

Effect of a preparation of *Saccharomyces cerevisiae* on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet¹

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ABSTRACT: Eight horses were allotted into pairs consisting of one cecum- and right ventral colon-fistulated animal and one cecum-fistulated animal. They were fed daily at the same level of intake either a high-fiber (HF) or a high-starch (HS) diet without or with 10 g of a *Saccharomyces cerevisiae* preparation, in a 4 × 4 Latin square design. The HS diet provided a starch overload (i.e., 3.4 g starch·kg⁻¹ BW·meal⁻¹) while maintaining a high amount of fiber intake (i.e., dietary NDF/starch ratio was 1.0). A 21-d period of adaptation to the treatments occurred before cecal and colonic contents were withdrawn 4 h after the morning meal to count total anaerobic, cellulolytic, and lactic acid-utilizing bacteria, lactobacilli, and streptococci. Lactic acid, volatile fatty acids, ammonia concentrations, and pH were measured on cecal and colonic fluid samples collected hourly during the first 12-h postfeeding. When the HS diet was fed, the concentration of total anaerobic and lactic acid-utilizing bacteria increased ($P < 0.001$), whereas that of cellulolytic bacteria decreased ($P < 0.05$) in the cecum. The concentration of lactobacilli and

streptococci increased ($P < 0.001$) in the cecal and colonic contents. These alterations of the microbial profiles were associated with decreases ($P < 0.001$) of pH, (acetate + butyrate)/propionate ratio and with an increase ($P < 0.001$) of lactic acid concentration. Supplementing the *S. cerevisiae* preparation increased ($P < 0.01$) the concentration of viable yeast cells, averaging 4.3×10^6 and 4.5×10^4 cfu/mL in the cecal and colonic contents, respectively. Yeast supplementation had almost no effect on microbial counts in the cecum and colon. The supplementation of *S. cerevisiae* appeared to modify ($P < 0.05$) pH, concentrations of lactic acid and ammonia, molar percentages of acetate and butyrate with the HS diet and [(acetate + butyrate)/propionate] ratio when the HF diet was fed. The effects of the *S. cerevisiae* preparation were greater in the cecum than in the colon, which coincided with the abundance of yeast cells. When the digestion of starch in the small intestine was saturated, the effect of the addition of a *S. cerevisiae* preparation appeared to limit the extent of undesirable changes in the intestinal ecosystem of the horse.

Key Words: Cellulolytic Microorganisms, Horses, Large Intestine Fermentation, *Saccharomyces cerevisiae*, Starch Digestion

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Introduction

The energy requirements of the athletic horse are usually satisfied by substituting one-third to two-thirds of the fibrous feeds (e.g., forages and pastures) with starchy feeds, mainly cereal grains. Limiting access to forage and increasing the intake of rapidly

fermentable carbohydrate make the horse more susceptible to colic or laminitis than when managed under pasture conditions (Kronfeld and Harris, 1997). Feeding 3.5 g of starch per kilogram BW exceeds the capacity of the small intestine to digest starch and allows nondegraded starch to reach the cecum (Potter et al., 1992), where it disturbs the lower gut microflora and their activity. A strategy to limit the negative consequences of cereal-based diets on the equine intestinal ecosystem is to increase digestion of starch in the small intestine (Kienzle, 1994). Few research trials have evaluated the consequences of a large starch intake combined with a high amount of fiber content in the diet (i.e., 3.4 g NDF·kg⁻¹ BW·meal⁻¹). Another strategy is based on the addition of direct-fed microbes

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to manipulate the microbial activities of the intestinal ecosystem. In ruminants, this practice has been shown to improve the environment of the rumen (Nagaraja et al., 1997). However, the ability of direct-fed microbes to modify the equine gastrointestinal tract environment and its population has not been extensively studied.

This paper presents a study designed to investigate two dietary formulations for limiting the negative impact of a starch overload (i.e., $3.4 \text{ g}\cdot\text{kg}^{-1}\text{BW}\cdot\text{meal}^{-1}$). The study measured the combined effects of two NDF/starch ratio in the diet and the supplementation with a live yeast culture preparation (YEA SACC¹⁰²⁶, Alltech, Dunboyne, Ireland) on the equine lower gut microflora and their metabolic activities.

Materials and Methods

Experimental Design and Treatments

Eight resting, crossbred male mature horses (mean age 12 yr; mean BW $305 \pm 18.4 \text{ kg}$) were allotted into pairs consisting of one cecum-and right ventral colon-fistulated animal and one cecum-fistulated animal (polyvinyl chloride cannula, i.d. 30 mm). Animals were surgically prepared by a certified large animal veterinarian, using the technique described in Drogoul et al. (2000a). The project was conducted under license from the Department of Health and Animal Care of the French Veterinary Authority. Pairs were randomly assigned to a replicated 4×4 Latin square experiment to evaluate two different dietary NDF/starch ratios fed daily without or with 10 g of a *Saccharomyces cerevisiae* (SC) preparation (YEA SACC¹⁰²⁶, Alltech, Dunboyne, Ireland) per animal.

The feedstuffs as well as their physical form were chosen to mimic French feeding practices conducted in horse barns. Each diet was formulated using either high fiber (HF) or high starch (HS) concentrates plus wheat straw in long form (Table 1). The ingredients of both HF and HS concentrates were ground through a 1.5 mm sieve and compressed into 3 mm diameter pellets. The HF diet contained 45.9% dehydrated alfalfa, whereas the HS diet contained 44.8% barley (Table 1). Both the HF and HS diets were fed at the same level of intake (i.e., $21.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) to avoid any effect of the size of meal on the rate of passage of digesta (Drogoul et al., 2001; Pearson et al., 2001) and to provide a similar amount of crude protein (Table 1). The HS diet was formulated to allow a starch intake of $3.4 \text{ g}\cdot\text{kg}^{-1}\text{BW}\cdot\text{meal}^{-1}$, in order to reach the upper limit of prececal starch digestion (Potter et al., 1992). The dietary NDF to starch ratio varied by a factor of 4 (3.5 vs 1.0 respectively for HF and HS diets). Therefore, the HF diet provided 120% of French energy requirement of horses (Martin-Rosset et al., 1994), whereas the HS diet provided 160%.

The SC preparation was a lyophilized flocculent strain of *Saccharomyces cerevisiae* plus growth me-

Table 1. Ingredient and mean chemical composition of diets

| Item | HF diet ^a | HS diet |
|---|----------------------|---------|
| Ingredient, % DM | | |
| HF concentrate ^b | 83.7 | 0 |
| HS concentrate ^c | 0 | 83.7 |
| Wheat straw ^d | 16.3 | 16.3 |
| Chemical composition, DM basis | | |
| Dry matter (DM), % | 88.7 | 88.0 |
| Ash, % | 11.9 | 9.6 |
| Neutral detergent fiber (NDF), % | 41.0 | 30.7 |
| Acid detergent fiber (ADF), % | 25.8 | 16.1 |
| Starch, % | 11.6 | 30.0 |
| Crude protein (CP), % | 12.6 | 12.8 |
| Digestible energy ^e (DE), Mcal/kg DM | 2.6 | 3.2 |
| Theoretic daily consumption | | |
| Dry matter intake, g/100 kg BW | 2,150 | 2,150 |
| NDF, g/100 kg BW | 882 | 660 |
| Starch, g/100 kg BW | 252 | 670 |
| Digestible energy ^e , Mcal/100 kg BW | 5.6 | 6.9 |

^aHF = High fiber, HS = High starch.

^bComposition HF concentrate (Lambey SA., Torpes, France): 54.8% dehydrated alfalfa, 25.5% wheat bran, 13.1% barley, 2.6% CaCO₃, 2% sugar cane molasse, 1% NaCl, and 1% horse premix (Central Soya, Trappes, France); (14.5% CP, 21.9% ADF, 34.1% NDF and 2.83 Mcal/kg^e).

^cComposition HS concentrate (Lambey SA., Torpes, France): 53.5% barley, 22.5% wheat bran, 10.0% dehydrated alfalfa, 7.6% soybean meal, 3.7% CaCO₃, 1% sugar cane molasse, 1% NaCl, and 1% horse premix (Central Soya, Trappes, France); (14.7% CP, 10.3% ADF, 21.8% NDF, and 3.50 Mcal/kg^e).

^dWheat straw (2.9% CP, 45.8% ADF, 76.4% NDF, and 1.63 Mcal/kg^e).

^eDE was calculated from equations reported by Fonnesebeck (1981).

dium. This specific strain of *S. cerevisiae* has been genetically defined by using PCR test associated with the $\delta 1$ (5'CAAATTCACCTATATCTCA3') and $\delta 2$ (5'GTGGATTTTATTCCAACA3') primers (Ness et al., 1993). It has been deposited and registered in an official culture collection under the following reference: CBS 493.94. The SC preparation has been registered as an additive in European Union: Commission Regulation (EC) No. 1436/98 (OJ L-191, 7.7.1998, p 15) under the number 5. The enumeration of the preparation used for the trial corresponded to 4.5×10^9 cfu/g.

Feeding and Management

The horses were maintained indoor in individual free-stalls bedded with flax shavings (ECOLit, Croissanville, France). They had access to a paddock 10 h/wk. Water and a block of trace-mineralized salt were offered free-choice. A double dose of Pyrantel (STRONGID, Laboratoire Pfizer, Orsay, France) followed a week later by a double dose of Ivermectin (EQVALAN, Laboratoire Merial, Lyon, France) were orally administered 15 d prior the beginning of the trial to control a wide range of gastrointestinal parasites (Herd, 1992; Hutchens et al., 1999).

The horses were weighed on two consecutive days before each diet adaptation period to adjust the feed

allowance to their metabolic body weight ($BW^{0.75}$). For both diets, animals were offered two equal meals (0800 and 1800) of 900 g DM of concentrate and 175 g DM of wheat straw per 100 kg BW each (Table 1). The wheat straw was fed 30 min after each pelleted meal. The SC preparation was top-dressed on the pellets (5 g per meal).

Experimental Period and Sampling

Each period consisted of 21 d of adaptation to the diet and 14 d of sampling. For microbial analyses, intestinal contents were collected anaerobically (Jullian et al., 1999), 4 h after the morning meal on d 22 from the cecum and colon and repeated on d 26 for the colon only. Samples held at 38°C in a water bath were diluted under O_2 -free CO_2 in an anaerobic mineral solution (Bryant and Burkey, 1953). For the first experimental period, live yeast cells were counted in both the cecum and colon from subsamples collected on d 22 from four horses fitted with cannula in the cecum and the right ventral colon. Because of the negligible amount of yeast cells in the intestinal content of horses not supplemented with the SC preparation (i.e., yeast count $< 10^3$ cfu/g), yeast counts in the subsequent three periods were determined only for subsamples from horses fed the SC supplemented diets. For biochemical measurements, a flexible tube (Cristallo extra, i.d. 10 mm; length 1.2 m) was inserted into each cannula of each animal, before the morning meal on d 33 of each period, as described in Drogoul et al. (2000b). Intestinal fluid contents were withdrawn hourly during the first 12 h after feeding. Three subsamples of the filtered intestinal contents were immediately frozen ($-20^\circ C$) for later analyses of concentrations of lactic acid (1 mL), VFA [(1 mL) added with a preservative (0.1 mL of a mixture of 5% H_3PO_4 + 1% $HgCl_2$)] and ammonia (3 mL).

Microbial Analyses

Concentrations of Total Viable Anaerobic Bacteria. Concentrations were determined with a modified complete agar medium (Leedle and Hespell, 1980; Jullian et al., 1999), after 96 h of incubation at 38°C, on four replicate roll tubes prepared with dilutions representing 10^{-6} , 10^{-7} , or 10^{-8} mL of intestinal contents.

Concentrations of Cellulolytic Bacteria. Concentrations were determined with a modified (Baruc et al., 1983) broth medium (Halliwell and Bryant, 1963; Jullian et al., 1999), after 15 d of incubation at 38°C, as the most probable number on four roll tubes inoculated with dilutions representing 10^{-5} , 10^{-6} , and 10^{-7} mL of intestinal contents.

Concentrations of Lactic Acid-Utilizing Bacteria. Concentrations were determined with a selective medium (Mackie and Health, 1979), after 96 h of incubation at 38°C, on four replicate roll tubes prepared with

dilutions representing 10^{-5} , 10^{-6} , or 10^{-7} mL of intestinal contents.

Concentrations of Streptococci spp. Concentrations were enumerated using an overlay method with a bile esculin azide agar medium (BK158HA, Biokar diagnostics, Beauvais, France). Three replicate Petri plates, prepared with dilutions representing 10^{-5} , 10^{-6} , or 10^{-7} mL of intestinal contents, were counted after 48 h of incubation at 38°C.

Concentrations of Lactobacilli spp. Concentrations were determined using an overlay method with a Rogosa agar medium (BK033, Biokar diagnostics, Beauvais, France). Three replicate Petri plates, prepared from dilutions representing 10^{-5} , 10^{-6} , or 10^{-7} mL of contents, were counted after 48 h of incubation at 38°C.

Concentrations of Saccharomyces cerevisiae. This enumeration was performed in another laboratory (Sigo, Vertou, France). Thirty millimeters of digestive samples were diluted with 270 mL sterile water and homogenized 3 min in a stomacher (Stomacher 400 LAB BLENDER, Seward Medical, London, United Kingdom). Live yeast cells were counted using a Sabouraud medium (BK025HA, Biokar diagnostic, Beauvais, France) modified with an ethanol (95%) solution plus 0.17% (vol/vol) of chloramphenicol (C0378, Sigma, Strasbourg, France). Three replicate Petri plates, prepared from each dilution representing 10^{-3} to 10^{-7} mL of contents, were counted after 48 h of incubation at 35°C.

Biochemical Analyses

Cecal and colonic pH were measured immediately after each collection, using an electronic pH meter (MP120, Mettler, Toledo, Spain). Ammonia concentration was measured with a NH_3 electrode (15 230 3000, Crison, Barcelona, Spain) connected to an Iono meter (GLP 22, Crison, Barcelona, Spain). Lactic acid and VFA concentrations were assayed, respectively, with an enzymatic reaction procedure (Lactate 735-10, Sigma, Strasbourg, France) quantified spectrophotometrically at 540 nm (MRX revelation, Dynatech Laboratories, Guyancourt, France) and by a gas-liquid chromatography (Gas chromatograph model 437 A, United Technologies Packard, Zurich, Switzerland) (Jouany, 1982). A ratio of acetate plus butyrate to propionate ($[(A + B)/P]$) was calculated according to Sauviant et al. (1994).

Statistical Analysis

The GLM procedures of SAS (SAS Inst. Inc., Cary, NC) were used to analyze the data. Logarithmic transformations were performed on microbial counts before statistical analysis. Differences in bacterial counts were tested with a model including horse as a randomized effect, diet and SC supplementation as fixed effects and the interaction between these two latter fac-

Table 2. Mean (\pm SD) cecal and colonic microbial concentrations^a in fistulated horses fed either HF or HS diets, without (+ 0) and with (+ SC) 10 g/d of *S. cerevisiae*

| Item and intestinal content | HF diet ^b | | HS diet ^b | | SEM | Effect ^d | | |
|--|--------------------------------|---------------------------------|--------------------------------|---------------------------------|-----|---------------------|----|------------------|
| | + 0 ^c | + SC ^c | + 0 | + SC | | Diet | SC | Diet \times SC |
| Total anaerobic bacteria, log ₁₀ cfu/mL | | | | | | | | |
| Cecum (n = 8) ^e | 7.9 (\pm 0.67) ^f | 7.8 (\pm 0.58) ^f | 8.6 (\pm 0.75) ^g | 8.6 (\pm 0.56) ^g | 0.7 | *** | ns | ns |
| Colon (n = 4) ^e | 8.8 (\pm 0.87) ^f | 8.2 (\pm 0.81) ^g | 8.7 (\pm 0.78) ^f | 8.6 (\pm 0.83) ^f | 0.8 | ns | ** | * |
| Cellulolytic Bacteria, log ₁₀ MPN/mL | | | | | | | | |
| Cecum (n = 8) | 6.1 (\pm 0.66) ^f | 5.3 (\pm 0.55) ^{fg} | 5.2 (\pm 0.76) ^g | 4.9 (\pm 0.81) ^g | 0.8 | * | ns | ns |
| Colon (n = 4) | 5.9 (\pm 0.93) | 5.4 (\pm 0.57) | 5.7 (\pm 0.45) | 5.7 (\pm 0.48) | 0.7 | ns | ns | ns |
| Lactic acid utilizing bacteria, log ₁₀ cfu/mL | | | | | | | | |
| Cecum (n = 8) | 7.0 (\pm 0.45) ^f | 7.1 (\pm 0.64) ^f | 7.7 (\pm 0.62) ^g | 7.8 (\pm 0.45) ^g | 0.6 | *** | ns | ns |
| Colon (n = 4) | 7.6 (\pm 0.94) | 7.5 (\pm 0.72) | 7.8 (\pm 0.87) | 7.6 (\pm 0.96) | 0.8 | ns | ns | ns |
| Lactobacilli, log ₁₀ cfu/mL | | | | | | | | |
| Cecum (n = 8) | 6.4 (\pm 0.55) ^f | 6.7 (\pm 0.82) ^g | 7.7 (\pm 0.60) ^h | 7.4 (\pm 0.56) ^{gh} | 0.6 | *** | ns | ** |
| Colon (n = 4) | 6.9 (\pm 1.04) ^f | 6.8 (\pm 0.94) ^f | 7.6 (\pm 0.79) ^g | 7.9 (\pm 0.77) ^g | 0.9 | *** | ns | ns |
| Streptococci, log ₁₀ cfu/mL | | | | | | | | |
| Cecum (n = 8) | 6.6 (\pm 1.15) ^f | 6.8 (\pm 1.14) ^f | 7.5 (\pm 0.75) ^g | 7.4 (\pm 0.76) ^g | 1.0 | *** | ns | ns |
| Colon (n = 4) | 6.9 (\pm 1.26) ^g | 6.3 (\pm 0.96) ^f | 7.3 (\pm 0.98) ^g | 7.9 (\pm 0.44) ^g | 1.0 | *** | ns | ns |

^aMicrobial concentrations were enumerated in cecal and colonic contents collected 4 h after the morning meal.

^bHF = High-fiber diet, HS = High-starch diet.

^c0 = No supplementation, SC = 10 g·d⁻¹ of *S. cerevisiae*.

^dLevel of significance: ns $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

^en = number of animals in each treatment.

^{fg}Least squares means within a row are different if superscript differ ($P < 0.05$).

tors. A repeated measures analysis of variance was performed to compare the profiles of fermentation parameters (VFA, lactic acid, NH₃ concentrations, and pH) using the repeated time option. The effect of post-feeding time was found to be significant for most of the parameters, therefore, biochemical data were analyzed for each hour individually in the bar charts. The standard deviation of the 13 sampling times after feeding was calculated for each horse and each fermentation parameter then tested with the model previously reported for bacterial data. Least squares means were calculated for all variables and separated using the pairwise *t*-tests (PDIF option of SAS). All statistical procedures were performed separately for the cecum and the colon with animal as experimental unit and the significance threshold for all tests was set at $P < 0.05$.

Results

Concentration of Microorganisms (Table 2)

No live yeast cell was detected at the lowest dilution (10⁻³ mL) prepared from the cecal and colonic contents of horses receiving both the HF + 0 and HS + 0 treatments. Concentrations of live yeast cells increased ($P < 0.01$) in both the cecum and colon of horses receiving the SC preparation and averaged 4.3×10^6 and 4.5×10^4 cfu/g, respectively (data not shown).

In the cecum, the SC supplementation had no overall effect on the bacterial counts (Table 2). However, a significant interaction between diet and SC ($P < 0.01$) was found for lactobacilli concentration: a positive effect ($P < 0.05$) of SC was registered on this spe-

cific group of bacteria when the HF diet was fed (Table 2). The HS diet increased ($P < 0.001$) the concentrations of total anaerobic, lactic acid-utilizing bacteria, lactobacilli, and streptococci in the cecum. On the contrary, the number of cellulolytic bacteria in the cecum decreased ($P < 0.05$) with the HS diet (Table 2). Although not statistically compared, the standard deviation was higher with HS + 0 than with HF + 0 in the cecum of the eight different horses, except for the streptococci. The standard deviations of total anaerobic, lactic acid-utilizing bacteria, and lactobacilli concentrations in the cecum had lower values, when SC was added in the HS diet.

In the colon, a significant ($P < 0.05$) interaction between diet and SC was observed for total anaerobes: the SC supplementation decreased ($P < 0.05$) their concentration with the HF diet. The concentrations of streptococci and lactobacilli were lower ($P < 0.05$) in the colon when HF diet was fed. A decrease ($P < 0.05$) of streptococci counts was found with the SC supplementation for the HF diet. The standard deviations of bacterial enumerations we observed, but did not analyze statistically, were different between HF and HS diets. The standard deviations of the bacterial counts in the colon appeared to decrease with the SC addition when the HF diet was fed (Table 2).

Biochemical Parameters

In the cecum, the fermentation parameters reported in Table 3 depended on the sampling time after feeding. Time interacted with the effect of the diet except for ammonia concentration and molar percentage of

Table 3. Cecal and colonic fermentation characteristics^a in fistulated horses fed either HF or HS diets, without (+ 0) and with (+ SC) 10 g/d of *S. cerevisiae*

| Item and intestinal content | HF diet ^b | | HS diet | | SEM | Effect ^d |
|-----------------------------|----------------------|--------------------|---------------------|--------------------|-------|---|
| | + 0 ^c | + SC ^c | + 0 | + SC | | |
| pH | | | | | | |
| Cecum (n = 8) ^e | 7.15 ^h | 7.12 ^h | 6.85 ^f | 7.01 ^g | 0.2 | T, T·D, t·sc |
| Colon (n = 4) ^e | 7.14 ^g | 7.06 ^g | 6.79 ^f | 6.88 ^f | 0.2 | d, T, t·d |
| Lactic acid, mg/L | | | | | | |
| Cecum (n = 8) | 167.9 ^f | 160.3 ^f | 407.7 ^g | 207.5 ^f | 160.7 | <u>d</u> , <u>sc</u> , d·sc, T, t·d, T·SC |
| Colon (n = 4) | 116.5 ^f | 137.9 ^f | 303.2 ^h | 235.7 ^g | 106.6 | <u>d</u> , <u>sc</u> , <u>d·sc</u> |
| Ammonia, mg/L | | | | | | |
| Cecum (n = 8) | 66.9 ^f | 67.2 ^f | 92.3 ^g | 65.1 ^f | 40.9 | d, <u>t</u> |
| Colon (n = 4) | 95.9 ^{fg} | 78.8 ^f | 102.1 ^{fg} | 110.9 ^g | 42.7 | T |
| Total VFA, mM | | | | | | |
| Cecum (n = 8) | 68.1 | 73.9 | 66.5 | 70.0 | 20.4 | T, <u>t·d</u> |
| Colon (n = 4) | 94.0 | 100.7 | 91.7 | 99.8 | 28.2 | T |
| Acetate (A), mM | | | | | | |
| Cecum (n = 8) | 50.9 ^{gh} | 55.4 ^h | 43.4 ^f | 47.1 ^{fg} | 14.2 | D, T, T·D, |
| Colon (n = 4) | 68.1 ^{fg} | 74.4 ^g | 60.2 ^f | 67.8 ^{fg} | 20.5 | T |
| A/total VFA, % | | | | | | |
| Cecum (n = 8) | 74.3 ^h | 74.9 ^h | 66.1 ^f | 68.0 ^g | 3.5 | D, sc, <u>t</u> , T·D |
| Colon (n = 4) | 72.1 ^h | 73.5 ^h | 65.8 ^f | 67.9 ^g | 3.0 | D, <u>sc</u> |
| Propionate (P), mM | | | | | | |
| Cecum (n = 8) | 12.8 ^f | 13.8 ^f | 17.7 ^g | 18.6 ^g | 6.3 | D, T, t·d |
| Colon (n = 4) | 17.6 ^f | 16.8 ^f | 20.1 ^{fg} | 22.9 ^g | 6.6 | <u>d</u> , T, t·d |
| P/total VFA, % | | | | | | |
| Cecum (n = 8) | 18.7 ^f | 18.3 ^f | 25.6 ^g | 25.3 ^g | 4.2 | D, T, <u>t·d</u> |
| Colon (n = 4) | 18.7 ^f | 16.9 ^f | 21.3 ^g | 22.6 ^g | 3.7 | D |
| Butyrate (B), mM | | | | | | |
| Cecum (n = 8) | 3.64 ^{fg} | 3.88 ^{fg} | 4.07 ^g | 3.39 ^f | 1.4 | d·sc, T, t·d |
| Colon (n = 4) | 6.27 ^f | 7.16 ^f | 9.28 ^g | 6.86 ^f | 3.3 | d·sc |
| B/total VFA, % | | | | | | |
| Cecum (n = 8) | 5.29 ^g | 5.44 ^g | 6.06 ^h | 4.73 ^f | 1.2 | SC, D·SC, T |
| Colon (n = 4) | 6.93 ^f | 7.11 ^f | 9.95 ^g | 7.08 ^f | 2.1 | D, sc, D·SC |
| [(A + B)]/P, % | | | | | | |
| Cecum (n = 8) | 4.37 ^g | 4.51 ^g | 3.06 ^f | 3.09 ^f | 0.7 | D, T, <u>t·d</u> |
| Colon (n = 4) | 4.27 ^g | 4.94 ^h | 3.67 ^f | 3.52 ^f | 0.8 | D, <u>d·sc</u> |

^aValues are means of fermentation parameters measured hourly during the first 12 h after feeding.

^bHF = High-fiber diet, HS = High-starch diet.

^c0 = No supplementation, SC = 10 g·d⁻¹ of *S. cerevisiae*.

^dEffect: D, d and d = diet ($P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively); SC, sc and sc = *S. cerevisiae* ($P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively); D·SC, d·sc and d·sc = diet × *S. cerevisiae* ($P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively); T and t = sampling time ($P < 0.001$ and $P < 0.01$); T·D, t·d and t·d = sampling time × diet interaction ($P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively); T·SC and t·sc = sampling time × *S. cerevisiae* ($P < 0.001$ and $P < 0.05$, respectively).

^en = number of animals in each treatment.

^{f,g,h}Least squares means within a row are different if superscript differ ($P < 0.05$).

butyrate. Time interacted with SC supplementation for pH and lactic acid concentration.

Cecal pH decreased rapidly during the first hours after feeding, reached a minimum between 5 to 7 h after the meal and then increased progressively until the subsequent meal (Figure 1). The rate and extent of the pH drop was more emphasized with the HS + 0 than with the HF + 0 treatments (Figure 1). The HS diet increased ($P < 0.05$) the standard deviation of the cecal pH (Table 4). *Saccharomyces cerevisiae* supplementation increased ($P < 0.05$) the mean cecal pH value (Table 3) and the pH at 4, 6, and 8 h after feeding, with the HS diet (Figure 1). Additionally, the standard deviation of cecal pH decreased ($P < 0.01$)

with the SC supplementation when the HS diet was fed (Table 4).

The concentration of lactic acid increased rapidly after feeding and reached 587 mg/L in the cecum when the horses were fed the HS + 0 treatment. It was no more than 275 mg/L in the cecum of the animals fed the HF + 0 treatment (Figure 2). In parallel, standard deviation of the lactic acid concentration increased ($P < 0.01$) when the horses received the HS diet (Table 4). The mean cecal lactic acid concentration of horses fed the HS diets decreased ($P < 0.05$) from 407.7 to 207.5 mg/L when SC was added (Table 3). This decreasing effect appeared ($P < 0.05$) at 2 h until 10 h postfeeding (Figure 2). On both diets, standard devia-

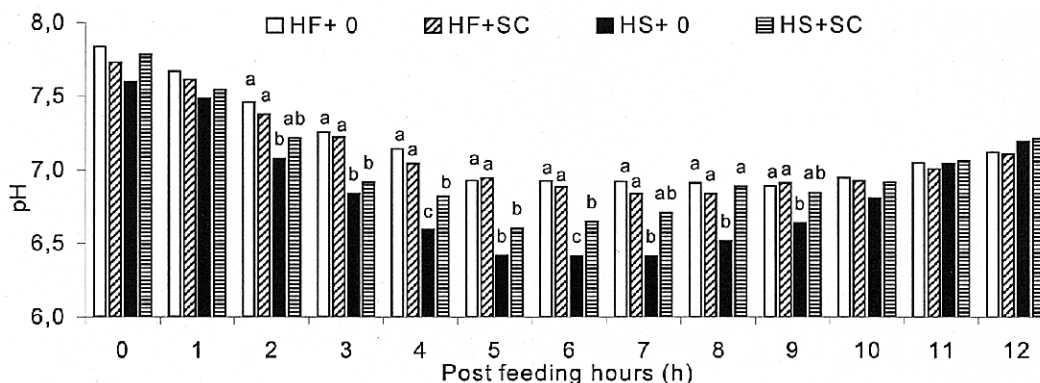


Figure 1. Mean postprandial changes in cecal pH of eight fistulated horses fed high fiber (HF + 0), HF supplemented with *S. cerevisiae* (HF + SC), high starch (HS + 0), or HS supplemented with *S. cerevisiae* (HS + SC) diets. ^{a,b,c}Within a postfeeding hour, bars without a common superscript letter differ ($P < 0.05$).

tion of the lactic acid concentration was lower ($P < 0.05$) in the cecum of horses supplemented with SC than that of unsupplemented horses (Table 4).

With HS + 0 treatment, the mean ammonia concentration was higher ($P > 0.05$) than those of the three other treatments (Table 3). *Saccharomyces cerevisiae* supplementation decreased ($P < 0.05$) the mean cecal concentration of ammonia (Table 3) and the concentra-

tion during the first 5 h postfeeding (data not shown) when horses were fed the HS diet but had no clear effect with HF diet. The standard deviation of the mean concentration of ammonia decreased ($P < 0.05$) in the cecum with the supplementation of SC in both diets (Table 4).

Total VFA were not affected by dietary treatments. The overall concentration of acetate was reduced in

Table 4. Standard deviations of cecal and colonic fermentation characteristics^a in fistulated horses fed either HF or HS diets, without (+ 0) and with (+ SC) 10 g/d of *S. cerevisiae*

| Item and intestinal content | HF diet ^b | | HS diet | | SEM | Effect ^d | | |
|-----------------------------|----------------------|--------------------|--------------------|--------------------|------|---------------------|----|-----------|
| | + 0 ^c | + SC ^c | + 0 | + SC | | Diet | SC | Diet × SC |
| pH | | | | | | | | |
| Cecum (n = 8) ^e | 0.34 ^f | 0.32 ^f | 0.46 ^g | 0.30 ^f | 0.07 | * | ** | ** |
| Colon (n = 4) ^e | 0.26 ^{fg} | 0.24 ^f | 0.50 ^h | 0.35 ^g | 0.07 | *** | * | ns |
| Lactic acid, mg/L | | | | | | | | |
| Cecum (n = 8) | 115.0 ^f | 86.2 ^f | 227.5 ^g | 147.2 ^f | 79.0 | ** | * | ns |
| Colon (n = 4) | 46.3 ^f | 59.4 ^f | 210.3 ^g | 116.2 ^f | 49.9 | ** | ns | * |
| Ammonia, mg/L | | | | | | | | |
| Cecum (n = 8) | 45.3 ^{fg} | 29.0 ^f | 67.4 ^g | 31.3 ^{fg} | 34.0 | ns | * | ns |
| Colon (n = 4) | 41.3 | 46.9 | 53.3 | 64.2 | 22.7 | ns | ns | ns |
| Total VFA, mM | | | | | | | | |
| Cecum (n = 8) | 30.1 | 25.2 | 21.8 | 23.4 | 8.1 | ns | ns | ns |
| Colon (n = 4) | 27.3 | 24.6 | 29.5 | 24.4 | 8.8 | ns | ns | ns |
| Acetate (A), mM | | | | | | | | |
| Cecum (n = 8) | 23.6 ^g | 18.0 ^{fg} | 14.3 ^f | 16.1 ^f | 5.7 | * | ns | ns |
| Colon (n = 4) | 19.8 | 19.0 | 19.2 | 16.8 | 5.8 | ns | ns | ns |
| Propionate (P), mM | | | | | | | | |
| Cecum (n = 8) | 7.02 ^{fg} | 5.07 ^f | 7.95 ^g | 6.10 ^{fg} | 2.3 | ns | * | ns |
| Colon (n = 4) | 5.26 | 5.0 | 8.29 | 7.32 | 2.4 | * | ns | ns |
| Butyrate (B), mM | | | | | | | | |
| Cecum (n = 8) | 1.52 | 1.32 | 1.34 | 1.35 | 0.6 | ns | ns | ns |
| Colon (n = 4) | 2.56 | 1.49 | 3.04 | 1.73 | 1.2 | ns | ns | ns |
| [(A + B)/P, %] | | | | | | | | |
| Cecum (n = 8) | 0.03 | 0.02 | 0.04 | 0.04 | 0.01 | * | ns | ns |
| Colon (n = 4) | 0.02 | 0.02 | 0.02 | 0.03 | 0.01 | ns | ns | ns |

^aFermentation parameters were measured hourly during the first 12 h after feeding.

^bHF = High-fiber diet, HS = High-starch diet.

^c0 = No supplementation, SC = 10 g·d⁻¹ of *S. cerevisiae*.

^dLevel of significance: ns $P < 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

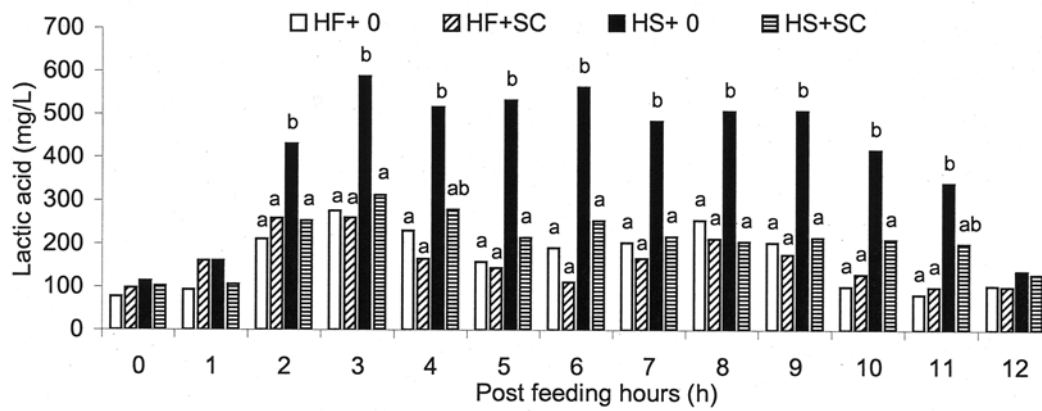


Figure 2. Mean postprandial changes in lactic acid concentration (mg/L) in the cecum of eight fistulated horses fed high fiber (HF + 0), HF supplemented with *S. cerevisiae* (HF + SC), high starch (HS + 0), or HS supplemented with *S. cerevisiae* (HS + SC) diets. ^{a,b}Within a postfeeding hour, bars without a common superscript letter differ ($P < 0.05$).

the cecum ($P < 0.001$), while the propionate concentration increased ($P < 0.001$) when the horses were fed HS diets (Table 3). The postfeeding [(A + B)/P] ratio decreased ($P < 0.05$) from 4.37 with the HF + 0, to 3.06 with the HS + 0 treatment in the cecum (Table 3), whereas the standard deviation of this ratio increased ($P < 0.05$) (Table 4). When the SC preparation was supplemented in the HF and the HS diets, the molar percentage of acetate increased ($P < 0.05$) in the cecum of horses (Table 3).

In the colon, sampling time after feeding affected ($P < 0.01$) pH and the concentrations of ammonia, total VFA, acetate, and propionate. Time interacted ($P < 0.01$) with the effect of diet for pH and propionate concentration. There was no interaction between time and SC supplementation. The changes in the profile of the colonic pH (Figure 3) was similar to the changes found in the cecum: HS diet increased ($P < 0.001$) the standard deviation of the colonic pH (Table 4). No effect of SC supplementation was reported on the over-

all pH value, but SC decreased ($P < 0.05$) the standard deviation of pH when the HS diet was fed.

The colonic lactic acid concentration increased ($P < 0.05$) during 6 h postfeeding to a maximum of 581 and 181 mg/L when the horses were fed the HS + 0 and HF + 0 treatments, respectively (Figure 4). The standard deviation of the lactic acid concentration increased ($P < 0.01$) in the colon when the horses received the HS diet (Table 4). When SC was added, the postfeeding lactic acid concentration in the colon of horses fed the HS diet decreased ($P < 0.05$) from 303.2 to 235.7 mg/L (Table 3), and the standard deviation was lower ($P < 0.05$) (Table 4).

The molar percentage of acetate and the [(A + B)/P] ratio were reduced in the colon ($P < 0.001$), while the concentration ($P < 0.01$) and the molar percentage ($P < 0.01$) of propionate increased when the horses were fed the HS diet (Table 3). The molar percentage of acetate increased ($P < 0.05$) when the SC preparation was supplemented in the HS diet (Table 3). *Sac-*

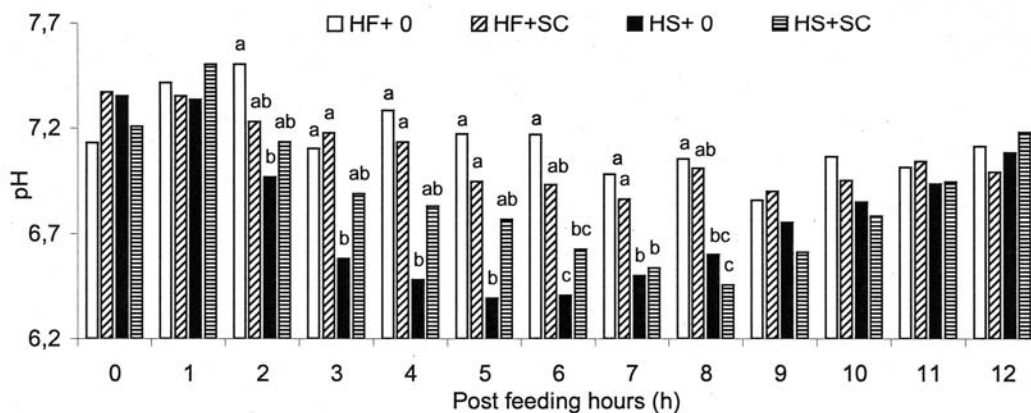


Figure 3. Mean postprandial changes in colonic pH of four fistulated horses fed high fiber (HF + 0), HF supplemented with *S. cerevisiae* (HF + SC), high starch (HS + 0), or HS supplemented with *S. cerevisiae* (HS + SC) diets. ^{a,b,c}Within a postfeeding hour, bars without a common superscript letter differ ($P < 0.05$).

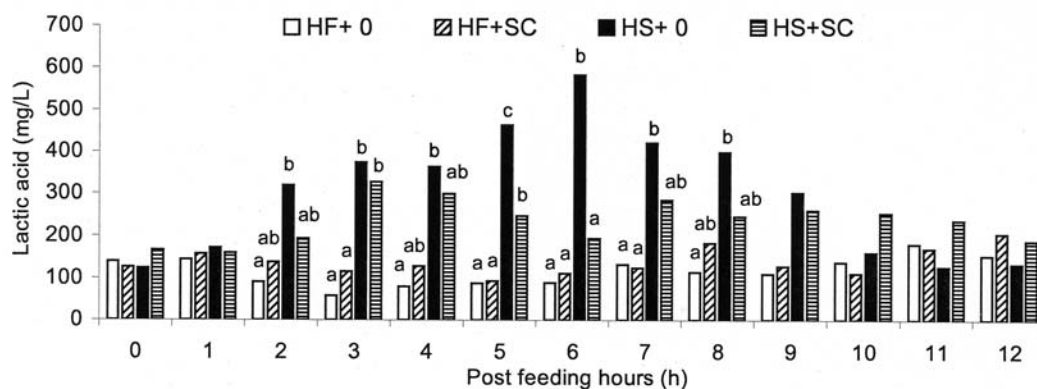


Figure 4. Mean postprandial changes in lactic acid concentration (mg/L) in the colon of four fistulated horses fed cerevisiae (HS + SC) diets high fiber (HF + 0), HF supplemented with *S. cerevisiae* (HF + SC), high starch (HS + 0), or HS supplemented with *S. cerevisiae*. ^{a,b,c}Within a postfeeding hour, bars without a common superscript letter differ ($P < 0.05$).

charomyces cerevisiae supplementation increased ($P < 0.05$) the colonic [(A + B)/P] ratio (4.27 vs 4.94) in horses fed the HF diet.

Discussion

Our data showed that both the profiles and the activities of the intestinal microflora in horses were modified by the NDF/starch ratio of the diet. Such alterations of the hindgut ecosystem have been already reported in the cecum (Willard et al., 1977; Garner et al., 1978; Goodson et al., 1988) and the colon (Hintz et al., 1971; Kern et al. 1974; Julliand et al., 2001) of horses as a result of changes in dietary carbohydrate sources. Interestingly, minimal pH values and maximal lactic acid concentrations were observed 5 and 6 h post-feeding in the cecum (6.45; 532 mg/L) and the colon (6.38; 582 mg/L), respectively. This showed that alteration in the colonic fermentation patterns occurred just 1 h after the one in the cecum. The ground and pelleted concentrates, given to horses, probably reduced the mean retention time of feeds in the cecum. In fact, Drogoul et al. (2000a) reported that fine particles of a ground and pelleted hay passed quickly through cecum and remained longer in the colon. In this way, when the high-starch diet was fed, a large amount of undegraded starch reached the colonic section. This confirmed that an overload of grain affected the colon (de fombelle et al., 2001; Juliand et al., 2001), which is known to be the major site for the occurrence of colic (Argenzio, 1975; Freeman, 1997).

In previous works (Willard et al., 1977; Goodson et al., 1988; Julliand et al., 2001), horses were fed at the same level of energy, which induced a significant decrease in the fiber amount in starchy diets. In the present study, both the high-fiber and high-starch diets were fed at the same level of dry matter intake. Therefore, the quantity of NDF provided by the high-starch diet represented only 25% less NDF than the high-fiber diet (i.e., 660 vs 882 g of DMI/100 kg BW).

In contrast, the intake of starch (252 vs 670 g of DMI/100 kg BW for HF and HS diets, respectively) increased by 166% with the high-starch diet. The larger quantity of starch provided with the HS diet allowed a greater amount of rapidly fermentable carbohydrates to reach the hindgut (Potter et al., 1992; Kienzle, 1994). Then, nondegraded starch was available for starch-utilizing bacteria (Garner et al., 1978; Goodson et al., 1988), such as lactobacilli and streptococci, and increased ($P < 0.001$) their concentrations in the intestinal contents. A 334% increase in the streptococci to total anaerobes ratio and a 66% decrease in the ratio of bacteria involved in the metabolism of lactic acid [i.e., lactic acid-utilizing bacteria/(lactobacilli + streptococci)] were noted in the colon of horses receiving the high-starch diet (data not shown). However, Julliand et al. (2001) reported a 95% decrease in the latter ratio, when the level of grain intake increased at the expense of fiber intake. Starch was readily used by streptococci and lactobacilli to produce lactic acid at low pH (Rowe et al., 1994; Garner et al., 1978). With the HS diet, the lowest pH (6.43) appeared 5 h after feeding, in the hindgut. At this time, the peak concentration of lactic acid (498 mg/L) in the large intestine was four times higher in the high-starch treatment than the one observed (123 mg/L) in horses fed the high-fiber diet. However, this increase in the concentration of lactic acid in equine hindgut was twice as low as that observed when the overload of starch was not balanced with a great amount of fiber (Julliand et al., 2001). The cecal concentration of cellulolytic bacteria decreased 4 h after feeding the HS diets compared to the HF diets, which was consistent with previous results reported by Garner et al., (1978) and Julliand et al., (2001). Although the mean concentration of total VFA did not differ between the two diets, we measured a significant increase in the concentration of propionate and a significant decrease in acetate concentration in the cecum and the colon when the HS diet was given to horses. Such an evolu-

tion resulted in a significant decrease of the [(acetate + butyrate)/propionate] ratio, suggesting a decline in the fibrolytic activity and an increase of the fermentation of starch in the large intestine of horses (Sauvant et al., 1994).

Viable yeast cells were found in the large intestine of supplemented horses 4 h after feeding. The concentration of yeast cells in the cecum remained at a level close to that initially supplemented (i.e., 10^6 cfu/g DM of feedstuff), which was in agreement with the results observed in the rumen (Durand-Chaucheyras et al., 1998; Fiems et al., 1993) and the ileum (Newbold et al., 1990) of sheep. However, only 4.5×10^4 cfu/g of viable yeast cells were detected in the colon contents. These data suggested that the strain of *S. cerevisiae* contained in the SC preparation was able to reach and survive in the cecum and the right ventral colon of the horse but not to colonize them. The supplementation of *S. cerevisiae* appeared to significantly modify pH, concentrations of lactic acid and ammonia, and molar percentages of acetate and butyrate when the high-starch diet was fed and [(acetate + butyrate)/propionate] ratio with the high-fiber diet. The interaction effect between *S. cerevisiae* and diet was greater inside the cecum than inside the colon. This was possibly related to the greater counts of yeast cells in the cecum than in the colon and suggests that the effects of *S. cerevisiae* are possible only if viable yeast cells are present in intestinal ecosystems (Nagaraja et al., 1997). With the high-starch diet supplemented with live yeast culture, the overall pH values increased, and its standard deviation decreased in the cecum. Such a buffering effect had been previously reported in ponies fed a 60:40 forage:grain ratio (Moore and Newman, 1993). Interestingly, the effect of *S. cerevisiae* on the cecal pH appeared from 4 to 9 h after feeding the high-starch diet, during a period when both pH values and lactic acid concentration were mainly affected by the diet. This was combined with an increase in the lactic acid-utilizing to lactic acid-producing bacteria ratio. The molar percentage of acetate was increased in the cecum and the colon with both SC-supplemented diets. This suggested an enhancement of the fibrolytic activity in the hindgut of horses supplemented with *S. cerevisiae*, although we did not observe any increase in the concentration of cellulolytic bacteria. This contrasts with the results reported by Moore et al. (1994), who found an increase in the cellulolytic bacterial concentration with the supplementation of *S. cerevisiae*. More studies are required to deeply understand the direct effects of the live yeast culture preparation on the lower gut microflora and their metabolic activities in horses.

The large standard deviations for microbial and biochemical parameters found in our study indicate the wide variation in the intestinal ecosystems of horses. This variation was greatest for horses fed the high-starch diet, which is consistent with the reports of Julliand et al. (2001) and Glinsky et al. (1976). There-

fore, practical nutritional recommendations should consider differences in the ability of horses to tolerate high-starch diets to minimize the occurrence of digestive disorders. Feeding *S. cerevisiae* in high-starch diets reduced variation in lactic acid concentration and pH of intestinal contents suggesting that yeast supplementation may allow some horses to better tolerate high-starch diets without developing digestive disorders.

Our investigation into the effects of a large supply of starchy feeds balanced with an equivalent amount of fiber (i.e., dietary NDF/starch = 1) on the complex interactions between dietary nutrients and enteric microflora provided some useful information on the relation between nutrition and health of the equine lower gut. However, further studies are needed to evaluate this feeding practice on the cellulolytic activity of the equine digestive ecosystem and its consequences on the total apparent digestibility of the ration.

Implications

In our study, the modifications observed on specific groups of bacteria, such as cellulolytics, lactic acid-utilizing, and lactic acid-producing bacteria, on pH and lactic acid concentration in the large intestine of horses were smaller than those reported when the overload of starch was not balanced with a high amount of fiber. The addition of *Saccharomyces cerevisiae* was shown to reduce the decrease in pH and the increase in lactic acid after feeding, in the large intestine of horses fed starchy diets. The strain of *S. cerevisiae* used was able to reach and survive in the cecum and the right ventral colon of the horse but not to colonize them. Consequently, yeast additive should be provided with each meal to modify microbial profiles and fermentation patterns. When the digestion of starch in the small intestine was saturated, the combined effect of a balanced dietary NDF/starch ratio and the addition of *S. cerevisiae* appeared to limit the extent of undesirable changes in the intestinal ecosystem of the horse.

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