Intra-caecal fermentation parameters in ponies fed botanically diverse fibre-based diets

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Received 20 January 1999; received in revised form 4 October 1999; accepted 17 February 2000

Abstract

Intra-caecal fermentation parameters in caecally fistulated ponies offered fibrous diets of diverse botanical origin were examined in two experiments, A and B. In Experiment A three ponies were offered ca. 4 kg dry matter (DM) per day of either ground, pelleted hay cubes (HC), a 67:33 mix of oat hulls:naked oats (OH:NO) or a 75:25 mix of unmolassed:molassed sugar beet feed (SBF), whilst in Experiment B the same ponies were offered ca. 4 kg DM of a 50:50 mix of unmolassed sugar beet:hay cubes (USB:HC). In Experiment A, DM intakes (kg per day) were 3.75, 4.03 and 1.72; (S.E.D. 0.26) for the HC, OHNO and SBF diets, respectively, and in Experiment B, average intakes were 4.03 kg per day. (S.E. 0.03). Intra-caecal fermentation parameters were measured in samples of caecal chyme obtained via a permanent in-dwelling caecal fistula. During the three 5-day collection periods, samples were removed 5 h after the morning feed on days 2, 3, 4 and 5; and on Day 4 of each collection period hourly samples were obtained commencing 1 h after the morning feed and ceasing 7 h later. In both experiments, each diet maintained the intra-caecal pH above 6.5 and molar proportions of acetate above 700 mmol mol\(^{-1}\) throughout the 8 h measurement period. When the entire diet consisted of SBF, voluntary feed intake was reduced, indicating that SBF should not be fed to ponies as the sole dietary ingredient. Each of the diets maintained caecal pH well above the critical level of pH 6, which is regarded as the threshold of sub-clinical caecal acidosis in equids. Thus, these three fibre-based feeds can be incorporated into equine diets,

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PII: S0377-8401(00)00117-6
offering alternatives to hay as the forage component of the diet without compromising hindgut function. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Ponies; Fibre; Intra-caecal fermentation parameters

1. Introduction

By-products of the human food industry which are high in dietary fibre (dietary fibre = non-starch polysaccharides, NSP and lignin), such as sugar beet pulp, soya hulls and oat hulls are used extensively in diets for farm animals. Such by-products may also be useful in diets for equids as alternatives to hay, which is often of poor quality frequently precipitating respiratory disease (Clarke, 1992). The potential energy-yielding portion of dietary fibre is the NSP fraction, which may be fermented in the large intestine of equids to yield volatile fatty acids (VFA) and lactate which are subsequently absorbed and metabolised to yield ATP. Compared with those of hay, the NSP fractions of some of these alternative feeds are highly fermentable and, as a consequence on a weight-for-weight basis, make a greater contribution to the energy balance of the animal (Glinsky et al., 1976). Moreover, feeding a source of fibre of greater energy concentration than hay will reduce the necessity for feeding high levels of cereal-based concentrates.

A number of studies have examined various intra-caecal fermentation parameters in equids when starch-based concentrates were used to supplement basal forage diets (Stillons et al., 1970; Wolter et al., 1978; Goodson et al., 1988; Radicke et al., 1991). In horses, fluctuating levels of fermentation products, frequently caused by feeding high levels of cereal starch, can lead to a number of metabolic disorders, such as acidosis, and colic (Clark et al., 1990). Such disorders are rarely found in horses which are fed fibre-based diets (Jackson, 1998).

The objective of the experiments reported here was to examine intra-caecal concentrations of pH, VFA and lactate in ponies offered a range of fibrous feeds of diverse botanical origin.

2. Materials and methods

2.1. Experiment A

Three mature Welsh cross pony geldings, each fitted with a permanent in-dwelling caecal cannula (Cottrell et al., 1998) at the top of the caecum (caecal base), were used in a 3×3 Latin square changeover design consisting of three 21-day periods. Ponies were individually loose housed in pens bedded with wood shavings with water available ad libitum. Ponies were offered 4 kg per day, on an as-fed basis, one of three diets comprising either ground, pelleted hay cubes (HC), a 67:33 mix of oat hulls:naked oats (OH:NO) or sugar beet feed (SBF) in a 75:25 unmolassed:molassed mixture. To avoid any potential problems of choking or colic associated with stomach distension, diets were soaked in 1.5 times their own weight in water and fed in two equal meals per day at 09:00
and 17:00 hours. Thirty grams of a mineral and vitamin supplement (Table 1) were added to each meal. The 21-day periods consisted of a 16-day adaptation phase and a 5-day collection phase.

2.2. Chemical analysis of feeds

Composite samples of the feeds, milled to pass a 1-mm mesh screen, were analysed for dry matter (DM), organic matter (OM), acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP) and gross energy (GE) contents according to the methods of the Association of Official Analytical Chemists (1990).

2.3. Intra-caecal fermentation parameters

On days 18–21 of each period, samples of digesta were collected, 5 h after the 09:00 h meal, by suction from the caecum through an indwelling plastic tube (internal diameter 13 mm) attached to the cap of the cannula. Similarly, on Day 20 of each period caecal digesta samples were taken at 09:00 h and hourly thereafter until the 17:00 h meal. Sample pH was determined immediately after withdrawal of chyme from the caecum, using a Mettler–Toledo 320 pH meter (Mettler–Toledo, Beaumont Leys, UK). Thereafter, a 9-ml sub-sample was preserved with the addition of 1 ml of 1.8 M H₂SO₄ and stored at −20°C until thawed for VFA and lactate analysis. Acetate, propionate and butyrate concentrations (mmol l⁻¹) in the caecal digesta samples were subsequently determined by gas chromatography, according to the method described by Merry et al. (1995). Lactate levels were measured according to the colorimetric method described by Merry et al. (1995).

2.4. Experiment B

After completion of Experiment A, the ponies were offered 2 kg of 50:50 unmolassed sugar beet:hay cubes (USB:HC) diet twice daily on an as-fed basis. Thirty grams of
Minerals were added to each feed (see Table 1) for the 21-day experimental period. Feed, and caecal samples were taken from days 18 to 21 as described for Experiment A; and analysed as indicated above.

2.5. Statistical analysis

All statistical analyses were carried out using Genstat 5 (Lawes Agricultural Trust, 1993). Intra-caecal fermentation parameters measured 5 h following the 09:00 h meal on days 18–21 were subjected to analysis of variance. Due to the repeated nature of the hourly measurements, the intra-caecal fermentation parameters measured between the 09:00 and 17:00 h meals were summarised to give average values for the 0–3 and 4–8 h following the 09:00 h meal and analysed by split plot analysis of variance. In Experiment A, one pony ate very little of the SBF diet, so this measurement was treated as a missing value in the statistical analysis. All data in Experiment B are expressed as simple arithmetic means with their standard errors.

3. Results

3.1. Feed composition and dry matter intake

The chemical composition of the four experimental diets offered in experiments A and B are presented in Table 1. The CP contents of all experimental diets were between 80 and 90 g kg⁻¹ DM. Average pony live weight (LW) and dry matter intakes (DMI) for both experiments, A and B, are given in Table 2. When given SBF as the sole dietary ingredient in Experiment A, ponies consumed less than half of the 4 kg DM per day

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment A</th>
<th>Experiment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>LW (kg)</td>
<td>265</td>
<td>265</td>
</tr>
<tr>
<td>DMI (kg day⁻¹)</td>
<td>3.75 a</td>
<td>4.03 a</td>
</tr>
</tbody>
</table>

Intra-caecal fermentation parameters (measured 5 h following the 09:00 meal)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment A</th>
<th>Experiment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.50</td>
<td>6.65</td>
</tr>
<tr>
<td>Lactate (mmol l⁻¹)</td>
<td>0.30 a</td>
<td>3.73 b</td>
</tr>
<tr>
<td>TVFAb (mmol l⁻¹)</td>
<td>55.7 a</td>
<td>45.3 ab</td>
</tr>
</tbody>
</table>

VFA molar proportions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment A</th>
<th>Experiment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate (mmol mol⁻¹)</td>
<td>764 ab</td>
<td>739 a</td>
</tr>
<tr>
<td>Propionate (mmol mol⁻¹)</td>
<td>172 a</td>
<td>228 b</td>
</tr>
<tr>
<td>Butyrate (mmol mol⁻¹)</td>
<td>64 a</td>
<td>33 b</td>
</tr>
</tbody>
</table>

a Values in the same row not sharing common letters differ significantly (p<0.05).

b Total volatile fatty acids.
offered. However, when given USB:HC in a 50:50 mix in Experiment B, the ponies consumed all of their ration.

3.2. Intra-caecal fermentation parameters

The average intra-caecal fermentation parameters measured 5 h after the 09:00 h meal on days 18–21 of each period in both experiments are given in Table 2. No statistically significant differences \((p>0.05)\) in the pH values at 5 h after the 09:00 h meal were seen between diets in Experiment A, with mean values ranging from 6.50 for HC to 6.65 for OH:NO. In Experiment B, pH measured 5 h after the 09:00 h meal averaged 6.58. In Experiment A, total VFA (TVFA) levels were significantly lower \((p<0.05)\) for SBF compared with the HC, but not with the OH:NO diet, reflecting the lower dry matter intake (DMI) when SBF was offered as the sole diet. Lactate levels were significantly lower when ponies were fed HC and SBF at 0.3 and 0.89 mmol \(l^{-1}\), respectively, than when the OH:NO diet was fed, which produced four times the amount \((3.73 \text{ mmol } l^{-1})\) of lactate than the other two diets. The USB:HC diet given in Experiment B resulted in the highest TVFA levels of 66.4 mmol \(l^{-1}\), and, in common with the SBF and HC diets fed in Experiment A, produced only low levels of lactate, 0.31 mmol \(l^{-1}\), in the caecal chyme. The acetate molar proportion was significantly higher \((p<0.05)\) for SBF compared with OH:NO, but not with HC, when measured 5 h after the 09:00 h meal. In contrast, propionate levels were significantly higher \((p<0.01)\) for OH:NO, compared with SBF or HC in Experiment A. Butyrate molar proportions were significantly lower \((p<0.05)\) when OH:NO was fed compared with the other diets fed in Experiment A.

Figs. 1–6 show the average hourly changes in pH, lactate \((\text{mmol } l^{-1})\), TVFA \((\text{mmol } l^{-1})\), acetate, propionate and butyrate molar proportions \((\text{mmol mol}^{-1})\), respectively, measured between the 09:00 and 17:00 h meals for all the experimental diets. Throughout the 8 h sampling period in both experiments, pH was maintained above 6.5 and acetate molar proportions above 700 mmol \(mol^{-1}\), regardless of the botanical source of the fibre. The OH:NO diet in Experiment A resulted in pH values above 7 and TVFA levels below 20 mmol \(l^{-1}\) for the first 3 h after the 09:00 h meal, whilst values for the remaining diets were comparable throughout the sampling period. In addition, propionate levels were higher throughout the 8 h sampling period when OH:NO were fed compared with the other two diets.

Table 3 details the intra-caecal fermentation parameters measured throughout the day, summarised as average values for 0–3 and 4–8 h following the 09:00 h meal for both experiments A and B. Average pH was significantly higher \((p<0.05)\) at 7.37 during the 0–3 h period when OH:NO was fed, compared with 6.86 obtained during the 4–8 h period. Likewise, higher levels of lactate were present in samples obtained 4–8 h after feeding compared with those collected between 0–3 h. By contrast, there were no significant differences in TVFA concentrations or individual VFA molar proportions between the 0–3 and 4–8 h time periods when the OH:NO diet was fed. Similarly, values for pH, TVFA and individual VFA molar proportion did not differ significantly between the 0–3 and 4–8 h time periods when either HC or SBF were fed. In general however, TVFA concentrations and acetate molar proportions were lower, whilst propionate molar proportions were higher when OH:NO was fed compared to feeding either HC or SBF in
Fig. 1. Intra-caecal changes in pH following the 09:00-h meal in ponies offered 2 kg hay cubes (■), a 67:33 ratio of oat hulls:naked oats (●●), or sugar beet feed (○○) in Experiment A or a 50:50 mix of unmolassed sugar beet:hay cubes mix (●●) in Experiment B.
Fig. 2. Intra-caecal changes in total volatile fatty acid (TVFA) concentration (mmol l$^{-1}$) following the 09:00-h meal in ponies offered 2 kg hay cubes (– ■ –), a 67:33 ratio of oat hulls:naked oats (– ▲ –), or sugar beet feed (– ◆ –) in Experiment A or a 50:50 mix of unmoiassed sugar beet:hay cubes mix (50:50) (– ○ –) in Experiment B.
Fig. 3. Intra-caecal changes in acetate molar proportion (mmol mol$^{-1}$) following the 09:00-h meal in ponies offered 2 kg hay cubes (– ■ –), a 67:33 ratio of oat hulls:naked oats (– ▲ –), or sugar beet feed (– ◆ –) in Experiment A or a 50:50 mix of unmolassed sugar beet:hay cubes mix (– ● –) in Experiment B.
Fig. 4. Intra-caecal changes in propionate molar proportion (mmol mol$^{-1}$) following the 09:00-h meal in ponies offered 2 kg hay cubes (--), a 67:33 ratio of oat hulls:naked oats (--), or sugar beet feed (-- --) in Experiment A or a 50:50 mix of unmolassed sugar beet:hay cubes mix (-- --) in Experiment B.
Fig. 5. Intra-caecal changes in butyrate molar proportion (mmol mol$^{-1}$) following the 0900-h meal in ponies offered 2 kg hay cubes (– –), a 67:33 ratio of oat hulls:naked oats (– –), or sugar beet feed (– –) in experiment A or a 50:50 mix of unmolassed sugar beet:hay cubes mix (– –) in experiment B.
Experiment A. In Experiment B, TVFA concentration 4–8 h after the 09:00 h meal was 43% higher than that recorded during the previous 3 h.

4. Discussion

4.1. Dry matter intake

Overall, the average daily DMI of SBF was only 0.43 of that of the other three diets across both experiments. A similar finding was reported by Smolders et al. (1990), who also recorded high levels of feed refusals when horses were fed a 70:30 sugar beet:hay diet. Additionally, Hyslop et al. (1998b) indicated that sugar beet products may suppress voluntary food intake (VFI) in ponies when included at 550–700 g kg$^{-1}$ DM of the diet. It has also been observed that VFI was lower when sugar beet products were included in the diet of dry sows compared with that of other fibrous foodstuffs (Brouns et al., 1995); these authors suggested that the lower intakes were due to pre-caecal gut distension, caused by the high water holding capacity of the sugar beet. The digestive system of equids is similar to that of pigs and it is, therefore, possible that sugar beet feed products restricted the VFI of the ponies in the current experiment in a similar manner. However, including USB in a 50:50 mix with HC in Experiment B did not result in any feed refusals and, as a consequence, the ponies consumed more sugar beet by weight than those in Experiment A.
4.2. Intra-caecal fermentation parameters

Radicke et al. (1991) suggested that when the intra-caecal pH of horses falls below 6.0, the animals may be regarded as exhibiting sub-clinical acidosis. A decline in intra-caecal pH has been reported when starch-based cereals have been used to supplement the basal forage in equine rations (Willard et al., 1977; Goodson et al., 1988). All the fibre-based diets examined here in the current programme, maintained the caecal pH above 6.5 at all time points measured, which is similar to the findings of Goodson et al. (1988) for ponies fed forage-based diets. Additionally, in studies using either one or two caecally fistulated ponies, Tisserand et al. (1977a, b) observed caecal pH values between 6.9 and 8.0 when ponies were fed hay and straw-based diets. However, the average intra-caecal pH of ponies fed the OH:NO diet, which contained >200 g starch kg\(^{-1}\) diet, declined significantly from 7.37 to 6.86 when measured between 0–3 and 4–8 h respectively, after presentation of the morning feed, whereas intra-caecal pH remained relatively stable in ponies offered the HC and SBF diets which contained a maximum of 20–30 g of starch kg\(^{-1}\) DM.

The mean intra-caecal lactate levels measured >4 h after the morning feed were significantly higher in ponies fed the OH:NO diet than in those fed either the SBF or HC
diets. Rapid fermentation of cereal starch in the hind-gut of horses causes an increase in intra-caecal levels of lactate (Pagan, 1998), and thus the elevated levels observed in the current programme may be attributed to the rapid fermentation of any naked oat starch which had escaped pre-caecal digestion. Pagan (1997) suggested that fibre in equid diets stimulates peristalsis causing a high rate of passage of digesta through the small intestine with a corresponding reduction in enzymatic digestion of starch and protein. This may well have occurred in the current programme with the oat hulls initiating a high rate of digesta passage, causing naked-oat starch to pass into the caecum. Alternatively, the naked oats may have contained some retrograded starch, which resisted amylolytic digestion in the small intestine (Englyst et al., 1987).

Although the overall TVFA production in caecal samples from ponies fed the HC diet was significantly higher than that produced on the SBF diet, when considered in terms of dry matter intake, levels of intra-caecal TVFA were higher at 20.46 mmol l\(^{-1}\) kg\(^{-1}\) SBF DMI compared with 14.85 mmol l\(^{-1}\) kg\(^{-1}\) HC DMI. This reflects a higher fermentability of SBF compared to HC as the intra-caecal lactate levels were similar for these diets. Furthermore, despite low intakes, all of the ponies maintained their body weight when fed SBF, thus suggesting that their energy requirements were largely being met. These results are in accordance with the finding that the coefficient of dry matter digestibility (DMD) of sugar beet feed is 0.72 compared with 0.3 for mature grass hay (Hyslop et al., 1998a, b). Low levels of TVFA were found in the caecal digesta of ponies fed the OH:NO diet during the 09:00 to 12:00 h collection period, and may reflect the comparatively low degradability of the oat hull fraction of the diet after rapid degradation of the naked oat starch from the previous meal. At all time points measured, the intra-caecal VFA molar proportions for ponies fed the HC and SBF diets were similar, being maintained at ca. 80:15:5 for acetate:propionate:butyrate, respectively. These VFA ratios are in agreement with those found in caecal digesta from ponies fed fibrous diets (Hintz et al., 1971; Argenzio et al., 1974; Willard et al., 1977). By contrast, the corresponding ratios for OH:NO were 73:23:4, and are similar to the 73:21:6 proportions recorded in the caeca of ponies fed a 3:1 forage:grain diet by Glinsky et al. (1976). However, intra-caecal proportions of acetate and propionate were significantly higher and lower, respectively, in ponies fed SBF compared with levels recorded when fed the OH:NO diet. The higher levels of propionate recorded in OH:NO fed ponies are consistent with starch fermentation in the caecum (McLean et al., 1998), and are compatible with the corresponding lactate levels.

5. Conclusion

Although the fibre-based diets offered to ponies in the current study resulted in significant differences in some intra-caecal fermentation parameters, these were insufficient to lower the pH below the critical threshold of pH 6. Therefore, each of the fibre-based diets examined could be used as partial replacements for hay forage in equine diets. Sugar beet feed when fed in conjunction with hay maintained dry matter intakes and stable intra-caecal fermentation parameters but, when fed as the sole dietary ingredient, dry matter intake was compromised. However, readily fermented fibrous
feeds, such as sugar beet, can increase the overall energy concentration of the diet and, when fed in conjunction with other forages, may avoid the necessity for feeding high levels of starch-based diets, thereby minimising the incidence of hind-gut dysfunction in horses.

Acknowledgements

We would like to thank D.H. Anderson and T. McHale for care of the animals, R.S. Lowman and B.M.L. McLean for assistance during the experimental work, R. Muirhead and E. Bakewell for laboratory analysis and M.S. Dhanoa for statistical advice. This work was part of project 612 supported by the Horserace Betting Levy Board.

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