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

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Summary

The gastro-intestinal tract of horses is inhabited by a significant amount of bacteria; the aim of this study was to assess the effect of short-chain fructo-oligosaccharides on these populations. Six gelding ponies were fed the same standard diet after being randomly allocated into two groups, of which one had its concentrate pelleted feed supplemented with 1% scFOS. Digesta from each part of the gastro-intestinal tract was collected and analysed for its microbial population, pH, lactate and volatile fatty acid concentrations. No Bifidobacteria was enumerated whatever diet was fed, or whatever part of the digestive tract was considered. In scFOS supplemented ponies, concentrations in total anaerobes, Streptococci and lactate-utilizers differed significantly, particularly in the stomach, where they were increased. On the other hand, concentrations in Lactobacilli and cellulolytic bacteria remained unchanged. The intra gastric pH in the scFOS group was higher than in the control group (5.0 vs 4.5; $p<0.05$). The concentration in lactate was the same in both groups. The scFOS had no effect on the concentration in VFA, nor on their proportion. The dietary supplementation with scFOS induced significant modifications in the microbial populations. Thus it may help to prevent some digestive disturbances associated with the management of performance horses.

Keywords: nutrition, fructo-oligosaccharides, prebiotic, digestive tract, microflora

Wirkung kurzkettiger Fructooligosaccharide auf das mikrobielle und biochemische Profil in den verschiedenen Segmenten des Gastrointestinaltraktes von Pferden

Der Gastrointestinaltrakt des Pferdes wird von einer komplexen Bakterienpopulation besiedelt; das Ziel dieser Studie war es, den Effekt von kurzkettigen Fructooligosacchariden (scFOS) auf diese Population zu bewerten. Sechs Ponywallache wurden, zufällig auf zwei Gruppen verteilt, mit der gleiche Basisration gefüttert. Die Versuchsgruppe erhielt ein pelletiertes Kraftfutter mit 1 % scFOS. Aus jedem Teil des Gastrointestinaltraktes wurden Ingesta gesammelt und auf mikrobielle Besiedlung, pH, Laktat- und flüchtige Fettsäurenkonzentationen untersucht. Unabhängig vom Darmabschnitt und der Ration konnten keine Bifidobakterien gefunden werden. Signifikante Veränderungen wurden in der Gesamtzahl an anaeroben Bakterien-, Streptokokken- und laktatverwertenden Bakterien beobachtet, insbesondere im Magen, wo sie durch die scFOS-Zulage erhöht war. Hingegen konnte kein Unterschied bei den Lactobazillen und den cellulolytischen Bakterien entdeckt werden. Der pH-Wert des Verdauungsbreis war zwischen den beiden Gruppen signifikant verschieden. Der Magen pH der scFOS-Gruppe war höher als bei der Kontrollgruppe (5,0 gegenüber 4,5; $p<0,05$). Die Laktatkonzentration unterschied sich zwischen den Gruppen nicht. Die scFOS-Zulage hatte weder einen Einfluss auf die Konzentration der flüchtigen Fettsäuren noch auf deren Verhältnis. Die Zulage von scFOS bewirkte eine signifikante Modifikation der mikrobiellen Population. Sie könnte damit helfen, Verdauungsstörungen, die bei der Haltung von Sportpferden auftreten, zu verhindern.

Schlüsselwörter: Fütterung, Fructooligosacchariden, Prebiotika, Verdauungskanal, Mikroflora, Gesamtkeimzahl

Introduction

Short-chain fructo-oligosaccharides (scFOS) are prebiotic compounds. They are fermentable food ingredients that beneficially affect the host by a selective stimulation the growth and/or activity of one or a limited number of bacterial species naturaly present in the colon (*Gibson* et al. 1995). Studies in different animal species showed that scFOS are able to stimulate the growth of beneficial bacteria, such as Bifidobacteria (*Swanson* et al. 2002, *Hsu* et al. 2004) and Lactobacilli (*Sparkes* et al. 1998, *Xu* et al. 2003) and to reduce potential pathogenic bacteria. When feeding scFOS, lower counts of faecal Clostridium perfringens were observed in pets (*Sparkes* et al. 1998, *Swanson* et al. 2002). The incidence of Salmonella typhimurium (*Bailey* et al. 1991) or Campylobacter jejuni (*Schoeni* et al. 1994) was lowered in poultry. In horses, scFOS reduced the concentration of E coli found in the faeces (*Pellegrini* et al. 1999, *Berg* et al. 2005). The dietary supplementation with scFOS is also associated with higher production of volatile fatty acids within the hindgut and improves its mucosal structure (*Swanson* et al. 2002, *Tsukahara* et al. 2003). At the beginning, prebiotics were thought to be effective only in the large intestine where the majority of the microflora is located. As the whole of the digestive tract is colonized by non-negligible concentrations of bacteria in horses (*Kern* et al. 1974, *deFombelle* et al. 2003), the aim of this study was to assess the effects of scFOS throughout the whole digestive tract on these populations, and their fermentation-related parameters.

Material and Methods

Animals and treatment diets

Six gelding ponies, with an average body weight of 374kg, were housed in individual free stalls with artificial bedding. They were fed a diet made of a pelleted concentrate (1.06kg dra matter/horse/day; UAR hippo 122, Evialis, France) and straw (2kg as fed/horse/day). The ponies were randomly allotted into two groups, fed the same standard diet. One group was supplemented with 1% of scFOS (Profeed®, Béghin-Meiji, France: a chain of up to four fructose molecules link to one glucose by β 2-1 bond) incorporated in the concentrate pellets.

Experiment 1

Ponies were given 21 days to adapt to their diet, prior to sampling. Then followed a six-day collection period of total faeces. After complete desiccation, faecal matter was ground (0.8mm) and analysed following the official procedure. Dry matter was analysed according to the NF V18-109 (1982), organic matter by a 5 hour combustion at 550°C, crude protein by the Kjeldahl method (1990) and the total lipids were determined by the NF V04-402 (1982) after acic hydrolysis.

For each nutrient, the total tract digestibility was calculated as nutrient intake (g/d) minus the faecal nutrient output (g/d) divided by the nutrient intake (g/d).

Experiment 2

Ponies were fed 21 days to adapt to their diet, prior to sampling. Ingesta were collected via abdominal laparotomy 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 sampling with an electronic pH-meter. Part of the sampled ingesta was used for microbiological analyses. Ten-fold dilution series were prepared under O_2-free CO_2, in anaerobic mineral solution for inoculation on specific media (*Bryant* et al. 1953). Total viable anaerobic bacteria counts were determined with a modified non-selective medium with an O_2-free CO_2 gas phase in anaerobic roll tubes (*Leedle* et al. 1980, *Julliand* et al. 1999a). Concentrations were determined from four replicate roll tubes. Lactic-acid-utilizing bacteria counts were determined on a selective medium (*Mackie* et al. 1979) in anaerobic roll tubes with O_2-free CO_2 gas phase. Lactobacilli spp. were grown on Rogosa agar (Biokar diagnostic, Beauvais, France) in Petri plates. Streptococci spp. were grown on a bile-esculin-azide agar medium (BK158HA, Biokar diagnostics, Beauvais, France). All plates and tubes were counted after a 48-hour incubation at 39°C. Bifidobacteria were enumerated with the selective medium of *Beerens* (1991) containing propionic acid and with a pH adjusted to 5.0.

The rest of the sampled ingesta was immediately frozen for further determination of scFOS, lactate and volatile fatty acids (VFA). ScFOS were analysed by ionic HPLC (DX500, Dionex, Voisins le Bretonneux, France). Lactate was analysed enzymatically (Lactate 735-10, sigma, Strasbourg, France), and quantified spectrophotometrically at 540nm (MRX Dynatex Laboratories, Guyancourt, France). VFA concentrations were assayed by gas-liquid chromatography (Gas chromatograph model 437 A, United technologies Packard, Zurich, Switzerland).

Statistical analysis

Results were analysed with the GLM procedure of SAS to assess 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 and the significance threshold for all tests was set at $p < 0.05$.

Results

Analysis of the pelleted concentrate fed during both experiments confirmed the presence of scFOS (0,88% in the control feed and 2.00% in the scFOS feed). Thus, the supplementation provided 0.06g scFOS/kg BW/day.

Experiment 1

No effect of scFOS was detected on the total tract digestibilities of dry and organic matter, crude protein or fat (Table 1).

Experiment 2

No scFOS were recovered from analysis of the content of the stomach or the small intestine. Bifidobacteria could not be enumerated in any part of the digestive tract, and whatever the diet. The localisation had a very significant effect on the other microbial counts (Table 2). ScFOS had no measurable effect on the microbial profile. However, scFOS and the digestive segment interacted significantly on the total anaerobic bacteria, the streptococci and the lactate-utilizing bacteria. Horses supplemented with scFOS had higher numbers of these microbes in their stomach in comparision to the control

Table 1 Total tract nutrient digestibilities by horses fed with or without scFOS for 21d.
Nährstoffverdaulichkeiten bei Pferden mit oder ohne scFOS Zulage über 21 Tage.

	Control in %	ScFOS in %	pooled SEM	P values
Dry Matter	54.0	53.7	3.6	0.899
Organic Matter	56.6	55.8	3.6	0.725
Crude Protein	59.3	60.5	4.2	0.647
Fat	19.8	26.6	9.1	0.304

Table 2 Microbial counts (logCFU/mL) in the different anatomic parts of the gastrointestinal tract of horses fed with or without scFOS for 21d. *Bakterienkeimzahlen (log KBE/mL) in den verschiedenen Abschnitten des Gastrointestinaltraktes von Pferden mit oder ohne scFOS Zulage über 21 Tage.*

	Anatomic part					pooled	P values		
	STO	JEJ	CAE	RVC	LDC	s.e.m	scFOS	Part	scFOS x Part
Total anaerobic bacteria									
Control [1]	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				
Lactate-utilizing bacteria									
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				

STO = Stomach; JEJ = Jejuno-ileum; CAE = Caecum; RVC = Right Ventral Colon; LDC = Left Dorsal colon

[1] Values are least-square means, ^{A,B} values within an item and a column are different if superscript differs ($p<0.05$)

animals. In the jejuno-ileum area Streptococci were lower for the scFOS supplemented horses while lactate utilizing bacteria were lowest in the control horses. The concentration of total anaerobic bacteria was lower in the right ventral colon of supplemented horses.

The pH of the ingesta differed accordingly to the site of the digestive tract and tended to be higher with the scFOS diet throughout the whole digestive tract (Table 3). The pH was significantly higher in the stomach of supplemented horses ($p<0.05$). The scFOS did not influence the concentration in lactate or VFA, nor their proportion (results not shown).

Discussion

As already observed in dogs, scFOS had no effect on the total tract digestibility of dry and organic matter, crude protein (*Swanson* et al. 2002) and fat (*Diez* et al. 1997).

In many species, the number of Bifidobacteria in the chyme increases with scFOS dietary supplementation. This phenomenon is associated with an indirect effect of scFOS on the health and/or welfare of the host. In accordance with previous studies (*Daly* et al. 2003, *Berg* et al. 2005) no Bifidobacteria were counted in the digestive tract of horses during this trial. Similarly to what happens in dogs, where Bifidobacteria are not always determined via classical culture methods, the prebiotic effect of scFOS can be shown on the Lactobacilli strains, also considered as probiotics (*Reid* 1999).

Interestingly, no scFOS was detected in the stomach of horses fed the supplemented diet, probably in relation with their fermentation inside this compartment. The retention time in the stomach was close to the half-emptying time measured with a similar concentrate (*Metayer* et al. 2004). Consequently, our observations probably reflected physiological conditions. It has been demonstrated that high concentrations of bacteria inhabit the horse's stomach (*Kern* et al. 1974, *Yuki* et al. 2000, et al. 2003, *Varloud* et al. 2004). The scFOS may

Table 3 pH, lactate and total VFA concentrations throughout the digestive tract of horses fed with or without scFOS for 21d. *pH, Laktat und flüchtige Gesamtazidität im Verdauungstrakt von Pferden mit oder ohne scFOS Zulage über 21 Tage.*

	Anatomic part					pooled	P values		
	STO [1]	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 (mmol/L)									
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				
Total VFA (mmol/L)									
Control	10,1	19,0	82,6	89,1	56,5				
scFOS	9,6	18,9	73,5	86,0	55,4	22,4	0,704	<0,001	0,989

[1] See Table 2 footnotes

have been fermented within the stomach. This is in accordance with the updated definition of prebiotics that extends the effects of prebiotics to the whole digestive tract and not only to the colon: prebiotics are compounds which are selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health (*Gibson* et al. 2004).

The stomach is the compartment where we measured the major differences between the two dietary treatments. The concentrations in total bacteria, Streptococci and lactate-utilizing bacteria increased when scFOS were added to the diet. The lactate concentration remained unchanged, probably due to the balance between lactate producers and lactate-utilizers, as shown in pigs and 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 studies in dogs (*Swanson* et al. 2002) and in horses (*Berg* et al. 2005), lactobacilli counts were unaltered. However, other studies showed that scFOS could modify the abundance profile of the different lactobacilli strains. They specially stimulate the growth of L. reuteri, without any difference on the total lactobacilli concentration, as observed in dogs (*Rastall* 2004), in piglets (*Konstantinov* et al. 2004) and in broilers (Arturo-Schaan, personal communication). L. salivarius, L. crispatus, L. reuteri and L. agilis have been identified in the equine gastric mucosa (*Yuki* et al. 2000). Thus, we may hypothesize that scFOS could influence the proportion of these different strains within the equine stomach, and that molecular tools would have detected this effect.

The other main effects of the scFOS supplementation were observed 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. 1999b). In piglets, compared to a control diet, a blend of sugar beet pulp and scFOS increased the diversity and the stability of the colonic microflora during the stress of weaning (*Konstantinov* et al. 2003).

The cellulolytic bacteria were not modified by the scFOS supplementation. The total count for anaerobic bacteria and the streptococci tended to decrease, whereas the lactate-utilizers tended to increase. As a result, the pH was significantly higher in 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 do not know if the scFOS reach the hindgut. Or, as suggested by *Fuller* et al. (1978), it could be an indirect effect on the hindgut, due to the arrival of bacteria stimulated in the upper part of the gastrointestinal tract.

Conclusions

It appears from this study that scFOS influenced to some extent the numbers and activities of the microbial populations in the gastrointestinal-tract in horses. Therefore, they might help to prevent some digestive disturbances associated with the management of performance horses. Further experiments, under conditions inducing digestive troubles are needed to explore this hypothesis.

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