# Effects of a gluco-oligosaccharide supplement on the morphological characteristics of the gastro-intestinal tract and growth performance in weaned piglets

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## ABSTRACT

This study was designed to evaluate the effects of a gluco-oligosaccharide (GOS), as an alternative to growth promoters in piglets, on: growth performance, blood parameters and morphological characteristics of the intestinal tract. Four week old weaned piglets (*n=128*) (7.2 ± 1.04 kg l.w.) were divided into four groups and fed for 77 days on different diets as follows: 1.- Basal diet (CTR); 2.- Basal diet supplemented with a 2% GOS; 3.- Basal diet supplemented with chlortetracycline and spiramicine at 1000 and 400 mg/kg, respectively, for 14 days and then fed the CTR basal diet (CTRM); or 4.-a GOS diet supplemented and administered as in group 3 (GOSM). Animals were individually weighed 5 times, on days 0, 14, 35, 56 and 77. At the same time, the feed intake and average daily gains (ADG) were recorded and the feed conversion ratio (FCR) was calculated. On days 0 and 77, plasma was sampled from 6 piglets/treatment group, whereas on 77$^{th}$ day, 4 piglets/treatment were slaughtered to assess the morphological characteristics of parts of their gastro-intestinal tracts (ileum and caecum). The results showed no effects of the medications on the ADG in all the experimental periods. However, from days 57 to 77 of the trial period, the ADG was found to be higher in the GOS-fed animals (P=0.0747). During the first 14 days of the trial, the piglets on the medicated diets showed a higher intake than the animals in the normal diet groups, but no differences were detectable in the FCR. The globulin concentration and the albumin/globulin ratio was found to be reduced by GOS treatment (P <0.01). The urea concentration in the blood was decreased (P<0.05), whereas the plasma concentration of phosphorous was increased (P<0.01), by GOS supplementation. We found that the heights of the villi in the ileum was higher in the piglets on the GOSM diet compared to CTRM diet: (188.66 mm *vs* 255.74 mm; P<0.01). We also observed that supplementing these diets with GOS lead to a higher caecum epithelial cell height (11.7 µm vs 14.7 µm; P 0.068) compared with animals on the non-supplemented diets.

Our current data indicate that the growth performance did not differ between piglets fed on control, antibiotic-supplemented or GOS-supplemented diets. A dietary supplementation of GOS does increase the villus length, but only in animals previously fed with medicated feeds. The use of GOS seems to exert anti-inflammatory effects upon these animals.

*Key words*: Piglets, Gluco-oligosaccharide, Growth, Blood parameters, Gut morphology.

## RIASSUNTO

### EFFETTO DI UN GLUCO-OLIGOSACCARIDE SULLE PERFORMANCE E LE CARATTERISTICHE MORFOLOGICHE INTESTINALI DI SUINETTI SVEZZATI

*Questo lavoro ha avuto la finalità di verificare gli effetti dell'integrazione alimentare con gluco-oligosaccaride (GOS) sulle performance e la morfologia intestinale di suinetti svezzati. E'stata utilizzata questa molecola in quanto è una potenziale alternativa agli antibiotici promotori di crescita. A tale scopo 128 incroci Duroc svezzati, dell'età di 4 settimane e del peso di 7,2 ± 1,04 kg, sono stati suddivisi in 4 trattamenti di 32 animali ciascuno e alimentati con uno dei seguenti regimi alimentari: 1.- Dieta controllo (CTR); 2.- Dieta controllo addizionata con gluco-oligosaccaride ad una concentrazione finale del 2% (GOS); 3.- Dieta controllo addizionata con clorotetraciclina e spiramicina, a dosaggi di 1000 e 400 mg/kg rispettivamente, nei primi 14 giorni di prova e poi alimentati con la dieta controllo non additivata (CTRM). oppure 4.-con la dieta GOS sino alla fine della sperimentazione GOSM). Gli animali sono stati pesati 5 volte: al giorno 0, 14, 35, 56 e 77; negli stessi periodi è stato registrato il consumo di alimento e calcolati l'accrescimento medio giornaliero (ADG) e l'indice di conversione (FCR). All'inizio e alla fine della prova è stato eseguito un prelievo di sangue da 6 animali/trattamento, mentre alla fine della prova 4 suinetti/trattamento sono stati macellati per procedere all'esame microscopico degli epiteli intestinali (cieco e ileo). In entrambi i periodi i risultati mostrano l'assenza di effetto del trattamento sugli accrescimenti, tuttavia dal 57° al 77° giorno l'ADG è risultato tendenzialmente superiore negli animali alimentati con la dieta GOS (P 0,0747). Nei primi 14 giorni l'integrazione con antibiotici ha determinato maggiori ingestioni, mentre nessun effetto è stato visto su FCR. La concentrazione plasmatica di globulina è stata ridotta dal trattamento con gluco-oligosaccaride (P< 0,01) e di riflesso anche il rapporto albumine/globulina (P< 0,01). L'urea ematica è stata diminuita dal trattamento con GOS (P< 0,05) che ha invece aumentato il fosforo plasmatico (P< 0,01). L'integrazione alimentare con GOS ha accresciuto la lunghezza dei villi intestinali ma unicamente nelle diete medicate (188,66 mm vs 255,74 mm; P< 0,01). L'integrazione alimentare con GOS ha determinato una tendenza verso una maggior altezza delle cellule epiteliali ciecali (11,7 µm vs 14,7 µm; P 0,068) rispetto al controllo negativo.*

*Le performance dei suinetti alimentati con una dieta integrata con GOS sono risultate analoghe a quelle delle diete medicate. L'integrazione alimentare con GOS aumenta la lunghezza dei villi intestinali ma solo negli animali che avevano ricevuto il mangime medicato nel primo periodo. L'impiego di GOS sembra esercitare un effetto anti-infiammatorio negli animali.*

*Parole chiave: Suinetti, Gluco-oligosaccaride, Accrescimenti, Parametri ematici, Morfologia intestinale.*

## Introduction

The intestinal tract harbours a large, active and complex community of microorganisms. The intestinal microbiota plays several roles in the ingestion of food, in addition to its function in immunoprotection and the prevention of colonisation by pathogenic bacteria in the gastro-intestinal tract. For these reasons, the microbiota is centrally involved in maintaining animal health and performance. After the EU-ban on the use of antibiotics as growth-promoters, the interest in the development of alternatives to these agents has increased.

The use of oligosaccharides as "feed addi-

tives" could play a prebiotic role, promote the development of certain microbial groups and be considered as alternatives to previously used drugs. In this regard, microorganisms are different amongst themselves for energy and protein sources, micronutrients and vitamins requirements are also different. It is therefore possible to stimulate the growth of some intestinal species of microbes through the supply of substrates such as fructo-oligosaccharides (FOS) (Houdijk *et al.*, 2002; Mikkelsen *et al.*, 2003), gluco-oligosaccharides (GOS) (Djouzi *et al.*, 1995; Gabert *et al.*, 1995), mannan-oligosaccharides (MOS) (Zhou *et al.*, 2002), galacto-oligosaccharides (GAS), xilo-oligosaccharides (XOS) and transgalacto–oligosaccharides (TOS) (Hidaka *et al.*, 1986; Katta *et al.*, 1993). The extent of the effects of these compounds is determined by numerous factors including the type of additive itself, the age and species of the animal, and the breeding conditions. The mechanism of action of these products is most likely a selective stimulation of special positive microbial clusters in the gut, such as *Bifidobacterium* (Unno *et al.*, 1993; Hirayama *et al.*, 1994; Howard *et al.*, 1995), *Bacteroides* and *Lactobacillus* (Takahashi *et al.*, 1996), *Pediococcus* spp. or *Enterococcus faecium*, but not Salmonella *typhimurium* (Oyarzabal and Conner, 1995).

The efficacy of the dietary integration of oligosaccharides is also modulated by the type of diet, and is for example greater in hamsters fed with a diet containing large amounts of bran versus those receiving feed with a larger meat component (Hirayama *et al.*, 1994). It is also not only the type of diet which affects the response to FOS administration and it has been shown that three different strains of *Bifidobacterium* (*B. infantis* ATCC 15697, *B. adolescentis* ATCC 15703, *B. longum* ATCC 15707) have different growth patterns in culture medium containing different types of fructane. Galacto-oligosaccharides are not as effective as FOS but can also promote the growth of *Bifidobacterium* and *Lactobacillus* (Morishita *et al.*, 1992). By administering GOS to axenic rats, Valette *et al.* (1993) have established previously that such molecules are resistant to intestinal digestion. However, if the same animals are inoculated with human intestinal microflora, the subsequent administration of GOS does not change the caecal pH, volatile fatty acids (VFA) production or lactic acid levels. Changes are in fact observed in terms of the VFA profile (reduced molar percentages of butyric, isobutyric, isovalerianic acids and an increased molar percentage of caproic acid) with the increased production of $H_2$ and $CH_4$ observed. A reduced production of branched chain VFAs may point to a decrease in the proteolytic activity of the large intestine. No effects on dry matter or protein digestibility were found in a study by Gabert *et al.* (1995) of piglets fed a dietary supplement of GOS, whereas the availability of alanine, a non-essential amino acid with a higher gluco-genetic capacity, was reduced. The gut morphology was also found to be affected by oligosaccharide supplementation in reports by Xu *et al.* (2002) and Van Nevel *et al.* (2003), which demonstrated that the villus length in piglets is increased by FOS supplementation.

The results of many studies of oligosaccharide-fed piglets were often conflicting. Results showing an improvement in the growth performance and reduction in the levels of post-weaning diarrhoea upon FOS and TOS administration were obtained by Hidaka *et al.* (1986), Katta *et al.* (1993) and Che *et al.* (2003). However, other studies have reported no such effects of a FOS diet (Mikkelsen *et al.*, 2003), FOS and TOS diets (Houdijk *et al.*, 1998) or a GOS diet (Gabert *et al*., 1995).

To further the development of alternatives to the use of antibiotics as growth pro-

moters, the aim of our current study was to evaluate the effects of GOS as a prebiotic feed supplement in weaned piglets. We assessed the impact of GOS supplements in the diets of these animals on their growth performance, the blood parameters observed to be linked to oligosaccharide action such as caecum protein fermentation (blood urea), their inflammatory status or mineral nutrition, and the morphological characteristics of their intestinal tracts.

## Material and methods

*Animals, diets and housing*

This study was carried out in accordance with the guidelines of Good Laboratory Practice. Commercial hybrid pigs that were 28 day old half-breed offspring of a Duroc boar (n= 128) were randomly distributed into four homogeneous groups each containing 32 animals (females and castrated males at a 1:1 ratio in each cohort). These piglets were born and weaned in the same farrowing house. The animals were weighed (7.2 ± 1.04 kg live weight; l.w.) and identified using an ear tag the day after their arrival in our facility (CERZOO). The animals were reared during the first 35 days of the current trial period in flat-deck cages and subsequently on slatted floors until the 77 day timepoint. Each pen contained four piglets of the same sex and 16 castrated males (M) and 16 females (F) were used for each of the four dietary treatments to obtain eight replicates in each case. All animals were reared according to Italian laws (D.L. 116/92) concerning animal welfare (care of experimental animals), as also set out by European Directive 86/609/CEE.

The piglets were housed in rooms under a controlled climate (set temperature: 25.9°C; set humidity: 67%) and natural daylight. The experimental trial period was 77 days and the experimental design comprised four test diets as follows: 1.- Basal diet (CTR); 2.- Basal diet supplemented with 20 g/kg (inclusion over 100% of the volume of the basal diet) of gluco-oligosaccharide (GOS); 3.- Basal diet supplemented with chlortetracycline and spiramicine at 1000 and 400 mg/kg feed, respectively (CTRM), which were administered during the first 14 days of the trial only, and replaced with the CTR or 4.- GOS diet from 15 to 77 days (GOSM from 0 to 14 d). The GOS preparation used during the current trial (Nutriose®, Roquette Freres, France) was in a white powder form characterised by 6.0% moisture, 4.1% reducing sugars and less than 0.5% protein and ash residue. The monosaccharide and disaccharide contents of the GOS were 0.9% and 0.1% respectively, whilst the total dietary fibre was 88%.

*Feed intake and growth performance*

The piglets were provided continuous access to feed and water *ad libitum*. The isonitrogenous and isoenergetic pellet diets were formulated according to the INRA (1989) requirements and given for 77 consecutive days. The composition and the analytical characteristics of the diets used in the first (0-14 d) and second (15-77 d) growing periods are shown in Tables 1 and 2, respectively. Animals were individually weighed without the removal of food or water on days 0, 14, 35, 56 from the start of the study. On day 77, the feed was withdrawn 12 hours before the end of the trial period. At the same time, the feed intake per pen was recorded (net weight without the unconsumed feed) to calculate the feed conversion ratio (FCR) and the average daily gain (ADG) for each replicate analysis. The adjusted FCR was then calculated by the total feed intake per pen divided by the sum of the animals' live weight gain, and the body weight of the animals that died during the two periods for each replicate experiment. For these evalu-

Table 1. Ingredients (g/kg as fed) of the experimental basal diets.

| | Post-weaning periods | |
|---|---|---|
| | 0 – 35 d | 36 – 77 d |
| Corn meal | 362.7 | 417.5 |
| Soybean meal 44% | 255.0 | 200.0 |
| Barley flakes | 100.0 | 100.0 |
| Barley meal | 90.0 | 90.0 |
| Wheat bran | 50.0 | 50.0 |
| Whey acid dry | 50.0 | 50.0 |
| Soybean oil | 30.0 | 30.0 |
| Potato protein | 20.0 | 20.0 |
| Dicalcium phosphate | 21.2 | 21.2 |
| Calcium carbonate | 6.7 | 6.7 |
| Sodium chloride | 3.4 | 3.4 |
| L-Lysine | 4.0 | 4.0 |
| L- Threonine | 1.0 | 1.2 |
| DL- Methionine | 1.0 | 1.0 |
| Premix[1,2] | 5.0 | 5.0 |

*[1]The premix provided ($kg^{-1}$ feed) in the first growing period: Vit. A: 15,500 U; Vit. $D_3$: 1200 U; Vit. E: 25 mg; Vit. $B_1$: 1.5 mg; Vit. $B_2$: 3 mg; Vit. $B_6$: 1.80 mg; Vit. $B_{12}$: 0.03 mg; Vit. H: 0.13 mg; Vit. $K_3$: 3.5 mg; Vit. PP: 17 mg; Choline chloride: 300 mg; Mn: 42 mg; Fe: 250 mg; Cu: 95 mg; Zn: 105 mg; I: 1.2 mg; Se: 0.17 mg; Co: 0.33 mg.*
*[2]The premix provided ($kg^{-1}$ feed) in the second growing period: Vit. A: 10,500 U; Vit. $D_3$: 1200 U; Vit. E: 25 mg; Vit. $B_1$: 1.5 mg; Vit. $B_2$: 3 mg; Vit. $B_6$: 1.80 mg; Vit. $B_{12}$: 0.03 mg; Vit. H: 0.13 mg; Vit. $K_3$: 3.5 mg; Vit. PP: 17 mg; Choline chloride: 300 mg; Mn: 42 mg; Fe: 250 mg; Cu: 95 mg; Zn: 105 mg; I: 1.2 mg; Se: 0.17 mg; Co: 0.33 mg.*

ations, all of the piglets that died during the course of the study were weighed within 24 hours of their death.

*Chemical analysis*

About 500 g of each experimental diet used in the two growing periods were sampled and then analysed for dry matter, crude protein, ether extract, crude fibre, ash and starch contents at the beginning of the study (AOAC, 1980).

*Blood sampling procedure and analysis*

Blood was collected from the same six animals/treatment (3 M and 3 F) on two occasions (0 and 77 d) during the course of this study via a jugular vein puncture using heparinised (lithium heparin) sterile tubes according to the Vacutainer method (Becton and Dickinson, Franklin Lakes, NJ, USA). An average of 5 to 9 ml of the total blood obtained from each animal was centrifuged for 15 min at 3000 x *g* at room temperature to obtain plasma for haematological analysis. The plasma samples were subsequently frozen at -22°C prior to analysis. Glucose, urea, albumin, globulin, Ca, and P were measured using a Syncron CX-5 analyzer (Beckman Coulter, Fullerton, CA, USA) and kits provided by Beckman. Serum was analyzed for haptoglobin using

Table 2. Proxymate analysis (g/kg as fed) of the experimental diets: control without antibiotics (CTR); GOS-supplemented without antibiotics (GOS); control with antibiotics in the first 14 d (CTRM); Nutriose-supplemented with antibiotics in the first 14 d (GOSM).

| | | Growing periods | | | | |
|---|---|---|---|---|---|---|
| | | 0 – 35 d | | | 36 – 77 d | |
| | | CTR | GOS | Medicated feed[1] | CTR | GOS |
| Dry matter | | 908.0 | | | 916.3 | |
| Crude protein | | 194.8 | | | 176.1 | |
| Ether extract | | 64.8 | | | 54.7 | |
| Crude fibre | | 33.6 | | | 27.6 | |
| Ash | | 62.3 | | | 60.3 | |
| Starch | | 383.2 | 418.5 | 418.5 | 395.4 | 406.0 |
| Net energy[2] | MJ $kg^{-1}$ | 10.85 | | | 10.96 | |
| Lys | | 14.1 | | | 13.0 | |
| Thr | | 9.3 | | | 8.8 | |
| Met | | 4.2 | | | 3.9 | |

*[1]Employed only in the first 14 days from the start of the study for the subsequent diets: CTRM and GOSM.*
*[2]According to the equation of Noblet et al. (1994).*

the Haptoglobin-assay supplied by Tridelta Development Ltd, (Maynooth, Ireland). Protein electrophoresis was performed using the semi-automated agarose gel electrophoresis system "Hydrasys" manufactured by Sebia Inc. (Norcross, GA, USA).

*Morphological examinations*

At the end of the growing phase, four animals/treatment (2 CM and 2 F) selected from the same group of animals used for blood sampling were slaughtered in the CERZOO facilities to sample the gastrointestinal tracts (ileum and caecum) for morphological examinations (villi length, crypt depth and gut thickness). Scanning Electron Microscopy (SEM) was used to evaluate the gut morphologies of these pigs. The specimen preparation methods can be divided in four phases: (1) fixation, where well-cleaned specimens were placed in fixative solution (1 to 2.5% glutaraldehyde) for more than 2 hours. After fixation, the specimens were washed with a buffer solution to remove reagent that has not reacted with the tissue; (2) a dehydration step in which the specimens were placed sequentially into 50, 60, 70, 80, 90, and 95% ethanol-water solutions in the ascending order of concentration. The time of immersion was 10 min for each solution and the specimens were eventually immersed in a 100% ethanol solution for 30 min. This step was repeated twice; (3) a drying step, in which the 100% ethanol was removed by placing the specimens into a sealed high pressure container filled with liquid $CO_2$ at room temperature. This container was heated at a temperature

above the critical point. Consequently the pressure rose, the solution entered a gaseous phase, and gas was then released; and (4) microscopic examination where the specimens were put on a slab, and air dried. The resulting dehydrated samples were then analysed by SEM.

*Statistics*

The data collected during this study (l.w., feed intake, ADG, FCR, blood parameters and morphological measurements) were statistically analysed to determine whether significant differences existed between the results obtained for each treatment. A pen containing four animals was designated as the experimental unit except when measuring blood parameters and gut morphologies where the single piglet was taken as the experimental unit. Statistical analysis was performed according to the GLM (General Linear Model) procedure of SAS Institute software package (1999-2001) release 8.2. The initial l.w. and initial blood parameter values were used as the co-variables. Sex effects were found not to be significant. The following model was adopted:

$Y = \mu + \alpha + \beta + \alpha * \beta + \varepsilon$

where:

Y = items
$\mu$ = overall mean
$\alpha$ = GOS effect
$\beta$ = antibiotic effect
$\varepsilon$ = experimental error

## Results and discussion

*Growth performance*

During the first 14 days of the experimental period, no significant effects of GOS upon the ADGs of the piglets were observed (Table 3). The medicated diets, when compared with the control diets, lead to a higher intake of food in the first 14 days (Table 4), which was not accompanied by a change in the feed to weight gain ratio or the average daily gain. This is consistent with the results of a study by Houdijk *et al.* (1998), which compared the effects of FOS and TOS or antibiotics in the diets also of piglets and demonstrated an improved intake of feed in the medicated diet groups.

At the end of the antibiotic treatments in our current experiments, during days 15 to 35, no differences in the ADG were detected. There were statistically significant differences observed between the effects of GOS and antibiotics upon the ADG during the periods from days 0-77 and 35-56. However, when these data were disaggregated on the basis of the use of medicated feed, no significant effect of GOS could be observed. In the 57-77 day period, the ADG was found to be reduced in all of the treatment groups, whilst at the same time a trend (P= 0.0747) towards an improved growth in animals fed on the GOS supplemented diets was evident (Table 3). It is not easy justify this reduction in animal growth. In the last period of the current trial, the feed intake dropped by about 15% in comparison with the 36-56 day period, but the ADG was found to be reduced by about 55%. Since our current experiment ended in the summer, we speculate that the higher temperatures reduced the feed consumption and consequently the animal growth. Although the temperatures in the piggery were controlled, the average temperature exceeded the threshold of discomfort for piglets (26°C) during the last experimental period for two days. However, at other times, the temperature exceeded 26°C for several hours (from 3 to 10 hours per day) which may explain the reduction in feed intake and the drop in the ADG and FCR. No mortality or disease was observed in the animals during the last 21 days of the experimental period, so these poor growth performances are unlikely to be attributable to health problems.

Table 3. Growth performance of the groups of piglets fed on the four different diets.

| | Without medicated feed in the first 14 d | | With medicated feed in the first 14 d | | SE | P value for the effects of : | | |
|---|---|---|---|---|---|---|---|---|
| | CTR | GOS | CTRM | GOSM | | GOS | Antibiotic | GOS* Antibiotic |
| Initial live weight (kg) | 7.09 | 7.19 | 7.25 | 7.14 | 0.222 | | | |
| Average daily gain (g/d): | | | | | | | | |
| 0 – 14 | 201.4 | 173.2 | 202.2 | 200.6 | 11.135 | 0.1923 | 0.2151 | 0.2441 |
| 0 – 35 | 298.1 | 269.6 | 321.4 | 303.4 | 17.618 | 0.1983 | 0.1165 | 0.7663 |
| 0 – 56 | 348.1 | 316.1 | 346.5 | 361.4 | 13.740 | 0.5477 | 0.1376 | 0.1135 |
| 0 – 77 | 299.4 | 284.8 | 293.4 | 318.0 | 8.571 | 0.5685 | 0.1397 | 0.0415 |
| 15 – 35 | 362.6 | 333.5 | 401.0 | 372.0 | 23.200 | 0.2207 | 0.1087 | 0.9979 |
| 36 – 56 | 431.3 | 393.9 | 388.1 | 458.0 | 23.375 | 0.4992 | 0.6631 | 0.0405 |
| 57 – 77 | 169.6 | 201.5 | 151.8 | 202.1 | 21.056 | 0.0747 | 0.6886 | 0.6695 |

The intake of feed in the other periods, and also the FCR, were unaffected by our dietary treatments (Table 4). However in the diets not supplemented with drugs, the use of GOS lead to a higher feed intake trend (P= 0.0975).

*Blood analysis*

GOS supplementation was further found to lead to a reduction in the globulin content with consequent changes in the albumin/globulin ratio (Table 5). The use of GOS increased the P plasma concentration but no effects were observed upon the Ca levels. Analysis of the plasma from piglets fed on the GOS-supplemented diets showed a lower urea content compared with the control animals. Other differences were also found for:

β-1 globulin: lower in piglets on the GOS diets. This protein is an inflammatory marker and an increment in its synthesis can indicate the presence of an inflammatory process;

Haptoglobin: lowered by GOS supplements in animals fed with the non medicated diets (P< 0.01). The synthesis of this protein is increased by inflammation.

Glucose: the supplementation of the piglet diets with GOS lead to a higher glucose level but only in animals who had not received the drug supplements (P< 0.01).

An improvement in the P absorption rate as a consequence of oligosaccharide supplementation, similar to that observed in our current analyses, was previously reported by Perez-Conesa *et al.* (2006) who fed rats with galacto-oligosaccharides, and by Ko *et al.* (2000) who studied piglets fed on B-glucan-supplemented diets. The hydrolysis of chemical bonds that entrap P into phytic acid, which is synthesised by GOS-fermenting bacteria, could explain these findings.

The plasma levels of urea in the piglets

Table 4. Feed intake and FCR of piglets fed on the four different diets.

| | Without medicated feed in the first 14 d | | With medicated feed in the first 14 d | | SE | P value for the effect of : | | |
|---|---|---|---|---|---|---|---|---|
| | CTR | GOS | CTRM | GOSM | | GOS | Antibiotic | GOS*Antibiotic |
| Feed intake (g/d): | | | | | | | | |
| 0 – 14 | 239.5 | 221.4 | 267.9 | 257.5 | 12.024 | 0.2459 | 0.0121 | 0.7496 |
| 0 – 35 | 412.4 | 380.8 | 443.1 | 417.4 | 17.913 | 0.1205 | 0.0704 | 0.8681 |
| 0 – 56 | 543.9 | 505.6 | 549.5 | 549.0 | 16.714 | 0.2687 | 0.1687 | 0.2808 |
| 0 – 77 | 561.1 | 541.1 | 562.4 | 581.8 | 13.939 | 0.9831 | 0.1587 | 0.1830 |
| 15 – 35 | 524.7 | 487.0 | 560.0 | 524.1 | 25.775 | 0.1643 | 0.1713 | 0.9712 |
| 36 – 56 | 762.9 | 713.7 | 726.7 | 768.1 | 22.397 | 0.8659 | 0.6920 | 0.0659 |
| 57 – 77 | 607.3 | 635.9 | 596.9 | 669.4 | 30.723 | 0.1256 | 0.7138 | 0.4898 |
| Feed/gain ratio: | | | | | | | | |
| 0 – 14 | 1.19 | 1.33 | 1.33 | 1.29 | 0.0588 | 0.4203 | 0.4085 | 0.1454 |
| 0 – 35 | 1.43 | 1.42 | 1.38 | 1.40 | 0.0635 | 0.9176 | 0.5839 | 0.8080 |
| 0 – 56 | 1.57 | 1.60 | 1.59 | 1.52 | 0.0382 | 0.6532 | 0.4227 | 0.1820 |
| 0 – 77 | 1.88 | 1.90 | 1.92 | 1.83 | 0.0384 | 0.4319 | 0.6913 | 0.1501 |
| 15 – 35 | 1.54 | 1.46 | 1.40 | 1.43 | 0.0843 | 0.7357 | 0.2950 | 0.5102 |
| 36 – 56 | 1.78 | 1.81 | 1.89 | 1.68 | 0.0682 | 0.2338 | 0.8847 | 0.1024 |
| 57 – 77 | 3.67 | 3.26 | 4.04 | 3.44 | 0.3560 | 0.1807 | 0.4647 | 0.7901 |

Table 5. Assay of plasma components in the dietary groups under study.

| | | Without medicated feed in the first 14 d of trial | | With medicated feed in the first 14 d of trial | | SE | P value for the effect of : | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CTR | GOS | CTRM | GOSM | | GOS | Antibiotic | GOS*antibiotic |
| Protein | g/l | 74.83 | 69.40 | 71.67 | 71.83 | 1.41 | 0.0952 | 0.8095 | 0.0774 |
| Albumin | " | 31.83 | 31.20 | 28.83 | 31.33 | 1.62 | 0.5234 | 0.3307 | 0.2888 |
| Globulin | " | 44.67 | 38.20 | 42.83 | 40.50 | 1.29 | 0.0064 | 0.8728 | 0.1669 |
| Albumin/globulin ratio | | 0.67 | 0.86 | 0.68 | 0.80 | 0.05 | 0.0061 | 0.6709 | 0.4545 |
| Urea | mmol/l | 4.31 | 3.08 | 4.08 | 3.45 | 0.41 | 0.0239 | 0.8428 | 0.4364 |
| Glucose | " | 3.72 | 4.89 | 4.26 | 3.71 | 0.44 | 0.4258 | 0.4137 | 0.0375 |
| Calcium | " | 2.40 | 2.30 | 2.29 | 2.29 | 0.37 | 0.2019 | 0.1807 | 0.2019 |
| Phosphorus | " | 2.63 | 3.09 | 2.65 | 2.98 | 0.10 | 0.0008 | 0.6144 | 0.5036 |
| α-1 globulin | g/l | 13.15 | 12.58 | 12.03 | 12.53 | 0.36 | 0.9229 | 0.1194 | 0.1500 |
| β-1 globulin | " | 8.90 | 4.90 | 8.45 | 7.03 | 0.89 | 0.0050 | 0.3369 | 0.1470 |
| β-2 globulin | " | 11.22 | 10.38 | 11.13 | 9.30 | 1.51 | 0.3884 | 0.7048 | 0.7454 |
| γ-globulin | " | 11.53 | 11.76 | 10.57 | 11.17 | 0.72 | 0.6063 | 0.3352 | 0.8155 |
| Haptoglobin | mg/ml | 5.78 | 2.05 | 4.27 | 4.35 | 0.78 | 0.0569 | 0.6397 | 0.0474 |

Table 6. Effect of the experimental diets on the piglet gut morphology.

| Items | | Without medicated feed in the first 14 d of trial | | With medicated feed in the first 14 d of trial | | SE | P value for effect of: | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CTR | GOS | CTRM | GOSM | | GOS | Antibiotic | GOS* antibiotic |
| Ileum: | | | | | | | | | |
| Epithelium thickness | mm | 1.57 | 1.97 | 2.13 | 1.91 | 0.156 | 0.5728 | 0.1345 | 0.0736 |
| Villus length | " | 235.88 | 205.98 | 188.66 | 255.74 | 18.747 | 0.3410 | 0.9473 | 0.0238 |
| Epithelial cells' height | µm | 20.43 | 18.73 | 19.29 | 22.81 | 1.528 | 0.5633 | 0.3566 | 0.1131 |
| Microvillus length | " | 1.22 | 1.25 | 1.32 | 1.32 | 0.137 | 0.8952 | 0.5274 | 0.9192 |
| Caecum: | | | | | | | | | |
| Epithelium thickness | mm | 1.22 | 1.20 | 1.01 | 1.31 | 0.151 | 0.3655 | 0.7288 | 0.3029 |
| Crypt depth | µm | 265.24 | 278.50 | 248.08 | 280.19 | 18.119 | 0.2345 | 0.6771 | 0.6123 |
| Height of epithelial cells | " | 11.77 | 16.67 | 11.58 | 12.72 | 1.460 | 0.0608 | 0.1809 | 0.2215 |

on GOS-supplemented diets were lower than the control animals, which was probably due to improved microbial growth in the gross intestine via GOS fermentation which leads to an increased level of $NH_3$ utilization during microbial protein synthesis and a reduction in the conversion of $NH_3$ to urea. A similar reduction in the blood urea content in piglets who were supplemented with oligo-saccharides, although not GOS in this case, was earlier reported by Liu *et al.* (2003) and Tang *et al.* (2005).

In our present study, supplementation with GOS led to a lower concentration of proteins involved in the inflammatory process, such as β-1 globulin and haptoglobin (only in piglets on the unmedicated feed; P= 0.0081). A higher glucose level in piglets on the FOS-supplemented diets was observed also by Xu *et al.*, (2002) and could be the result of a reduction in inflammatory signals and lower levels of the hypercatabolic metabolism that is characteristic of inflammation.

*Intestinal morphology*

*Small intestine.* Our current dietary treatments were found to have an impact upon the gut morphology of the piglets (Table 6). The villus lengths in piglets on medicated diets were increased by GOS supplementation (P= 0.0088), whereas no effects were observed when non medicated diets were given. In our present trial, we were unable to detect any effects of our dietary

treatments upon the ileum thickness.

As already reported by Xu *et al.* (2002) and Van Nevel *et al.* (2003), the villus lengths in piglets is increased by FOS supplementation. The production of volatile fatty acids resulting from the fermentation of oligosaccharides by bacteria may underlie this higher development of the villus. As described earlier by McCracken *et al.* (1999) and Touchette *et al.* (2002), the villus length is reduced by inflammatory processes. Furthermore, there is a negative correlation between a higher expression of inflammatory markers such as globulin and haptoglobin and the villus length, whereas this correlation is positive in the case of the ileum thickness (Eckersall *et al.* 1996; Magnusson *et al.*, 1999).

Inflammatory processes affect the gut morphology by reducing the villus length and increasing the ileum thickness. In our current trial, we detected an increase in the villus length but no effects upon the ileum thickness as a result of the intake of our dietary supplements. The haptoglobin levels were found to be reduced by GOS supplementation in animals that never received drug-supplements in their diet ($P< 0.01$), but across whole group of animals, this effect was not significant.

These higher levels of inflammatory markers could have arisen from an imbalance in the gut microflora which was prevented by the GOS intake. In a previous report by Kostantinov *et al.* (2003), piglets fed with fermentable carbohydrates showed a higher bacterial diversity and a more rapid stabilisation of the bacterial communities in the gut compared with the control animals. In addition, the villus lengths, and consequently the surfaces of the villi, could have been reduced by alterations in the microbial profile of the small intestine. As highlighted by Delzenne *et al.* (2003), animal studies as well as data from *in vitro* cell culture have underlined the potential of oligosaccharides to protect against inflammatory processes in the gut.

*Large intestine.* No significant effects of GOS on the caecum morphology in the subject piglets were observed (Table 6). However a trend ($P= 0.068$) towards an increased epithelial cell height following GOS intake was evident, whilst the effects of antibiotics alone, and the interaction of GOS and these drugs, were not found to be significant (Table 6). Our current findings are thus more or less consistent with the hypothesis previously formulated by Galfi and Bokori (1990) that there are stimulating effects of the VFAs (butyrate in particular) generated by GOS fermentation upon the growth of gut cells. Van Nevel *et al.* (2003) have also observed a doubling of the acetate and butyrate concentration in the guts of piglets receiving dietary supplements of GOS, and an increase in butyrate production following the administration of Nutriose in rats was also observed by Bakker-Zierikzee (2004). However, the opposite results were obtained by Valette *et al.* (1993) who reported a decrease in the butyric acid content as a result of feeding rats with a GOS-supplemented diet. We did not determine the VFA levels produced in the caecum in our current study, so the existence of a GOS-positive effect on caecum cells mediated by the butyrate that results from GOS fermentation should still be considered only as a working hypothesis.

## Conclusions

We conclude from our current findings that the growth performance of piglets is not affected by antibiotic GOS dietary supplements. The use of gluco-oligosaccharides does appear to increase the villus length, but only in animals that have been previously given medicated feeds. It is not clear if this positive effect of GOS is due to an imbalance in the intestinal microflora of these animals as a result of antibiotic treatment.

Furthermore, piglets that had been fed a GOS-supplemented diet showed a tendency towards a height increase in their colonic epithelial cells. GOS thus seems to exert anti-inflammatory effects in animals.

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