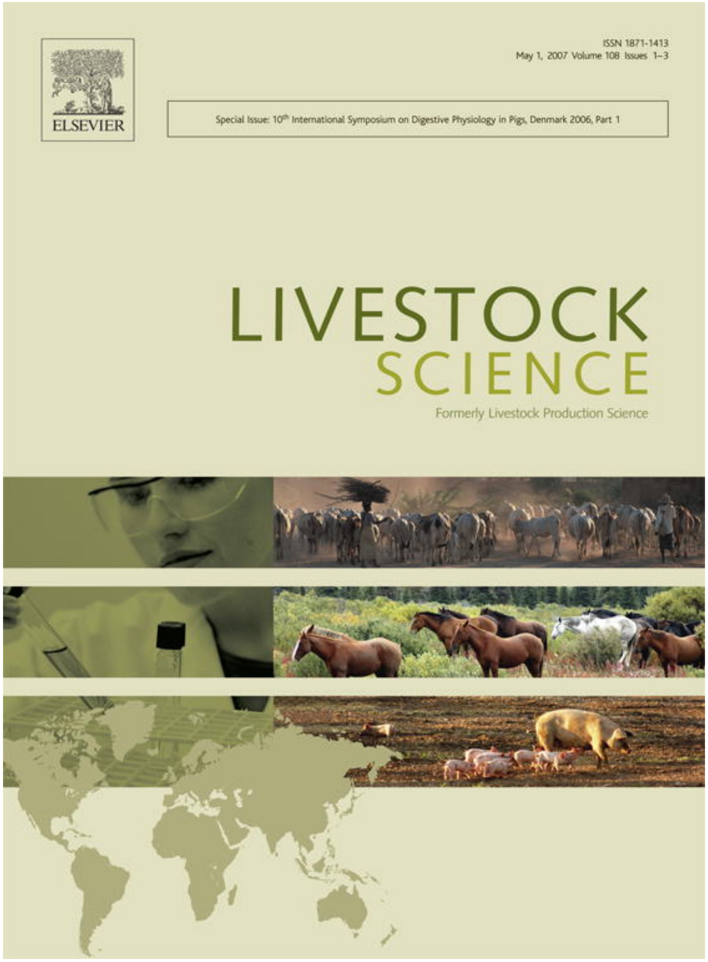
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Microbial activity in the gut of piglets: I. Effect of prebiotic and probiotic supplementation ☆

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Abstract

Four groups of six 21 days-old piglets were used to evaluate the effect of a prebiotic or probiotic on the intestinal fermentative activity. In each group, piglets received one of the following diets: basal diet (C); basal diet supplemented with xylo-oligosaccharide (C-XOS); basal diet supplemented with *Saccharomyces cerevisiae* (C-SC); and basal diet supplemented with xylo-oligosaccharide and *S. cerevisiae* (C-XOS+SC).

The short chain fatty acids in the colon of piglets were decreased with the inclusion of *S. cerevisiae* in the diet ($P<0.01$). The xylanolytic activity was higher ($P<0.05$) in the small intestine of piglets fed C-XOS+SC diet, but no significant differences were found in the caecum and colon. In the caecum contents, the cellulolytic activity was increased ($P<0.05$) by the C-XOS and C-SC diets, but remained similar when the diet was supplemented with the two additives combined.

Keywords: Piglets; Xylo-oligosaccharides; *Saccharomyces cerevisiae*; Intestinal fermentation

1. Introduction

One potential way to control the intestinal fermentation process is using prebiotics or probiotics as feed additives. Prebiotics are non-digestible feed ingredients that beneficially affect the host by stimulating the growth and activity of specific bacteria groups mostly in the colon. Xylo-oligosaccharides are considered prebiotics presents in different by-products like corn cob or peanuts shell (Maxwell et al., 2004).

Oligosaccharides are hydrolysed to their monomeric units (glucose, galactose, xylose or arabinose) and then fermented to short chain fatty acids (SCFA), mainly (C2), (C3) and (C4) by gut bacteria. They are an important energy source and help to maintain a balanced intestinal ecosystem (Midtvedt, 1994). In fact, acetate and propionate are used by the pig as energy source (Montagne et al., 2003) and butyrate stimulates the development and growth of large and small intestine, by improved epithelial cell proliferation (Sakata, 1987).

The supplementation with *Saccharomyces cerevisiae* can improve microbial balance in the gut of young pigs (Heugten et al., 2003), due to either the yeast cell wall components (mannan-oligosaccharides) effect on the immune modulation (Newman and Newman, 2001), or a direct effect to reduce pathogenic bacteria and toxic metabolites (Anderson et al., 1999).

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Table 1
Effect of xylo-oligosaccharide (XOS) or *Saccharomyces cerevisiae* (SC) supplementation on total concentration ($mmol \cdot l^{-1}$) and molar proportions of SCFA in intestinal contents

	C	C-XOS	C-SC	C-XOS+SC	XOS	SC	XOS×SC	RSD [(2)]
Small intestine								
C2+C3+C4[(1)]	22.8	20.7	23.0	15.1	0.1946	0.4742	0.4501	9.1
C2:C3	3.44	3.30	3.99	3.93	0.7359	0.0617	0.8968	0.73
C2:C4	5.05	4.10	5.20	4.91	0.1164	0.2163	0.3807	0.92
C3:C4	1.45	1.33	1.32	1.29	0.5291	0.4860	0.6900	0.37
Caecum								
C2+C3+C4	53.9	61.4	58.8	53.3	0.8837	0.8173	0.3580	17.0
C2:C3	2.04	1.91	2.04	2.04	0.3828	0.3952	0.3588	0.17
C2:C4	3.13	2.98	2.87	3.04	0.9600	0.5954	0.3937	0.44
C3:C4	1.54	1.56	1.41	1.49	0.5471	0.2310	0.7049	0.20
Colon								
C2+C3+C4	118.4^{a}	81.6^{ab}	55.4^{b}	49.4^{b}	0.1155	0.0016	0.2503	31.9
C2:C3	2.96^{ab}	3.50^{a}	2.53^{b}	2.71^{ab}	0.1693	0.0285	0.4939	0.63
C2:C4	9.49^{a}	10.31^{a}	4.72^{b}	4.31^{b}	0.8655	0.0002	0.6124	2.94
C3:C4	3.14^{a}	2.95^{a}	1.89^{b}	1.61^{b}	0.4232	0.0003	0.8797	0.72

[(1)]C2: Acetic, C3: Propionic, C4: Butyric. [(2)]For effects: Probability of significance of the *F* value. RSD: Residual standard deviation. In the same row, values with different letters superscripts differ significantly ($P<0.05$).

It is known that probiotics act mostly in the upper compartments of the gastrointestinal tract; while prebiotics are suppose to be used as substrate for potentially beneficial bacteria in the hindgut of monogastric animals. Therefore it is expected that the association of a specific prebiotic and probiotic may have a synergetic positive effect on intestinal microflora Nemcova et al. (1999).

The objective of this work was to evaluate the effect of xylo-oligosaccharide and *S. cerevisiae* supplementation, alone or combined, on the microbial activity in the gut of the piglet.

2. Materials and methods

Four groups of six 21 days-old weaning piglets were used. Piglets were individually housed in metabolism cages, during 4 weeks. In each group piglets received one of the following diets: (C) basal diet (wheat: 600 $g \cdot kg^{-1}$; maize starch: 140 $g \cdot kg^{-1}$; soybean oil: 50 $g \cdot kg^{-1}$; soluble fish protein concentrate: 150 $g \cdot kg^{-1}$), (C-XOS) basal diet supplemented with xylo-oligosaccharide (20 $g \cdot kg^{-1}$), (C-SC) basal diet supplemented with *S. cerevisiae* (6×10^8 $CFU \cdot kg^{-1}$) and (C-XOS+SC) basal diet supplemented with xylo-oligosaccharide and *S. cerevisiae*. At the end of the experimental period the piglets were slaughtered and pooled samples from the small intestine (SI), caecum and colon contents were collected. The SCFA concentration (C2, C3 and C4) were quantified by gas chromatography following Jouany (1982). The microbial enzymes activity was analysed by spectrophotometry following the method described by Falcão-e-Cunha et al. (2004). Data were subjected to analyses of variance using the GLM procedure of SAS (2001).

Table 2
Effect of xylo-oligosaccharide (XOS) or *Saccharomyces cerevisiae* (SC) supplementation on enzymatic[(1)] activities in intestinal contents of piglets

	C	C-XOS	C-SC	C-XOS+SC	XOS	SC	XOS×SC	RSD [(2)]
Small intestine								
Cellulolytic	132.9	58.6	156.0	52.6	0.0675	0.8540	0.7539	106.6
Xylanolytic	46.1^{a}	96.5^{a}	56.3^{a}	229.8^{b}	0.0189	0.1173	0.1757	107.4
Caecum								
Cellulolytic	102.2^{a}	354.6^{b}	523.6^{c}	125.0^{a}	0.2246	0.1164	<0.0001	131.1
Xylanolytic	288.1	329.2	273.5	253.2	0.8550	0.4291	0.5903	134.1
Colon								
Cellulolytic	74.2	92.4	92.9	76.7	0.9358	0.9069	0.1914	31.2
Xylanolytic	387.5	636.8	507.0	404.2	0.4170	0.5296	0.0602	216.5

[(1)]Enzymatic activities: mg hydrolysed substrate/g DM intestinal contents/h. [(2)]For effects: probability of significance of the *F* value. RSD: Residual standard deviation. In the same row, values with different letters superscripts differ significantly ($P<0.05$).

3. Results

The results of SCFA are showed in Table 1, and the microbiological enzymatic activities data are presented in Table 2. The inclusion of xylo-oligosaccharide or *S. cerevisiae* in the piglet diet did not affect the levels or molar proportions of SCFA in the SI and caecum. However, the level of total SCFA (mmol·l^{-1}) in the colon, decreased with the inclusion of *S. cerevisiae* on the diet ($P=0.0016$). Similarly, molar proportions of SCFA were affected by *S. cerevisiae* supplementation only in the colon contents. Molar proportions of acetic:butyric and propionic:butyric acids were higher in piglets fed the control diet, than on those fed diets supplemented with *S. cerevisiae* ($P=0.0002$; $P=0.0003$, respectively). Compared to the control diet, the cellulolytic activity in the caecum contents was increased by the prebiotic or probiotic supplementation, but remained similar when the diet was supplemented with the two additives combined, thus a very significant interaction between the effect of these additives ($P<0.0001$). Furthermore, no significant differences were found in the SI and colon contents for the cellulolytic activity. The xylanolytic activity was higher ($P=0.0189$) in the small intestine of piglets fed xylo-oligosaccharides, but the difference was statistically significant only in case of the C-XOS+SC diet. On the other hand, no significant differences were found in the caecum and colon.

4. Discussion

As the xylo-oligosaccharides can be degrade by the intestinal microflora of the pig (Maxwell et al., 2004), it was expected that the inclusion of this prebiotic in the diet could increase the levels of SCFA in the intestinal contents. However, in our experiment, the xylo-oligosaccharides mixture was included at the level of 20 g·kg^{-1}, while Mikkelsen et al. (2003) have tested prebiotic mixtures (FOS, TOS) at levels of 40 g·kg^{-1}. Therefore, considering the level of total non-digestible oligosaccharides (NDO) of the control diet, the amount of additive we have used was probably too low to have an effect on the SCFA levels. The low level of total SCFA in the colon of piglets receiving the *S. cerevisiae* supplemented diets suggests a decrease in the microbial activity in the hindgut. Previous results from Mathew et al. (1998) have showed no effects of live yeast supplementation on SCFA concentrations along the gastrointestinal tract of the weaning piglets. It is likely that *S. cerevisiae* could degrade dietary NDO in the ileum, reducing the level of fermentable substrates reaching the hindgut and consequently the microbial population in the colon. Nevertheless, the absence of effect of *S. cerevisiae* on the concentration of SCFA in the SI does not confirm this hypothesis. The low SCFA concentration found in the colon contents of piglets receiving the C-XOS+SC diet failed to demonstrate a positive effect, on the intestinal microbial population, of combining the two additives. According to Nemcova et al. (1999) the benefit of associating prebiotics to probiotics in the diet is a complex problem, and depends on the prebiotic composition and the specific live micro organisms used.

The effect of the two tested additives on the enzymatic activities of intestinal contents must be overlooked with caution, because of the high residual standard deviation of these results. Nevertheless, the higher xylanolytic activity in the SI of C-XOS+SC fed piglets and the higher cellulolytic activity in the caecum of the piglets fed C-XOS or C-SC diets, suggest a greater microbial activity in these compartments, although these results are not confirmed by the SCFA concentrations.

In conclusion, the inclusion of *S. cerevisiae* in the diet has significantly decreased the SCFA concentration and the molar proportion of butyric acid in the colon. Results on the effect of xylo-oligosaccharides or *S. cerevisiae* supplementation on the intestinal fermentative activity have show high variability. A positive effect of combining the two additives on microbial enzymes was found only in the case of xylanolytic activity of the small intestine contents.

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