0.02	0.6
	SBP	5.3	5.6[A]	4.9[a]	4.4				

[a,b; A,B]*Mean values between two consecutive rows within a column with unlike superscript letters were significantly different to orthogonal contrasts; with small letters:*
P<0.05; with capital letters: P<0.01.
Each value is the mean of 16 observations (2 obs. x 8 pens).

Table 7. DNA adducts (OHdG/10^5 dG) and MPO (Myeloperoxidase activity, units/mg) measured in fragments of rectum mucosa of piglets fed the control (C) and the sugar beet pulp (SBP) diets.

Items	Diet	Age, days				P value for main effects			SEM
		21 to 28	29 to 35	36 to 49	50 to 64	Diet	Period	Diet x Age	
DNA adducts	C	124[1]	106	46	60	0.15	<0.01	0.43	5.9
	SBP	88	100	51	47				
MPO	C	1.19[2]	1.16	1.00	1.10	0.19	0.85	0.55	0.64
	SBP	0.58	0.81	0.94	1.14				

[1] Mean values between the two consecutive rows for 21 to 28 d of age had a P value of 0.062.

[2] Mean values between the two consecutive rows for 21 to 28 d of age had a P value of 0.088.

Each value is the mean of 16 observations (2 piglets x 8 pens).

Discussion

### Feed intake and growth performance

According to previous experiments (Longland *et al.*, 1994; Lizardo *et al.*, 1997; Gill *et al.*, 2000), the replacement of 120 g/kg of cereals with sugar beet pulp in the diet for weaned piglets had limited effects on feed consumption and on the live weight at different ages. However, for increasing ages the daily live weight gain of piglets receiving SBP was lower with respect to control. This probably reflected a lower availability of digestible nutrients. Live weights of piglets fed the SBP diet could certainly have been influenced by higher gut contents (Just *et al.*, 1983; Pluske *et al.*, 1998). On the other hand, the ability of piglets to digest the fibrous fractions increased markedly with age, in particularly for SBP. From 29 to 64 days of age the proportion of NDF from sugar beet pulp apparently digested increased from 516 to 878 g/kg, reaching values very high and similar to those obtained on piglets of similar age (Longland *et al.*, 1994), as well as to those obtained on growing and adult pigs (Longland *et al.*, 1993; Noblet and Bach Knudsen, 1997; Galassi *et al.*, 2003). This reflects the progressive establishment of an effective gut microflora able to ferment quickly large amounts of the sugar beet pulp constituents (Figure 2). Thus, the lack of remarkable effects of SBP on feed consumption and growth over time was considered to be mainly due to the considerable proportions of sugar beet pulp digested in the gut, to the corresponding reduction of the bulky properties of this ingredient (Guillon *et al.*, 1998) and to the contribution of the end products of fermentations on the energy balance of piglets (Le Goff and Noblet, 2001). This does not exclude that some adaptations on feed passage rate and gut capacity have occurred, in particularly during the first period after weaning when the digestive ability of piglets was not fully exploited (Just *et al.*, 1983; Pluske *et al.*, 2001; Freire *et al.*, 2000).

End product of fermentation and microbial community

The higher moisture, acetic and propionic acids contents found in the feces of the SBP group with respect to the control group also confirmed the fact that during the first two weeks after weaning the piglet's ability to digest sugar beet pulp was not fully exploited. High proportions of acetic acid in the feces of piglets receiving sugar beet pulp were expected (Salvador *et al.*, 1993; Sunvold *et al.*, 1995; Bauer *et al.*, 2001). However, for increasing ages the differences between treatments in the fecal moisture and VFA fecal contents gradually disappeared. These results are clear evidence that piglets exposed to sugar beet pulp had progressively enhanced, not only their ability to digest considerable amounts of fiber

Figure 2. Amount of hemicellulose and ADF apparently digested by piglets fed the control (hemicellulose △, -------; ADF ○, -------) and the sugar beet pulp (hemicellulose ▲, ———— ; ADF ● , ————) diets.
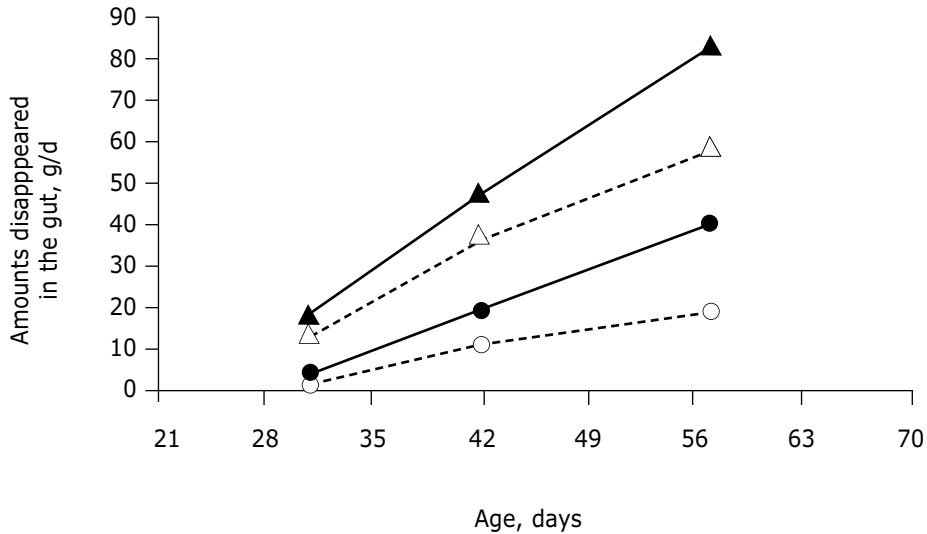
compounds, but also their ability to absorb, more rapidly, the corresponding end products of degradation. It cannot be excluded that with increasing age larger proportion of fiber could have been degraded in the upper portions of the large intestine. An early degradation of fiber in the gut can promote a larger VFA absorption because cecum and colon have a greater ability to absorb VFA with respect to other more distal parts of the gut (Freire *et al.*, 2000), and because the intestinal content could have more time and surface to be absorbed (Bach Knudsen *et al.*, 1991). The regulation of VFA production and absorption in different parts of the gut can play positive roles in maintaining a less pathogenic microflora and improving the health of intestinal tissues (Velazquez *et al.*, 1997; Williams *et al.*, 2001; Piva *et al.*, 2002). Some evidence of different biochemical patterns of fermentation of sugar beet pulp due to the site of fermentation are suggested by the results of Tagliapietra *et al.* (2003). They observed that sugar beet pulp incubated with an ileal *inoculum* produced VFA with a glucogenic to chetogenic fatty acids ratio significantly higher than that obtained by using a fecal *inoculum*. It has been suggested that fermentation acids could play a role in resisting colonization by opportunistic bacteria, including *E. coli*, in the gastrointestinal tract (Mathew *et al.*, 1996). However there is a clear lack of knowledge on these issues.

The dietary treatment had also some significant effects on the viable cell count for many microbial categories, particularly during the second week of feed exposure. Piglets of 29 to 35 days of age receiving the control diet showed higher cell counts for *Enterobacteriaceae*, coliforms, *E. coli*, sulphite-reducing clostridia (spores), *Staphylococcus* spp., yeast and molds, with respect to those receiving SBP diet. Increased coliforms are normally observed when young animals have been subjected to stressing conditions such as weaning, diet change and transportation (Kenworthy and Crabb, 1963; Tannock and Savage, 1974; Ewing and Cole, 1994). A higher metabolic activity of some strains of *E. coli* in converting protein to amines can increase intestinal peristalsis and produce diarrhea, since amines are irritating and toxic. *Staphylococcus* spp. and sulphite-reducing clostridia have also been associated with toxin production and intestinal disorders (Gibson, 1998).

Health status

In this experiment there were no evident signs of diarrhea or other pathologies, except for meningitis probably due to *Streptococcus suis*. This bacterium is thought to reside in the tonsils, from whence it can set up bacteraemia and meningitis; thus this pathology can hardly be associated with intestinal disorders. There is an ample debate about the role of fiber in preventing the development of pathogenic bacteria, in reducing the intestine inflammatory status, in protecting the integrity of the intestinal mucosa and in improving the host immune system (Rumney *et al.*, 1993; Mao *et al.*, 1996; Mathew *et al.*, 1996; Zunft *et al.*, 1997; Wachtershauser and Stein, 2000; Williams *et al.*, 2001). This explorative experiment failed to find a clear evidence of alterations of DNA adducts and of the inflammatory status of the rectum mucosa of sample subjects due to the dietary treatment. However, the significant reduction observed in the number of dead and removed piglets suggested that sugar beet pulp could have had some protective effects on the health status of the host. This issue clearly needs more specific investigation.

Conclusions

This experiment described the development after weaning of the digestive ability of piglets exposed to antibiotic-free diets with or without the inclusion of a fermentable source of fiber such as the sugar beet pulp. The partial replacement (120 g/kg) of wheat and maize ingredients with sugar beet pulp had little effect on feed consumption and growth performance with respect to a low-fiber conventional diet. With respect to the control group, SBP also had significant effect on the evolution over time of the microbial and chemical characteristics of the feces, indicating the possibility of manipulation of the gut environment. Significant reductions of the number of dead piglets and of the number of piglets showing serious lack of growth were observed when the piglets received the SBP diet. The fermentable fiber supplied by the sugar beet pulp could have had some protective effects on the health status of the host. Research is needed to confirm these findings. The inclusion of sugar beet pulp up to 120 g/kg in diets for weaned piglets can be recommended.

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