Open access • Journal Article • DOI:10.1080/17450390512331387936

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Published on: 01 Apr 2005 - Archives of Animal Nutrition (TAYLOR & FRANCIS LTD)

Topics: Locust bean gum and Guar gum

Archives of Animal Nutrition
April 2005; 59(2): 123–138

Taylor & Francis
Taylor & Francis Group

Incorporation of galactomannans in the diet of newly weaned piglets: Effect on bacteriological and some morphological characteristics of the small intestine

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(Received 26 October 2004; accepted 10 February 2005)

Abstract

In search of substances replacing antibiotics as growth promoters for farm animals, non-digestible oligosaccharides (NDO) or non-starch polysaccharides (NSP) have been proposed as possible alternatives. In this context, the influence of galactomannans on bacteriological and morphological aspects of the gastrointestinal tract in weanling pigs was investigated. Four groups of five newly weaned piglets received one of the following diets: control feed (C), C supplemented with guar gum (1%), C supplemented with locust bean gum (1%) and C supplemented with 10% of carob tree seeds meal as source of locust bean gum. The animals were euthanized after 11–12 days and digesta were sampled in stomach, jejunum (proximal and distal) and caecum, while mucosal scrapings and ring shaped tissue samples were taken of proximal and distal jejunum. On these samples bacteriological, biochemical and morphological determinations were carried out. Total count of bacteria in digesta and mucosal scrapings was not influenced by the different diets, with the exception of the proximal jejunum where a small decrease (0.5 $\log_{10}$ CFU) was noted with the guar gum and carob tree seeds diet. The number of *E. coli* increased by feeding both gums and carob tree seeds. With the latter diet, higher counts of streptococci were observed. In agreement with the lower concentration of lactic acid in jejunal contents, guar gum decreased the number of lactobacilli. Locust bean gum decreased the molar proportion of acetate in caecal contents while butyrate and valerate were augmented. Feeding the carob tree seeds resulted in shorter villi and a lower villus height/crypt depth ratio in the jejunum mucosa, which was an indication for a faster renewal rate of the epithelium. Both locust bean gum feeds significantly lowered the mitotic index in the crypts of the small intestine. Only with the carob tree seeds diet, viscosity of jejunal contents was increased. In conclusion, the effects of the addition of 1% of pure guar gum or locust bean gum were inconsistent and not very outspoken, whereas 10% of carob tree seeds meal in the diet resulted in influences on intestinal characteristics at the bacteriological and morphological level.

Keywords: *Piglets, NDO, galactomannans, intestinal flora, gut morphology, viscosity*

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ISSN 1745-039X print/ISSN 1477-2817 online
DOI: 10.1080/17450390512331387936

1. Introduction

Because of the forthcoming EU ban on the use of antibiotics as growth promoting substances in farm animals, considerable research has been going on with the aim of developing alternatives having comparable overall effects on animal performance. It has been proposed that the underlying mechanisms for the growth promoting effect of antibiotics are related to interventions in the gastrointestinal tract and more particular in the small intestine, at the bacteriological, physiological and immunological level (Visek 1978, Vervaeke et al. 1979, Corring et al. 1981, Anderson et al. 1999, Hillman 2001, Verstegen & Williams 2002, Gaskins et al. 2002). Based on these considerations, several substances as possible alternatives for nutritional antibiotics have been put forward: probiotics, non-digestible oligosaccharides (NDO) with or without prebiotic properties, organic acids, enzymes (proteases, NSP-ases), herbs, bacteriocins, antimicrobial peptides and bacteriophages (Partanen & Mroz 1999, Hillman 2001, Dierick et al. 2002, 2004; Reid & Friendship 2002, Verstegen & Williams 2002, Joerger 2003, Ricke 2003, Montagne et al. 2003). NDO and non-starch polysaccharides (NSP) are not digested by the host enzymes in the small intestine, thus reaching the hindgut where they can be used for metabolism and growth of favourable bacteria (e.g., lactobacilli or bifidobacteria) to the detriment of harmful species. In this case, they are considered as prebiotics (Gibson & Roberfroid 1995). However, they are fermented to a certain extent in the small intestine, and therefore the importance of effects on the bacterial flora present in the small intestine certainly should also be investigated (Van Nevel et al. 2003).

Guar gum and locust bean gum are highly viscous galactomannan NSP, not digested in the small intestine and capable of increasing the viscosity of intestinal contents, accompanied by decreasing nutrient (glucose, lipids) and water absorption in pigs and chickens (Blackburn & Johnson 1981, Rainbird & Low 1986). Considerable research efforts have been devoted to gums, but mainly based on their ability to improve glucose tolerance in animals (rat, chicken) and man, while plasma cholesterol was also lowered (Blackburn & Johnson 1981, Ellis et al. 1981, Johnson et al. 1984, Evans et al. 1992). NDO or NSP can also affect intestinal immunity (defense) and health, and the exact mechanism(s) remain(s) unknown, but may be related to changes in the bacterial flora (numbers) or in its composition, e.g. proportion of gram positive to gram negative species (Moreau & Coste 1993, Gaskins 1997, Hamann et al. 1998). Such changes can result in increased immunity through direct effects of intact bacteria via Peyer's patches or by bacterial cell by-products (Schley & Field 2002). Modulation of the immune system by mannans has been reported in literature (see Swanson et al. 2002). Galactomannans are also interesting soluble fiber substances, because it has been argued that mannan residues are able to inhibit fimbrial adhesins of *E. coli* and *Salmonella* (Swanson et al. 2002). Finally, the prebiotic effect of partially hydrolyzed guar gum has been demonstrated in a human volunteer study, where in the faeces a sizable increase in lactobacilli was observed while the increase in bifidobacteria was rather small (Okubo et al. 1994).

In earlier work we investigated the effect of β- 1,3-1,6 glucans on bacteriological and morphological aspects of the small intestine of newly weaned piglets (Van Nevel et al. 2003). In this paper, we report and discuss results of a similar experiment where guar gum, locust bean gum or meal of carob tree seeds was added to the diet of newly weaned piglets. Again, piglets were used as experimental animals because the weaning process causes profound modifications in intestinal morphology resulting in a temporal vulnerabilty of the animal (Cera et al. 1988, Nabuurs et al. 1993). So, any beneficial effect of galactomannans would be at its most obvious and easier to detect and demonstrate.

2. Material and methods

2.1. Animals

A group of 24 cross-bred piglets (Seghers hybrid x Piétrain) was weaned at five weeks of age and divided into four homogenous (sex, weight) subgroups of six animals, of which five were actually predestined for euthanasia and one animal was kept as reserve. They were housed in a clean, temperature (28°C) and relative humidity (55%) controlled facility, and each subgroup received one of the four experimental diets. The initial live weight of the piglets was 9.49 ± 1.52 kg (SD). The animals were fed in groups and had free access to feed and water. After 11 days, a first group of ten piglets was taken at random from the four subgroups and the animals were euthanized (overdose of Nembutal, natrii pentobarbitalum; Ceva Santé Animale, Brussels, Belgium). On day 12, the remaining ten piglets were sacrificed. Immediately after euthanasia, the gastrointestinal tract was removed and divided in sections: stomach, proximal jejunum (J1: 0–3 m distal to pylorus), distal jejunum (J2: 3–0 m proximal to caecum) and caecum. The different segments were emptied and the contents weighed. The jejunum segments were then flushed with saline (0.85 g of NaCl l^{-1} of water) until the effluent was clear. The segments were cut longitudinally and the mucosa was completely scraped off with a microscopy slide. After determination of pH the contents were homogenated (Ultra-Turrax T25, Janke & Kunkel GmbH & CoKG, Staufen, Germany; 30 s at 13500 rpm) and samples for bacterial counts and biochemical parameters were taken. Mucosal scrapings were also homogenized and sampled for bacterial enumeration. From proximal and distal jejunum tissue, a sample (10 cm of length) was transversally cut and divided in four adjacent subsamples of approximately 2 cm of length and fixed in neutral buffered formalin for 24 h. The sampling site was always at 3 m distal to pylorus for the proximal jejunum and 3 m proximal to the caecum for the distal jejunum.

2.2. Diets

Four diets were formulated: control feed (C), C supplemented with guar gum (1%), C supplemented with locust bean gum (1%) and C supplemented with 10% of carob tree seeds meal as a commercial source of locust bean gum. Their composition is shown in Table I. Diets were balanced for total protein content, net energy value (9.66 MJ kg^{-1}), Ca, P and some essential amino acids (based on small intestinal digestibility) using current standards and calculation. All ingredients were ground and diets fed in the dry form. Guar gum (G 4129) and locust bean gum (G 0753) were bought from Sigma Biochemicals and Reagents (Bornem, Belgium). Guar gum is extracted from seeds of *Cyanopsis tetragonoloba* and consists of a (1→4)-linked β-D-mannopyranose backbone with branchpoints from their 6-positions linked to α-D-galactose (1→6-linked-α-D-galactopyranose). Locust bean gum is extracted from seeds of the carob tree (*Ceratonia siliqua*) and the structure is similar to guar gum, but there are less mannose residues for every galactose. For the fourth diet it was decided to increase the percentage of locust bean gum to 5% (w/w), not as the pure product, but as a more commercial source of locust bean gum already used in the petfood industry, namely carob tree seeds meal, obtained by grinding the seeds of the carob tree (Caraton PFTG; Alimcarat S.L., Mallorca, Spain) of which 10% was mixed in the diet. Samples of feeds and the carob tree seed meal were analysed for dry matter, ash, crude protein, crude fat according to EU standard methods (Anonymous 1971a, 1971b, 1992, 1993, 1998). Total dietary fibre (TDF) analysis was carried out as described by Prosky et al. (1985). On a sample of the gums and carob tree seeds meal NSP monomers were determined following Theander et al. (1989).

Table I. Composition of the experimental diets [g kg^{-1}].

Ingredients	Control diet	Guar gum diet	Locust bean gum diet	Carob tree seeds diet
Barley	368.40	350.00	350.00	350.00
Maize (press. cooked)	100.00	100.00	100.00	100.00
Soya beans Danex ⋆	127.00	132.60	132.60	147.00
Wheat feed flour	150.00	149.91	149.91	43.63
Soya bean meal †	150.00	150.00	150.00	117.70
AA Protamyl SF ‡				25.00
Soya bean oil	6.60	9.50	9.50	18.40
Methionine	2.00	2.00	2.00	2.10
Lysine	4.40	4.30	4.30	3.80
Threonine	2.00	2.00	2.00	1.80
Tryptophane	0.24	0.23	0.23	0.31
NaCl	0.17	0.17	0.17	0.17
Mono Ca–phosphate	6.40	6.50	6.50	7.30
Whey permeate	57.70	57.70	57.70	57.70
Vit. E 50%	0.09	0.09	0.09	0.09
Premix vit. pigs 3/2.5 #	25.00	25.00	25.00	25.00
Guar gum		10.00		
Locust bean gum			10.00	
Carob tree seeds §				100.00

⋆ Expanded full fat soya beans (Danis N.V., Belgium); † Solvent extracted; ‡ Potato protein; #Provided per kg feed: vitamin A 10000 IU, vitamin D_3 2000 IU, vitamin E 60 mg, vitamin K 1 mg, vitamin B_1 1.25 mg, vitamin B_2 3.25 mg, , vitamin B_6 2 mg, vitamin B_{12} 23.75 μg, pantothenic acid 12 mg, niacin18.38 mg, folic acid 0.8 mg, choline 200 mg, Fe 120.00 mg, Cu 20 mg, Zn 100 mg, Mn 81.75 mg, I 1.13 mg, Co 1.13 mg, Se 0.38 mg, antioxidant 37.5 mg; § Caraton PTFG (Alimcarat, Spain).

2.3. Enumeration of viable bacteria and biochemical parameters

Bacterial counts on intestinal contents and mucosal scrapings were done using the ring-plate technique described by Van Der Heyde and Henderickx (1963). The selective media and incubation conditions (aerobic or anaerobic ; incubation time) of the inoculated plates used for the determination of the number of total bacteria, lactobacilli, streptococci and *E. coli* were exactly as described earlier (Van Nevel et al. 2003). On caecal contents, only the number of total bacteria was determined.

Contents sampled in J1, J2 and caecum were acidified (2% w/v of H_2SO_4 18N final concentration) after measuring pH and stored at − 18°C until further analysis. Lactic acid, short chain fatty acids (SCFA) and ammonia-N were determined as described elsewhere (Van Nevel and Demeyer 1977).

2.4. Gut morphology and histology

2.4.1. Morphometry. After fixation in neutral-buffered formalin, intestinal tissue samples were processed under standard conditions in an automatic tissue processor (Shandon, Pittsburgh, PA, USA). Processing consisted of serial dehydration with ethanol, clearing with xylene and impregnation with paraffin wax. In order to assure a sufficient number of well-oriented villi and crypts present on a specimen, for each jejunal tissue sample (sampling site), two adjacent segments (approx. length 1 cm) were embedded per paraffin block, resulting in two sections per specimen cut at 4 μm of thickness. Then, per tissue sample, four slides (silane-prep™ slides, Sigma, St Louis, MO 63178, USA) were prepared and each slide thus contained a specimen with two jejunal sections. One slide

was used for hematoxylin-eosin (HE) staining. Another one was stained with periodic acid and Schiff reagent (PAS) for counting goblet cells. The remaining slides were used for determination of the apoptotic and mitotic index. Per specimen stained with HE, villus length (V, from tip to base) and crypt depth (C, from base to opening) of all well-oriented villi and adjacent crypts were measured, using a microscope equipped with a camera and computer with appropriate software (Image Archiving Plus 4.51; Lucia, Laboratory Imaging s.r.o., Praha 4 – Hája, Czechia). The ratio V/C was calculated and per piglet mean values for V, C and V/C were calculated, finally resulting in five repetitions (piglets) per diet ($n=5$). Per piglet, the number of villi with adjacent crypts suitable for measurement varied between eight (one specimen) and 24.

2.4.2. Enumeration of intra-epithelial lymphocytes (IEL). On the HE stained specimen, 10 villi were selected for counting IEL, based on morphological characteristics (dark and densely stained). Series of 50–150 enterocytes were defined in the optical field and IEL present in that area were counted. Finally, the sum was made of all counted enterocytes and IEL per specimen, and the latter expressed as number of IEL per 100 enterocytes. Following this procedure, one value per piglet was obtained or 5 repetitions per diet. In this experiment, the number of enterocytes counted per animal (10 villi) varied between 582 and 1565 while the number of corresponding IEL was 64 - 215.

2.4.3. Enumeration of goblet cells. On the specimen stained with PAS reagent, goblet cells on the villi and in the crypts were counted. Therefore, ten villi and crypts were selected and positive stained cells counted. For villi, numbers of goblet cells were expressed per 100 μm of circumference while for the crypts, cells were expressed per 100 μm of crypt depth. As far as the crypts were concerned, this counting procedure did not make distinction between genuine goblet cells or stained mucin granules present in the crypt. Also, the size of the stained spots was not taken into account (see *Discussion*).

2.4.4. Determination of the apoptotic index. Apoptotic enterocytes on the villi were visualized using the TdT-FragEl DNA Fragmentation Detection Kit (Oncogene Research Products, Boston, MA 02118, USA). The protocol provided by the manufacturer was strictly applied. As apoptotic (positive) cells were generally very scarce, all intact villi present on a section were counted as well as the apoptotic enterocytes thereupon and the latter expressed per 100 villi. Mean apoptotic index ($n=5$) was calculated and used for statistical treatment. Per piglet (specimen), the number of intact villi taken into consideration for counting apoptotic cells varied between 144 and 231.

2.4.5. Determination of mitotic index. Proliferating cells in the crypts were determined by immunohistochemical labelling utilizing the Ki67 Antigen Kit (Novocastro Lab. Ltd, Newcastle upon Tyne, England). The protocol provided by the supplier had to be slightly changed: incubation with the primary antibodies (clone MM1) was performed overnight instead of during 60 min. Cells in mitosis were then clearly stained and could be counted, on condition that the crypt was perfectly oriented (base to mouth). Positive and negative cells were counted in 8–12 crypts per specimen. Per crypt, mitotic cells were expressed as% of total cells (mitotic index). This procedure resulted in five observations per diet and mean values were calculated and used for statistics.

2.5. Viscosity measurements

Viscosity of the diets was measured as described by Johansen et al. (1996). An aliquot of the diet (10 g) was suspended in centrifuge tubes in 50 ml of distilled water and put in a shaking waterbath at 38°C for 1 h. After centrifugation (30000 *g*; 15 min; 3°C) viscosity was measured on the supernatant using a digital viscometer (Model DV – II+, Brookfield Engineering Laboratories Inc., Staughton, UK). The shear rate was 73.42 s^{-1} (speed 60 rpm) and the spindle chamber was kept at 28°C. Jejunal contents sampled in the middle part of the jejunum, i.e., between the proximal (J1) and the distal (J2) part were centrifuged and viscosity was determined as described above.

2.6. Statistical analysis

For statistical evaluation of the results, one pig was used as an experimental unit, which was in agreement with other authors (see e.g., Manzanilla et al. 2004, Mikkelsen & Jensen 2004). The GLM ANOVA procedure was used and mean values were compared by a LSD test. All calculations were carried out using the SPSS 7.5 program for Windows (SPSS Inc., Chicago IL, USA). In view of the limited numbers of animals, significancy of differences was given at $p < 0.05$ or $p < 0.1$.

3. Results

3.1. Diets and animals

The chemical composition of the diets and the carob seed meal is presented in Table II. The crude protein and lipid contents were slightly higher for the supplemented diets when compared to the control ration, but these differences can be considered as being not very important in view of the fact that comparison of effects on growth parameters was not the aim of this experiment. By NSP monomer analysis it was found that in the carob tree seeds meal NSP and mannose content was 37.3% and 21.7% respectively. For locust bean gum, these figures were 61.6% and 41.4%, respectively. From these data, it could be calculated that the locust bean gum content of the feed supplemented with the carob tree seeds was 5.3%. Mean daily weight gain of the piglets (0–12 d post weaning) was 158 ± 56 g (SD) and no differences between the groups were observed. Daily feed intake was 240–260 g and feed conversion ratio varied between 1.47 and 1.77 (calculated per group). Although not measured, it was observed that faeces of the animals receiving carob tree seeds seemed to be less consistent, containing more moisture than the other groups.

Table II. Proximate analysis of diets and carob tree seeds meal [g kg^{-1}].

Diet	DM	OM	Ash	CP	EE	TDF[†]
Control diet	891	834	57.1	174.1	59.0	167.4
Guar gum diet	895	834	55.9	175.8	64.3	170.9
Locust bean gum diet	898	839	59.0	182.5	65.0	166.1
Carob tree seeds diet	904	846	57.8	186.4	73.3	178.1
Carob tree seeds meal	923	894	29.5	128.6	15.5	n.d.[‡]

* After acid hydrolysis; [†] Total dietary fibre (corrected for ash and protein in TDF); [‡] n.d.: not determined; NSP content from monomer analysis, excluding uronic acids, was 372.8 g kg^{-1}.

3.2. Bacterial counts

In Table III, the results of the viable counts of the different bacterial groups are presented. Compared with the control diet, total bacterial count was not affected by the supplemented diets, except in the luminal contents of the proximal jejunum (J1) where a rather small decrease (0.5 $\log_{10}$ CFU) was observed in the groups fed guar gum and the carob seeds diet. The gum containing diets caused clear increases in the number of *E. coli*, but this effect was only significant in the contents of the distal jejunum (J2) and in mucosal samples of both sites. The effect of the gums on the count of streptococci was rather complex and not very consistent. With guar gum, higher numbers were found in contents of both jejunal sites (although only significant in J1), but no changes could be observed in the stomach or in mucosal scrapings (J1 and J2). With the locust bean gum only an increase was noted in stomach and J1. The most pronounced and unequivocal effects were seen in the animals fed the carob tree seeds: compared with the control diet, the number of streptococci was higher in contents of the stomach, J1 and J2, and also on the mucosa but only in J2. Counts of lactobacilli were only influenced by the guar gum diet, where decreased numbers were counted in contents of stomach and J1 and J2. The lower numbers observed on the jejunual mucosa were not statistically significant.

3.3. Biochemical parameters in intestinal tract

Concerning the pH measurements in the differents parts of the gastrointestinal tract, the only result worth mentioning was a significant lower value in the stomach of animals fed carob tree

Table III. Effect of various diets on viable counts [$\log_{10}$ CFU g^{-1}] in digesta and mucosa ★ ‡.

Bacteria	Diet	Stomach	Jejunum 1	Jejunum 2	Mucosa 1	Mucosa 2
Total count	Control	7.8 ^{A}	6.9 ^{a}	7.2 ^{A}	5.3 ^{AB}	5.9 ^{A}
	Guar gum	7.3 ^{A}	6.4 ^{b}	7.8 ^{A}	5.0 ^{A}	6.1 ^{A}
(RCM)	Locust bean gum	7.6 ^{A}	6.5 ^{ab}	7.6 ^{A}	5.4 ^{AB}	5.5 ^{A}
	Carob tree seeds	7.1 ^{A}	6.3 ^{b}	7.3 ^{A}	6.1 ^{B}	6.2 ^{A}
	RSD †	0.8	0.4	0.8	1.1	0.8
E. coli	Control	2.6 ^{A}	3.4 ^{A}	3.6 ^{a}	1.9 ^{A}	1.7 ^{A}
	Guar gum	3.6 ^{A}	3.9 ^{A}	6.5 ^{b}	2.2 ^{AB}	4.9 ^{B}
(EMB)	Locust bean gum	4.2 ^{A}	4.1 ^{A}	5.7 ^{b}	2.4 ^{AB}	3.5 ^{B}
	Carob tree seeds	4.0 ^{A}	4.4 ^{A}	6.5 ^{b}	3.9 ^{B}	4.8 ^{B}
	RSD	2.2	1.3	1.5	1.8	1.7
Streptococci	Control	4.9 ^{AC}	4.0 ^{A}	4.7 ^{a}	4.4 ^{A}	4.7 ^{A}
	Guar gum	4.8 ^{A}	5.1 ^{B}	5.9 ^{ab}	4.2 ^{A}	5.2 ^{A}
(Slan. &	Locust bean gum	6.1 ^{B}	5.0 ^{B}	4.9 ^{a}	4.1 ^{A}	4.0 ^{B}
Bartley)	Carob tree seeds	6.1 ^{BC}	5.6 ^{B}	6.6 ^{b}	4.6 ^{A}	5.9 ^{C}
	RSD	1.1	0.9	1.2	0.5	0.6
Lactobacilli	Control	8.2 ^{a}	7.3 ^{A}	8.1 ^{ac}	5.4 ^{AC}	5 .6 ^{AC}
	Guar gum	7.0 ^{b}	6.7 ^{B}	7.0 ^{b}	4.9 ^{A}	5.4 ^{A}
(Rogosa)	Locust bean gum	8.2 ^{a}	7.2 ^{AB}	8.6 ^{a}	5.5 ^{BC}	6.1 ^{BC}
	Carob tree seeds	7.7 ^{a}	6.8 ^{AB}	7.4 ^{bc}	5.4 ^{AC}	5.5 ^{AC}
	RSD	0.6	0.5	0.8	0.6	0.6

★Mucosa 1 and 2 sampled in jejunum 1 and 2, respectively; † RSD: residual standard deviation; ‡ Mean values per column and per bacterial group bearing different superscripts are significantly different at $p < 0.05$ (a,b,c) or $p < 0.1$ (A,B,C).

seeds: a value of 2.5 compared to 3.2 – 3.8 for all other groups. SCFA determination in jejunal contents was not satisfactory as the acetate peak was not separated from a quantitatively important but unknown component. Therefore, data are not shown. The concentrations of lactic acid and ammonia-N in contents from J1 and J2 are presented in Table IV. Only with the guar gum diet an effect on lactate was noted as concentrations were lower in J1 and J2. In comparison with the control feed, the supplemented diets showed higher concentrations of NH_3-N in both J1 and J2 but the effect lacked significance. Biochemical parameters determined in caecal contents are summarized in Table V. Only with the diets containing locust bean gum (pure product or seed meal), the molar proportions of the SCFA were influenced: acetate was decreased accompanied by an increase in n-butyrate and n-valerate.

3.4. Morphometrical and histological parameters of small intestine

Compared with the control diet, only in the case of the carob tree seeds feed clear differences were noted: villi (V) in J1 and J2 were considerably shorter although significance was only reached in J1, while the crypts (C) were deeper, resulting in rather low values for the V/C ratio (Table VI). The decrease in V/C was only significant in J1 as for one of the five J2 samples very long villi (mean value of 637 μm) were observed. Enumeration of goblet cells revealed no differences between the diets as far as the crypts were concerned. Mean values varied between 9.0 and 10.8 cells per 100 μm of crypt depth. The number of goblet cells on the villi in the control group was 1.3 and 1.6 cells per 100 μm circumference for J1 and J2, respectively. This

Table IV. Lactic acid and ammonia-N [mmol kg^{-1}] in jejunal contents †.

	Jejunum 1*		Jejunum 2	
Diet	Lactic acid	Ammonia-N	Lactic acid	Ammonia-N
Control	18.44 ^{AC}	4.61 ^{A}	51.61 ^{A}	2.34 ^{A}
Guar gum	13.33 ^{B}	4.04 ^{A}	23.34 ^{B}	4.38 ^{BC}
Locust bean gum	20.13 ^{C}	3.93 ^{A}	65.65 ^{A}	3.87 ^{AC}
Carob tree seeds	15.95 ^{AB}	4.87 ^{A}	39.57 ^{AB}	4.01 ^{AC}
RSD ‡	3.82	0.93	25.48	1.56

* Jejunum 1: 0 – 3 m distal to pylorus; jejunum 2: 3 – 0 m proximal to caecum; † Mean values ($n = 5$) per column bearing different superscripts are significantly different at $p < 0.1$; ‡ RSD: residual standard deviation.

Table V. Effect of the diets on fermentation parameters in caecal contents †.

	SCFA [mol%]				Total SCFA [mmol kg^{-1}]	Lactic acid [mmol kg^{-1}]	NH_3-N [mmol kg^{-1}]
Diet	Acetic acid	Propionic acid	n-butyric acid	n-valeric acid			
Control	60.21^{A}	29.81 ^{A}	9.08 ^{A}	0.91 ^{a}	121.0 ^{A}	18.00 ^{A}	6.03 ^{AC}
Guar gum	62.19 ^{A}	27.45 ^{A}	9.15 ^{AC}	1.21 ^{a}	135.5 ^{A}	6.09 ^{A}	7.13 ^{BC}
Locust bean gum	55.41 ^{B}	27.94 ^{A}	13.48 ^{B}	3.17 ^{b}	126.5 ^{A}	22.14 ^{A}	4.65 ^{AC}
Carob tree seeds	55.56 ^{B}	29.62 ^{A}	12.45 ^{BC}	2.37 ^{b}	135.4 ^{A}	6.54 ^{A}	2.25 ^{A}
RSD ‡	4.24	3.34	3.07	1.00	23.11	19.38	4.09

† Mean values ($n = 5$) per column bearing different superscripts are significantly different at $p < 0.05$ (a,b,c) or $p < 0.1$ (A,B,C); ‡ RSD: residual standard deviation.

parameter was not influenced by the guar gum diet, but with the locust bean gum and the carob seeds feed, a lower value was observed in J2 samples, namely 1.0 cell per 100 μm although this decrease was only statistically significant for the latter diet, due to the rather high variability of the counts. The following considerations urged us to determine the number of intraepithelial lymphocytes (IEL). They are part of the mucosal immune system (first line of defence under the protective mucus layer) and play also a role in the elimination of damaged or infected cells (Cerf-Bensussan & Guy-Grand 1991, Gaskins 1997). Furthermore, the number of IEL is influenced by microbial colonization (bacterial load) and hence by diet composition (Gaskins 1997). Also, a positive relationship between the number of IEL in the small intestinal mucosa and its turnover rate has been reported earlier (Guy-Grand et al. 1998). On the villi of J1 and J2 samples from the piglets on the control diet, the number of IEL per 100 enterocytes was 14.4 $\pm$ 4.4 (SD; $n = 5$) and 13.5 $\pm$ 3.2, respectively. None of the supplemented diets exerted an effect on this parameter (data not shown). Apoptotic and mitotic index can provide useful information concerning the renewal rate of the intestinal mucosa. Both parameters were determined and the results are shown in Table VII. The variability of the determination of the apoptotic index in the proximal jejunum (J1) was very high, which was not different from the other diets. However, in J2 samples the very low values determined with the carob tree seeds and guar gum diets were significant. Compared with the

Table VI. Effect of the diets on villus length [μm] and crypt depth [μm] in jejunum *.

Diet	Jejunum 1			Jejunum 2		
	Villi (V)	Crypts (C)	V/C	Villi (V)	Crypts (C)	V/C
Control	432 ^{a}	342 ^{a}	1.30 ^{a}	465 ^{A}	323 ^{a}	1.48 ^{AB}
Guar gum	432 ^{a}	348 ^{a}	1.26 ^{a}	459 ^{A}	351 ^{ab}	1.34 ^{AB}
Locust bean gum	429 ^{a}	357 ^{a}	1.23 ^{a}	474 ^{A}	316 ^{a}	1.55 ^{B}
Carob tree seeds	318 ^{b}	441 ^{b}	0.77 ^{b}	376 ^{A}	384 ^{b}	1.06 ^{A}
RSD†	62	23	0.21	109	40	0.41

* Mean values ($n = 5$) bearing different superscripts are significantly different at $p < 0.05$ (a,b) or $p < 0.1$ (A,B); † RSD: Residual standard deviation.

Table VII. Apoptotic [villi] and mitotic [crypts] index in jejunum as influenced by experimental diets †.

	Apoptotic index*		Mitotic index§	
Diet	Jejunum 1	Jejunum 2	Jejunum 1	Jejunum 2
Control	66.8 ^{A}	49.7 ^{A}	61.1 ^{a}	60.8 ^{A}
Guar gum	47.0 ^{A}	8.9 ^{B}	61.0 ^{a}	61.3 ^{A}
Locust bean gum	57.2 ^{A}	46.9 ^{A}	52.4 ^{b}	52.3 ^{B}
Carob tree seeds	23.7 ^{A}	8.2 ^{B}	47.8 ^{b}	45.9 ^{B}
RSD‡	63.9	28.9	6.3	7.7

† Mean values ($n = 5$) per column bearing different superscripts are significantly different at $p < 0.05$ (a,b) or $p < 0.1$ (A,B); * Apoptotic index: Number of apoptotic enterocytes were counted on all intact villi on a specimen (2 sections) and then expressed per 100 villi; §Mitotic index: Mitotic cells in the crypts were counted and expressed as % of total cells in the crypt; ‡ RSD: Residual standard deviation.

control and guar gum diet, significantly lower values for mitotic index were found with both locust bean gum feeds (see Table VII).

3.5. *Viscosity of feeds and digesta*

The viscosity measurements on feed extracts and jejunal contents are presented in Table VIII. Only in the case of the carob seeds diet, a clear increase in viscosity of the jejunal digesta was noted.

4. Discussion

4.1. *Bacteriological enumeration*

Numerous research reports deal with the effect of incorporating NDO or NSP in the diets of man and rats whereby emphasis was put on the composition of the bacterial flora in the large intestine. The aim was to demonstrate a possible prebiotic effect of these compounds. However, in case of investigating growth promoting effects in farm animals caused by this class of substances, much attention should be paid to influences on different aspects of the small intestine, because by far the most important part of the digestion takes place in this part of the gastrointestinal tract.

As numbers of total bacteria and lactobacilli were of the same order of magnitude, it is clear that lactic acid bacteria were the most important fraction of the flora. One of the most consistent effects of the gum containing diets or the carob tree seeds diet on the small intestinal flora was an increase in the numbers of *E. coli* in contents and on the mucosa, although statistical significancy was not reached in the proximal jejunum samples. This is not necessarily in disagreement with the fact that mannose residues were thought to inhibit adhesion of *E. coli* having type I fimbriae (Swanson et al. 2002) as not all *E. coli* strains express fimbriae. Also, Gibson (1998) mentioned that oligosaccharides (mannanoligosaccharides) having mannose side chains might be effective against *E. coli* and related organisms, through blocking of adhesion sites otherwise available for pathogens. Compared with the control feed, the effect of the three gum containing diets on streptococci can be summarized as follows: lower counts of total bacteria or specific groups were never noted and in the majority of samples, higher counts were observed indicating the possibility of a growth stimulating effect. Effects on digesta were not always reflected in the corresponding mucosal samples. The lower lactobacilli numbers after feeding guar gum can be related to a reduced adherence to

Table VIII. Viscosity measurements [mPa s] on the diets and jejunal contents.

	Viscosity	
Diet	Diet	Jejunal contents *
Control	1.12	1.53 [a]
Guar gum	1.72	2.29 [a]
Locust bean gum	1.33	1.51 [a]
Carob tree seeds	2.45	4.64 [b]
RSD[†]		1.23

* Mean values ($n = 5$) per column bearing different superscripts are significantly different at $p < 0.05$; [†] Residual standard deviation.

enterocytes (Lynn et al. 1994a), although the decrease in numbers in mucosal scraping samples was not significant. Compared with literature data (Jensen 1999), viable counts of total bacteria in caecal contents were too low (7.8–8.2 $\log_{10}$ CFU) and are therefore not included in Table III. The ring-plate method used in this work needs careful adaptations, especially concerning the maintenance of strict anaerobic conditions during sampling and diluting of caecal or colon contents, and inoculation on the plates. For the sake of completeness, it should also be mentioned that the effect of carob tree seeds meal cannot be solely ascribed to the presence of the higher amounts of locust bean gum, as it has been shown that carob tree seeds can contain polyphenols with antibacterial properties (Mangan 1988, Papagiannopoulos et al. 2004).

4.2. Biochemical parameters

The lower pH value observed in the stomach of piglets fed the carob tree seeds diet may be related to a slower gastric emptying, as Rainbird (1986) reported reduced gastric emptying in growing pigs receiving a high-energy meal containing 6% of guar gum. Feeding the guar gum diet decreased significantly the lactate concentration in both jejunal sampling sites which was in agreement with lower viable counts of lactobacilli. However, Maisonnier et al. (2003) found an increase in lactic acid in the small intestine of broiler chickens when the diet contained 0.5% of guar gum. The comparison with our data is perhaps not very designated due to the difference in animal species and amount of guar gum supplied. Although effects of the different supplemented feeds on total SCFA, lactate and ammonia-N in caecal contents were absent, the molar proportions of SCFA were affected but only by both feeds containing locust bean gum. Percentages of n-butyrate and n-valerate were increased while acetate was lowered. In view of the metabolic importance of n-butyrate in the large intestine, this increasing effect can be considered as being favourable (Sakata 1987, 1997, Salminen et al. 1998, Bach Knudsen et al. 2003). Higher n-valerate proportions can be the result of an enhanced proteolysis but the tendency to lower ammonia-N concentrations in caecal contents on both locust bean gum diets is in contradiction with this hypothesis. When rats were fed diets containing gums (8–10% in feed), SCFA pattern in caecum was affected, but in contrast with our results, the proportion of propionate was increased at the expense of acetate and n-butyrate. A positive relation between the proportion of galactose in the gums and the intensity of the effect was seen, while we observed the opposite (Evans et al. 1992, Henningsson et al. 2002). Furthermore, in incubations with cat faeces as inoculum and several NSP as sole substrate, high proportions of propionate were determined with guar gum and locust bean gum (Sunvold et al. 1995). However, McDonald et al. (1999) supplemented a diet based on white rice with 10% of guar gum, and when fed to newly weaned piglets, the effect on SCFA in caecal contents agreed well with our results when locust bean gum diets were administered: the percentage of acetate and propionate was decreased, but n-butyrate increased from 6.5–16.0%. It is also worth mentioning that the amount of gums added in the present study was rather low compared with the work mentioned above. Thus, from results published in literature, it seems that the effect of gums on SCFA pattern in caecum varied with animal species and with the kind of gum used.

4.3. Morphometry and histological determinations

In an experiment with newly weaned piglets receiving a white rice based diet, the supplementation with 10% guar gum had no effect on villus height or crypt depth in the small intestine (McDonald et al. 1999). The lower V/C in our piglets fed the carob tree seeds

is an unfavourable aspect as it is an indication for an increased turnover rate of the intestinal mucosa. When crypt cell proliferation rate remains unchanged, shorter villi and deeper crypts result in a faster migration rate of enterocytes along the villus and increased loss of enterocytes from the villus tip, with possible consequences on digestion due to immature enterocytes and lower brush border enzyme activity (Hampson 1986, Hampson & Kidder 1986, Li et al. 1990, Pluske et al. 1997). A faster turnover rate of the intestinal epithelium results in a higher maintenance requirement, which can finally lead to lower growth rate or growth efficiency of the animal.

It is difficult to interpret the smaller number of goblet cells present on the villi of piglets receiving the carob tree seeds diet. If such a decrease is indeed indicative for lower secretion of mucins, then it can be reasoned that precursors, otherwise needed for formation of mucous substances, can now be used for maintenance or growth purposes. Mucins are only digested to a minor extent in the small intestine, but they are fermented by bacteria in the hindgut which is a less efficient process for the animal (Neutra & Forstner 1987, Dierick et al. 1990). Counting goblet cells in the crypts was not an optimal procedure as no distinction was made between genuine cells and mucin granules, while also the size of the stained objects was not taken into account. The latter can be avoided by the use of an appropriate image analysis program. Comparison with literature data showed that the number of IEL in our experiment was within the normal range (Nunez et al. 1996, Rooke et al. 1997, Pabst & Rothkötter 1999, Gu et al. 2002, Gu and Li, 2004). However, in an earlier experiment we observed considerably higher values (Van Nevel et al. 2003). As the diets fed in both experiments were basically very similar, the lower number of IEL could be related to the fact that in the current experiment, weaning age was five instead of four weeks. It has been shown that weaning age and age in general have clear influences on the number of IEL in the small intestine of piglets (Pabst & Rothkötter 1999, Gu et al. 2002, Gu and Li 2004). Supplementation of the control diet had no effect on the IEL number, which can be explained by the fact that the influence on the number of total bacteria (bacterial load) was absent or negligible (Gaskins 1997). Indeed, higher numbers of *E. coli* and streptococci were counterbalanced by a lower lactobacilli count.

Homeostasis of the intestinal epithelium is maintained by cell proliferation in the crypts and loss of cells (apoptosis) at the villus tip (Hall et al. 1994, Jin et al. 1994, Potten et al. 1997). The count of apoptotic enterocytes on the villi was very low and highly variable and the effect of the supplemented diets was not very consistent. Lower numbers in the distal jejunum when guar gum or carob tree seeds were fed should normally be related to an increased length of the villi which was not the case in this experiment. Feeding guar gum (4–10% of the diet) to rats resulted in an increased cell proliferation rate or mitotic index in the crypts of the small intestine (Johnson et al. 1984, Pell et al. 1992, Lynn et al. 1994b). It has been suggested that such an increase might be the result of interactions between viscosity of luminal contents and trophic effects of SCFA (Sakata 1988, Mathers 1998). In our piglet experiment, the guar gum diet did not affect the mitotic index in jejunal samples, but the amount of gum mixed in the feed was rather small (1%). On the contrary, mitotic index was clearly decreased when locust bean gum diets were fed and a reasonal explanation for this cannot be offered. Further research on this subject seems to be indicated. The decreased mitotic index seen with the latter diets can be very important in connection with the renewal rate of the intestinal epithelium. As mentioned above, the lower villus length and increased crypt depth in the jejunum of animals fed the carob tree seeds feed was indicative for a higher turnover rate of intestinal mucosal cells. However, when the decreased rate of cell proliferation is also taken into account, it is possible that compared to the control diet, the renewal rate of the mucosa remained unchanged or was even slower, although this reasoning is highly speculative.

In connection with the influence of different galactomannans on morphological and histological characteristics of the intestinal tract of piglets, much more research needs to be done before conclusions can be formulated.

4.4. Viscosity measurements

Only with the carob tree seeds diet, a higher viscosity in luminal contents sampled in the jejunum was determined. In general, higher viscosity can be accompanied by shorter villi and a lower V/C ratio with reduced overall capacity of digestion and absorption of nutrients, increased endogenous secretions and water content of the digesta (Ehrlein & Stockmann 1998, McDonald et al. 2001, Bartelt et al. 2002). It seems that in order to assure an optimal digestion and absorption of nutrients in piglets and pigs, viscosity of luminal contents should not exceed a treshold value, but exact quantitative aspects are not yet known.

5. Conclusion

It can be concluded from this experiment that an addition of 10% of carob tree seeds meal to a diet for newly weaned piglets effected intestinal characteristics at the bacteriological and morphological level. Also, viscosity of jejunal contents was increased. Effects after addition of 1% of pure guar gum or locust bean gum were much less pronounced and inconsistent. Because in the control feed NSP such as $\beta-1,3-1,4$ glucans, arabinoxylans and polymers with galactose and mannose as predominant monomers were already present, they perhaps partially masked the effects of the galactomannan supplements. It remains possible that by using a synthetic diet, effects of the addition of 1% of the gums could have been demonstrated, but this procedure is very different from conditions used in practice. Therefore, it cannot be concluded whether galactomannans can be considered as valuable alternatives for the use of antibiotics in piglet feeding. For that reason, studies more directed towards animal performances and health and carried out in properly designed experiments need to be done.

Acknowledgements

Grants were received from the former Federal Ministry of Agriculture, DG6, Brussels and VITAMEX NV, Drongen, Belgium. C. Vermander, A. Ovijn, A. Maes, E. Turtelboom, D. Bayens and S. Coolsaet are thanked for skillful technical assistance.

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