Effect of live yeast culture supplementation on hindgut microbial communities and their polysaccharidase and glycoside hydrolase activities in horses fed a high-fiber or high-starch diet[1]

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ABSTRACT: Four cecum and right ventral colon-fistulated horses were assigned in a 4 × 4 Latin square design and fed a high-fiber (HF) or a high-starch (HS) diet with or without 10 g of *Saccharomyces cerevisiae* (SC; CBS 493.94) containing 4.5×10^9 cfu/g. The HF and HS diets consisted of pelleted feeds and long wheat straw (18.0 and 3.5 g of DM·kg^{-1} of BW·d^{-1}, respectively) given in 2 equal meals to provide an NDF:starch ratio of 3.5 and 1.0, respectively. After a 21-d adaptation period intestinal contents were collected 4 h after the morning meal on d 23 and 25 to determine bacterial and SC concentrations. Polysaccharidase activities (CMCase, xylanase, amylase) and activities of glycoside hydrolases (α-L-arabinosidase, β-D-cellobiosidase, β-D-glucosidase, β-D-xylosidase) were determined in liquid-associated bacteria (LAB) and solid-adherent bacteria (SAB) isolated from both compartments. Lactobacilli were increased in the cecum ($P = 0.012$) and colon ($P = 0.086$) when starch intake increased, whereas total anaerobes, cellulolytics, and streptococci did not change in either compartment. In yeast-supplemented horses, SC concentrations were greater in cecum (4.4×10^6 cfu/mL) than in right-ventral colon (5.6×10^4 cfu/mL) and did no change with diet. Concentrations of lactobacilli and lactic-acid utilizers were greater ($P = 0.099$ and 0.067, respectively) in the cecum but remained similar in the colon of SC-supplemented horses. The CMCase activities of SAB were not affected by diet. Colonic xylanase activities of SAB were reduced ($P = 0.046$) by starch addition, but no change was seen in the cecum. All SAB glucoside hydrolase activities in the cecum and colon, except β-D-xylosidase in the cecum, were decreased when starch intake was increased. The LAB CMCase ($P = 0.049$ in the colon) and xylanase ($P = 0.021$ in the cecum; $P < 0.001$ in the colon) activities decreased with starch intake. No effect of starch on LAB or SAB amylase activity was observed. Addition of SC improved SAB CMCase in the cecum ($P = 0.019$) and colon ($P = 0.037$) as well as β-D-cellobiosidase ($P = 0.002$) and β-D-glucosidase ($P = 0.041$) in the cecum. Only xylanase in the cecum ($P = 0.015$) and β-D-xylosidase in the cecum ($P = 0.028$) were improved with SC, whereas colonic LAB α-amylase activity was significantly decreased ($P = 0.046$). Most enzymes involved in plant cell wall digestion were increased after SC addition. This fact may contribute to explain a better digestion of fiber that has been previously reported in SC-supplemented horses.

Key words: cellulolytic, enzymatic, fiber digestion, horse, large intestine, *Saccharomyces cerevisiae*

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INTRODUCTION

Horses have developed a specialized digestive system that allows them to utilize cellulosic and hemicellulosic materials via microbial degradation that occurs in the hindgut and provides them with energy via the production of VFA. Although the microbial breakdown of plant material occurs at the beginning of the digestive tract of ruminants and at the end of the digestive tract of horses, the mechanisms involved in the digestion of cellulosic material have great similarities in their basic approach in both types of animals. Thus, in horses, like ruminants, the efficiency of microbial digestion of cellulosic feed is greatly dependent on feeding practices. For example, the addition of grain concentrates to the diet can alter the microbial population and its enzymatic

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activities in different ways depending on the botanical origin of cereals, feed processing, or the amount of starch that is ingested. In horses, a level of starch intake greater than 2% $BW \cdot meal^{-1}$ enhances the quantity of starch reaching the hindgut, which can have a negative effect on the microbial fibrolytic activity and affect fiber degradation (Julliand et al., 2006).

Because the addition of live yeasts has now been recognized as a way to improve the digestive efficiency of rumen microbes (Jouany et al., 1998a,b; Chaucheyras-Durand et al., 2007) attempts have been made to determine a similar effect on the digestive efficacy of the microbial ecosystem of horses offered the same probiotic, especially when animals are fed high-starch (**HS**) diets. Previous studies in equines indicated that live yeasts can increase the concentration of total anaerobic bacteria, change the pattern of hindgut fermentation (Medina et al., 2002), and improve the digestibility of dietary cellulose (Glade, 1991a,b; Jouany et al., 2008). In ruminants fed a high concentrate diet, the effect of live yeast on fiber degradation has been associated with increased polysaccharide-degrading activities of the solid-associated bacterial fraction of ruminal contents (Jouany et al., 1998a; Chaucheyras-Durand and Fonty, 2006). Such an impact on microbial fibrolytic activities in the cecum or colon of equines remains ignored. Therefore, the objective of the present work was to evaluate the effect of supplementing the diet of horses with a yeast culture *Saccharomyces cerevisiae* CBS 493.94 (**SC**) on cecal and colonic concentrations of solid-adherent bacteria (**SAB**) and liquid-associated bacteria (**LAB**) in the digestive content of the equine hindgut and their polysaccharidic and glycoside hydrolase activities when animals were fed a high-fiber (**HF**) or HS diet.

MATERIALS AND METHODS

The experiment was conducted in the animal research unit of ENESAD (Etablissement National d'Enseignement Supérieur Agronomique de Dijon) under a license delivered by the Health and Animal Welfare Department of the French Veterinary Authority.

Animals

Four crossbred male horses (12 ± 5 yr) with an average BW of 387 ± 19.1 kg at the start of the experiment were used. Animals were fitted with cecum and right-ventral colon polyvinyl chloride cannulae (i.d. 30 mm) at least 6 mo before the beginning of the experiment.

Horses were wormed 2 wk before the experiment started with a double dose of pyrantel (Strongid, Laboratoire Pfizer, Orsay, France) followed 1 wk later by a single dose of ivermectin (Eqvalan, Laboratoire Merial, Lyon, France). Animals were kept inside the barn during the entire experimental period and were given access to a sandy paddock for 10 h per wk during the diet adaptation period (21 d) preceding each experimental period. Indoor housing consisted of concrete-floored individual boxes (2.0 × 2.5 m) bedded with flax shavings (ECOLIT, Croissanville, France).

Table 1. Composition of the high-fiber (HF) and high-starch (HS) diets

Item	HF diet[1]	HS diet[2]
DM, % of fresh matter	88.7	88.0
OM, % of DM	88.2	90.3
CP, % of DM	12.6	12.7
NDF, % of DM	41.0	30.7
ADF, % of DM	25.8	16.1
ADF-ADL,[3] % of DM	20.5	13.1
NDF-ADF,[4] % of DM	15.2	14.6
Starch, % of DM	11.6	30.1
DE,[5] Mcal/kg of DM	2.63	3.19

[1]The HF and HS diets had the following composition, respectively, on a DM basis (%): long wheat straw (16.3 and 16.3), dehydrated alfalfa (46.0 and 8.3), wheat bran (21.3 and 18.8), barley (10.9 and 44.8), soybean meal (0.0 and 6.4), $CaCO_3$ (2.12 and 2.98), sugar cane molasses (1.63 and 0.80), NaCl (0.81 and 0.80), and mixture of vitamins and trace elements (5.4 and 5.4).

[2]Supplied per kilogram of mixture: vitamin A, 15,000 IU; vitamin D_3, 2,500 IU; vitamin E, 135 IU; biotin, 0.2 mg; and Cu, 25 mg.

[3]The difference ADF-ADL was used to estimate the cellulose content.

[4]The difference NDF-ADF was used to estimate the hemicellulose content.

[5]Digestible energy was calculated from equations reported by Fonnesbeck (1981).

Diets

The physical form and composition of diets were chosen to mimic normal French feeding practices used in horse riding schools. Animals were fed the 2 following diets: a pelleted HF diet or a pelleted HS diet (Table 1); both pellets were offered in a mixture of long wheat straw as a coarse ingredient. The HF and HS diets were pressed into 3-mm diameter pellets after grinding the ingredients to a particle size of 1.5 mm. The 2 diets were supplemented (**HF+SC**; **HS+SC**) or not (**HF+0**; **HS+0**) with a lyophilized culture of *Saccharomyces cerevisiae* strain CBS 493.94, plus the growth medium (lot 22700, Yea-Sacc, Alltech Inc., Lexington, KY). The live yeast culture supplement contained 4.5×10^9 cfu/g and was given at a dose of 10 $g \cdot d^{-1} \cdot horse^{-1}$.

The minimum daily voluntary intake of wheat straw (3.5 g of $DM \cdot kg$ of $BW^{-1} \cdot d^{-1}$), which was determined on all horses during a pre-experimental period using the same HF and HS pellets, was then given for the entire duration of the experiment. The 4 diets were distributed at the same level 21.5 g of $DM \cdot kg^{-1}$ of $BW \cdot d^{-1}$ (i.e., 18.0 g of pelleted feeds + 3.5 g of long wheat straw) to minimize its impact on the rate of passage of digesta (Drogoul et al., 2001; Pearson et al., 2001) and minimize its impact on microbial activities. This intake level provided 100 and 130% of energy requirements for HF and HS fed animals, respectively (Martin-Rosset et al., 1994).

The HF and HS diets provided a range of NDF:starch ratios that were large enough (3.5 and 1.0 for the HF

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and HS diets, respectively) to assess possible interactions between diet composition and yeast content. Daily rations were offered as 2 equal meals in individual troughs at 0800 and 1700 h. Mean ingestion of starch was equal to 2.7 and 1.0 $g \cdot kg^{-1}$ of $BW \cdot meal^{-1}$ with HS and HF diets, respectively. Pelleted diets were initially distributed with or without the SC culture top-dressed (5 g/meal), and the wheat straw fraction of the diet was provided 30 min later.

Horses were weighed on 2 consecutive days before each adaptation period to adjust feed allowances to according to BW. Animals were given individual free access to clean potable drinking water and a lick block of trace-mineral salt (composition per kg of mixture: vitamin A, 15,000 IU; vitamin D_3, 2,500 IU; vitamin E, 135 IU; biotin, 0.2 mg; and Cu, 25 mg).

Experimental Design

The 4 horses were distributed in a 4 × 4 Latin square design [4 animals, 4 periods, 2 diets (HF and HS) and 2 treatments within each diet (with and without SC)]. Animals were adapted to diet for a period of 21 d preceding each period.

Collection of Digesta Samples

Approximately 200 mL of digesta were sampled on d 23 and 25 of each experimental period 4 h after the morning meal through the cecal and colonic cannulae for microbial and enzymatic analysis, respectively. The fraction of digesta trapped inside the cannulae was carefully removed; then the fluid fraction of the digesta was collected in a sterile bottle saturated with CO_2 and maintained at 39°C.

Enzyme Extraction from SAB and LAB

Bottles containing cecal and colonic digesta were quickly transferred to an anaerobic chamber kept under CO_2 where the following procedures were undertaken. Samples of digesta were homogenized by hand agitation of the bottles and then strained through a 100-μm nylon filter (Blute, SAATI Inc., Sailly Saillisel, France) to separate the solid phase from the liquid phase. The filtrate called Filtrate 1 was placed under anaerobic conditions into a flask for further treatment.

Exactly 15 g of solid phase was washed in 200 mL of anaerobic buffer prewarmed at 39°C to remove nonadherent bacteria. The SAB were recovered by filtration (100 μm). The filtrate called Filtrate 2 was placed into a flask for further treatment. All filtrations were made under anaerobic conditions.

Five grams of the previously washed digesta containing the SAB was suspended at 4°C into 20 mL of a MESDTT solution consisting of an anaerobic MES buffer solution made of 0.025 *M* of sulfonic acid 2-(*N*-morpholin) ethane at pH 6.5 containing 1 m*M* dithiothreitol (**DTT**) and stored at −80°C before enzyme extraction. Two successive cycles of freezing to −20°C and thawing were applied to the microbial suspension according to the methods of Noziere and Michalet-Doreau (1994) to release the attached bacteria. The suspension was then placed in ice and sonicated under anaerobic conditions (4 cycles for 30 s with 30-s intervals) to break microbial cells. Finally, the suspension was centrifuged at 15,000 × *g* for 15 min at 4°C to remove unbroken cell material, and analyses of enzymatic activities of SAB were made on supernatants. As indicated by Noziere and Michalet-Doreau (1994), the enzymes of feed origin were considered as negligible because they represent less than 5% of total nitrogen of enzymes in the supernatants.

Filtrates 1 and 2 were combined and centrifuged at 15,000 × *g* for 15 min at 4°C. The pellets that contained the LAB were subjected to the same treatments as the SAB fraction. They were suspended into a MESDTT solution, then frozen and thawed, sonicated, and finally centrifuged. All steps from filtrates until enzyme preparation were performed under anaerobic conditions. Analyses of enzymatic activities of LAB were made on supernatants.

Determination of Enzyme Activities

Polysaccharidase activities involved in the digestion of plant cell walls (CMCase, xylanase) and starch (amylase) were determined according to the methods of Martin and Michalet-Doreau (1995). The amounts of reducing sugars liberated from purified polymers (Birchwood-xylan, Sigma X-0502; carboxymethylcellulose, Sigma C-5678; potato starch, Sigma S-2002; Sigma-Aldrich Chemie, Lyon, France) after incubation with cellular extracts, were used to calculate enzyme activities. The volumes of LAB and SAB extracts, the amounts of substrates, and the incubation times are given in Table 2. The reaction was stopped by heating at 100°C for 5 min. Reducing sugars were quantified spectrophotometrically at 410 nm (Lever, 1977).

Activities of glycoside hydrolases (α-L-arabinosidase, β-D-cellobiosidase, β-D-glucosidase, β-D-xylosidase) were estimated through the release of para-nitrophenol from para-nitrophenol derivated substrates (para-nitrophenol-α-L-arabinofuranoside, Sigma N-3641; para-nitrophenol-β-D-cellobioside, Sigma N-5759; para-nitrophenol-β-D-glucopyranoside, Sigma N-7006; para-nitrophenol-β-D-xylopyranoside, Sigma N-1232; Williams et al., 1984). Incubations were carried out as described by Williams et al. (1984; 45 min at 39°C under agitation in a water bath). The reaction was stopped by increasing pH with a 0.4 *M* solution of glycine/soda (1/1 vol/vol). The released paranitrophenol was dosed by spectrophotometry at 420 nm. Blanks were made for enzymes and substrates to assess the real activities of extracted enzymes.

Protein content of enzyme preparations was determined according to the method of Pierce and Suelter (1977) using BSA as the standard. Enzyme activities were expressed as specific activities [i.e., as the quan-

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Table 2. Protocol used for the determination of enzymatic polysaccharidase activities in equine intestinal content

Enzyme	Volume of solution, mL		Incubation time, min	Standard solution, range of sugar concentrations (number of points on the standard curve)
	Bacterial extract	Substrate		
Amylase	0.2	2.0	80	0 to 30 µg of glucose/mL (6)
CMCase	0.5	2.0	70	0 to 30 µg of glucose/mL (6)
Xylanase	0.2	2.0	60	0 to 30 µg of xylose/mL (6)

tity of reducing sugar (for polysaccharidases) or paranitrophenol (for glycosidases) released per mg of protein per unit of time].

Concentrations of Bacterial and Yeast Populations

Total Viable Anaerobic Bacteria. Bacteria were cultivated for 96 h at 38°C in roll tubes prepared with a modified complete agar medium (Leedle and Hespell, 1980; Julliand et al., 1999). Four replicated tubes were used for each 10^{-6}, 10^{-7}, and 10^{-8} dilution of intestinal digesta.

Cellulolytic Bacteria. Cellulolytic bacteria were cultivated with a broth medium (Halliwell and Bryant, 1963; Julliand et al., 1999) for 15 d at 38°C. The concentrations were calculated by the most probable number method on 4 tubes inoculated with 10^{-5}, 10^{-6}, and 10^{-7} dilutions of each intestinal digesta.

Lactic Acid-Utilizing Bacteria. Lactic acid utilizing bacteria were selectively cultivated for 96 h at 38°C on the medium used by Mackie and Health (1979). Four replicated roll tubes were prepared for each 10^{-5}, 10^{-6}, and 10^{-7} dilution of intestinal digesta.

Streptococci spp. An overlay method with bile esculin azide agar medium (BK158HA, Biokar diagnostics, Beauvais, France) was used to enumerate *Streptococcus*. Three replicated Petri plates prepared with 10^{-5}, 10^{-6}, and 10^{-7} dilutions of intestinal digesta were counted after 48 h of incubation at 38°C.

Lactobacilli spp. The overlay method with a Rogosa agar medium (BK158HA, Biokar diagnostics, Beauvais, France) was applied to 3 replicated Petri plates prepared from 10^{-5}, 10^{-6}, and 10^{-7} dilutions of intestinal digesta and incubated for 48 h at 38°C.

Saccharomyces cerevisiae. Thirty milliliters of digesta were diluted with 270 mL of sterile water and homogenized for 3 min in a stomacher (Stomacher 400 Lab Blender, Seward Medical, London, UK). Yeasts were numerated in a Sabouraud medium (BK025HA, Biokar diagnostics, Beauvais, France) with an ethanol solution of chloramphenicol (0.17%). Three replicated Petri plates were counted for each dilution from 10^{-3} to 10^{-7} of digestive content after 48 h of incubation at 35°C. The genetic profile of live yeasts were systematically checked according to the PCR method with specific δ1 primers (Ness et al., 1993) and was compared with the profile of the CBS 493.94 strain of the product Yea-Sacc[1026].

Calculations and Statistical Analyses

Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC) for each digestive compartment. The model used, $Y_{ij} = \mu + A_i + P_j + (D_k + SC_l) + D_k \times SC_l + E_{ijkl}$, included the animal (A), period (P), diet (D), yeast (SC) as single effects, the D × SC interaction, and E as residual error.

Only diet and yeast effects, as well as their interaction, will be discussed in the present paper. Logarithmic transformations were performed on microbial counts for statistical analyses. Means were compared by Fisher's LSD when a significant overall treatment *F*-value was observed. Two levels of significance ($P < 0.10$ and $P < 0.05$) were tested.

RESULTS

Concentration of Yeasts

The strain CBS 493.94 was not detected in the hindgut of nonsupplemented horses. Yeasts with the same genetic profile (*S. cerevisiae* strain CBS 493.94) as the strain added in the diet were recovered alive in the cecum and colon of supplemented horses. Their concentrations were greater ($P < 0.001$) in the cecum (4.4×10^6 cfu/mL) than in the right-ventral colon (5.6×10^4 cfu/mL) and were not affected ($P > 0.10$) by diet (HS or HF; Table 3).

Concentration of Total Anaerobic Bacteria and Cellulolytic Bacteria

The concentrations of total anaerobic bacteria or cellulolytic bacteria in the cecum and the colon were not affected ($P > 0.01$) by SC addition or by dietary conditions (Table 4).

Concentration of Bacteria Involved in Lactic Acid Metabolism

There was no effect of diet on the concentration of lactic acid-utilizing bacteria present in the cecal and the colonic content, whereas lactobacilli were more numerous in the cecum ($P = 0.012$) and colon ($P = 0.086$) of horses fed the HS diet. *Saccharomyces cerevisiae* CBS 493.94 addition increased concentrations of lactic acid-utilizing bacteria and lactobacilli ($P = 0.067$ and 0.099, respectively) in the cecum but had no effect ($P > 0.10$)

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Table 3. Concentrations (log cfu/mL) of live *Saccharomyces cerevisiae* CBS 493.94 strain in the cecum and colon of fistulated horses (n = 4/treatment) fed a high-fiber (HF) or a high-starch diet (HS) with (+SC) or without (+0) live yeast supplementation

Digestive compartment	Diet				SEM	P-value		
	HF + 0	HF + SC	HS + 0	HS + SC		Diet	Yeast	Diet × yeast
Cecum	ND[a]	6.4[b]	ND[a]	6.8[b]	0.7	>0.10	<0.001	>0.10
Colon	ND[a]	4.9[b]	ND[a]	4.5[b]	0.5	>0.10	<0.001	>0.10

[a,b]Means with different superscripts on the same line are different ($P < 0.05$); ND: cells of *S. cerevisiae* (strain CBS 493.94) were not detected.

on bacteria in the colon. Concentrations of streptococci were not affected ($P > 0.10$) by diet or SC addition for cecal and colonic digestive contents (Table 4).

Polysaccharidase Activities of SAB

No dietary or yeast effects were observed on amylase activity of SAB isolated from the colon or cecum of horses. The CMCase activities of SAB were improved with SC supplementation in the cecum ($P = 0.019$) and colon ($P = 0.037$) but were not affected ($P > 0.10$) by diet in either digestive compartment.

Xylanase activities of SAB isolated from the colon were greater ($P = 0.046$) with HF diet than HS diet, but did not change with SC supplementation ($P > 0.10$). Xylanase activities of cecal SAB were not affected ($P > 0.10$) by diet or yeast supplementation (Table 5).

Glucoside Hydrolase Activities of SAB

Values of β-D-cellobiosidase activities of SAB were greater in both digestive compartments ($P < 0.001$) in the colon ($P = 0.072$ in the cecum) when HF diets were compared with HS. A significant and positive effect of SC on β-D-cellobiosidase activities was observed in the cecum ($P = 0.002$) but not in the colon ($P = 0.954$).

The β-D-glucosidase activities of SAB were greater in horses fed the HF diet than the HS diet ($P = 0.001$ in the cecum; $P < 0.001$ in the colon). A significant and positive effect of SC addition ($P = 0.041$) was observed on β-D-glucosidase activities of SAB isolated from the cecum but not in those isolated from the colon ($P > 0.10$).

The α-L-arabinosidase activities of SAB from the colon and cecum had greater values ($P < 0.001$) in HF than HS diet. The addition of SC improved arabinosidase activities of SAB in the colons of horses fed the HF diet ($P = 0.056$) but had no effect on arabinosidase activities of SAB extracted from the cecum regardless of diet.

The β-D-xylosidase activities of SAB had greater values ($P = 0.015$) in the colon when animals were fed HF diet than HS. *Saccharomyces cerevisiae* CBS 493.94 stimulated bacterial β-D-xylosidase activities in the colon and cecum ($P < 0.05$) of horses fed the HS diet, which reached the values observed with HF diet (Table 5).

Polysaccharidase Activities of LAB

Amylase activities of LAB isolated from the cecum and colon were not affected ($P > 0.10$) by diet. *Sac-*

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Table 4. Cecal and colonic bacterial concentrations in fistulated horses (n = 4/treatment) fed a high-fiber (HF) or a high-starch diet (HS) with (+SC) or without (+0) live yeast supplementation

Item	Digestive compartment	Diet				SEM	P-value		
		HF + 0	HF + SC	HS + 0	HS + SC		Diet	Yeast	Diet × yeast
Total anaerobic bacteria, log_{10} cfu/mL	Cecum	7.4	7.7	7.9	8.5	0.34	0.105	0.210	0.693
	Colon	7.6	7.8	8.2	7.9	0.20	0.139	0.722	0.206
Cellulolytic bacteria, log_{10} MPN[1]/mL	Cecum	4.3	5.3	4.7	4.2	0.40	0.410	0.568	0.137
	Colon	4.7	5.1	5.4	4.7	0.44	0.803	0.728	0.271
Lactic-acid utilizing bacteria, log_{10} cfu/mL	Cecum	6.5	7.0	6.9	7.5	0.25	0.118	0.067	0.792
	Colon	6.5	6.5	7.2	6.7	0.28	0.170	0.465	0.391
Lactobacillus spp., log_{10} cfu/mL	Cecum	6.5[b]	6.6[b]	6.8[ab,B]	7.2[a,A]	0.13	0.012	0.099	0.337
	Colon	6.1[B]	6.2[B]	6.7[A]	6.8[A]	0.29	0.086	0.884	0.983
Streptococcus spp., log_{10} cfu/mL	Cecum	6.9	6.7	6.5	6.9	0.18	0.633	0.711	0.123
	Colon	6.4	6.5	6.8	6.8	0.21	0.166	0.870	0.682

[a,b]Means with different superscripts within a row are different ($P < 0.05$).
[A,B]Means with different superscripts within a row are different ($P < 0.10$).
[1]MPN = most probable number.

Table 5. Enzymatic activities of solid-adherent bacteria (SAB) in the colon and cecum of fistulated horses (n = 4/treatment) fed a high-fiber (HF) or a high-starch diet (HS) with (+SC) or without (+0) live yeast supplementation

		Diet					*P*-value		
Item	Digestive compartment	HF + 0	HF + SC	HS + 0	HS + SC	SEM	Diet	Yeast	Diet × yeast
α-Amylase[1]	Cecum	513.5^{A}	381.1^{A}	329.7^{B}	529.0^{A}	73.2	0.808	0.652	0.033
	Colon	410.7	366.0	398.8	517.3	112.0	0.540	0.745	0.474
CMCase[1]	Cecum	136.6^{b}	411.7^{a}	118.0^{b}	192.7^{b}	69.4	0.101	0.019	0.162
	Colon	232.9^{AB}	357.3^{AB}	193.2^{B}	422.4^{A}	80.0	0.874	0.037	0.518
Xylanase[1]	Cecum	371.6	484.7	458.7	539.1	66.4	0.298	0.159	0.807
	Colon	813.6^{ab}	$1,015.0^{a}$	547.2^{b}	670.5^{ab}	144.6	0.046	0.273	0.789
β-D-Cellobiosidase[2]	Cecum	715.9^{b}	$1,381.3^{a}$	$486.1^{b,B}$	$974.1^{ab,A}$	170.4	0.072	0.002	0.598
	Colon	$1,112.7^{a}$	$1,018.5^{a}$	546.8^{b}	627.8^{b}	112.6	<0.001	0.954	0.444
β-D-Glucosidase[2]	Cecum	$3,432.2^{ab}$	$4,319.0^{a}$	$1,817.9^{c}$	$2,784.5^{bc}$	428.3	0.001	0.041	0.926
	Colon	$3,733.8^{a}$	$3,856.2^{a}$	$1,794.2^{b}$	$2,115.5^{b}$	278.3	<0.001	0.433	0.724
α-L-Arabinosidase[2]	Cecum	$2,360.1^{a}$	$2,326.8^{a}$	$1,593.0^{b}$	$1,845.6^{b}$	119.5	<0.001	0.368	0.244
	Colon	$2,173.0^{b}$	$2,766.9^{a}$	$1,674.6^{c}$	$1,709.9^{c}$	156.4	<0.001	0.056	0.088
β-D-Xylosidase[2]	Cecum	$1,829.5^{a}$	$1,838.7^{a}$	$1,141.2^{b}$	$2,104.7^{a}$	223.1	0.354	0.040	0.043
	Colon	$1,840.9^{a}$	$1,978.6^{a}$	$1,080.2^{b}$	$1,803.0^{a}$	164.6	0.015	0.025	0.115

[a–c]Means with different superscripts within a row are different ($P < 0.05$).
[A,B]Means with different superscripts within a row are different ($P < 0.10$).
[1]Polysaccharidase activities are expressed in nmoL of sugar·min^{-1}·mg of protein^{-1}.
[2]Glucoside hydrolase activities are expressed in μmol of paranitrophenol·min^{-1}·mg of protein^{-1}.

charomyces cerevisiae CBS 493.94 addition reduced ($P = 0.046$) amylase activity in the colon of animals fed the HS diet.

The CMCase activities of LAB isolated from the cecum were not affected by diet ($P = 0.940$). Those isolated from the colon had greater values when horses were fed the HF diet rather than the HS diet ($P = 0.049$). *Saccharomyces cerevisiae* CBS 493.94 addition had no significant effect on CMCase activities in the colon ($P = 0.554$) or in the cecum ($P = 0.551$) irrespective of diet.

Xylanase activities of LAB were greater with the fiber-rich diet than in the diet rich in starch ($P = 0.021$ in the cecum; $P < 0.001$ in the colon). The addition of SC increased ($P < 0.05$) xylanase activities of LAB in the colon of horses fed HF diet and in the cecum of those fed HS diet (Table 6).

Glucoside Hydrolase Activities of LAB

No effects of diet or treatment on β-D-cellobiosidase activities of LAB isolated from the colon and cecum (*P*

Table 6. Enzymatic activities of liquid-associated bacteria (LAB) in the colon and cecum of fistulated horses (n = 4/treatment) fed a high-fiber (HF) or a high-starch diet (HS) with (+SC) or without (+0) live yeast supplementation

		Diet					*P*-value		
Item	Digestive compartment	HF + 0	HF + SC	HS + 0	HS + SC	SEM	Diet	Yeast	Diet × yeast
α-Amylase[1]	Cecum	196.5	63.7	88.9	116.9	49.1	0.585	0.297	0.115
	Colon	158.6^{A}	97.5^{A}	172.8^{A}	80.2^{B}	36.4	0.965	0.046	0.670
CMCase[1]	Cecum	96.5	138.2	116.0	113.7	32.7	0.940	0.551	0.507
	Colon	236.2^{a}	159.1^{ab}	119.3^{b}	164.3^{ab}	26.9	0.049	0.554	0.033
Xylanase[1]	Cecum	312.6^{a}	352.8^{a}	194.3^{b}	317.4^{a}	31.1	0.021	0.015	0.196
	Colon	329.0^{a}	411.4^{b}	242.7^{c}	241.6^{c}	25.8	<0.001	0.128	0.119
β-D-Cellobiosidase[2]	Cecum	$1,281.6^{a}$	706.8^{bc}	632.4^{c}	$1,108.8^{ab}$	149.5	0.411	0.741	0.001
	Colon	766.3	703.9	646.5	783.8	140.5	0.888	0.792	0.485
β-D-Glucosidase[2]	Cecum	2,506.2	1,850.6	1,828.7	2,152.9	268.3	0.491	0.543	0.081
	Colon	1,862.4	2,655.0	1,739.3	1,953.5	266.7	0.136	0.072	0.290
α-L-Arabinosidase[2]	Cecum	1,575.5	1,704.9	1,157.8	1,629.7	198.7	0.228	0.144	0.398
	Colon	1,673.9	1,805.6	1,514.3	1,559.6	167.2	0.238	0.602	0.798
β-D-Xylosidase[2]	Cecum	832.5^{B}	$1,169.8^{A}$	893.3^{AB}	$1,195.6^{A}$	136.0	0.753	0.028	0.899
	Colon	1,115.1	1,159.4	1,264.2	1,419.4	154.7	0.235	0.557	0.743

[a–c]Means with different superscripts within a row are different ($P < 0.05$).
[A,B]Means with different superscripts within a row are different ($P < 0.10$).
[1]Polysaccharidase activities are expressed in nmoL of sugar·min^{-1}·mg of protein^{-1}.
[2]Glucoside hydrolase activities are expressed in μmol of paranitrophenol·min^{-1}·mg of protein^{-1}.

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> 0.05) were observed. The β-D-glucosidase activities of LAB in the colon, which were not different between the 2 diets ($P = 0.136$), were improved by SC supplementation ($P = 0.072$). In the cecum, β-D-glucosidase activities of LAB were not affected by diet or yeast addition ($P > 0.10$).

The α-L-arabinosidase activities of LAB were not affected ($P > 0.10$) by diet or SC addition irrespective of the digestive compartment. The β-D-xylosidase activities of LAB increased with SC supplementation ($P = 0.028$) in the cecum. Those obtained from the colon did not change ($P > 0.10$) regardless of diet or yeast supplementation (Table 6).

DISCUSSION

The results of this study confirm that a large part of ingested live SC can survive during transit through the digestive tract of horses reaching the cecum and colon (Newman and Spring, 1993; Moore et al., 1994). Gobert et al. (2006) reported that live SC fed at similar concentrations to those of the present study appeared in the colonic content of horses 3 h after their ingestion. In the present study, live SC concentration found in the cecum (10^6 cfu/mL) is equivalent to the concentration usually found in the rumen (Fiems et al., 1993; Durand-Chaucheyras et al., 1998) or in the ileum (Newbold et al., 1990) of ruminants fed approximately the same oral dose. In agreement with previous data (Gobert et al., 2006), the concentration of live SC in the colon was less, and below the threshold of efficacy (10^5 cfu/mL) already set for the rumen (Brossard et al., 2006). This may explain why changes due to live SC supplementation were observed more frequently within cecal contents than colonic contents.

For the first time, the technique of separation of LAB and SAB described for rumen bacteria (Martin et al., 1993) has been applied to cecal and colonic digestive contents of horses, with each type of bacteria being analyzed in terms of bacterial concentration and enzymatic activities. Like the rumen of cows and sheep (Martin et al., 1993; Eugène et al., 2004) the activity of polysaccharidases involved in cell-wall degradation originates mainly from bacteria bound to particles (SAB fraction) isolated from colon or cecum content. Amylase activities were also greater in SAB than in LAB.

Similarly, glucoside hydrolases showed a lesser specific activity in the liquid fraction when compared with the solid fraction, but the differences between the 2 bacterial fractions varied according to the enzyme being considered. For example, β-D-cellobiosidase activities of LAB and SAB were comparable, whereas β-D-glucosidase, β-D-xylosidase, and α-L-arabinosidase were much greater in SAB than LAB. The values found here for β-D-xylosidase in the liquid fraction of cecal contents are similar to those previously published for horses (Bonhomme-Florentin, 1988).

Comparison of polysaccharidase activities in the cecum and colon of horses were close to and agreed with the concentrations of cellulolytic bacteria, which were also very similar in both digestive compartments. This result agrees with previous numeration of cellulolytic bacteria determined simultaneously in the cecal and the colonic contents, which showed that the microbial densities were not significantly different between the 2 digestive compartments (Moore et al., 1994; Julliand et al., 2001; Medina et al., 2002; de Fombelle et al., 2003). However, the proportion of cultivable cellulolytics among total anaerobes appeared to be greater in the cecum than in the lower parts of the hindgut and confirmed that this blind pocket was probably the most propitious digestive compartment for cellulolysis (de Fombelle et al., 2003).

The profiles and activities of the intestinal microflora in horses have been reported to be modified by the NDF:starch ratio of the diet (Medina et al., 2002). Activity of most polysaccharide depolymerases and glycoside hydrolases relating to fiber degradation were greater in HF than in HS diet, whereas the number of cellulolytic bacteria remained unchanged regardless of diet. This result with enzymes implies that more fiber was digested in the HF diet. In a previous study conducted with the same experimental design and similar diets, the digestibility of ADF was greater in HF diet than HS diet, which corroborates the present data (Jouany et al., 2008). These data also agree with the greater acetate concentration reported by Medina et al., (2002) who observed that the concentration of cellulolytic bacteria remained unchanged between the 2 diets. This suggests that the diet affected the activity of cellulolytic (and hemicellulolytic) bacteria without affecting their total concentration when measured with conventional cultural techniques. A similar study conducted in the rumen of cows showed that the addition of a large amount of starch to a hay-based diet similarly decreased the fibrolytic activity of the 3 main cellulolytic bacteria (*Ruminococcus albus*, *R. flavefaciens*, and *Fibrobacter succinogenes*) without modifying their number (Martin et al., 2001).

Amylase activities of LAB and SAB isolated from the hindgut of horses were not affected by diet although the amount of dietary starch was significantly different between HF and HS diets. Such a result is likely explained by the digestion of starch in the small intestine, which was probably complete for the HF diet (intake = 1.0 g of starch·kg^{-1} of BW·meal^{-1}) but allowed a small amount of starch to reach the hindgut for the HS diet (intake = 2.7 g of starch·kg^{-1} of BW·meal^{-1}). The increase in starch-digesting bacteria concentration such as lactobacilli confirms this hypothesis. However, the supply of starch to the large intestine of HS-fed horses was not sufficient enough to significantly modify bacterial amylase activities.

Like the rumen, starch is converted in the hindgut of horses by amylolytic bacteria into lactic acid (Garner et al., 1978; Rowe et al., 1994; Medina et al., 2002), which is then metabolized into propionate by lactic acid-utilizing bacteria. Using similar diets, Julliand et al.

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(2001) and Medina et al. (2002) noted that lactic acid and propionate did not accumulate in the hindgut of horses, which confirms that the amount of starch escaping the digestion in the small intestine and reaching the hindgut was probably small, even for the HS diet.

Saccharomyces cerevisiae CBS 493.94 supplementation had no effect on the concentrations of all the tested bacterial communities in the colon of horses. In contrast, the populations of lactobacilli and lactic acid-utilizing bacteria in the cecum increased when SC was present. This specific result on bacteria involved in the metabolism of lactic acid is likely explained by the greater concentrations of live yeasts found in the cecum than in the colon of animals (4.4×10^6 cfu/mL vs. 5.6×10^4 cfu/mL) as previously mentioned. The same reason could explain why SC improved more CMCases, cellobiosidases, β-D-glucosidases, and xylosidases of SAB in the cecum than in the colon.

These data underline that large similarities exist between the rumen and the hindgut of horses with regard of the effects of live yeasts on the digestive microbial ecosystem and its activities (Wallace and Newbold, 1992; Chaucheyras et al., 1996; Doreau and Jouany, 1998; Chaucheyras-Durand and Fonty, 2006). Therefore, live yeasts can be used in horses to balance and stabilize the digestive microbial ecosystem as they are in ruminants. Their main positive effect is due to increases in the enzymatic activities of bacteria involved in the digestion of cellulosic material rather than as a direct effect on bacterial biomass. This effect is likely magnified in the case of a high supply of fermentative carbohydrates (sugars or starch) in the digestive tract. Although prececal digestion, which occurs in horses, tends to limit the risk of acidosis in the hindgut, an overload of grain in the diet can affect the colonic ecosystem and generate microbial and digestive disorders leading to decreased digestion of cell wall carbohydrates (de Fombelle et al., 2001; Julliand et al., 2001), which can be corrected by addition of live yeasts to the diet of animals.

The increase in the major enzymes involved in plant cell wall digestion (e.g., CMCases, β-D-cellobiosidases, β-D-xylosidases, β-D-glucosidases, and α-L-arabinosidases in the cecum or colon of horses after SC addition to HS diet) partially explain why the fiber fraction is better digested by SC-treated horses fed with a high-cereal diet (see Jouany et al., 2008). The same mechanism as described in the rumen by Callaway and Martin (1997) who indicated that yeasts stimulate growth of bacteria that utilize lactate and digest cellulose and can be applied to horses fed an HS diet [because SC tended ($P = 0.067$) to increase the population of lactic-acid utilizing bacteria]. In addition, our experiment showed significant and positive effects of SC supplementation on CMCases, α-L-arabinosidases, β-D-cellobiosidases in horses fed an HF diet. Although no explanation could be given for such evolution, it can be concluded that yeasts have positive effects on the utilization of fiber in horses regardless of diet.

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