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Effect of live yeast supplementation on gastric ecosystem in horses fed a high-starch diet

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ABSTRACT

Starch/grain intake has been reported to be at risk for equine gastric lesions of the squamous mucosa (Equine Squamous Gastric Disease – ESGD). Exposition to VFAs and lactic acid, end-products of the fermentation occurring in the gastric ecosystem, can cause ESGD. As in the hindgut, supplementing yeast to high starch/grain diet could increase lactate-utilizing bacteria and thus limit the production of lactic acid and eventually the acidity. The objective of this study was to investigate the effect of two levels of *Saccharomyces cerevisiae* (Sc47) supplementation on gastric ecosystem in horses fed high-starch diet.

Six horses were assigned in a 3*3 Latin square design with three supplement treatments: 10.10^9 (dose 1) or 10.10^{10} (dose 10) cfu of zootechnical additive Sc47 per day or a control. Each experimental period lasted 21 days and was separated from the next one by a wash-out period of 21 days. During experimental periods, horses received a 70:30 ratio hay:barley (2.3 kg DMI / 100 kg BW / day) and 50 g per day of the additive. Gastric content samples were collected on day 18 of each experimental period 3 h and a half after morning barley meal to measure: pH, bacterial populations (total anaerobic, amylolytic and lactate-utilizing bacteria), and fermentation products (VFAs and lactic acid).

The observation of the stomach with the video-endoscope did not reveal any alteration of the squamous gastric mucosa. Total anaerobic bacteria and amylolytic concentrations were lower with Sc47 dose 10 compared to placebo ($P = 0.03$ and 0.04, respectively). With dose 10 of Sc47, lactate-utilizing bacteria concentrations decreased compare to Sc47 dose 1 ($P = 0.03$) and placebo ($P = 0.004$). No treatment effect was observed on total VFAs and lactic acid concentrations. Butyric and valeric acids concentrations showed higher concentration with Sc47 dose 1 compared to other treatments during period 1. Although, the pHs were numerically less acidic with live yeast, they were not significantly modified by the treatment. Globally, there was a dose effect of Sc47 on bacteria concentrations which all decreased or tended to decrease when the level of yeast increased in the diet. Further investigation is required to know whether live yeast supplementation could be part of the strategy to reduce gastric fermentation of starch and thereby help reducing the risk of ESGD.

1. Introduction

Starch/grain intake has been reported to be at risk for equine gastric lesions of the squamous mucosa (Equine Squamous Gastric Disease – ESGD). Stabled horses entering training and fed 6 kg of a concentrate feed per day within 14 days of being taken from pasture, all developed moderate to severe ESGD (Vatistas et al., 1999). It was later confirmed that feeding grain at 1% BW once a day, one hour before the morning hay was distributed, was an ulcerogenic diet for stabled horses (Frank et al., 2005). More recently, an epidemiological study quantified that starch intake exceeding 0.2% BW per day or 0.1% BW per meal was associated with an increase in severe gastric lesions regardless of the location and that feeding starch over 0.1% BW per meal was at risk for severe ESGD (Luthersson et al., 2009).

The most important cause of ESGD is the exposition to acids: hydrochloric acid but also volatile fatty acids (VFAs) and lactic acid (Nadeau and Andrews, 2009). VFAs in association with a pH ≤ 4 have been shown to reduce mucosal integrity and to affect the bioelectric properties of the mucosal tissue in vitro. These functional damages can eventually lead to sloughing and ulcers of the mucosa (Nadeau et al., 2003a, 2003b). VFAs and lactic acid are end-products of the bacterial fermentation occurring in the stomach. In the gastric content of horses fed high starch/grain diet, De Fombelle et al. (2003) reported lower concentration of lactic acid and higher concentration of VFAs compared

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to horses given the fiber/hay diet. As they enumerated concomitantly higher proportion of lactate-utilizing bacteria, the authors suggested that more lactic acid had been fermented into VFAs in the stomach of horses receiving the higher intake of starch, ensuring a less acidic gastric pH.

In the caecum and right ventral colon, live yeast supplementation was reported to increase lactate-utilizing bacteria and thus to limit the production of lactic acid and eventually the acidity in case of high-starch diets (Medina et al., 2002). Such effect could happen in the gastric ecosystem and be beneficial for limiting the pH decrease due to higher supply of dietary starch. To our knowledge, no data has been published on the impact of live yeast supplementation on gastric bacteria structure and activity. The objective of this study was thus to investigate the effect of two levels of *Saccharomyces cerevisiae* (Sc47) supplementation on gastric ecosystem in horses fed high-starch diet.

2. Material and methods

The project was approved by the French Minister of Research (file no. 05175.01) and the local ethical committee (C2EA Grand Campus Dijon).

2.1. Animals, health management and housing

After examination by the veterinary practitioner in charge of the stable, six geldings Trotteurs Français 7–12 years old, 530 ± 37 kg BW, average body condition score 3.25 on the INRA-IFCE's scale) entered the experimental trial. All horses were dewormed one month before the beginning of the trial with Equimax® (Virbac, France).

Along the trial, horses were housed in 13.5 m^2 individual boxes. The stable was organized to avoid cross contamination between horses belonging to different groups during the experimental periods. Boxes were bedded with wood shavings during the experimental periods and with straw during the wash-out periods.

Horses had access to dry paddocks for five hours daily during experimental periods and for nine hours daily during wash-out periods. Only horses of a same group had access to the same paddock. During experimental periods, horses were exercised in an automatic walker 1 h per day, five days per week at 2.5 m/s.

2.2. Experimental design

The six horses were allocated into three homogenous groups according to their age, weight and body condition in a 3 periods x 3 treatments Latin square design. Each experimental period lasted 21 days and was separated from the next one by a 21 days wash-out period. The treatments were double-blind tested. All pairs of horses went through the three treatments: dose 1, dose 10 and placebo. During wash-out periods, horses did not receive any treatment.

2.3. Diets

Diets were chosen to mimic French feeding practices conducted in horse riding schools and were formulated to provide 100% energy according to the French requirements for horses at light work (Martin-Rosset, 2012).

During experimental periods, horses were fed 2.3% BW DM per day of grass hay (70% of total DMI) and rolled barley (30% of total DMI) (Table 1). Barley was gradually introduced in the ration over 7 days to minimize dietary stress. Daily starch intake represented 0.33% BW per day. The morning (8:00) barley meal was twice larger than the evening (17:30) one. Hay was distributed in two equal meals at 10:00 and 16:30. In each box, horses had free-access to water and a salt block. During wash-out periods, horses were fed grass hay only ad libitum.

During the three experimental treatments horses received either 10.10^9 cfu (dose 1) or 10.10^{10} cfu (dose 10) of *S. cerevisiae* Sc47 (Actisaf®, Lesaffre, Marcq-en-Barœul, France), as decided by the yeast manufacturer, or a placebo. The feed additive was mixed with 50 g corn cobs and added top-dress to the morning barley meal.

Table 1
Biochemical composition of hay and barley (on a DM basis).

	Hay	Barley
DM (%)	90.7	89.4
DE (MJ/kg DM)	8.37	14.76
CP[a]	5.66	11.88
CF[a]	1.70	2.11
Starch[a]	0.55	54.69
NDF[a]	67.25	17.85
ADF[a]	41.75	6.87
ADL[a]	6.17	1.67

DE: Digestible Energy, CP: Crude Protein, CF: Crude Fat.
[a] Percentage of dry matter.

2.4. Samples collection

On day 18 of each experimental period, a minimum of 20 mL gastric content was collected via a nasogastric tube 3:30 h after the morning barley meal (Varloud et al., 2006). The day of sampling the hay morning meal was given after gastric collection.

After homogenization, part of gastric sample was placed into a 60 mL flask filled to its maximum capacity to guarantee anaerobic conditions 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.

2.5. Microbial analyses

Decimal dilutions of fresh gastric content were prepared under O_2-free CO_2 in an anaerobic mineral solution (Bryant and Burkey, 1953). Total anaerobic bacteria counts were performed in roller tubes under CO_2 gas phase on a non-selective medium (Julliand et al., 1999; Leedle and Hespell, 1980). Lactate-utilizing and amylolytic bacteria concentrations were determined by numeration in roller tubes under CO_2 gas phase on selective media containing lactic acid and starch as the sole energy source respectively (Grimm, personal communication; 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).

2.6. Biochemical analyses

Gastric content pH was measured immediately after collection with an electronic pH-meter (Cyberscan 500, Eutech Instruments, Strasbourg, France) on fresh juice (Jouany, 1982). L- and D-lactic acid were assayed with an enzymatic reaction procedure (Megazyme, Wicklow, Ireland) and quantified spectrophotometrically at 340 nm (MRX Revelation, Dynatech Laboratories, Guyancourt, France). VFAs concentrations in gastric content samples were measured by gas chromatography with a Clarus® 500 gas chromatograph (PerkinElmer, Courtabœuf, France). All measurements were duplicated.

2.7. Statistical analyses

Logarithmic transformations were performed on bacterial counts before statistical analysis. For each parameter (microbial counts, pH, VFAs, L- and D-lactic acid), statistical analysis was realized using the mixed procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC, USA).