Interaction between the sequence of feeding of hay and concentrate, and boiling of barley on feed intake, the activity of hydrolytic enzymes and fermentation in the hindgut of Arabian mares

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Summary
The interaction between the sequence of feeding of hay and concentrate and the hydrothermal processing of barley in alleviating concentrate effects on intake, and hindgut fermentation in horses was tested. Six Arabian mares (4–10 years of age, 410 ± 35 kg body weight) were used to evaluate the effects of feeding sequence (FS) and type of barley (TB) on intake, and faecal volatile fatty acids (VFA), activities of α-amylase (AA: EC 3.2.1.1), carboxymethyl cellulase (CMCase: EC 3.2.1.4), microcrystalline cellulase (MCCase: EC 3.2.1.91) and general filter paper degrading activity (FPD).

Mares were offered a ration of air-dried alfalfa and concentrate (70:30 as-fed) in four subsequent periods of 14 days including 8 days of adaptation and 6 days of sampling. In each period and each meal, mares received concentrate either 30 min after (HC) or 30 min before (CH) alfalfa hay. Barley was either milled or boiled in water. Rectal samples were grabbed directly from rectum once per period. Mares subjected to CH had higher dry matter intakes than mares under HC regime. The acetate:propionate ratio (A:P ratio) in rectal content was higher with CH than HC. The AA activity was higher under CH than under HC. Mares fed boiled barley had lower rectal concentrations of VFA and propionate and a higher A:P ratio than mares fed milled barley. Furthermore, the rectal content showed a higher MCCase activity but a lower AA activity when mares were fed boiled compared with milled barley. Interactions between FS and TB were observed with respect to CMCase activity, and concentrations of propionate and valerate. In conclusion, the present results suggest that both, feeding concentrate before hay and boiling the barley, might improve the hindgut environment in Arabian mares, and that the two measures were mostly additive and sometimes even synergistic.

KEYWORDS
α-amylase, boiled barley, carboxymethyl cellulase, hindgut fermentation, horse, microcrystalline cellulase
Horses are grazing animals adapted to forage-based diets (Harris et al., 2017). As post-gastric fermenting mammals, horses utilize dietary plant fibre with the help of enzymes of gut microorganisms (Frape, 2001). Feeding horses with forages alone is best suited to sustain an efficiently functioning hindgut microbial population (Harris et al., 2017). Still, concentrates based on cereals such as barley are indispensable due to the high energy demand of athletic horses. However, diets with high concentrate proportion fed to horses facilitate acidification of the hindgut digesta (Hudson, Cohen, Gibbs, & Thompson, 2001; Sadet-Bourgeteau, Philippeau, & Julliand, 2016) and perturbation of the caecal ecosystem (Hudson et al., 2001). Concentrate feeding was therefore reported to cause changes in the hindgut microbial ecosystem in horses (Grimaldi, Philippeau, & Julliand, 2017; Julliand, De Fombelle, Drogoul, & Jacotot, 2001; Moore & Dehority, 1993). In particular, the cellulolytic bacteria (Julliand et al., 2001) and the total population of functional bacteria in the colonic contents of horses (Philippeau, Sadet-Bourgeteau, Varloud, & Julliand, 2015) are compromised by concentrates like barley. In the literature, it has been shown that feeding concentrate after or along with the forage portion of the diet could, to some extent, help maintaining an appropriate hindgut physiochemical environment (Jensen, Austbø, & Tauson, 2012; Sadet-Bourgeteau et al., 2016; Zeyner, Geijller, & Dittrich, 2004). Another attempt to alleviate potential adverse effects of concentrate on hindgut fermentation could consist of the hydrothermal treatment of cereals to make their starch better accessible to digestion in the small intestine and thus reduce the amount of starch arriving in the hindgut and its adverse influence. Julliand, De Fombelle, and Varloud (2006) reported that hydrothermally processed and thus gelatinized starch of cereal grains through steam cracking of the membranes of the starch granules makes the starch well available to small intestinal digestion. Philippeau et al. (2015) demonstrated that using thermomechanically treated cereals limits the potential negative impact of starch on fibrolytic activity in horses. To our knowledge, the combination of a strategic feeding sequence (FS) and a hydrothermal processing of cereals with the purpose of improving hindgut fermentation in concentrate-fed horses has not yet been tested. Thus, it is still unknown whether or not there are interactions between these two approaches.

Therefore, the hypothesis tested in this study was that combining a feeding regime where concentrate is provided after the forage and boiling of the concentrate are additive in alleviating adverse concentrate effects on hindgut fermentation in adult healthy Arabian mares. The model feeds selected to test this hypothesis were hay as forage and barley either milled or boiled as concentrate. The state of hindgut fermentation was described by VFA concentration and profile as well as the activities of key hydrolytic enzymes, namely α-amylase (AA: EC 3.2.1.1), carboxymethyl cellulase (CMCase: EC 3.2.1.4), microcrystalline cellulase (MCCase: EC 3.2.1.91) and as an indicator of general fibre degradation activity, the filter paper degrading (FPD) activity in rectal contents.

2 | MATERIALS AND METHODS

2.1 | Animal, diets and treatments

All the procedures and protocols employed in this study were approved by the Institutional Animal Ethics Committee (IAEC) of Lorestan University, Iran.

Six Arabian mares (5–10 years of age; 410 ± 35 kg body weight; mean ± standard deviation) were used in a cross-over design in four consecutive 2-week experimental periods. Horses were kept in individual free stalls (3 × 4 m) with concrete floor bedded with straw. During the experiment, the horses had access to a sand paddock for 1 hr/day. Mares were fed individually with diets that were formulated to meet the recommendation for horses subjected to 1 h of light work according to NRC (2007). Experiment consisted of four consecutive 2-week experimental periods. Each experimental period consisted of 8 days for adaptation and 6 days for sampling. Horses were randomly divided into two groups of three (A and B). In period one, horses of group A were fed concentrate after (sequence HC) and those of group B prior the hay (sequence CH). In the subsequent period, this was reversed between groups A and B. The horses were offered dried alfalfa hay (per kg DM: 919 g organic matter [OM]; 147 g crude protein [CP]; 25 g ether extract [EE]; 695 g neutral detergent fibre [NDF]; 319 g acid detergent fibre [ADF]; 313 g crude fibre [CF]; 8.7 MJ/kg digestible energy [DE]; 6.8 MJ/kg metabolizable energy [ME]) at ad libitum access. As part of the concentrate, each horse was offered 1,950 g/day of barley grain.

The barley was composed as analysed in per kg of dry matter (DM of 948 g OM; 523 g starch; 98 g CP; 22 g EE; 291 g NDF; 203 g ADF; 61 g CF; 13.5 MJ/kg DE; 12.7 MJ/kg ME). In addition, each horse received 650 g/day of soybean meal (on g/kg DM: 935 g OM; 440 g CP; 16 g EE; 395 g NDF; 268 g ADF; 74 g CF; 13.8 MJ/kg DE; 10.2 MJ/kg ME). Barley grain for both treatments originated from a single batch and was either subjected to hammer mill (Poosam Industrial Group, Karaj, Iran) treatment (“milled barley”) or boiled in water without any previous processing (“boiled barley”). The barley grain was boiled in a big pan (ca. 30 L) where the grain was completely covered with water. It took between about 30 min to reach the boiling point. After 1 hr, the heater was turned off and the barley remained in the pot to expand, and gradually cool down for about 3 hr. As the final step, any remaining water in pan was filtered by one layer of cheese cloth and the starch retained on the cloth was re-added to the barley. The boiled barley was kept at room temperature to cool down and was offered to the horses thereafter. The horses had free access to fresh water and salt blocks. The horses were fed in two equal meals at 0800 and 1700 hr. In both meals, concentrate was offered either 30 min after (sequence HC) or 30 min prior the air-dried alfalfa hay (sequence CH).

The amount of feed offered, and orts were weighed per horse per day during the data collection time (between Day 9 and Day 14 of each period). Each day a representative sample of feed and about 10% of the totally collected feed refusals (if any) were taken. The samples