

Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet

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ABSTRACT: Prebiotic compounds, such as short-chain fructooligosaccharides (scFOS), have been shown to improve health, welfare, or both, in several species, but few studies have been conducted in horses, despite the sensitivity of their hindgut microflora. We hypothesized that prebiotic oligosaccharides, known to be able to stabilize the intestinal microflora in other species, would be of importance in horses. Our study was designed to evaluate the effect of scFOS supplementation on the equine intestinal microflora and to assess its effectiveness in reducing hindgut microbial disturbances related to sudden diet changes. Four adult geldings were allotted by weight into 2 groups and assigned to diets with and without (control) scFOS supplementation for 21 d in a crossover design. Cecal and colonic contents were collected through cannulas to assess the effect of

an abrupt incorporation of barley in the diet of horses on microbial populations and fermentation variables. The addition of barley to the control diet caused substantial changes in the colonic microflora, such as increases ($P < 0.05$) in the concentration in total anaerobes, lactobacilli, streptococci, and lactate-utilizing bacteria. The scFOS supplementation reduced the barley intake-related changes. In contrast to the control diet, *Lactobacillus* and *Streptococcus* populations did not increase. Although the colonic D-lactate concentration increased ($P < 0.05$) after the meal of barley in the control group, it did not accumulate with scFOS supplementation. These data indicate that a scFOS supplementation would be effective in reducing disruptions of the microbial populations in the equine hindgut under stressful situations like acute starch overloads.

Key words: fructooligosaccharide, horse, large intestine, microflora, prebiotic

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INTRODUCTION

Prebiotics are selectively fermented ingredients that allow specific changes in the composition, activity, or both, of the gastrointestinal microflora that would benefit well-being and health of the host (Gibson et al., 2004). Short-chain fructooligosaccharides (scFOS), produced by transfructosylation of sucrose, are composed of a molecule of glucose linked to 1 to 4 molecules of fructose by a $\beta(2-1)$ linkage. The scFOS are fermented by bifidobacteria and lactobacilli populations in the digestive tract of humans and other nonruminant animals. Bifidobacteria have not yet been detected in the equine gastrointestinal tract, and large lactobacilli concentrations are generally not expected. However, studies in rats (Le Blay et al., 1999; Hashizume et al., 2003) indicated that the effects of scFOS on microbial populations are more diverse and affect several populations directly or indi-

rectly. Short-chain fructooligosaccharides have already been shown to modify the fecal microflora of horses (Pellegriani et al., 1999; Berg et al., 2005).

Horses are sensitive to environmental stress and may develop colic or laminitis in response to sudden diet changes or a carbohydrate overload (Garner et al., 1977; Hudson et al., 2001). Abrupt changes in the diet of horses were associated with such drastic modifications in their microbial population in the large intestine, and microbial population in the colon seemed to be more sensitive than the population in the cecum (Goodson et al., 1988; de Fombelle et al., 2001). Konstantinov et al. (2004) demonstrated in piglets that prebiotics could increase the stability of the colonic microflora. Thus, we conducted a study to investigate the effects of scFOS on the microbial populations and fermentation variables within the large intestine of horses subjected to a sudden change in diet.

MATERIALS AND METHODS

Animals and Diets

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Table 1. Ingredients and nutrient composition of the pelleted concentrate feed

Item	Amount
Ingredient, g/kg (as-fed basis)	
Roughages and by-products (hay, alfalfa, wheat bran, and straw)	525
Cereals and sugars (barley, oat, and carob)	430
Soybean meal	20
Mineral and vitamin premix ¹	25
Analyzed composition (DM basis)	
DM, g/100 g	88.96
OM, g/100 g	81.82
Ash, g/100 g	7.14
CP, g/100 g	11.16
Fat, g/100 g	1.91
Crude fiber, g/100 g	17.24
NDF, g/100 g	40.4
Starch, g/100 g	20.3
GE, kJ/g	17.6

¹Provided the following per kilogram of pelleted concentrate: 8,000 IU of vitamin A, 2,400 IU of vitamin D₃, 25 IU of vitamin E, 2 mg of vitamin B₁, 2 mg of vitamin B₂, 4 mg of vitamin D-pantothenic acid, 0.7 mg of vitamin B₆, 2 mg of niacin, 11.75 mg of vitamin C, 0.004 mg of vitamin B₁₂, 125 mg of choline chloride, 935 mg of Mg (as MgO), 17 mg of Fe (as Fe₂SO₄), 10 mg of Mn (as MnO), 31.875 mg of Zn (as ZnO), 15 mg of Cu (as Cu₂SO₄), 0.35 mg of Co (as CoCO₃), and 0.07 mg of Se (as Na₂SeO₃).

Four 7-yr-old crossbred geldings with an average BW of 425 ± 27 kg, each fitted with polyvinyl chloride cannulas (30-mm i.d.) in the cecum and right ventral colon, were used in the study. Horses were individually housed in 3 × 3 m free stalls bedded with wood shavings. Horses were given free exercise in an outdoor paddock for 30 min every other day, dewormed (Pyrantel, Pfizer, Orsay, France; Ivermectin, Janssen-Cilag SA, Issy-les-Moulineaux, France) before the trial. Horses were allotted into 2 groups according to their BW. All horses were fed a commercial pelleted feed (1.17 kg/100 kg of BW; HIPPO 122, UAR, Villemoisson, France; Table 1) and wheat straw (0.5 kg/100 kg of BW). The roughage:concentrate ratio was approximately 30:70. Amounts fed met maintenance requirements, as defined by Institut National de la Recherche Agronomique (Martin-Rosset, 1990). The quantity of pelleted concentrate feed was divided in 3 equivalent meals (fed at 0800, 1200, and 1730), allowing a starch intake per meal equivalent to 0.08% of BW, and the straw was given in 2 other meals (1000 and 1500). In a crossover design, the treatment group was supplemented with 30 g of scFOS/d per horse (PROFEED P95, Béghin-Meiji, Marckolsheim, France, 95% of scFOS with molecular chain length from 3 to 5), which was top-dressed on the concentrate during the morning meal. After 21 d, horses were fed 2.25 kg of barley calculated to provide 0.28% BW of starch (Sauvant et al., 2004) instead of their usual morning concentrate meal. The addition of the barley meal was to mimic a feeding mistake and trigger a digestive stress. Changing the botanical origin of starch or increasing its intake above 0.2% BW per meal can induce a digestive stress in horses (Julliand et al.,

2006). Subsequently, supplementation was switched between the 2 groups of horses, and a second meal of barley was fed after another 21-d period.

Sample Collection

Digestive contents were collected for 3 consecutive days, corresponding to -19 h, +5 h, and +29 h after the meal of barley. Cecal and colonic digesta samples (200 mL) were collected by gravity via the cannulas into CO₂-saturated sterile flasks, which were then resaturated with CO₂. Flasks were held at 38°C until inoculation. About 10 mL of each digesta sample was filtered (Blutex nylon filter 100 µm, Tripette and Renaud, Paris, France) and divided into 2 subsamples and immediately frozen (-20°C) for later determination of D- and L-lactate (1-mL sample) and VFA [1-mL sample together with a preservative (0.1-mL mixture of 5% H₃PO₄ + 1% HgCl₂)].

Microbial Analyses

Ten-fold dilution series were prepared under O₂-free CO₂ in an anaerobic mineral solution (Bryant and Burkey, 1953) for inoculation on specific media. Total viable anaerobic bacteria counts were determined with a modified nonselective medium, with an O₂-free CO₂ gas phase in anaerobic roll tubes (Leedle and Hespell, 1980; Julliand et al., 1999). Concentrations were determined from 4 replicate roll tubes. Lactic acid-utilizing bacteria counts were determined on a selective medium (Mackie and Heath, 1979) in anaerobic roll tubes with O₂-free CO₂ gas phase. *Lactobacilli* spp. were cultured on Rogosa agar (Biokar Diagnostic, Beauvais, France) in petri plates. Streptococci spp. were cultured on a bile-esculin-azide agar medium (BK158HA, Biokar Diagnostics). All plates and tubes were counted after a 48-h incubation at 39°C.

Biochemical Analyses

Cecal and colonic pH were measured immediately after sample collection using an electronic pH meter (MP 120 pH-meter, Metter-Toledo, Barcelona, Spain). The L- and D-lactic acids were assayed with an enzymatic reaction procedure (D-lactic acid/L-lactic acid Enzymatic BioAnalysis, Food analysis kit, Boehringer Mannheim, R-Biopharm, Darmstadt, Germany) and quantified spectrophotometrically at 340 nm (MRX revelation, Dynatech Laboratories, Guyancourt, France). The total VFA, acetate, propionate, and butyrate concentrations were assayed by GLC (gas chromatograph model 437 A, United Technologies Packard, Zurich, Switzerland).

Statistical Analysis

An ANOVA with the GLM procedure (SAS Inst. Inc., Cary, NC) was used to assess the effect of scFOS supplementation (with or without) and time (-19 h; +5 h or

Table 2. Colonic microbial populations of horses with or without dietary supplementation of short-chain fructooligosaccharides (scFOS) for 21 d and subjected to a digestive stress¹

Item	Dietary supplementation		SEM	S × T ²
	Control	scFOS		
Total anaerobes, log ₁₀ cfu/mL				
-19 h	6.8 ^a	8.0 ^b		
+5 h	7.3 ^A	7.7	0.5	<0.001
+29 h	7.4 ^A	7.8		
Lactate-utilizing bacteria, log ₁₀ cfu/mL				
-19 h	5.8 ^a	7.0 ^b		
+5 h	6.4 ^A	7.0	0.4	<0.001
+29 h	6.8 ^A	6.6 ^A		
Lactobacilli, log ₁₀ cfu/mL				
-19 h	5.5 ^a	6.1 ^b		
+5 h	6.0 ^A	6.2	0.4	<0.001
+29 h	6.7 ^A	5.7 ^A		
Streptococci, log ₁₀ cfu/mL				
-19 h	5.7 ^a	6.7 ^b		
+5 h	6.0 ^A	6.3 ^A	0.3	<0.001
+29 h	6.4 ^A	6.1 ^A		

^{a,b}At -19 h, values within the same item and the same row are different if superscripts differ ($P < 0.05$).

^AWithin the same item and the same column values are different from the -19 h sampling time ($P < 0.05$).

¹Values are least squares means ($n = 4$).

²S × T = P -value for interaction between supplementation and time.

+29 h) and their interaction on the response variables (microbial counts, pH, VFA, and D- and L-lactic acids). The microbial counts data were subjected to a logarithmic transformation before statistical analysis. Least squares means were calculated for all variables. The effect of FOS supplementation was assessed at -19 h, and the effect of time was assessed at +5 h and +29 h, which was separated from -19 h, within control or supplementation group using PDIFFF option of SAS, and the significance threshold was set as $P < 0.05$.

RESULTS

Concentrations of Microorganisms

We observed an interaction between the supplementation and the time ($P = 0.001$) on the total anaerobes, the lactate-utilizing bacteria, the lactobacilli, and the streptococci concentrations within the colon (Table 2). For the control diet (without supplementation), the concentrations of total anaerobes, lactate-utilizing bacteria, lactobacilli, and streptococci increased over time ($P < 0.05$). For scFOS supplementation, the concentrations of total anaerobes, lactate-utilizing bacteria, and lactobacilli remained stable after 5 h, and the concentrations in lactate utilizers and lactobacilli ($P = 0.05$) decreased 29 h after the stress. Streptococci decreased ($P = 0.05$) after 5 and 29 h in both the control and scFOS-supplemented groups.

Table 3. The pH and D- and L-lactate concentrations in the cecum and in the colon of horses supplemented or not with short-chain fructooligosaccharides (scFOS) for 21 d¹

Item	Sampling time	Dietary supplementation		SEM	S × T ²
		Control	scFOS		
pH					
Cecum	-19 h	7.05	7.00		
Cecum	+5 h	7.26 ^A	6.84	0.15	0.005
Cecum	+29 h	7.05	6.87		
Colon	-19 h	6.85	6.75		
Colon	+5 h	6.76	6.96	0.24	0.015
Colon	+29 h	6.69	6.50 ^A		
L-Lactate, mmol/L					
Cecum	-19 h	0.35 ^a	2.89 ^b		
Cecum	+5 h	1.14	6.10 ^A	1.46	<0.001
Cecum	+29 h	0.00	1.13 ^A		
Colon	-19 h	1.31	3.50		
Colon	+5 h	6.17	6.11	3.51	0.065
Colon	+29 h	5.46	2.87		
D-Lactate, mmol/L					
Cecum	-19 h	0.28	0.68		
Cecum	+5 h	0.78	1.50 ^A	0.39	<0.001
Cecum	+29 h	0.00	0.64		
Colon	-19 h	1.06	2.24		
Colon	+5 h	8.16 ^A	2.19	3.91	0.007
Colon	+29 h	7.88 ^A	2.87		

^{a,b}At -19 h, values within the same item and the same row are different if superscripts differ ($P < 0.05$).

^AWithin the same item, compartment and column values are different from the -19 h sampling time ($P < 0.05$).

¹Values are least squares means for cecum ($n = 3$) and colon ($n = 4$).

²S × T = P -value for interaction between supplementation and time.

Biochemical Characteristics

Because it was not possible to collect cecal content from 1 horse along the whole study, only 3 horses were used to study the cecal biochemical characteristics. In the cecum, there was an interaction between dietary supplementation and the time on the pH ($P = 0.005$) and the L- ($P < 0.001$) and D-lactate ($P < 0.001$) concentrations (Table 3). With the scFOS supplementation, the pH remained unchanged after the meal of barley, whereas it was increased ($P < 0.05$) after 5 h with the control diet. The cecal concentrations of L- and D-lactate were very low with the control diet and were not modified by the meal of barley. With the scFOS supplementation, the concentration of L-lactate was greater ($P < 0.05$) 5 h after the meal but decreased ($P < 0.05$) after 29 h to below the concentration observed before feeding barley. In the colon, there was also an interaction between the supplementation and time on the pH ($P = 0.015$) and the D-lactate ($P = 0.007$) concentration. With the control diet, the pH remained stable, whereas it was slightly decreased with the scFOS supplementation 29 h after the meal of barley. The colonic D-lactate concentration did not alter over time with the scFOS supplementation, whereas it increased ($P < 0.05$) 8-fold 5 h

Table 4. Concentrations of VFA in the cecum of horses supplemented or not with short-chain fructooligosaccharides (scFOS) for 21 d

Item	Sampling time	Dietary supplementation				Concentrations		Proportion	
		Control		scFOS		SEM	S × T ²	SEM	S × T
		mmol/L	% ¹	mmol/L	%				
Total VFA ³									
Cecum	-19 h	52.15		50.19					
Cecum	+5 h	38.43		50.15		8.97	0.082		
Cecum	+29 h	55.88		69.54					
Acetate									
Cecum	-19 h	36.72	70.7	35.65	71.0				
Cecum	+5 h	25.97	66.6	32.57	65.0	5.42	0.054	2.5	0.167
Cecum	+29 h	38.68	69.1	46.67	67.3				
Propionate									
Cecum	-19 h	12.71	24.1	11.71	23.3				
Cecum	+5 h	9.95	26.6	14.07	27.9	3.41	0.172	2.5	0.354
Cecum	+29 h	13.71	24.7	19.02	27.1				
Butyrate									
Cecum	-19 h	2.16	4.1	2.31	4.6				
Cecum	+5 h	1.42	3.5	2.11	4.3	0.53	0.064	0.8	0.480
Cecum	+29 h	2.59	4.6	3.37	4.9				

¹The percentage of acetate, propionate, and butyrate.

²S × T = *P*-value for interaction between supplementation and time.

³Total VFA (mmol/L) = acetate + propionate + butyrate + isobutyrate + valerate + isovalerate; values are least squares means (n = 3).

after the meal of barley with the control diet and remained greater after 29 h (*P* < 0.05).

In the cecum, we observed trends for increased concentrations of total VFA (*P* = 0.082), acetate (*P* = 0.054), and butyrate (*P* = 0.064) over time with the dietary supplementation of scFOS, whereas it remained stable with the control diet (Table 4). The percentage of acetate, propionate, and butyrate did not change. In the colon, no interaction between the supplementation and time and was observed in VFA concentrations, and only a trend (*P* = 0.100) was observed in the proportion of acetate (Table 5). The ratio [(acetate + butyrate)/propionate] remained unchanged regardless of the diet and averaged 2.89 ± 0.41 in the cecum (data not shown; calculated from Table 4). In the colon, the ratio decreased from 4.15 to 3.52 (*P* = 0.02) 5 h after the meal of barley (data not shown; Table 5).

DISCUSSION

Microbial analyses were performed with colonic contents, because it is the main site of microbial disturbances. Fine particles can flow quickly through the cecum and remain longer in the colon, allowing greater variations in the colon than in the cecum during a diet change (de Fombelle et al., 2001). The microbial concentrations in the colon as well as the lactate and VFA concentrations in the cecum and colon were within the lower limits reported in a review by Julliand (2005). Our results were lower than values obtained with a similar diet based on straw and concentrate pellets (Medina et al., 2002), even though the diet used in this study provided more starch than their study (237 g/100

kg of BW vs. 136 g/100 kg of BW, respectively). In the current study, horses were fed 3 times per day, whereas they were fed 2 times per day in the study by Medina et al. (2002), resulting in lower starch intakes per meal (0.08 vs. 0.13% BW). This difference underlines the great effect of feeding management on microbial activity within the digestive tract and the quantity of starch per meal (Potter et al., 1992).

The digestive stress induced in this study is similar to what may happen under normal conditions and did lead to substantial changes in the hindgut. The meal of barley altered the concentration and the activity of microbial populations in the large intestine in control horses as reported by Goodson et al. (1988) and de Fombelle et al. (2001). The reaction to a greater amount of starch reaching the hindgut was the increase in concentration of total anaerobes, lactobacilli, streptococci, and lactate-utilizing bacteria. The increase in lactate-utilizing bacteria is contrary to the results reported by Julliand et al. (2001) but similar to the results reported by Goodson et al. (1988). In the study of Julliand et al. (2001), horses were fed a starch-free basal diet based on roughage. Horses in our study were already adapted so that limited amounts of starch were reaching the hindgut. With the control diet, the accumulation of D-lactate induced by the digestive stress was consistent with results obtained by de Fombelle et al. (2001). The small intestinal capacity of starch digestion has been reported to be 0.4% of BW per meal (Potter et al., 1992). Beyond this level of starch intake, the microbial disruption in the hindgut can result in greater lactate production because of excessive starch reaching the colon. Our results indicated that even 0.3% BW of barley starch

Table 5. Concentrations of VFA in the colon of horses supplemented or not with short-chain fructooligosaccharides (scFOS) for 21 d

Item	Sampling time	Dietary supplementation				Concentrations		Proportion	
		Control		scFOS		SEM	S × T ²	SEM	S × T
		mmol/L	% ¹	mmol/L	%				
Total VFA ³									
Colon	-19 h	60.34		64.30					
Colon	+5 h	65.57		46.79		15.65	0.155		
Colon	+29 h	70.00		83.23					
Acetate									
Colon	-19 h	44.05	72.5	47.15	73.2				
Colon	+5 h	46.53	69.7	32.43	68.3	10.10	0.120	2.7	0.100
Colon	+29 h	50.87	72.0	56.40	68.3				
Propionate									
Colon	-19 h	11.67	20.2	11.95	19.3				
Colon	+5 h	13.83	23.1	10.44	24.0	3.97	0.266	2.6	0.195
Colon	+29 h	14.37	21.8	18.61	22.2				
Butyrate									
Colon	-19 h	2.72	4.5	3.31	4.9				
Colon	+5 h	3.07	4.4	2.29	4.8	1.84	0.244	1.3	0.476
Colon	+29 h	3.31	4.5	5.85	6.5				

¹The percentage of acetate, propionate, and butyrate.

²S × T = *P*-value for interaction between supplementation and time.

³Total VFA (mmol/L) = acetate + propionate + butyrate + isobutyrate + valerate + isovalerate; values are least squares means (n = 4).

per meal could induce such disruption. Instead of 0.4% BW, the level of starch intake per meal should not be greater than 0.2% BW as reported by Kienzle et al. (1992) and supported in a recent review by Julliand et al. (2006). However, an intake of dietary starch from sorghum, equivalent to 0.32% BW per meal, induced only very low concentrations of D- and L-lactate within the hindgut of horses with no difference between dry-rolled or steam-flaked sorghum diets (Al Jassim, 2006). All these results indicate that the extent of foregut digestion depends more on botanical origin vs. starch content per se as suggested by Moore-Colyer et al. (2005).

A daily intake of more than 8 kg of grain (Rowe et al., 1994), as well as grazing from May to September when the content of fructans in pastures can reach more than 30% of the *Lolium perenne* DM (Longland et al., 1999), can lead to laminitis. A large intake of starch is commonly considered a risk factor for laminitis and has been associated with increased concentrations of lactobacilli and streptococci in the hindgut, resulting in an increased production of lactate. Populations of *Lactobacillus* and *Streptococcus* can reach 10 log cfu/mL in the cecum of horses after a carbohydrate overload (Garner et al., 1978). This rapid and sharp increase in lactic bacteria is considered to be responsible for the accumulation of lactate and the decrease in pH (5.8 after 8 h), leading to bacterial death. Endotoxins released from dying bacteria can lead to the onset of laminitis (Garner et al., 1977; Bailey et al., 2003). Laminitis can be experimentally induced by the administration of 7.5 g/kg of BW of oligofructose (French and Pollitt, 2004) or by the administration of 15 g/kg of BW of

cornstarch via a stomach tube (Garner et al., 1978). These levels equal to an intake of 3.75 kg of oligofructose or 7.5 kg of starch for a 500-kg horse, which is far greater than any recommended dosage of fructooligosaccharides (30 g/d) or starch (0.2% of BW/meal) in a horse diet. In the experimental laminitis model, *Lactobacillus salivarius* was 1 of the main L-lactate producers identified by 16S rDNA methodology applied to colonic and rectal digesta samples. *Lactobacillus delbrueckii* and *Mitsuokella jalaludinii* were the main D-lactate producers identified in the colon and rectum of experimentally induced laminitic horses that had a greater concentration of blood D-lactate than healthy control horses (Al Jassim et al., 2005). Because a substantial increase in D-lactate was observed after the meal of barley, we concluded that the aforementioned strains were involved in this response.

Similarly to what happens in other species (e.g., dogs and poultry; Swanson et al., 2002; Xu et al., 2003), intestinal microbial populations in horses can be changed when the diet is supplemented with scFOS. We observed greater concentrations of lactobacilli in the colon of horses fed the diet supplemented with scFOS. No bifidobacteria have been detected (Daly and Shirazi-Beechey, 2003; Berg et al., 2005; Respondek et al., 2007); however, lactobacilli could support the prebiotic concept in horses (Reid, 1999; Weese et al., 2004). Effects of dietary scFOS on the *Lactobacillus* populations can be observed both in quantity and quality (profile of strains). In human studies, the bifidogenic effect of scFOS depends on the dosage of scFOS and the level of bifidobacteria already present in the colon (Rycroft et al., 2001). Increases in the concentration of

lactobacilli might be due to the relatively low concentration observed in horses fed the control diet. Also, microbial counts with biomolecular technologies might have highlighted modifications of the relative proportions of the different strains within the whole *Lactobacillus* population. Indeed, studies using dogs and pigs demonstrated that scFOS will particularly stimulate the growth of *Lactobacillus reuteri*, whereas they will be poor promoters of *Lactobacillus mucosae* (Konstantinov et al., 2004; Rastall, 2004). Moreover, a reduction of potentially pathogenic bacteria (e.g., *Escherichia coli* because of the competition with lactobacilli) has already been observed from fecal samples of adult horses (Pellegrini et al., 1999) and yearlings (Berg et al., 2005) with no modification of the lactobacillus concentration. Even if fecal ecosystem contains greater concentrations of bacteria than in the colon, it is an appropriate marker of intestinal changes occurring in the colon ecosystem (Julliard and Goachet, 2005). Thus, similar reduction of *E. coli* could be expected within the colon without necessarily increasing concentrations of the total population of lactobacilli but by modifying the proportion among various strains within the population.

In other nonruminant species, an increase of about 1 log cfu/mL in lactic acid-producing bacteria such as lactobacilli can be considered beneficial. Lactate is an intermediate metabolite in the hydrolysis of carbohydrates, because it can be fermented into acetate, propionate, or butyrate by lactate-utilizing bacteria such as *Selenomonas ruminantium*, *Veillonella parvula*, *Desulfovibrio desulfuricans*, and *Megasphaera elsdenii*. It has already been shown that *M. elsdenii* can manage high lactate production in rats (Hashizume et al., 2003) by fermenting and converting it to butyrate as observed in swine (Tsukahara et al., 2002). We observed around 7 log cfu/mL of lactate-utilizing bacteria in the colon. Such populations as *Selenomonas*, *Veillonella*, and *Megasphaera* have already been isolated from the equine cecum (Baruc et al., 1983; Maczulak et al., 1985). We may assume that, like in rats (Le Blay et al., 1999; Hashizume et al., 2003), scFOS would indirectly promote the growth of lactate-utilizing bacteria in the large intestine and thus decrease the risk of lactate accumulation. The intake of scFOS (0.05 to 0.07 g·kg of BW⁻¹·d⁻¹) may enhance the stability of the microbial populations under stressful conditions. Supplementation of scFOS prevented the increase in both lactobacilli and streptococci, which was observed with the control diet after the dietary stress. The stabilization of the colonic microflora with a diet enriched with prebiotic fiber was already observed in weaned piglets by using the denaturing gradient gel electrophoresis analysis (Konstantinov et al., 2004).

Neither the cecal pH nor the concentration in L- and D-lactate was affected by the scFOS supplementation in the current study, as reported earlier (Respondek et al., 2007). This agrees with the concomitant increase in lactate-producing and lactate-utilizing populations observed with the scFOS supplementation. Contrary to

an increase in individual and total VFA concentrations observed in the feces of yearling horses supplemented with scFOS (Berg et al., 2005), we did not observe changes in acetate and butyrate concentrations in the cecum and no significant effect in the colon in the current study. This difference might be due to the nature of sample. If the fecal ecosystem contains greater concentrations of bacteria than intestinal samples, greater concentrations of VFA and lactate are also expected. In rats, scFOS induced a short-term increase in lactic acid-producing bacteria and a persistent increase in cecal butyrate (Le Blay et al., 1999). In humans, as in swine and dogs, butyrate is associated with an improved gut health through its involvement in immune mechanisms, enterocyte metabolism, and the mucosal structure. Butyrate has been identified in man (Finnie et al., 1995; Young and Gibson, 1995) and swine (Tsukahara et al., 2003) as the main VFA that is capable of increasing normal cell proliferation and mucin secretion by the intestinal epithelium. We can assume that scFOS could lead to a beneficial increase in VFA production, such as butyrate, in the equine hindgut, but the results would need to be confirmed with a greater number of horses. The trend we observed for a lower proportion of acetate in the colon supports a shift of the fermentation profile toward a greater propionate and butyrate production. Contrary to the control diet and according to the results on bacterial populations, D-lactate concentration remained stable after the meal of barley. Even though scFOS may indirectly stimulate the growth of lactate-utilizing bacteria, they may not support the growth of D-lactate producers like *Mitsuokella*, as demonstrated by in vitro study with strains from human origin (Hidaka et al., 1986).

The upper gastrointestinal tract of horses is also colonized by substantial bacterial populations. Therefore, it may be possible that the effects observed in this study were not only the result of what had taken place in the hindgut. The stimulation of the foregut microflora, as reported earlier (Respondek et al., 2007), may also have limited the disruption of microbial populations after the barley meal by enhancing prececal polysaccharide digestion. It was shown in broilers that scFOS were able to increase the total amylase activity within the small intestine by increasing *Bifidobacteria* and *Lactobacillus* concentrations (Xu et al., 2003). Further investigation on the beneficial effect of scFOS in the digestive upset in the upper part of the digestive tract, like gastric ulcers, would be of great interest. In accordance with the reduction of digestive problems in performance horses housed in free stalls (Wolter, 1999), results of this study support the fact that supplementing the diet of the horse with 0.05 to 0.07 g·kg of BW⁻¹·d⁻¹ of scFOS should be beneficial in preventing digestive disorders associated with starch intake. It would also be interesting to test their efficiency in every situation that can induce stress and disruptions in the microbial population in the hindgut (e.g., during the feed change, transport, competition, and weaning).

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