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Effect of dehydrated alfalfa on equine gastric and faecal microbial ecosystems

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ABSTRACT

Disturbances of the gastric and hindgut ecosystems can lead to damage of the mucosa in the stomach and to intestinal colic. Some studies suggested that alfalfa could have preventive and healing capacities against gastric ulcerations. Preliminary data suggest that buffering capacity of alfalfa could also be beneficial for the hindgut ecosystem. The objective of this study was thus to assess the effect of dehydrated alfalfa pellets on the bacterial composition and the microbial activity of the equine gastric and faecal ecosystems. Six adult horses allotted in three pairs were studied in a 3 diets x 3 periods Latin square design experiment. During the 18-days experimental period, horses were fed grass hay (57% of total DMI) plus rolled barley and high-protein pellets (27% and 16% of total DMI) that represented a total intake of 1.8% BW / day. High-protein pellets consisted of alfalfa (obtained from 2 different processes, ALF – 2.35 mcal/kg DM, 16.9% DM CP, 27.2% DM NFC, 10.8% DM ash, 2.1% DM Ca) and sunflower meal (SFM – 2.26 mcal/kg DM, 29.1% DM CP, 13.8% DM NFC, 7.2% DM ash, 0.5% DM Ca) pellets. Daily starch intake (289 g) was divided into 2 equal meals. During the 5-day wash-out period, horses were fed grass hay and rolled barley (93% and 7% of total DMI respectively). On day 18 of the experimental period, faeces and gastric content were collected 3:00 and 3:30 h respectively after morning barley + high-protein pellets meal to assess microbial ecosystem parameters. Horses fed ALF had significantly lower gastric concentrations of amylolytic bacteria than the ones fed SFM ($P = 0.04$). On the contrary, amylolytic bacterial concentrations were higher in the faeces of horses fed ALF ($P = 0.01$) compared to SFM diets, as well as lactate-utilizing and pectinolytic bacterial concentrations ($P = 0.05$ and $P = 0.04$ respectively). However, no significant modification of the pH, VFAs proportions, VFAs and lactic-acid concentrations were measured in gastric content and faeces. Regarding gastric health, minimizing carbohydrate fermentation could be beneficial, although VFAs concentrations were not different in this study between ALF and SFM diets. In the hindgut, higher lactate-utilizing concentrations could be advantageous to limit hindgut acidosis. Further work is required to understand the mechanisms that altered gastric and faecal bacterial ecosystems in horses fed the alfalfa diet and to investigate whether other proportions or processed forms of alfalfa in the diet would be more beneficial.

1. Introduction

Along the different digestive segments, the equine gastro-intestinal tract (GIT) harbours rich and diverse microbial communities that play crucial roles for the nutrition and health of the host. Maintaining the delicate balance of this microbiota is essential to prevent microbial dysbiosis, and ensuing problems in terms of microbial fermentation which can contribute to acidifying the ecosystem and increase the occurrence of diseases. In fact, in the horse stomach, exposition to acidity has been identified as a primary cause of the squamous mucosa damage (Nadeau and Andrews, 2009). Similarly, in the horse hindgut, acidosis has been reported as a major cause of intestinal dysbiosis that could lead to laminitis or colic (Sadet-Bourgeteau and Julliand, 2010).

Diet is an important factor in determining gastrointestinal microbial community structure and thus affects the extent of microbial fermentation. Various strategies are pursued to minimize the acidity due to carbohydrate fermentation, like addition of antacids, pre- or probiotics (Sykes et al., 2015). Some studies have assessed the use of alfalfa (*Medicago sativa*) on gastric mucosa health. Compared to most raw materials fed to horses, alfalfa has a high intrinsic buffering capacity due to its high calcium and protein concentrations (Giger-Reverdin et al., 2002). When horses were fed an alfalfa hay-grain diet, gastric pH appeared higher during five hours after feed removal compared with horses fed bromegrass hay (Nadeau et al., 2000). Later work concluded

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that alfalfa hay exhibited preventive and therapeutic capabilities against gastric ulcers in horses compared to coastal Bermuda grass hay (Lybbert, 2007). More recently, it was suggested that alfalfa based feeds could be used to prevent 40–60% DMI fermented alfalfa) and heal 70–100% DMI fermented alfalfa) gastric ulcerations (Stowers et al., 2013). However, the mechanisms that confer alfalfa a beneficial effect on gastric health still remain to be elucidated.

To the authors' knowledge the impact of alfalfa on the equine hindgut microbial ecosystem has received little attention. Using an *in vitro* gas production technique, recent work measured substrate fermentation with equine faecal inocula and reported that inclusion of alfalfa negated the pH decrease observed with starch or inulin (Garber et al., 2016). Further work is required to clarify the mechanisms that explain *in vivo* the beneficial impact of alfalfa and its potential buffering effects in the hindgut.

The objective of this study was thus to assess the effect of dehydrated alfalfa pellets on the bacterial composition and the microbial activity of the equine gastric and faecal ecosystems.

2. Materials and methods

This protocol was approved by the French Minister of Research and the local ethics committee for animal experiments (C2EA Grand Campus Dijon).

2.1. Animals and management

Prior to the experimental trial, six French Trotters geldings (7–12 years of age; 511 ± 43 kg bodyweight; 3.3 ± 0.3 body condition score on the INRA-IFCE's scale) were examined by the veterinary practitioner in charge of the stable. All horses were considered healthy and able to enter the trial. Their deworming (Equimax®, Virbac, Carros, France) was updated one month before the experiment began. They were housed in 3.5 × 4.0 m individual free stalls bedded with straw and had free access to water. Horses were turned out in a dry paddock for 4.5 h during experimental periods and for 9.0 h during wash-out periods. During experimental periods, horses were trained six days per week in an automatic walker for 1.5 h at 2.5 m s^{-1}.

2.2. Experimental design

Before the trial horses were allotted in three homogeneous groups according to their age, weight and body condition in a 3 periods × 3 diets Latin square design. Each experimental period lasted 18 days and was separated from the next one by a 5-day wash-out period. Diets were double-blind tested. The three pairs of horses went through the three diets. During wash-out periods, horses did not receive any high-protein pellets. Each pair of horses was turned out in a separate dry paddock.

2.3. Diets

Diets were formulated to meet 100% of energy requirements for medium exercised horses (Martin-Rosset, 2012). During experimental periods, horses received a daily ration composed of 57% meadow hay (1.0 kg DM hay /100 kg BW), 27% rolled barley (0.5 kg DM /100 kg BW) and 16% high-protein pellets (0.3 kg DM /100 kg BW). Levels of high-protein pellets included in the diet were designed to mimic the average rate of inclusion in commercial feeds. Three different high-protein pellets were tested: two dehydrated alfalfa (ALF1 = low-protein alfalfa and ALF2 = high-protein alfalfa, Désialis, Châlons-en-Champagne, France) and sunflower meal (SFM) as decided by the alfalfa pellets manufacturer (Table 1). Barley and pellets were distributed together in two equal meals at 8:00 and 17:30. The increase in concentrate proportion in the ration was spread over one week. Hay was fed in two equal meals at 10:30 and 16:30. In each stall, horses had free access to a salt block. During wash-out periods, horses were fed grass hay and rolled barley (93% and 7% of total DMI respectively).

Table 1
Biochemical composition of the three high-protein pellets and mean total daily intake.

	Biochemical composition of the pellets				Mean daily intake depending on the diet (per 100 kg BW)		
	ALF1	ALF2	SFM		Diet ALF1	Diet ALF2	Diet SFM
DE (MJ / kg DM)	9.9	9.8	9.5	DE (MJ)	18.0	18.0	17.9
CP[a]	16.2	17.6	29.1	CP (g)	170	174	206
CF[a]	1.8	2.6	1.5	CF (g)	33	35	32
Starch[a]	2.2	1.3	1.2	Starch (g)	299	296	296
NFC[a]	29.2	25.2	13.8	NFC (g)	554	544	512
NDF[a]	41.4	44.5	48.4	NDF (g)	900	909	921
ADF[a]	31.3	34.7	34.7	ADF (g)	528	538	539
ADL[a]	8.2	8.3	10.7	ADL (g)	80	80	87
Ash[a]	11.4	10.1	7.2	Ash (g)	114	111	103
Ca[a]	2.22	2.07	0.48	Ca (g)	11.0	10.6	6.2
DCAD (mEq / kg DM)	347	224	183				

DE: digestible energy, CP: crude protein, CF: crude fat, NFC: non fibre carbohydrate, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, DCAD: dietary cation-anion difference.
[a] Percent of DM.

2.4. Samples collection and pH measurement

Gastric contents and faeces were sampled on day 18 of each experimental period. 300 g of faeces were collected in the rectal ampulla 3:00 h after the morning concentrate meal. The day of sampling the hay morning meal was given after gastric collection. A minimum of 100 mL gastric contents were sampled via a nasogastric tube according to the methodology described in Varloud et al. (2006), 3:30 h after the morning concentrate meal, which has been reported to be a discriminative sampling time (Varloud et al., 2007). After homogenization, part of each collected (gastric and faecal) sample was placed into a 60 mL flask filled to its maximum capacity to avoid the presence of oxygen and immediately inoculated for bacterial cultivation. The remaining part of each sample was filtered (100 µm diameter) and filtrates were immediately frozen in microtubes Eppendorf at −20 °C for further lactic acid (1.0 mL) and VFAs (1.0 mL added to a 0.1 mL preservative solution composed of 4.25% H_3PO_4 and 1.0% $HgCl_2$) analyses. pH was measured on fresh filtered gastric content and faeces with an electronic pH-meter (Cyberscan 500, Eutech Instruments, Strasbourg, France).

2.5. Bacterial cultures

Total anaerobes, amylolytic and lactate-utilizing bacterial concentrations were estimated both on gastric contents and faeces. Pectinolytic, xylanolytic and cellulolytic bacterial concentrations were assayed on faeces only.

Serial decimal dilutions of fresh gastric content and faeces were prepared under O_2-free CO_2 continuous flow in an anaerobic mineral solution (Bryant and Burkey, 1953). Total anaerobic bacterial counts were performed in roll tubes on a non-selective medium (Julliand et al., 1999; Leedle and Hespell, 1980). Lactate-utilizing, amylolytic, pectinolytic and xylanolytic bacterial concentrations were determined by numeration in roll tubes on selective media containing lactic acid, starch, pectin and xylan as the sole energy source respectively (Grimm, personal communication; Leedle and Hespell, 1980; Mackie and Heath, 1979). The number of viable bacteria was determined after 48 h of incubation at 38 °C as the average of colony counts from four replicates roll tubes (Hungate and Macy, 1973). The most probable number of