Effects of short-chain fructo-oligosaccharides on the microbial and biochemical profile of different segments of the gastro-intestinal tract in horses

Frédérique Respondek[1], Anne-Gaëlle Goachet[2], Florence Rudeaux[1] and Véronique Julliand[2]

Beghin-Meiji, Thumeries[1] and ENESAD, Dijon[2], France

Introduction

Short-chain fructo-oligosaccharides (scFOS) belong to the family of the prebiotic compounds. These are non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a number of bacterial species already present in the colon (*Gibson* et al. 1995). Studies in different species (e.g. dogs and pigs) showed that scFOS are able to enhance the production of volatile fatty acids within the hindgut and improve the mucosal structure (*Swanson* et al. 2002, *Tsukahara* et al. 2003).

As the whole digestive tract of horses is colonized by non-eligible concentrations of bacteria (*Kern* et al. 1974, *deFombelle* et al. 2003), the objective of the study was to evaluate the effects of scFOS on these populations and their fermentation-related parameters throughout the whole digestive tract.

Material and Methods

Six unbroken gelding ponies, with an average body weight of 374kg, were housed in individual free stalls with artificial bedding. They were fed a diet of concentrate pellets (1.06kg of DM/horse/day; UAR hippo 122, Evialis, France) and straw (2kg/horse/day). The ponies were randomly allotted into two groups, which received the same basal diet. One group was supplemented with 1% scFOS (Profeed®, Béghin-Meiji, France) incorporated in the concentrate pellets. Ponies were adapted to their diet for 21 days prior to measurements. Following this, digesta was collected from each part of the gastro-intestinal tract two hours after the morning meal of pellets (*deFombelle* et al. 2003). The pH was measured immediately after the collection of all samples with an electronic pH-meter.

One part of the digesta samples were prepared under O_2-free CO_2[6] in an anaerobic solution then ten-fold diluted and analysed by usual cultural methods for the determination of total anaerobes, cellulolytics, lactobacilli, streptococci and lactate utilizers concentrations. The remaining samples were immediately frozen for further determination of scFOS, lactate and volatile fatty acids (VFA). ScFOS were analysed by HPLC, lactate by spectrophotometry, and VFA by gas-liquid chromatography.

Results were analysed with the glm procedure of SAS to evaluate the variables' response to the scFOS supplementation, to the digestive compartment and to their interaction. The model included horse, nested in the diet, as a randomized effect. Least-square means were calculated for all variables and separated using the pairwise t-tests, the significance threshold for all tests was set at $p<0.05$.

Results

Analysis of concentrate pellets confirmed the presence of scFOS (0,88g%g in the control feed and 2.00g%g in the scFOS feed). Thus, the supplementation provided 0.09g scFOS/kg BW/day. However no scFOS was recovered from analysis of the stomach and the small intestine digesta contents.

Digestive compartment had a significant effect on the different microbial concentrations (Table 1). No effect of scFOS was measured. A significant interaction between the scFOS and the digestive part was measured for the total anaerobic bacteria, the streptococci and the lactate-utilizing bacteria. These populations were present at higher concentrations in the stomach from horses supplemented with scFOS than from the control horses. Lactate-utilizing bacteria were also present at higher concentrations in the jejuno-ileum. The concentration of total anaerobic bacteria was lower in the RVC of supplemented horses.

Table 1 Microbial concentrations in the different anatomic parts.

	Anatomic part					pooled	*P values*		
	STO[1]	JEJ	CAE	RVC	LDC	s.e.m	scFOS	Part	scFOS x Part
Total anaerobic bacteria									
Control [2]	9.1^A	8.3	7.6	8.1^A	9.1	0.6	0.673	<0.001	0.017
scFOS	9.6^B	8.0	7.5	7.6^B	8.9				
Cellulolytic bacteria									
Control	1.4	1.8	6.0	6.0	5.6	0.5	0.564	<0.001	0.837
scFOS	1.2	1.4	6.3	5.7	5.7				
Lactobacilli									
Control	7.8	6.4	6.2	6.5	7.4	0.4	0.328	<0.001	0.522
scFOS	7.9	6.1	6.0	6.5	7.4				
Streptococci									
Control	7.3^A	7.7^A	6.2	7.1	7.4	0.6	0.419	<0.001	<0.001
scFOS	8.5^B	7.1^B	6.2	6.7	7.1				
Control	6.8^A	6.6^A	6.1	5.8	6.5	0.5	0.147	<0.001	0.021
scFOS	7.3^B	7.0^B	6.1	6.2	6.1				

[1] *STO = Stomach; JEJ = Jejuno-ileum; CAE = Caecum; RVC = Right Ventral Colon; LDC = Left Dorsal colon*
[2] *Values are least-square means.*
[A,B] *values within an item and a column are different if superscript differs (p<0.05)*

The pH of the digesta differed according to the digestive part of the gut and was significantly higher with the scFOS diet than with the control diet (Table 2). The scFOS did not influence the concentration of lactate or VFA, neither did it affect their proportion (results not shown).

Discussion

It has been demonstrated indeed that high concentrations of bacteria inhabit the horse's stomach (*Kern* et al. 1974, *deFombelle* et al. 2003, *Varloud* et al. 2004). Interestingly, no scFOS was detected in the stomach of horses fed the sup-

Table 2 pH, lactate and total VFA concentrations throughout the digestive tract.

	Anatomic part					pooled	P values		
	STO	JEJ	CAE	RVC	LDC	s.e.m	scFOS	Part	scFOS x Part
pH									
Control	4,5	7,1	6,2	6,2	6,7	0,2	0,001	<0,001	0,079
scFOS	5,0	7,2	6,3	6,5	6,8				
Lactate									
Control	7,3	6,0	0,0	0,0	0,0	0,5	0,489	<0,001	0,807
scFOS	7,1	5,5	0,1	0,0	0,0				
Control	10,1	19,0	82,6	89,1	56,5	22,4	0,704	<0,001	0,989
scFOS	9,6	18,9	73,5	86,0	55,4				

plemented diet, possibly related to fermentation levels. In our study, the retention time in the stomach was close to the half-emptying time measured with similar concentrate pellets (*Metayer* et al. 2004). Consequently, our observations reflect physiological situations. This is in accordance with the updated definition of prebiotics which are selectively fermented ingredients that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon the hosts wellbeing and health (*Gibson* et al. 2004) The effect of prebiotics is extended to whole the digestive tract instead of only the colon.

Therefore the stomach appeared to be the compartment where we measured the major differences between the two dietary treatments. The concentrations of total bacteria, Streptococci and lactate-utilising bacteria increased when scFOS were supplemented. The lactate concentration remained unchanged, probably explained by the balance between lactate producers and lactate utilizers as demonstrated in pigs and in rats (*Tsukahara* et al. 2003). The pH was higher in the scFOS group than in the control one. This environment may decrease the risk of gastric ulcers (*Nadeau* et al. 2000).

Similarly to some studies in dogs (*Swanson* et al. 2002), we observed no change in lactobacilli counts. scFOS under some circumstances do not influence the concentration of Lactobacilli but could modify the abundance profile of the different strains, specifically stimulating the growth of L. reuteri (*Rastall* 2004). *Yuki* et al. (2000) using molecular techniques identified L. salivarius, L. crispatus, L. reuteri and L. agilis from the equine gastric mucosa. We may hypothesize that scFOS could have an influence on the proportion of these different strains within the stomach, and that molecular tools would have detected this effect.

The second main effects we observed with the scFOS supplementation were focused in the right ventral colon. This part of the hindgut has been reported as playing an essential role in the digestion (*Drogoul* et al. 2000) and is also very sensitive to colic (*Julliand* et al. 1999).

The cellulolytics were not altered by the scFOS supplementation. The total anaerobes counts decreased, the streptococci concentration tended to decrease whereas the lactate utilizers tended to increase. As a result the pH was significantly higher for animals fed the supplemented diet. Several hypotheses may be drawn to explain the effect of scFOS in the colon. It could be due to a direct action of the daily scFOS supplementation but we ignored whether the scFOS reached the hindgut. Or it could be an indirect impact due to the arrival in the hindgut of bacteria stimulated in the upper part of the gastrointestinal tract as suggested by (Fuller et al., 1978).

Conclusions

It appears from this study that scFOS influenced to some extent the numbers and the activities of the microbial populations in the gastrointestinal-tract of horses. Though this they might help to prevent some digestive disturbances associated with the management of performance horses.

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F. Respondek
Beghin-Meiji
59239 Thumeries
France
frespondek@tereos.com