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Effect of transportation on fecal bacterial communities and fermentative activities in horses: Impact of *Saccharomyces cerevisiae* CNCM I-1077 supplementation[1]

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ABSTRACT: This study evaluated the effect of transportation on fecal bacterial communities and activities in horses with or without supplementation of live yeast and attempted to link those effects with changes in blood stress markers. Four mature horses were assigned to a crossover design and fed a basal diet (60:40 forage to concentrate; 1.45% BW on a DM basis), with or without supplementation, of 2×10^{10} cfu/d of *Saccharomyces cerevisiae* CNCM I-1077. After a 14-d adaptation to dietary treatments, the 5-d experiment started 1 d before transportation (d –1). At d 0, horses were simultaneously transported in a truck for 2 h. Feces were sampled 4 h after the morning meal of concentrate at d –1, 0 (immediately after transportation), and 3 for enumeration of the main functional bacterial groups and determination of fermentative variables. Within each dietary treatment, feces were pooled before DNA extraction and molecular analysis of the bacterial communities, using temporal temperature gradient electrophoreses (TTGE). Blood samples were collected at the same time for determination of white blood cells (WBC) counts and glucose and total protein concentrations. Regardless of dietary treatment, the neutrophil to lymphocyte ratio increased during transportation ($P < 0.01$), indicating that horses were stressed. In both treatments, TTGE profiles were clearly different before and 3 d after transportation, and the percentage of similarity between profiles at d –1 and 3 was greater in supplemented horses compared with the controls. From d 0 to 3, the molar percentage of propionate increased and total concentration of VFA and the acetate + butyrate to propionate ratio decreased, regardless of dietary treatment ($P < 0.01$, $P = 0.02$, and $P < 0.01$, respectively), whereas pH decreased only in control horses ($P = 0.03$). Regardless of day of sampling, fecal concentrations of lactate-utilizing bacteria and cellulolytic bacteria were greater in supplemented horses than in control horses ($P = 0.04$ and 0.08, respectively). Our results indicate that transportation for 2 h disturbed the fecal bacterial ecosystem in horses that could increase the risk of triggering microbial dysbiosis on a longer term in the equine large intestine. Supplementing *Saccharomyces cerevisiae* CNCM I-1077 could help reduce the negative impact of transportation on the fecal bacterial ecosystem.

Key words: bacterial ecosystem, feces, horse, *Saccharomyces cerevisiae,* temporal temperature gradient electrophoresis

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INTRODUCTION

Because of purchasing, races, or reproduction issues, horses are frequently subjected to transportation. Long transportation has been shown to create a stressful situation that negatively affects the health of horses (Friend 2001; Stull et al., 2008). Even short transportation may result in detrimental changes of the intestinal bacterial community (Goachet et al., 2003; Boensma et al., 2006). Because alterations of the intestinal microbiota have been associated with colic (Durham, 2008) or laminitis (Millinovich et al., 2007) in horses, transportation may induce such troubles.

Several strategies have been developed to prevent microbial disturbances in the equine intestine. One strategy consists of supplementing the diet with probiotics, such as live yeast (Julliand and Zeyner, 2009;

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Zeyner, 2010). These microorganisms are used in ruminant nutrition, particularly when animals are fed high amounts of readily fermentable carbohydrates (Chaucheyras-Durand et al., 2008). In horses fed high-starch diets, live yeast supplementation appeared to limit the extent of undesirable changes in the intestinal microbial ecosystem (Medina et al., 2002; Jouany et al., 2009).

Until recently, equine intestinal microbiota has mainly been studied using conventional cultural techniques to investigate the behavior of the main functional bacterial groups (Sadet-Bourgeteau and Julliand, 2010). Molecular techniques, such as temporal temperature gradient gel electrophoresis (**TTGE**) or denaturing gradient gel electrophoresis that allow for monitoring changes in bacterial composition, have been applied to study the intestinal microbiota of the horse (Ince et al., 2008; Grønvold et al., 2010). The aim of this study was to test the impact of transportation on fecal bacterial communities, using both conventional and molecular techniques, and on fecal fermentation variables in horses supplemented with or without live yeast, and eventually to link these data with changes in blood stress markers.

MATERIALS AND METHODS

The experiment was conducted at the animal research facility of AgroSup Dijon, Saint-Nicolas-Lès-Cîteaux, France, under license from the Department of Health and Animal Care of the French Veterinary Authority.

Animals, Management, and Diets

Four crossbred adult horses (3 geldings and 1 mare) with an average BW of 411 ± 53 kg were maintained in indoor, individual, free stalls bedded with shavings (Doulit, Sud Ouest Absorbants, Anglet, France). Clean, potable drinking water and a block of trace-mineralized salt were offered free choice. The diet (Table 1) and dietary schedule corresponded to feeding practices commonly used in French horse riding schools. Horses were fed meadow hay, representing 60% of their daily DM intake (900 g DM/100 kg BW), pelleted feed (250 g DM/100 kg BW), and barley (300 g DM/100 kg BW). Hay and pelleted feed were distributed in 2 equal meals at 1000 and 1600 h, and 0800 and 1730 h, respectively. Barley was given with the morning meal of pelleted feed, providing 180 g starch/100 kg BW. Before each diet adaptation period, horses were weighed on 2 consecutive days to adjust the feed allowance to their metabolic BW ($BW^{0.75}$) as defined by the Institut National de la Recherche Agronomique (Martin-Rosset, 1990).

The daily basal diet was supplemented or not with 1 g of live yeast corresponding to supplier recommendations, providing an average of 4.87×10^9 cfu of live yeast/(100 kg BW · d). Live yeasts (Levucell SC20, *Saccharomyces cerevisiae* CNCM I-1077; Lallemand Animal Nutrition, Blagnac, France) were mixed into the morning meal of pellets given to supplemented horses. Control horses did not receive any yeast additive.

Experimental Design, Treatments, and Transport Procedure

Four horses were randomly assigned by pairs in a crossover design at the beginning of the study. The 2 pairs of animals were physically separated to avoid cross contamination and received 1 of 2 dietary treatments: basal diet (control) or basal diet supplemented with 1 g/d of yeast additive (Levucell SC20). Each experimental period lasted 19 d, split into a 14-d adaptation period to the experimental diets, followed by a 5-d period of measurement beginning the day before transportation. Between the 2 experimental periods, horses were fed the basal diet for a 23-d period to allow the complete washout of yeasts from the intestinal tract of horses (Gobert et al., 2006). During each period of measurement, the 4 horses were transported simultaneously in a truck for 2 h from 1000 to 1200 h on d 0.

Collection Procedure and Sampling

At each period of measurement, fecal and blood samples were collected 4 h after the morning meal 1 d before transport (d –1), day of transport, and 3 d after transport (d 3). Blood samples were taken via venipuncture of the jugular vein into one 5-mL dry tube for serum total protein determination, one 5-mL pretreated potassium oxalate/sodium fluoride tube for plasma glucose determination, and one 5-mL pretreated liquid K_3EDTA tube for determination of blood cell counts. Serum and plasma were obtained by centrifugation (450 x g for 10 min at room temperature) of dry tubes and tubes containing oxalate/sodium fluoride, respectively, and transferred into new dry

Table 1. Dry matter content and composition of experimental feedstuffs given to horses (%, DM basis)

Item	Pelleted feed[1]	Barley	Hay
DM	90.2	86.0	89.7
OM	91.0	97.4	94.1
CP	15.3	11.7	nd[2]
NDF	46.5	nd	57.5
ADF	37.3	nd	34.5
ADL	4.7	nd	6.5
Starch	20.5	51.6	nd

[1]On a DM basis (%), the pelleted feed had: wheat bran (49%), barley (26%), and dehydrated alfalfa (16%), and others (9%; sugar cane molasses, sunflower meal, extruded linseed, calcium carbonate, NaCl, and fructooligosaccharides).

[2]nd = not determined.

tubes. Tubes containing K_3EDTA did not undergo any processing. All tubes were then immediately mailed to the laboratory (Laboratory of Equine Biology, La Chapelle en Serval, France) for further analysis. Fecal samples were collected manually from the rectum, using single-use gloves. A first subsample of ~30 g of fecal material was collected for microbial analysis and immediately transported to the laboratory in a sterile flask, saturated with CO_2 and maintained at 38°C. A second subsample of ~100 g was filtered (Blutex 100 µm) and pH of the filtrate was immediately measured with an electronic pH meter (340i/SET, WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany). The filtered content was divided into 2 aliquots and immediately frozen (–20°C) for further determination of D- and L-lactate (1 mL), and VFA concentrations [1 mL mixed with 0.1 mL of a preservative solution consisting of 4.25% (vol/vol) H_3PO_4 and 1.0% (wt/vol) $HgCl_2$]. A third subsample of ~30 g of fecal material was immediately frozen at –20°C in hermetically sealed plastic boxes for further molecular analysis of the bacterial community.

Blood Analyses

Plasma concentrations of glucose and total protein were determined enzymatically, using commercial test kits (kits n° 61269 and 61974, respectively, from BioMérieux, Craponne, France). Whole blood was used for determination of white blood cell (**WBC**) differential counts, using standard laboratory techniques and the neutrophil/lymphocyte ratio was calculated.

Bacterial Analyses

Serial decimal dilutions of the fecal samples were prepared under O_2-free CO_2 in an anaerobic mineral solution (Bryant and Burkey, 1953) for inoculation on specific enumeration media. Total viable anaerobic bacteria and lactic acid-utilizing bacteria were enumerated, using the roll tubes procedure under O_2-free CO_2 in a nonselective medium (Leedle and Hespell, 1980; Julliand et al., 1999) and a selective medium containing 2% (vol/vol) lactate as the sole energy source (Mackie and Heath, 1979), respectively. Bacterial counts were determined after 48 h of incubation at 38°C from 4 replicate roll tubes prepared per dilution. *Streptococci* spp. were enumerated on an agar medium (Bile-Esculin-Azide, BK158HA; Biokar Diagnostics, Beauvais, France) and *Lactobacilli* spp. on an agar medium (Rogosa, BK033; Biokar Diagnostics, Beauvais, France). Three replicate Petri plates were inoculated per dilution. Bacterial colonies were directly counted after 48 h of incubation at 38°C. Cellulolytic bacteria were enumerated in a modified (Baruc et al., 1983) complex liquid medium (Halliwell and Bryant, 1963; Julliand et al., 1999), containing filter paper cellulose strips as the sole energy source. After 15 d of incubation at 38°C of 4 replicate tubes per dilution, the most probable number of cellulolytic bacteria was determined using McGrady's tables (Clarke and Owens, 1983). Viable yeasts were enumerated on an agar medium (Sabouraud, BK025HA; Biokar Diagnostic, Beauvais, France), supplemented with 0.2% (vol/vol) chloramphenicol (Sigma, Strasbourg, France). Colonies were counted on 3 replicate Petri plates per dilution after 48 h of incubation at 30°C.

Biochemical Analyses

All samples were analyzed in duplicate. The L- and D-lactic acid concentrations were measured with an enzymatic reaction procedure (D-lactic acid/L-lactic acid Enzymatic kit, No. 1002891, ENZYTEC/SCIL; Diagnostics GmbH, Martinsried, Germany), quantified spectrophotometrically at 340 nm (MRX Revelation; Dynatech Laboratories, Guyancourt, France). The VFA concentrations were assayed by gas-liquid chromatography (Gas Chromatograph Model 437 A; United Technologies Packard, Zurich, Switzerland; Jouany, 1982). The molar proportions of acetate, propionate, and butyrate, and the ratio (acetate + butyrate/propionate) was calculated according to Sauvant et al. (1994).

Molecular Analysis of Fecal Bacterial Communities

The DNA Extraction and 16S Ribosomal DNA Amplification. Within each experimental period, feces of the 2 horses receiving the same treatment were pooled. At the end of the experiment, the 2 fecal pools corresponding to horses receiving the same treatments were mixed. This procedure was done to avoid inter-individual variations (He et al., 2011; Mosoni et al., 2011; Montagne et al., 2012), which have been previously reported in horses (Ince et al., 2008; Grønvold et al., 2010). By pooling individual samples before fingerprint analysis, the overall change in fecal microbiota could be compared between dietary treatments.

Ten grams of each final pool were homogenized (Stomacher 80; Seward Ltd, Norfolk, UK) with 10 mL of sterile distilled water (high speed for 10 min at 4°C), centrifuged to sediment microbes and plant residues (6500 × *g* for 15 min at 4°C), and the supernatant was discarded (Mosoni et al., 2007). Total DNA was then extracted in duplicate, using 0.25 g of the solid phase, according to the bead-beating procedure described by Yu and Morrison (2004). The DNA concentration was determined by measuring absorbance at 260 nm. The DNA integrity was checked by electrophoresis of an ethidium bromide stained 1% (wt/vol) agarose gel in 1X Tris-Borate-EDTA.

The V6-V8 variable region of the 16S-rRNA bacterial gene was amplified, using primers U968-GC-f (5' GC clamp-AA CGC GAA GAA CCT TAC) and L 1401-r (5' GCG TGT GTA CAA GAC CC; Zoetendal et al., 1998). The PCR reactions were performed in a final volume of 50 µL, containing 200 µM of each dNTP, 0.8 µM of each primer, 1X PCR buffer, 2.5 mM $MgCl_2$, 1.5 U of Hot Master Taq DNA polymerase (Eppendorf, Le Pecq, France), and 100 ng of genomic DNA. The PCR program was 94°C for 2 min, 30 cycles of PCR (94°C for 1 min, 58°C for 1 min, 70°C for 1 min 30 s), and 70°C for 15 min. The PCR products (434 bp) were visualized and quantified by densitometry on ethidium bromide stained 2% (wt/vol) agarose gel.

Temporal Temperature Gradient Gels Procedure. Electrophoreses were performed (DCode Universal Mutation Detection System; Bio-Rad, Marne-La-Coquette, France) on 10% polyacrylamide gels, in Tris (50 mM)-Acetate (25 mM)-EDTA (1.25 mM) buffer with pH 8.3. Three hundred nanograms of each PCR product were loaded on the gel. The TTGE was run at 60 V for 16 h with a temperature ranging from 66.0 to 69.2°C and a ramp rate of 0.2°C/h (Batisson et al., 2007). After electrophoresis, gels were stained for 1 h in the dark with a nucleic acid gel stain bath (Gel Star; Cambrex Bioscience, Rockland, ME). Bacterial profiles were visualized (Gel Doc Imaging System; Bio-Rad) under UV transillumination and analyzed (Diversity Database 2.1; Bio-Rad). The total number of visual bands on the bacterial profiles was used to indicate the number of molecular species. Comparisons between TTGE profiles were performed using Dice's Similarity Coefficient analysis. A dendrogram was obtained by the unweighted pair group method using algorithm averages (Fromin et al., 2002).

Statistical Analyses

Logarithmic transformations were performed on microbial counts before statistical analysis. The mixed procedure (SAS Inst. Inc., Cary, NC) was used to analyze the data, with a model including animal as a random effect, period, dietary treatment, and day of sampling as a repeated measure, and the interaction between dietary treatments and day of sampling as fixed effects. Only dietary treatment, day of sampling, and their interaction are discussed in the present paper. Least squares means were calculated for all variables and separated using the pairwise *t* test (PDIFF option of SAS). Main effects and interaction were significant if $P < 0.05$, and trends were considered at $P < 0.10$.

RESULTS

Blood Measurements

The interaction between day of sampling and dietary treatment tended to be statistically significant for WBC count ($P = 0.07$; Table 2). From d –1 to 0, WBC count increased in control horses ($P < 0.01$), whereas it did not change in supplemented horses. From d 0 to 3, WBC count decreased in both dietary groups ($P < 0.01$) and reached similar values initially measured at d –1 for supplemented horses only. No effect of the dietary treatment on the other blood measurements and their evolution over time was detected. From d –1 to 0, the percentage of neutrophils and neutrophil/lymphocyte ratio increased ($P < 0.05$), whereas the percentage of lymphocytes decreased ($P < 0.01$). From d 0 to 3, the percentages of neutrophils and lymphocytes and neutrophil/lymphocyte ratio returned to their initial value ($P < 0.05$), and serum concentration of total protein decreased ($P = 0.02$). Serum concentration of glucose remained stable from d –1 to 3.

Fecal Bacterial Counts

An interaction between day of sampling and dietary treatment was detected for the concentration of total anaerobes ($P = 0.02$) and tended to be statistically significant for the concentration of *Streptococci* spp. ($P = 0.09$; Table 3). In supplemented horses, fecal counts of total anaerobes were greater at d –1 than in control horses ($P < 0.01$) and decreased from d –1 to 0 ($P < 0.01$), whereas they remained stable over time in control horses. *Streptococci* spp. counts decreased between d –1 and 0 in supplemented horses ($P < 0.01$), whereas they remained stable in control horses. Fecal concentrations of cellulolytic bacteria, lactate-utilizing bacteria, and *Lactobacillus* spp. were not affected by day of sampling. In supplemented horses, fecal concentrations of lactate-utilizing bacteria were greater and those of cellulolytic bacteria tended to be greater compared with control horses ($P = 0.04$ and 0.08, respectively). Fecal concentrations of *Lactobacilli* spp. were not affected by the dietary treatment. Live yeast cells were never detected in feces of control horses. When horses received yeast additive daily, yeast cells were recovered at an average concentration of 3.9×10^3 cfu/g of feces (data not shown).

Fecal Fermentative Variables

An interaction between dietary treatment and day of sampling was detected for fecal pH ($P = 0.04$; Table 4). At d –1 and 0, pH was lower in supplemented horses

Table 2. Blood measurements in horses with or without (control) supplementation, with live yeasts and subjected to transportation[1]

Item	Dietary supplementation	Day of sampling[2]			SE	*P*-value		
		−1	0	3		Day	Diet	Interaction
White blood cell (WBC) counts, × 10^6/L	Control	10.5[a]	11.9[Ab]	9.7[c]	0.6	<0.01	0.30	0.07
	Live yeasts	10.5[ab]	11.0[Bb]	9.9[a]				
Neutrophils, % WBC	Control	47.2	54.5	48.2	2.1	<0.01	0.24	0.78
	Live yeasts	45.7	51.75	47.7				
Lymphocytes,% WBC	Control	48.2	41.2	47.2	2.1	<0.01	0.13	0.90
	Live yeasts	50.7	43.7	48.5				
Neutrophil to lymphocyte ratio	Control	1.0	1.4	1.0	0.1	<0.01	0.44	0.89
	Live yeasts	0.9	1.3	1.0				
Glucose, g/L	Control	0.99	1.00	0.99	0.05	0.75	0.38	0.86
	Live yeasts	1.00	1.06	1.04				
Total protein, g/L	Control	74.5	75.2	72.0	2.1	0.07	0.43	0.72
	Live yeasts	72.2	75.5	71.0				

[a,b]Within a row, means with same letter do not differ ($P > 0.05$).

[A,B]Within a variable, means in a column with same letter do not differ ($P > 0.05$).

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

[2]Fecal samples were collected before transportation (d –1), day of transportation (d 0), and 3 d after transportation (d 3).

than in control horses ($P < 0.05$). In supplemented horses, fecal pH increased between d –1 and 0 ($P = 0.02$), and remained stable from d 0 to 3, whereas it decreased from d 0 to 3 in control horses ($P = 0.03$). The dietary treatment did not affect the other fermentative variables and their evolution over time. From d –1 to 0, no change was observed. From d 0 to 3, total VFA concentration and acetate + butyrate/propionate ratio decreased ($P = 0.02$ and $P < 0.01$, respectively), and the proportion of propionate increased ($P < 0.01$), whereas the proportions of acetate and butyrate remained stable. The concentrations of D-lactate, L-lactate, and total lactate remained stable throughout the experiment and were on average 1.20, 0.42, and 1.62 mmol $\cdot L^{-1}$, respectively.

Molecular Analysis of Fecal Bacterial Communities

Within each dietary treatment, patterns generated from pooled samples obtained at d –1 and 0 clustered together, and the pattern from pooled samples obtained at d 3 appeared to be clearly different (Fig. 1). The percentage of similarity between profiles at d –1 and 3 was greater in supplemented horses than control horses (63% vs. 22%, respectively). In yeast-supplemented horses, the number of molecular species in the fecal microbiota was 14 to 25% greater than control horses, regardless of day of sampling.

Table 3. Bacterial counts in feces of horses with or without supplementation (control), with live yeasts and subjected to transportation[1]

Item	Dietary supplementation	Day of sampling[2]			SE	*P*-value		
		−1	0	3		Day	Diet	Interaction
Total anaerobic bacteria, $\log_{10}$ cfu/g fresh weight	Control	7.8[B]	8.0	8.0	0.1	0.14	0.05	0.02
	Live yeast	8.5[aA]	7.8[b]	8.1[ab]				
Cellulolytic bacteria, $\log_{10}$ MPN[3]/g fresh weight	Control	3.0	2.9	2.9	0.23	0.66	0.08	0.87
	Live yeast	3.3	3.3	3.1				
Lactate-using bacteria, $\log_{10}$ cfu/g of fresh weight	Control	7.0	7.1	7.3	0.2	0.56	0.04	0.54
	Live yeast	7.5	7.3	7.5				
Lactobacillus spp., $\log_{10}$ cfu/g of fresh weight	Control	5.7	5.6	6.0	0.3	0.81	0.20	0.50
	Live yeast	6.3	6.0	5.9				
Streptococcus spp., $\log_{10}$ cfu/g of fresh weight	Control	7.5	7.5	7.6	0.2	0.10	0.55	0.09
	Live yeast	7.9	7.2	7.7				

[a,b]Within a row, means with same letter do not differ ($P > 0.05$).

[A,B]Within a variable, means in a column with same letter do not differ ($P > 0.05$).

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

[2]Fecal samples were collected before transportation (d –1), day of transportation (d 0), and 3 d after transportation (d 3).

[3]MPN = most probable number.

DISCUSSION

The present work was conducted to assess the effect of an abiotic factor (transportation stress) and a biotic factor (yeast supplementation) on the fecal bacterial communities, using both cultural and molecular approaches, and microbial activities in horses. Feces are often used for assessing the condition of the intestinal ecosystem in both humans and animals (Lamendella et al., 2011, Hildebrand et al., 2012). Although colonic and fecal microbiota may differ in terms of composition and fermentative activities (Da Veiga et al., 2005; Sadet-Bourgeteau et al., 2010), changes in feces appear to be appropriate markers for changes in the colon of horses (Julliand and Goachet, 2005; Müller et al., 2008). In our study, fecal samples were used to assess the impact of transportation on the intestinal bacterial ecosystem in horses. Fecal samples at d –1 served as a baseline for each horse. The TTGE analysis revealed that bacterial profiles obtained at d 0 were not different from those obtained at d –1. As clear differences between profiles appeared later at d 3, sampling feces immediately after transport may not have been totally appropriate to detect a response of the intestinal bacterial populations to transportation in the feces. However, empirical evidence indicated that the colon transit time was accelerated in horses during transportation. Given the fact that intestinal transit duration has been described to range between 18 and 42 h in normal conditions (Cuddeford et al., 2010; Goachet et al., 2009), collecting fecal samples at 12, 24, and 48 h after transportation would have been more appropriate to provide an acute response. At this stage, there is no clear explanation for the differences we observed between d –1 and 0. As the DM of feces has been reported to decrease during transport (Goachet et al., 2003), it is possible that the biochemical conditions were altered in the feces, and, thereby, partly changed some bacterial populations. Further analyses on feces are required to confirm this.

Effects of transportation on the bacterial communities were previously reported by Boensma et al. (2006), in which 1 group of bacteria disappeared after transportation, using microbial community profiling and characterization but was not described further. In other respects, Goachet et al. (2003) reported greater concentrations of total anaerobes and lactate utilizers, and decreased concentration of streptococci in colonic contents of horses 3 d after transportation. In our study, we measured changes in fecal concentrations of some fermentation end products between d 0 and 3, concomitantly with alterations in bacterial community structure. This indicated that transportation had an impact on the intestinal bacterial communities and its fermentative activity, which was measurable in feces 3 d after transportation. As reported in horses fed high-starch diets (Julliand et al., 2001; Medina et al., 2002) or after an abrupt incorporation of barley in the diet (de Fombelle et al., 2001), the increase in the proportion of propionate after transportation could result from greater conversion of lactate into propionate by lactate-using bacteria. As lactate concentration was not altered by transportation, we suggest that more lactate would be produced but would be more efficiently converted into propionate. Moreover, concomitantly with the decrease in total VFA concentration from d 0 to 3, the lower acetate + butyrate/propionate ratio could also reflect a negative impact of transportation on the activity of the plant cell wall-degrading bacterial group. In horses fed a high-starch diet, the activity of most

Table 4. The pH, concentration of total VFA, molar proportions of acetate, propionate, and butyrate, and the acetate + propionate:butyrate ratio measured in the fecal juice of horses with and without supplementation (control), with live yeasts and subjected to transportation[1]

Item	Dietary supplementation	Day of sampling[2]			SE	*P*-value		
		−1	0	3		Day	Diet	Interaction
pH	Control	6.83^{Aab}	7.03^{Aa}	6.76^{b}	0.09	0.02	<0.01	0.04
	Live yeasts	6.43^{Ba}	6.74^{Bb}	6.80^{b}				
Total VFA, mmol/L	Control	47.33	48.38	27.25	6.64	0.02	0.15	0.17
	Live yeasts	40.50	62.67	42.81				
Acetate/total VFA, %	Control	73.6	72.0	71.2	1.0	0.11	0.12	0.29
	Live yeasts	73.5	75.0	72.1				
Propionate/total VFA, %	Control	15.2	14.8	17.7	0.8	<0.01	0.31	0.93
	Live yeasts	14.7	14.3	16.8				
Butyrate/total VFA, %	Control	6.4	6.8	6.6	0.6	0.98	0.27	0.60
	Live yeasts	6.4	5.8	6.2				
Acetate + butyrate/ propionate	Control	5.3	5.3	4.4	0.3	<0.01	0.13	0.80
	Live yeasts	5.4	5.7	4.9				

[a,b]Within a row, means with same letter do not differ ($P > 0.05$).

[A,B]Within a variable, means in a column with same letter do not differ ($P > 0.05$).

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

[2]Fecal samples were collected before transportation (d –1), day of transportation (d 0), and 3 d after transportation (d 3).

enzymes involved in fiber degradation decreased (Jouany et al., 2009) and acetate concentration was decreased in intestinal content without changes in cellulolytic bacteria concentration (Medina et al., 2002). These data illustrated that cellulolytic bacteria could be highly susceptible to changes in the intestinal ecosystem after transportation, which may have an impact on the fermentation pattern. Our results indicate that a transportation of 2 h could disturb the intestinal microbial activity, increasing the risk of triggering microbial dysbiosis on a longer term in the equine large intestine.

The changes we observed in the structure of the bacterial communities and in some fecal fermentative variables could result from a physiological stress response of the host because of the communication between the neuroendocrine system, gastrointestinal tract physiology, and commensal microflora (Freestone et al., 2008). Our horses were indeed stressed by the process of transportation as demonstrated by the increase in WBC counts and neutrophil/lymphocyte ratio (Stull et al., 2008). Several animal studies have indicated recently that environmental changes can cause stress and modify the composition of intestinal microbiota (O'Mahony et al., 2009; Bailey et al., 2010). These modifications are associated with increased susceptibility to inflammatory stimuli in the intestinal tract. Moreover, germ-free animal studies showed that experimental perturbation of the microbiota can alter behavior (Collins and Bercik, 2009). Live yeast supplementation induced a lower physiological response to transport stress of 1 blood variable (i.e., WBC counts), which could indicate that supplemented horses were less stressed. Further research is needed to confirm these findings, as several studies have reported these types of effects on the gut-brain axis for different probiotics (Collins and Bercik, 2009).

Our data showed that *Saccharomyces cerevisiae* CNCM I-1077 were able to reach and survive in the large intestine of horses. As no more yeast were found in the feces of supplemented horses after the end of supplementation, we suggest that the supplemented yeast strain did not establish in the large intestine, in agreement with results observed with other strains of *S. cerevisiae* (Medina et al., 2002; Desrochers et al., 2005, Gobert et al., 2006). When yeasts were fed to horses (3.4×10^6 cfu/g DM of feedstuff on average), the mean concentration of viable yeast cells was 3.9×10^3 cfu/g of fecal content, a result lower than that previously reported in the feces of horses (7.8×10^4 cfu/g of feces) receiving daily 4.5×10^6 cfu/g DM of feedstuff (Gobert et al., 2006).

At d –1, fecal pH was lower in supplemented horses, which is not in agreement with previous studies that reported cecal pH did not change when live yeast was supplemented to horses receiving a similar quantity of starch (252 g/100 kg BW · d^{-1} vs. 180 g/100 kg BW · d^{-1} in our study; Medina et al., 2002) or even increased when supplemented horses received similar hay:concentrate (Moore et al., 1994). Concomitantly, we observed a slight increase in total anaerobe counts (+0.7 $\log_{10}$ cfu/g fresh feces) in supplemented horses compared with controls. However, no changes in VFA and lactate were observed in supplemented horses at d –1. Because we worked on feces, alterations of fermentative variables might be less important because of their absorption through the intestinal wall or their utilization by other microorganisms along the large intestine.

Regardless of day of sampling, fecal bacterial concentration of lactate utilizers was greater when live yeast was fed. Although these differences were quite small, this could indicate that lactate utilizers were greater in the large intestine of horses, in accordance with data obtained in cecal contents of horses supplemented with yeast (Jouany et al., 2009). As demonstrated in in vitro studies (Chaucheyras et al., 1996), stimulation of lactate-utilizing bacteria in the large intestine of horses could be explained by the supply of growth factors by yeasts. In control horses, fecal pH decreased after transport, whereas it did not change in supplemented horses. Although no

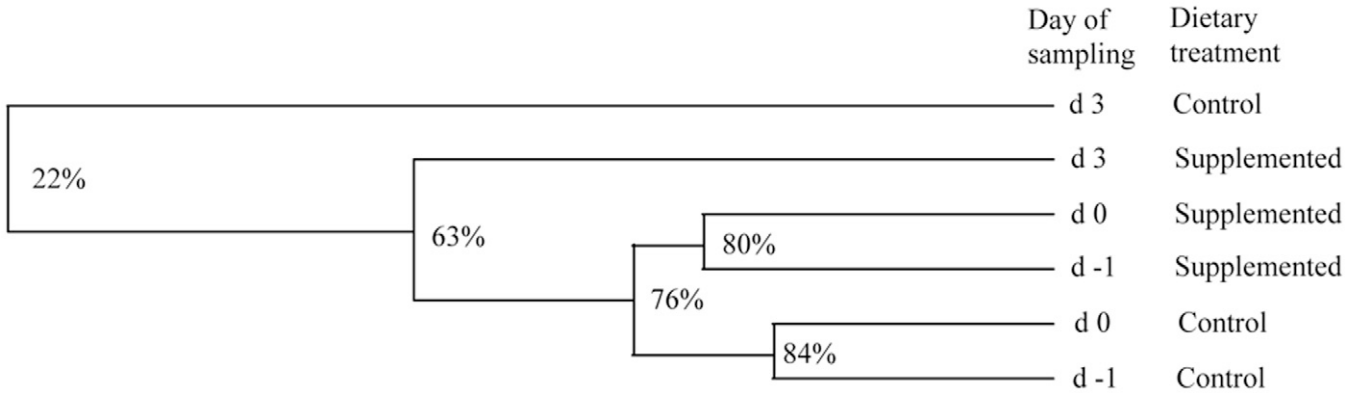

Figure 1. Temporal temperature gradient electrophoreses-derived dendrogram of fingerprint similarities, illustrating the effects of day of sampling (d –1, 0, and 3) and dietary treatment (supplemented and control) on the bacterial communities. The similarity among profiles was calculated with the Dice's Similarity Coefficient and clustering was done with the unweighted pair group method, using algorithm averages.

change in fecal lactate concentration could be observed in supplemented horses, we hypothesize that the growth of lactate-utilizing bacteria in supplemented horses may have limited alterations in microbial activity associated with transportation and, thereby, helped intestinal pH to stabilize as reported in the cecum of horses (Medina et al., 2002) and also in the rumen (Chaucheyras-Durand et al., 2008). Such positive effects could support the slight increase in cellulolytic bacterial concentrations in yeast-supplemented horses as reported previously (Moore et al., 1994). We presumed that this live yeast additive could be used in horses to help stabilize the digestive ecosystem during transportation, as demonstrated when horses are fed a high-starch diet (Medina et al., 2002; Jouany et al., 2009). This could be supported by the bacterial structure that appeared less affected by transportation when yeast was supplemented as shown by the greater percentage of similarity between profiles at d –1 and 3 in supplemented horses. Moreover, regardless of day of sampling, the number of molecular species was greater in supplemented horses compared with control horses, signifying a greater bacterial diversity. In clinical human trials, probiotic therapy was associated with increased intestinal bacterial diversity and maintenance of remission in patients suffering from inflammatory bowel diseases (Kühbacher et al., 2006), indicating that greater diversity may confer a beneficial effect for the host. In our study, we chose to pool samples before molecular analyses to test the global effect of transportation with or without yeast supplementation on fecal bacterial populations. Sample pooling before fingerprint analysis appeared to be an effective strategy to monitor changes in the dominant bacterial communities on external factors. Nevertheless, as individuals can react differently in stressful situations, such as during transportation for horses, our TTGE results need to be confirmed by taking into account the potential individual effect. Further fingerprint analyses on a greater number of horses are required.

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