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Effects of different amounts of *Saccharomyces cerevisiae* supplementation on apparent digestibility and faecal parameters in horses fed high-roughage and high-concentrate diets

F.M.P. Taran [a,b,*], A.A.O. Gobesso [b,**], I.V.F. Gonzaga [b], R. Françoso [b], T.N. Centini [b], C.G. Moreira [b], L.F.P. Silva [b]

[a] *Departamento de Nutrição Animal e Pastagens, Instituto de Zootecnia, Universidade Federal Rural do Rio de Janeiro, BR 465-Km 7, 23851-970 Seropédica, Rio de Janeiro, Brazil*

[b] *Departamento de Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo-USP, Av. Duque de Caxias Norte, 225, 13635-900 Pirassununga, São Paulo, Brazil*

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ABSTRACT

The aim of this study was to evaluate the influence of different amounts of *Saccharomyces cerevisiae* (*SC*) supplementation in high-roughage (HR) and high-concentrate (HC) diets on apparent total tract digestibility (ATTD), faecal microbial profile and faecal pH. Eight gelding miniature horses, about 36-months-old with an average weight of 113 ± 12 kg were randomly assigned into a double 4×4 Latin Square. Two distinct experiments of 4 periods each were conducted with *SC*-supplementation of 0 (control), 10, 20 and 30 g (5×10^8 cfu/g), per animal per day. Experiment 1 used a HR diet (70% grass hay, 30% concentrate) and experiment 2 used a HC diet (30% grass hay, 70% concentrate). Each experimental period consisted of 23 days: 15 adaptation days, 5 days for data collection and a 3-day-wash-out interval between periods. Nutrient digestibility was evaluated by total faecal collection for each animal. The cellulolytic and lactic acid bacteria populations in faeces were calculated and faecal pH was measured. In the HR diet, *S. cerevisiae* supplementation was not associated with any changes in ATTD of nutrients, microbial profile in faeces and did not increase faecal pH values. In the HC diet, only the addition of 20 g *SC* reduced crude protein digestibility when compared with the control group and 30 g *SC*. For the other variables of digestibility the amounts of *SC* supplementation did not differ from control group. Furthermore, the microbial profile in faeces and faecal pH were not affected by *S. cerevisiae* supplementation. The present study showed that the *S. cerevisiae* strain used was not able to induce any changes in the equine hindgut and did not improve the fibrolytic activity with high-roughage and high-concentrate diets.

1. Introduction

During evolution, horses developed an efficient digestive system to convert structural carbohydrates of plants into energy by volatile fatty acids, resulting from microbial fermentation and used in metabolic processes. However, the energy requirements of horses in activity are usually supplied by adding grain cereals rich in starch. Variations in diet composition, nutritional fibre quality, neutral detergent fibre (NDF)/starch ratio have been linked to changes in the gastrointestinal tract of horses (Drogoul et al., 2001; Julliand et al., 2001; Medina et al., 2002; Jouany et al., 2008). The impact of changing high-fibre to high-starch diets is known to increase amylolytic bacteria (e.g., *Streptococci and Lactobacilli*) and lactate concentration as well as to decrease pH and cellulolytic activity causing gastrointestinal disorders, such as colic and laminitis (Julliand et al., 2001; Medina et al., 2002). Therefore, the addition of live yeast (*Saccharomyces cerevisiae)* has been used in horse diets to improve the digestibility of nutrients, the development of the microbial fibrolytic population and to limit the extent of undesirable changes in the intestinal ecosystem when horses are fed a high-starch diet (Medina et al., 2002; Morgan et al., 2007; Jouany et al., 2008, 2009; Agazzi et al., 2011). However, some studies have reported that the inclusion of live yeast to horse diets has no advantages (Glade and Biesik, 1986; Hall et al., 1990; Furtado et al., 2010; Mackenthun et al., 2013). The contradictory

* Corresponding author at: Departamento de Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo-USP, Av. Duque de Caxias Norte, 225, 13635-900 Pirassununga, São Paulo, Brazil.
** Corresponding author.
E-mail addresses: fernanda.taran@gmail.com (F.M.P. Taran), cateto@usp.br (A.A.O. Gobesso).

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results on the effects of adding dietary yeast may be associated with changes in diet composition, strain viability and yeast concentration used in the different studies. This study aimed to evaluate the effect of different amounts of *S. cerevisiae* supplementation in two distinct diets (high-roughage and high-concentrate) on the total apparent digestibility of nutrients, microbial profile in faeces and faecal pH. We hypothesised that increasing amounts of live yeast would lead to a decrease in the concentration of lactic acid bacteria, promote the development of cellulolytic bacteria, increase pH values and the digestibility of dietary components, especially the fibre fraction.

2. Material and methods

2.1. Animals

Eight gelding miniature horses, about 36-months-old with an average weight of 113 ± 12 kg were used. The animals were individually housed in box stalls, bedded with wood shavings and turned out into a sand paddock for 2 h/day, except during the collection period. Water and mineral salt were provided ad libitum. The study was conducted under the license from the "Ethics Committee for Animal use" (No. 189/2009) of the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (FMVZ/USP).

2.2. Experimental design and treatments

Two distinct experiments were conducted to use different NDF/starch ratios and to evaluate the impact of different amounts of live yeast supplementation: 0 (control), 10, 20 and 30 g of *S. cerevisiae* (*SC*) preparation with 5×10^8 cfu/g (Equihealth-Yes Sinergy®, strain Y-904, AB Vista, Jandaia do Sul, Brazil), per animal per day. Horses were assigned into a double 4 × 4 Latin square in each trial period. *Experiment 1* used a high-roughage diet (HR) consisting of 70% grass hay (*Cynodon dactylon, cv.* Tifton-85) and 30% commercial concentrate pellets (Corcel Tradicional-Presence®, São Paulo, Brazil). *Experiment 2* used a high-concentrate diet (HR) consisting of 70% commercial concentrate pellets and 30% grass hay (*Cynodon dactylon, cv.* Tifton-85). The compositions of the diets are shown in Table 1. Both the HR and HR diets were offered at an intake level of 20 gDM kg^{-1} body weight (BW) per day (NRC, 2007) and divided into two equal meals at 07:00 and 19:00 h. The *SC* preparation was top-dressed on the pellets (the total quantity divided by the two meals). The HR diet was formulated to allow a starch intake of 2.05 g starch kg^{-1} BW per meal, in order to reach the limit of prececal starch digestion proposed by Kienzle (1994). Horses were weighed before each diet adaptation period to adjust the feed allowance to their body weight.

2.3. Experimental period and sampling

Each experiment consisted of four periods of 23 days: 15 adaptation days and 5 days for data collection and there was a 3-day-wash-out interval between periods (Gobert et al., 2006). Feed samples were collected for each initial experimental period, mixed, homogenised and frozen (−20 °C) for further analysis. Apparent total tract digestibility (ATTD) of nutrients was estimated by the total faecal collection method with the animals kept in pens with concrete floors and no bedding. The faeces collected every 24 h were placed in plastic bags, weighed and identified by animal. After the data collection period the total faeces excreted per animal was homogenised and 10% was removed, wrapped in plastic bags and frozen at −20 °C for further analysis. To determine the microbial profile in the faeces and faecal pH, samples were collected four hours after the morning meal, directly from the rectum, once a day during the data collection period.

Table 1
Ingredients and mean chemical composition of high-roughage (HR) and high-concentrate (HC) diets.

Variable	Diets	
	HR[a]	HC[b]
Ingredients, %		
Concentrate[c]	30	70
Hay	70	30
Bromatological Composition, %DM		
Dry Matter (DM)	88.3	88.9
Crude protein (CP)	11.6	13.9
Neutral detergent fibre (aNDFom)	63.1	50.2
Acid detergent fibre (ADFom)	26.4	19.9
Ash	8.4	10.7
Starch	10.5	20.5
Organic matter (OM)	91.6	89.3

[a] Composition HR hay: 9.51% CP, 74.08% aNDFom, 35.32% ADFom, 1.79% starch.
[b] Composition HC hay: 7.69% CP, 79.64% aNDFom, 42.58% ADFom, 063% starch.
[c] Basic composition HR and HC concentrate: Wheat bran, corn germ meal, corn gluten meal-21, rice bran, soybean bran, broken rice, sugar cane molasses, calcitic limestone, sodium chloride, sodium bicarbonate, iron sulphate, copper sulphate, manganese monoxide, zinc oxide, calcium iodate, cobalt sulphate, vitamins (A, B1, B2, B6, B12, D3, E), folic acid, niacin, pantothenic acid, lysine, methionine, propionic acid (16.58% CP, 37,61% aNDFom, 10,15% ADFom, 29.01% starch).

2.4. Microbial analyses and faecal pH

For microbial analysis, approximately 20 g of fresh faeces were collected, placed into sterile falcon tubes and immediately frozen at −80 °C until analysis. Samples were subjected to DNA extraction. Total cellular DNA was extracted from 0.2 g samples using the QIAamp DNA Stool Mini Kit (QIAamp® DNA Stool Handbook, 2012) and the genetic profile of each species were systematically checked using the real-time PCR method (qPCR) and specific primers. The specific primers used were for *Fibrobacter succinogenes* (F:5′-GGTATGGGATGAGCTTGC-3′, R:5′-GCCTGCCCCTGAACTATC-3′), *Lactobacillus* genus (F:5′-AGCAGTAGGGAATCTTCCA-3′, R:5′-CACCGCTACACATGGAG-3′) and *Ruminococcus flavefaciens* (F:5′-TCTGGAAACGGATGGTA-3′, R:5′-CCTTTAAGACAGGAGTTTACAA-3′). Total bacteria population was quantified with the primer (F:5′-GTGSTGCAYGGYTGTCGTCA-3′, 5′-ACGTCRTCCMCACCTTCTC-3′) (Maeda et al., 2003) and the relative populations of each bacteria were calculated using the ΔΔCt method (Livak and Schmittgen, 2001). Faecal pH was assessed using 50 g of fresh faeces mixed with 50 ml of deionized water and pH was measured using a portable pH metre (Quimis®, Diadema, São Paulo, Brazil) (Berg et al., 2005).

2.5. Analytical methods

Hay, concentrate and faecal samples were analysed for content of dry matter (DM), organic matter (OM), ash, crude protein (CP) (micro-Kjeldahl, $N \times 6.25$) and starch in accordance with the AOAC (2000). Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom) and acid detergent fibre expressed exclusive of residual ash (ADFom) were analysed according to Van Soest et al. (1991) and Robertson and Van Soest (1981). Apparent total tract digestibility of nutrients was calculated by the following formula: ATTD (%)=(intake of nutrient