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Effect of yeast supplementation on hindgut microbiota and digestibility of horses subjected to an abrupt change of hays

P. Grimm*, V. Julliand, C. Philippeau, S. Sadet-Bourgeteau

Agrosup Dijon, URANIE, USC1335 Nutrition du cheval athlète, 26 Bd Docteur Petitjean, F-21079 Dijon, France

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ABSTRACT

According to epidemiological studies, abrupt change of hay is a major risk factor of equine colic. Disturbances of the microbial ecosystem can lead to colic. However, it is not known if the hay change disturbs the hindgut microbial ecosystem. This study aimed to assess the effect of *Saccharomyces cerevisiae* (SC) supplementation during an abrupt change of hay on the hindgut microbial ecosystem and digestibility. Six cecum and colon fistulated horses allotted in two groups were subjected to a 2 × 2 Latin-square design experiment. Horses received a 80/20 ratio forage/concentrate (1.3 DMkg/day/100 kgBW meadow hay) diet. During each experimental period, one of the two groups was supplemented with 1 g/d of Yea-Sacc preparation in the morning meal. In each experimental period, groups were first fed hay1 (88.1%DM, 62.6%NDF, 34.9%ADF, 5.3%ADL, 33.1%CF, 8.6%CP) for four weeks and then hay2 (87.5%DM, 60.6%NDF, 35.0%ADF, 5.0%ADL, 33.4%CF, 7.8%CP) for the next three weeks without an adaption period. Cecal and colonic samples were collected 4 h after the morning meal 6 days before (d-6) and one (d1), eight (d8) and 15 days after (d15) the abrupt change to assess the microbial ecosystem parameters. The digestibility was also measured before (pool1: d-4 to d-1), immediately (pool2: d0 to d3) and after (pool3: d10 to d13) the hay change. There was no effect of either SC supplementation or SC supplementation × sampling day interaction on any parameter (microbial ecosystem/digestibility). In the two experimental groups, the abrupt change of hay caused at d1 a significant increase in Copy Number of Target Gene (CNTG) of *Bacteroidetes* ($P=0.037$) and a trend for increasing CNTG of *Firmicutes* ($P=0.089$) in the cecum, as well as a significant increase in total volatile fatty acids ($P=0.019$) and acetate concentrations ($P=0.026$) in the colon. At d8, a significant decrease of the colonic CNTG of *Bacteroidetes* was noted ($P=0.019$), as well as a tendency to increase for CNTG of colonic *Firmicutes* ($P=0.056$). The cecal pectinolytic bacteria ($P=0.036$) and colonic propionate concentration ($P=0.009$) significantly increased, respectively, at d8 and d15 after the hay change. The DM, OM, NDF and ADF digestibility increased significantly in pool 2 and 3 ($P<0.01$). Despite the close composition of the two hays, changes were measured after the abrupt hay change suggesting a sensitive response of the hindgut microbial ecosystem. Further investigations are needed to evaluate the extent to which hindgut microbial ecosystem disturbances can lead to colic and to understand how yeast could be supplemented to minimize their effect.

1. Introduction

Disturbances of the hindgut microbiota composition and/or activity may alter digestive health (Sadet-Bourgeteau and Julliand, 2012) and potentially lead to the onset of colic, which is a major concern in the horse industry. Diet is the most quoted risk factor of colic in epidemiological studies as shown in the review of Gonçalves et al. (2002). A major dietary risk factor is a recent change (within two weeks) of hay type without adaptation (Tinker et al., 1997; Cohen et al., 1999). Two studies have investigated the impact of a sudden change of forage on the hindgut microbial ecosystem. A change between two silages with different crude protein content did not provoke modification in bacterial counts nor in microbial activity after 24 h. But within the subsequent three weeks, it was associated with a decrease in pH and an increase in VFA concentration (Muhonen et al., 2008). A change from hay to haylage or silage from the same botanical origin did not induce alterations after 28 h but it was responsible for modification of the colonic *lactobacilli* and *streptococci* bacterial counts within the next three weeks (Muhonen et al., 2009). To our knowledge no data have been published regarding the impact of a change between two hays with different fiber content on the hindgut microbial

* Corresponding author.
E-mail address: pauline.grimm@agrosupdijon.fr (P. Grimm).

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ecosystem, although, in the literature, hays associated with increased risk of colic had high fiber contents (Cohen et al., 1999; Hudson et al., 2001).

In addition, when the microbiota composition and/or activity are disturbed, digestibility can be modified (Drogoul et al., 2001; Julliand et al., 2001). Yeast supplementation can be used to limit hindgut dysbiosis (Jouany et al., 2009) and improve cell-wall digestibility (Jouany et al., 2008). Therefore, the objective of our study was to evaluate the effect of *Saccharomyces cerevisiae* yeast supplementation (Yea-Sacc, Alltech, Dunboyne, Ireland) during an abrupt change between hays with different fiber contents on the composition and the activity of the equine hindgut microbiota and the digestibility of the parietal contents.

2. Materials and methods

The project was conducted under license from the Department of Health and Animal Care of the French Veterinary Authority no. A21002 (ethic committee approval no. 21 CAE 004).

2.1. Animals and management

Six adult crossbred geldings (from 453 to 501 kg of BW, aged from 11 to 19 years), each fitted with polyvinyl chloride cannulas in the cecum and right ventral colon were individually housed in 4×3.5 m^2 free stalls bedded with wood shavings. Animals were allotted into two homogeneous groups according to their age, their weight, and their body score condition. They had access to a sand paddock for 5 h, 5 days per week. Horses were exercised twice weekly in an automatic walker 1 h per day at 6–7 km/h. Their vaccinations against tetanus and influenza (ProteqFlu TE, Merial) and their deworming (Equest Oral Gel (moxidectin), Fort Dodge Animal Health) were updated before the start of the experiment.

2.2. Diets

The diet was formulated to meet the French recommendations for horses subjected to a very light work. All along the experiment, horses received a ration composed of 80% meadow hay (1.3 DM kg hay/day/100 kg BW) and 20% of pelleted concentrate (DP Evasion Loisir, DP Nutrition, France; 88.8% DM, 38.9% NDF, 18.2% ADF, 17.1% CF, 3.5% ADL, 13.9% CP). Two hays were tested (hay1 and hay2, Table 1) at the same intake level. Both were first cuts of meadows in low mountain region (altitude of 350 m and 240 m for hay1 and hay2 respectively) composed exclusively of grass hay, harvested in good weather conditions and preserved for 6 months in a shed. hay2 was harvested three weeks later than hay1. Hay and pelleted concentrate were distributed simultaneously in two equal meals daily, at 0800 h and 1700 h. Water and a block of trace-mineralized salt were offered free-choice. As chosen by the yeast manufacturer, supplemented horses received 1 g/d of a preparation of *Saccharomyces cerevisiae CBS 493.94* (Yea-Sacc Alltech, Dunboyne, Ireland) top dressed on the concentrate of each morning meal. This product is a dried live yeast containing a minimum active substance level of 1×10^9 CFU/g, and was in the form of free flowing prills.

Table 1
Biochemical composition of the two hays.

	hay1	hay2
DM (%)	88.06	87.52
NDF[a]	62.57	60.55
NDF–ADF (hemicellulose)[a]	27.71	25.52
ADF[a]	34.86	35.03
ADF–ADL (cellulose)[a]	29.61	30.05
ADL[a]	5.25	4.98
CF[a]	33.07	33.39
CP[a]	8.59	7.83

[a] Percentage of dry matter.

2.3. Experimental design

The two groups of animals were randomly assigned to a 2×2 Latin square experiment consisting of two periods of 7 weeks each and separated by a wash out (WO) period of 6 weeks. In each experimental period, the two groups of horses were fed hay1 for the first four weeks then hay2 for the next three weeks, without an adaption period in order to create an abrupt change. During the first period (P1) group 1 was supplemented with Yea-Sacc, while during period 2 (P2) group 2 was supplemented. During the WO period, the six horses received hay1 without yeast supplementation, in order to avoid any carry-over effect of the yeast and diet into the P2.

2.4. Digestibility measurements

For each period, total tract apparent digestibility was determined using partial collection of feces over four consecutive days (Goachet et al., 2009) before the abrupt change (d-4 to d-1), immediately (d0 to d3) and later (d10 to d13) after the change of hay. Fresh feces (300 g) were taken by rectal sampling at 0830 h and 1730 h every day from each horse for the determination of digestibility coefficients. Feces were weighed, dried in a 75 °C air-forced oven until a constant weight and then finely ground. For each horse, three pools (Pool1: d-4 to d-1; Pool2: d0 to d3; Pool3: d10 to d13) of 200 g DM of fecal content were prepared. Samples of hay and concentrate were collected at each change of hay bale and opening of a new bag of concentrate. Samples were weighed, dried in a 75 °C air-forced oven until a constant weight and then finely ground. For each horse and for every 24 h period (at 0800 h prior to feeding), the amounts of feed offered and refused were weighed.

2.5. Microbial ecosystem measurements

During each period, cecal and colonic contents were collected six days before (d-6) and one (d1), eight (d8) and 15days (d15) after the abrupt change of hay. All samples were collected 4 h after the morning meal (i.e. 1200 h) by gravity via the cannulas.

Part of the contents was filtered through a 100 µm filter (1 mL, storage at −20 °C until analysis) and added to a preservative (0.1 mL of a solution of 5% H_3PO_4 + 1% $HgCl_2$) for further VFA analysis. An aliquot of the unfiltered contents (solid and liquid phase) was sampled for qPCR molecular biology analysis (1 mL, storage at −20 °C until analysis). Finally, another aliquot of unfiltered content was also sampled in a container filled to capacity (to avoid the presence of oxygen) for microbiological analyses performed immediately after the sample collection.

2.6. Digestibility analyses

Feed and fecal DM were determined after drying the samples in an oven at 75 °C during 24 h. Organic matter was determined after incineration at 550 °C for 5 h (71/250/CEE). Analyses of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and lignin (NF V18-122) (Van Soest et al., 1991) and Crude Protein content (CP) using Dumas method (NF EN ISO 16634-1) were performed in a private laboratory (In Vivo Labs, Château Thierry, France).

The total apparent digestibility was calculated using Lignin (ADL) as an indigestible internal marker (Pys, 2000; Santos et al.,