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Administration of loperamide and addition of wheat bran to the diets of weaner pigs decrease the incidence of diarrhoea and enhance their gut maturation

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The influence of fibre inclusion and transit time regulation on the performance, health status, microbial activity and population, physico-chemical characteristics of the hindgut digesta and intestinal morphology in early weaned pigs were examined. For these experiments, wheat bran (WB) was used as fibre source and loperamide as a drug (LOP) to increase the digesta transit time. In Expt 1, a total of 128 early weaned pigs were randomly distributed in a 2×2 factorial combination of WB inclusion (0 *v.* 40 g/kg) and LOP administration (0 *v.* 0·07 mg/kg body weight) during 13 d. For Expt 2, a total of twenty-four piglets were allotted to three dietary treatments for 15 d with the same basal diet (control diet) as Expt 1; a diet with 80 g/kg of WB and the combination of WB and LOP. In Expt 1, LOP improved the average daily feed intake and average daily gain of the animals ($P=0{\cdot}001$ and 0·007, respectively). The same result was obtained when WB was combined with LOP. The WB–LOP group also showed a higher concentration of SCFA ($P=0{\cdot}013$), acetic acid ($P=0{\cdot}004$) and propionic acid ($P=0{\cdot}093$). On the other hand, WB inclusion reduced the organic matter and crude protein digestibility ($P=0{\cdot}001$) and tended to decrease the enterobacteria population ($P=0{\cdot}089$). In Expt 2, WB increased the butyric acid concentration ($P=0{\cdot}086$). We concluded that the inclusion of WB to modify the intestinal microbiota activity combined with LOP may be beneficial to animal health and performance.

Wheat bran: Loperamide: Microbiota: Piglets: Digesta transit time

Weaning is a critical phase for piglets; it is associated with a variable period of anorexia during the first days after weaning, the deterioration of the digestive function and accumulation of undigested feed as a result of inefficient digestion(1). During this period, piglets are more susceptible to suffer post-weaning diarrhoea, with the proliferation and attachment to the intestinal mucosa of B-haemolytic strains of *Escherichia coli*(2). Previous studies have demonstrated that adding sources of dietary fibre in the piglet diets may reduce post-weaning diarrhoea(3).

There is a physiological rationale to support the addition of dietary fibre to young animals. Fermentable carbohydrates constitute the major energy source for microbial fermentation and therefore may act as a link between the piglet and its enteric commensal microbiota(4). Adding dietary fibre into the diet can reduce the protein fermentation in the digesta(5), and may normalise the colonic function and the small intestine and colonic mucosa architecture. However, there is conflicting evidence whether NSP promotes a beneficial effect or a detrimental effect on pig health. Thus, some studies have demonstrated that adding sources of mostly insoluble or slowly fermentable NSP(6) or soluble NSP that do not increase viscosity(7) reduce infection-associated symptoms and enhance intestinal structure and function. On the other hand, diets containing soluble NSP sources, which promote increases in the digesta viscosity, such as pearl barley or guar gum, were associated with increased incidence of enteric disorders(8).

In earlier studies, we observed that adding wheat bran (WB) in the diet of weaned piglets promoted a beneficial shift in the microbial colonisation of the digestive tract, with a higher production of butyrate in the large intestine and lower enterobacteria counts in the colonic digesta(3) and intestinal mucosa(9). The WB is a source of insoluble NSP that is fairly resistant to microbial degradation in the gastrointestinal tract (GIT) of monogastric animals and reduces the digesta transit time in the small and large bowel(10,11). We suggested that fermentable carbohydrates from WB were likely influencing bacterial cell growth and activity. However, we were not able to exclude that other changes on the physico-chemical properties of digesta or the digesta kinetics might have a role on the changes observed on the intestinal microbial populations. It might be hypothesised that WB might normalise

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Abbreviations: ADG, average daily gain; BW, body weight; CP, crude protein; CT, control diet; FM, fresh matter; GIT, gastrointestinal tract; LOP, loperamide; MTT, minimum transit time; OM, organic matter; WB, wheat bran; WRC, water retention capacity.

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the digestive function and reduce enterobacteria counts by stimulating fermentation and the propulsive digestive motility. In this respect, butyrate, which is considered the main oxidative fuel for colonocytes, is known to increase with decreased digesta transit time in the GIT(12) or by higher dilution rates *in vitro* (13).

We designed the present studies to elucidate the role of the digesta transit time in the gut health. To this end we used loperamide (LOP) as a drug to increase digesta transit time. LOP works by decreasing peristalsis and fluid secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the GIT(14). It has been used extensively to delay the oro-caecal transit time in human studies(15), in rats(16) and also in pigs(17). With the present study, we aimed to confirm the likely beneficial effects of including WB in the diet of early weaned piglets and to assess which, if any, of the productive, digestive or microbial effects of WB are dominant in changing the digesta transit time.

Material and methods

Animals and housing

Two experiments were performed at the Animal Facilities of the Universitat Autònoma de Barcelona and received prior approval from the Animal Protocol Review Committee of this institution. The treatment, management, housing, husbandry and slaughtering conditions conformed to the European Union Guidelines(18). In Expt 1, a total of 128 commercial crossing piglets ((Large White × Landrace) × Pietrain), which had been excluded from receiving creep feed, were weaned at the age of 24 d with an average body weight (BW) of 6·4 (SEM 1·17) kg. Pigs were transported from a commercial farm to the animal facilities and placed into thirty-two pens (four animals per pen). Each pen had a feeder and a water nipple to ensure *ad libitum* feeding and free water access. The pens were allotted to four treatments (eight replicates for each treatment, Table 1) in a 2 × 2 factorial design that included two levels of WB in the diet (0 *v.* 40 g/kg, control diet (CT) *v.* WB, respectively) and two levels of LOP administration (0 or 0·07 mg/kg BW, named 0 *v.* LOP, respectively). For Expt 2, a total of twenty-four piglets of 7·4 (SEM 1·17) kg from the same origin, breed and age as the previous one were randomly distributed into twelve pens (two animals per pen). The pens were allotted to three treatments (Table 1) that included the same basal diet (CT) as Expt 1; but was modified by adding 8 % of WB and adding WB with LOP (0·07 mg/kg BW, LOP).

Experimental procedures and sampling

In Expt 1, animals received the diets from the first day of the experiment until day 13. LOP (Fortasec®, Esteve, Barcelona, Spain) was administered every morning to the LOP group at a dose of 0·07 mg/kg BW as an oral solution (0·2 mg/ml LOP). The rest of the animals received the same dose of water. The solutions were carefully administered by a 5 ml plastic syringe fitted with an oesophageal tube. Two experimental periods (0–7 and 7–13 d) were selected to register individual BW, pen feed consumption and piglet health status. On day 10, 0·15 % of chromic oxide was added in the diet to determine the total tract apparent digestibility. On day 12, faeces samples were taken to determine the Cr and SCFA concentrations, and lactobacilli and enterobacteria counts. On day 12, 0·25 % of ferric oxide was also included in the diet to determine the minimum transit time (MTT), which is defined as the time between the administration and the appearance of the red marker in the faeces per pen(19). For Expt 2 all the animals received the experimental diets during 15 d. On day 15, animals were euthanised with an intravenous injection of sodium pentobarbital (200 mg/kg BW). Animals were bled, and the abdomen was immediately opened to tie and remove the whole GIT. Samples from the colon consisted of a pool of all colonic contents. Half of the collected samples were freeze-dried and then dried at 103°C

Table 1. Diet composition and chemical analysis

	Trial 1		Trial 2	
Diet	CT	WB	CT	WB
Raw ingredients (g/kg)				
Maize	332·1	290·3	331·1	280·0
Barley	211·6	210·0	238·2	210·0
Whey powder	130·0	130·0	130·0	130·0
High fat whey	100·0	100·0	100·0	100·0
Soya protein	90·0	90·0	92·5	87·1
Wheat gluten	58·1	55·7	30·0	30·0
Fish meal LT*	40·0	40·0	40·0	40·0
Wheat bran	–	40·0	–	80·0
Sunflower oil	–	6·5	–	6·5
Dicalcium phosphate	10·3	9·6	9·1	8·4
Calcium carbonate	9·3	9·7	11·7	10·6
L-Lys HCl	6·8	6·7	5·2	5·2
DL-Met	1·5	1·5	4·1	4·1
L-Thr	2·1	2·1	2·3	2·3
L-Trp	0·7	0·7	0·6	0·6
Vitamin and mineral premix†	4·0	4·0	5·0	5·0
Salt	3·4	3·3	–	–
Chromic oxide	–	–	0·15	0·15
Calculated composition (g/kg as fed)				
ME (MJ/kg)	14·6	14·6	14·4	14·3
CP	208·8	209·1	189·6	190·9
SID Lys	13·75	13·71	12·14	12·12
SID Met	4·84	4·79	6·94	6·91
SID Thr	8·72	8·68	8·28	8·23
SID Trp	2·67	2·67	2·27	2·29
SID Ile	7·77	7·73	7·02	6·96
SID Val	9·07	9·05	8·3	8·26
Chemical analysis (g/kg as fed)				
DM	908·2	907·6	903·0	903·0
GE (MJ/kg)	17·1	17·5	17·8	17·7
CP (N × 6·25)	200·8	205·6	208·0	210·8
Neutral-detergent fibre	74·0	87·0	85·3	106·3
Acid-detergent fibre	25·0	29·0	18·9	22·9
Diethyl ether extract	67·4	80·5	71·0	75·0
Ash	55·0	57·0	64·0	66·0

CT, control diet; WB, wheat bran diet; LT, low temperature; ME, metabolisable energy; CP, crude protein; SID, standardised ileal digestible; GE, gross energy.

* Fishmeal LT: product obtained by removing most of the water and some or all of the oil from fish by heating at low temperature (<70°C) and pressing.

† Supplied per kg of feed: 5000 IU (1500 μg) vitamin A; 1000 IU (25 μg) vitamin D_3; 15·0 mg vitamin E; 1·3 mg thiamine; 3·5 mg riboflavin; 1·5 mg pyridoxine; 0·025 mg cyanocobalamin; 10·0 mg calcium pantothenate; 15·0 mg niacin; 15·0 mg biotin; 0·1 mg folic acid; 2·0 mg vitamin K_3; 80·0 mg Fe; 6·0 mg Cu; 0·7 mg Co; 60·0 mg Zn; 30·0 mg Mn; 0·7 mg iodine; 0·1 mg Se and 0·15 mg etoxiquín as antioxidant (Capsoquin, Itpsa, Barcelona, Spain).

for complete water removal. The other half were divided into four aliquots: 3 g was collected into previously weighed 10 ml screw cap tubes for water retention capacity (WRC) analysis; the remainder was collected in tubes for water swelling capacity; SCFA; microbial population. Finally, a section of 4 cm from mid-jejunum and 4 cm from the medium colon were removed, opened longitudinally and fixed by immersion in 10 % (v/v) buffered formalin for histological study.

Analytical procedures

Chemical analyses of the diets (Table 1) were performed according to the Association of Official Analytical Chemists(20) standard procedures. The chromium oxide concentration in feed and digesta was determined by atomic absorption spectrophotometry following the method of Williams *et al.* (21). The WRC of fresh colon digesta contents was determined by centrifugation (2500 ***g***/25 min) following the method of Anguita *et al.* (22); the water swelling capacity was determined as the ratio of liquid phase to solid phase obtained after allowing fresh colon digesta to stand for 3 h at room temperature in a test tube. DNA from faeces and colon was extracted and purified using the commercial QIAamp DNA Stool Mini Kit (Qiagen, West Sussex, UK) with some modifications as described by Castillo *et al.* (23). Enterobacteria and lactobacilli were quantified by real time PCR using SyBR Green dye following the method of Castillo *et al.* (23). The lactobacilli:enterobacteria ratio was calculated by subtracting log 16S rDNA gene lactobacilli copies/g fresh matter (FM) minus log 16S rDNA gene enterobacteria copies/g FM. SCFA and lactic acid concentrations were determined by GC, after submitting the samples to an acid–base treatment followed by diethyl ether extraction and derivatisation, as described by Jensen & Jørgensen (24). Tissue samples for the histological study were dehydrated and embedded in paraffin wax, sectioned at 4 μm and stained with haematoxylin and eosin. Morphometric measurements were performed with a light microscope (BHS, Olympus, Spain). Villus height and crypt depth, and the globet cell number in crypts were measured. Measurements were taken in ten well-oriented villi and crypts from each intestinal section of each animal. The villus height and crypt depth were measured using a linear ocular micrometer (Olympus, Ref. 209-35 040; Microplanet, Barcelona, Spain). On the basis of the cellular morphology, differences between globet cells and lymphocytes were clearly distinguishable at 400 × magnification. Cell density was expressed as the number of lymphocytes per 1000 μm^2. All morphometric analyses were done by the same person, who was blind to the treatments.

Statistical analyses

In Expt 1, results on productive performance, microbial counts, organic matter (OM) and crude protein (CP) digestibility, MTT and SCFA in the faeces were subjected to ANOVA using the generalized linear model procedure(25). Data were analysed as a 2 × 2 factorial arrangement of treatments, with diet and LOP treatment as the factors in four randomised blocks. Productive performance data were adjusted for initial live weight by covariance analysis. In Expt 2, results on OM and starch digestibility, physico-chemical characteristics, SCFA and lactic acid and microbial population of the colonic digesta and morphometry of the intestinal mucosa were subjected to ANOVA with diet as the classification factor, using the generalized linear model procedure(25). In both the experiments means presented in the tables are least square means, the pen was considered as the experimental unit. Differences were considered significant at $P < 0{\cdot}05$. Tendencies for $0{\cdot}05 < P < 0{\cdot}15$ were also presented.

Results

Expt 1

Animal performance, health status and nutrient digestibility. Data on feed intake and growth performance are shown in Table 2. The pigs receiving the LOP treatments showed a higher average daily feed intake ($P = 0{\cdot}001$) than animals without it. Differences were more pronounced in animals fed on the WB diet, reflecting the tendency in the interaction with

Table 2. Performance of pigs fed on the experimental diets early after weaning (Trial 1)

		Diets							
		CT		WB			*P*-diet		
Item	Period	0	LOP	0	LOP	SEM (*n* 8)	DIET	LOP	D × L
Body weight (g)	Initial	6430	6400	6390	6390	11·4	0·952	0·970	0·969
	Final	8590	8850	8610	9630	12·8	0·382	0·168	0·408
Average daily feed intake (g/animal and d)	Week 1	189	221	183	248	33·3	0·408	0·001	0·210
	Week 2	354	382	328	424	50·6	0·660	0·001	0·070
	Overall	271	293	253	325	37·5	0·610	0·001	0·069
Average daily gain (g/animal and d)	Week 1	82	127	95	191	40·0	0·017	0·001	0·101
	Week 2	234	248	233	327	71·3	0·131	0·050	0·129
	Overall	157^{b}	170^{b}	156^{b}	238^{a}	47·0	0·050	0·007	0·047
Gain:feed ratio	Week 1	0·43	0·58	0·53	0·77	0·171	0·033	0·005	0·454
	Week 2	0·66	0·63	0·70	0·77	0·147	0·095	0·678	0·366
	Overall	0·57	0·57	0·62	0·77	0·115	0·014	0·173	0·174

CT, control diet; WB, wheat bran diet; LOP, loperamide; DIET, effect inclusion CT or WB in diet; D × L, effect diet and loperamide treatment.

^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

Table 3. Mortality, pigs with diarrhoea per treatment and coefficient of total tract apparent organic matter and crude protein digestibility in early weaned pigs (Trial 1)

		Diets					*P*-diet		
		CT		WB					
Item	Period	0	LOP	0	LOP	SEM (*n* 8)	DIET	LOP	D × L
Animal health status (no. of pigs)									
Mortality	Overall	2/32	2/32	2/32	1/32	0·44	0·690	0·690	0·690
Pigs with diarrhoea	Overall	9/30	8/30	13/31	4/32	0·24	0·969	0·029	0·096
Total tract apparent digestibility									
Organic matter	Final	83·2	83·7	72·0	78·0	6·75	0·001	0·061	0·116
Crude protein	Final	83·8	84·6	68·7	76·0	6·96	0·001	0·026	0·074

CT, control diet; WB, wheat bran diet; LOP, loperamide; DIET, effect inclusion CT or WB in diet; D × L, effect diet and loperamide treatment.

WB and LOP during the second week ($P=0·070$) and the overall period ($P=0·069$). A significant effect of the experimental treatments was also observed for the average daily gain (ADG) of the animals during weeks 1 and 2 after weaning. LOP and WB increased the ADG of the animal, with LOP pigs fed on the WB diet showing a much larger increase in ADG than the rest of the experimental treatments (interaction between WB and LOP, $P=0·047$). As a result of differences on the average daily feed intake and the ADG, pigs fed on the WB diet increased the feed efficiency ($P=0·013$) during the 2-week period, while LOP administration increased feed efficiency during week 1 ($P=0·005$).

Table 3 shows the number of pigs with diarrhoea and the mortality rate, as well as the total tract apparent digestibility of OM and CP. The LOP treatment reduced the number of pigs suffering diarrhoea ($P=0·029$). This effect was essentially observed with the WB diet (tendency for an interaction, $P=0·096$). However, no significant differences in the mortality were observed between treatments. The incorporation of WB in the diet reduced the total tract digestibility of OM ($P=0·001$) and CP ($P=0·001$). On the other hand, LOP tended to improve the coefficient of OM ($P=0·061$) and improved the CP ($P=0·026$) digestibility, especially with the WB diets (P interaction$=0·074$ and 0·116 for CP and OM digestibility, respectively). No significant differences among diets were observed in the MTT registered on day 13 after weaning, averaging 13.7, 15.5, 13.4 and 14.4 hours for the CT-0, CT–LOP, WB-0 and WB–LOP treatments, respectively. However, LOP tended ($P=0·070$) to increase the MTT compared with the non-treated animals.

Fermentation end products and quantitative changes in the microbial population of faeces. Total SCFA concentration and microbial counts in faeces are shown in Table 4. A significant interaction was observed between WB and LOP groups ($P=0·013$) on the total SCFA concentration. The LOP administration increased the faecal SCFA concentration in piglets fed on the WB diet, whereas no differences were observed in the CT group. LOP treatment increased the concentration of acetic acid ($P=0·004$) and tended to increase the propionic acid ($P=0·093$) in piglets fed on the WB diet and increased ($P=0·009$) the butyric acid in both the groups of animals (CT and WB). On the other hand, piglets fed on the WB diets tended to reduce ($P=0·062$) concentration of isoacids. The piglets fed on the WB diet tended ($P=0·089$) also to show lower counts of enterobacteria in the faeces compared with the CT diet. No significant differences were observed in the lactobacilli counts or associated with the LOP treatment.

Table 4. Concentration of SCFA and bacterial population (enterobacteria and lactobacilli) on faeces of piglets, 13 d after weaning (Trial 1)

	Diets					*P*-diet		
	CT		WB					
Item	0	LOP	0	LOP	SEM (*n* 8)	DIET	LOP	D × L
Concentration (μmol/g FM) of SCFA								
Total SCFA	103·0^{a,b}	102·2^{a,b}	90·9^{b}	114·8^{a}	18·35	0·642	0·021	0·013
Acetic	66·1^{a,b}	63·3^{a,b}	57·1^{b}	73·3^{a}	11·85	0·641	0·037	0·004
Propionic	22·2	22·1	19·9	23·5	4·106	0·454	0·109	0·093
Butyric	8·9	10·5	8·8	11·7	3·147	0·625	0·009	0·401
Isoacids	3·5	3·9	2·8	3·1	1·436	0·062	0·384	0·824
Branched chain ratio	0·035	0·041	0·034	0·031	0·0142	0·189	0·769	0·263
Bacterial population measured by real-time PCR (log 16S rDNA gene copies/g FM)								
Enterobacteria	9·0	9·3	8·8	8·5	0·68	0·089	0·965	0·295
Lactobacilli	9·6	9·4	9·4	9·1	0·46	0·291	0·216	0·818

CT, control diet; WB, wheat bran diet; LOP, loperamide; DIET, effect inclusion CT or WB in diet; D × L, effect diet and loperamide treatment.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

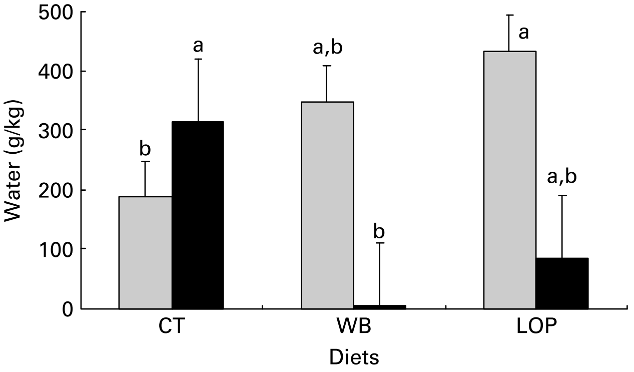

Fig. 1. Water retention capacity (WRC; ▢) and unbound water (■) of colonic digesta in piglets fed experimental diets (Trial 2). Diets: CT, control diet; WB, wheat bran diet; LOP, animals treated with loperamide. Values are least square means (n 4), with standard errors represented by vertical bars. [a,b] Mean values with unlike letters were significantly different ($P < 0.05$). The P-value for diet was 0·001 for the WRC and 0·018 for the unbound water.

Expt 2

Digestion and morphometry of the intestinal mucosa. LOP increased ($P=0.010$) the digestibility of OM (data not shown). LOP also promoted significant changes in the morphometry of the jejunum, with an increase ($P=0.031$) in the villus height:crypt depth ratio compared with the WB diet (data not shown). No other significant differences were observed in any of the variables studied in the jejunum. In the colon no significant differences were observed among the dietary treatments in the morphometric and the cellular measurements.

Physicochemical characteristics, fermentation parameters and microbial population of the colonic digesta. Data related to the physico-chemical characteristics of digesta (WRC and unbound water) are presented in Fig. 1. Feeding animals with WB diet and LOP administration increased ($P=0.001$) the WRC compared with the CT diet. On the other hand, animals fed on the WB diet showed the lowest concentration ($P=0.018$) of unbound water compared with the CT diet. No significant differences between treatments were observed on the total concentration of SCFA and the concentration of acetic, propionic, isoacids and lactic acid (data not shown). On the other hand, the butyric acid concentration tended ($P=0.086$) to be higher in pigs fed on the WB diet (35·9 and 20·3 μmol/g FM for the WB and the WB–LOP groups, respectively) compared with the CT diet (11·7 μmol/g FM). No significant differences were observed on the enterobacteria and lactobacilli counts (data not shown).

Discussion

The influence of wheat bran on the adaptation of piglets after weaning

Dietary fibre has become one of the dietary components, which has attracted much interest in connection with the nutrition of young animals. Previous studies have demonstrated that adding sources of mostly insoluble low-fermentable NSP (such as oat bran) to the diets for weaned pigs can ameliorate the incidence of diarrhoea and the animal performance(6,26). The basis for this protective effect is still uncertain, but the authors suggested that it could be related to changes in the numbers and metabolic activity of selected components of the intestinal microbiota.

The WB (the coarse outer membrane of the wheat kernel) was chosen for the present study because of its high proportion of NSP as insoluble cellulose and arabinoxylans(27) and its large particle size. Our results show that pigs fed on the WB treatment showed a reduction in the total tract digestibility of the OM and CP, but underwent an increase in the feed efficiency. Numerous reports indicate that dietary fibre reduces the total tract digestibility of protein and energy. In practice, fibrous diet components dilute the nutrient in feed because the NSP fraction is digested to a lower extent than other fractions, such as those of starch, CP or fat(28,29). Moreover, changes in the physical characteristics of the intestinal contents due to the presence of specific fibre components may increase the viscosity, influence gastric emptying or slow the diffusion or mobility of enzymes, substrates and nutrients to the absorptive surface. The consequence is that fibre may reduce nutrient digestibility of fat(30) or increase the endogenous nitrogen excretion(31). This effect was quantitatively described by Le Goff *et al.* (32). They found that the impact of the neutral-detergent fibre fraction on the digestibility coefficient of energy is significant, with approximately 0·1 % reduction per 1 g neutral-detergent fibre/kg DM.

In the literature, it is also generally accepted that fibre in the growing pig diet may also reduce the voluntary intake and the BW gain of the animals. In the present study, the pigs fed on the WB diets did not lower their voluntary intake and even increased the gain:feed efficiency. Some authors(3,6,33) have reported that moderate levels of WB or oats hulls in post-weaning diets increased the feed consumption of pigs. The authors suggested that young piglets may have a minimum requirement of fibre for correct functioning of the digestive tract. However, we should not exclude the possibility that the increased weight gain efficiency observed in the present study could be due, at least in part, to the increased weight of the internal organs, possibly by a higher weight of the gut contents(34).

Including WB in the diet reduced the branched-chain fatty acid concentration and tended to decrease the enterobacteria population in the faeces. Previous results from our group have also indicated that incorporation of WB to the diet also decreased the enterobacteria counts in the caecum digesta(3) and the K88 *E. coli* attachment to the ileum mucosa after an experimental infection(9). Although enterobacteria contain numerous species of bacteria, its reduction may indicate a beneficial shift in the composition of the microbial population. In this respect, different authors have demonstrated that the inclusion of fermentable carbohydrates in weanling diets may reduce the protein fermentation along the GIT(4,35) being related with the reduction in isoacids observed here. Protein fermentation in the digestive tract is considered as a potential risk for dysbiosis and proliferation of pathogenic bacteria(36). Pigs fed on the WB diet tended also to show a higher concentration of butyric acid in the colon. In this respect, butyrate is considered an important metabolite because it is the principal oxidative fuel for the colonocytes and may have beneficial trophic effects on the inflamed caeco–colonic mucosa(13). It is accepted that starch and bran from wheat or oat stimulate the formation of butyrate(37), while xylans and pectin rich fractions are all associated with a related low formation of butyrate(38).

On the other hand, fibre is also able to modify the physicochemical properties of digesta. WB, due to its high content of insoluble fibre, is known to improve constipation in human subjects(39) and reduces the mean retention time of digesta in the small intestine of pigs(11). In the present study, we were not able to detect differences in the MTT with the WB supplementation, but we observed a significant increase in the WRC and a reduced percentage of unbound water in the colonic digesta. The results demonstrate the higher water-binding capacity of the insoluble long-chain NSP as compared with other compounds, such as starch or protein(22). Moreover, these changes could suggest that the physicochemical properties of digesta could have a role in some GIT processes, such as the gastric emptying, the small intestine motility or the hindgut fermentation. Some reports have suggested that a coarse diet may modify the physico-chemical and microbial properties of digesta contents, with decreases in the survival level of some enterobacteria, such as *Salmonella* (40). The authors speculated that processes in the foregut, such as distribution of HCl within the stomach content, is favoured when a diet has a coarse structure and a higher WRC, so that lower counts of *Salmonella* reach the small intestine.

The influence of loperamide on the adaptation of piglets to the diet

LOP is a synthetic opiate derivative frequently used as antidiarrhoeal drug in human subjects. It decreases the motility of the circular and longitudinal smooth muscles of the intestinal wall, slows down the flow entering the colon and stimulates colonic water absorption(41). While LOP is widely used in adults, there has been concern about the safety of using this drug for young children and during the course of infectious diarrhoea. The contraindications in cases of invasive bacterial infections come from the risk of aggravating the symptoms per digesta stasis allowing bacterial translocation. Results from the present study indicate that LOP tended to increase the MTT along the GIT and increased the total tract and foregut digestibility of OM and CP, especially with the WB diet. These results were associated with a significant increase in the villus height:crypt depth ratio in the jejunum (Expt 2), which is considered a useful criterion for estimating the digestive capacity in the small intestine(42). In contrast, other authors have reported that LOP strongly inhibits pancreaticobiliary secretion (bilirubin and amylase), acting on the nerve supply to the pancreas and gallbladder(43,44).

Treating animals with LOP also increased the average daily feed intake and the ADG during the first 2 weeks after weaning. LOP is known to affect not only the opioid receptors related to inhibition of intestinal motility, but also those related to analgesia that are present on the peripheral sensory nerves and is up-regulated during the development of inflammation(45). After weaning, the stresses that the animals suffer lead to a period of anorexia that may contribute to a local inflammation in their small intestine(46). Moreover, the reduced enteral feeding during the first days after weaning is considered to be the cause of the impairment of the piglet gut barrier and the shortened villi. It appears that treating animals with LOP early after weaning may reduce the intestinal inflammation and improve the behaviour of the animal; this resulted in a higher feed consumption, weight gain and improved mucosa integrity. This is in good agreement with Bowden *et al.* (47), who reported positive effects of anti-inflammatory analgesic drugs and muscarinic receptor blocking agents on appetite in the pig.

The increase in the digestibility of WB diets with LOP was also associated with a significant increase in the concentration of SCFA, acetic, propionic and butyric acids in the faeces, which likely result from an advanced maturation of the colonic digestive tract. Graham *et al.* (48) and Le Goff *et al.* (32) suggested that the fibre degrading capacity in the pig intestine increases with age, likely due to increases on the transit time and the metabolic activity of the microbiota.

Conclusions

The inclusion of a moderate level of an insoluble fibre ingredient such as WB that could modify the intestinal microbiota activity, together with a drug like LOP, that has effects on the intestinal motility and peripheral analgesia, to the post-weaning diet, may have beneficial effects with regard to animal health and performance.

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