Short communication

The effect of feeding a low- or high-starch diet on equine faecal parameters

Jo-Anne MD Murray, Annette Longland, Meriel Moore-Colyer, Catherine Dunnett, Annette Longland

a Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Roslin, Midlothian EH25 9RG, UK
b Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth SY23 3EB, Wales, UK
c Institute of Rural Sciences, University of Wales, Aberystwyth, Llanbadarn Campus, Aberystwyth, SY23 3AL Wales, UK
d Dengie Crops Limited, Heybridge Business Centre, 110 The Causeway, Maldon, Essex CM9 4ND, UK

A R T I C L E   I N F O

Article history:
Received 6 February 2013
Received in revised form
11 October 2013
Accepted 13 October 2013

Keywords:
Equine
Starch
Faeces
Diet

A B S T R A C T

Seven mature Welsh-cross pony geldings provided the faecal inocula in a cross-over design experiment, consisting of two 14-day periods. In period 1, four ponies (group 1) were fed a low-starch fibre-mix (LS), and three (group 2) were fed a conventional high-starch coarse-mix (HS), both groups were fed these mixes in a 50:50 ratio with mature grass hay, to give a total daily dry matter (DM) intake of 17.5 g/kg live weight per day. Diets were then switched in period 2. At the end of each experimental period freshly voided faeces were collected from each animal and analysed for cellulolytic and amylolytic bacterial numbers, volatile fatty acid (VFA) and lactate concentration. There was no effect of diet on the number of cellulolytic and amylolytic bacteria, VFA or lactate present in the faeces of the experimental ponies. Consequently, it would appear that the effect of feeding LS or HS on faecal parameters is minimal; however, further work is required to determine the accuracy of faeces as a model for changes in the hindgut environment of the horse.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Horses have evolved to survive on a diet consisting of large quantities of low-quality fibre, ingested on an almost continual basis. However, the nutrient, and in particular energy demands of performance horses have necessitated the incorporation of large amounts of energy-dense cereal grains in the diets of these animals, which are high in starch and low in fibre. It is well known that the small intestine of the horse has a limited capacity for starch digestion (Kienzle et al., 1994; Meyer et al., 1995) and thus when high levels of starch are fed in a single meal, undigested starch enters the hindgut. Excess starch entering the hind-gut favours the proliferation of Gram positive lactic acid producing bacteria in the hindgut of the horse at the expense of the Gram negative fibre-degrading bacterial population. The increase in lactate, volatile fatty and acids (VFA) and consequent reduction in gut pH elicited through feeding high-starch diets can cause a number of intestinal and metabolic disorders in horses such as colic and laminitis (Rowe et al., 1994). Therefore, the present study was undertaken to examine the effects of feeding a low- or high-starch diet on faecal parameters.

2. Materials and methods

2.1. Experimental design

Seven mature Welsh-cross pony geldings (280 kg ± 17.6 LW) provided the faecal inocula in a cross-over design experiment whereby four ponies (group 1) were fed a low-starch fibre-mix (LS) containing 186 g/kg DM of starch, and
three (group 2) were fed a conventional high-starch coarse-mix (HS) containing 512 g/kg DM of starch. Both mixes were proprietary mixes and there was no information on how the ingredients were processed. Both mixes were fed in a 50:50 ratio with mature grass hay (Hay), to give a total daily dry matter (DM) intake of 17.5 g/kg LW per day. Each diet was fed in two equal meals, giving a starch intake per meal of 1.2 and 2.2 g/kg LW for LS and HS, respectively. Each diet was fed for a period of 14 days, after which group 1 was offered diet HS and group 2 was offered diet LS. At the end of each 14-day period freshly voided faeces were collected immediately after defaecation from each animal and analysed for cellulolytic and amylolytic bacterial numbers, VFA and lactate concentration.

2.2. Chemical analyses of feedstuffs

Feed samples were analysed for DM, neutral detergent fibre (NDFom), acid detergent fibre (ADFom), starch, water soluble carbohydrate (WSC), gross energy (GE) sodium (Na), calcium (Ca), phosphorus (P), potassium (K) and magnesium (Mg) according to the methods described by (Murray et al., 2008).

2.3. Faecal most probable number (MPN) counts

At the end of each of the 14-day period, freshly voided faeces were collected before the morning feed (09:00 h) from each pony and placed separately in pre-warmed (39 °C) flasks for transportation to the laboratory. Faeces were tightly packed in the collection vessels to maintain anaerobic conditions. Each faecal sample was processed separately for MPN counts according to the technique described by Davies et al. (1993). Cellulolytic and amylolytic anaerobic bacteria were enumerated by MPN procedures using grass hay (ground through a 1 mm dry mesh screen) and soluble starch (BDH, Poole, Dorset) as substrates, respectively. The chemical composition of the feedstuffs is given in Table 1.

Enumerations were performed on 10 g (fresh weight) quantities of fresh faecal samples. Faecal samples (10 g) were combined with 90 ml of anaerobic culture medium as described by Davies (1993) and homogenised in a stomacher (Laboratory blender stomacher 400, Seward, London, UK) for 2 min. This resultant suspension 10 ml of was sub-sampled and serially diluted in 90 ml of culture medium. Positive and negative tubes were employed for MPN enumerations using three replicates per dilution and $10^{-7}$ to $10^{-13}$ dilutions.

Growth of cellulolytic and amylolytic bacteria was estimated by increased turbidity after seven days incubation at 39 °C. MPN counts were expressed on a faecal DM basis. The data were transformed to $\log_{10}$ since the logarithmic distribution tends to be more symmetrical in comparison to that of the estimated density values (Cochran, 1950).

2.4. Faecal VFA and lactate analyses

VFA and lactate contents were determined on water extracts of freshly voided faeces. Extracts were prepared by placing a 20 g fresh matter sample of faeces in 100 ml of distilled water. Samples were then shaken and filtered through a sieve (1 mm pore size). The resultant filtrate was then acidified with 0.5 ml $\text{H}_2\text{PO}_4$ and stored at 4 °C for 24 h prior to freezing at −20 °C. Samples were then defrosted and analysed for VFA and lactate content according to the method of Merry et al. (1995).

2.5. Statistical analyses

Values for the most probable number counts, and VFA and lactate concentrations were analysed for significant differences as a cross-over design using restricted maximum likelihood (REML) in Genstat 5 Lawes Agricultural Trust, 1993). Comparisons between treatment groups were made by least significant difference equations.

3. Results

Starch levels were lower for the hay in comparison to the FMix and SMix; the SMix contained almost three fold the amount of starch (512 g/kg DM; Table 1). Consequently, the starch content of the HS diet (274 g/kg DM) was 2.5 times higher than that of the LS diet, which contained 111 g/kg DM. In terms of starch intake, ponies that were fed the HS diet received 2.2 g starch/kg LW, whilst those fed with the LS diet were offered 1.2 g/kg LW. ADFom and NDFom contents were the greatest in the hay, the lowest in the SMix and intermediate in the FMix (Table 1).

There were no horse–diet interactions for microbial counts, VFA or lactate concentrations. There was no effect of diet on the number of cellulolytic and amylolytic bacteria present in the faeces of the experimental ponies (Table 2). Moreover, there was little inter-animal variation in the number of cellulolytic and amylolytic bacteria present in the faeces (Table 3). Faecal VFA concentration was also similar across both diets (Table 2); however, there was an inter-animal variation in total VFA concentration, attributable to differences in the concentration of acetate and butyrate present in the faeces of these animals (Table 3). Faecal lactate concentration was similar across both diets and for all ponies.

| Chemical composition of the dietary ingredients; grass hay (Hay), fibre-mix (FMix), starch-mix (SMix) and the two experimental diets consisting of Hay fed in a 50:50 ratio with either fibre-mix (LS) or starch-mix (HS) (g/kg DM unless otherwise stated). |
|---|---|---|---|---|---|
| **Hay** | **FMix** | **SMix** | **Diets** |
| DM (g/kg) | 912 | 900 | 885 | 906 | 898 |
| OM | 848 | 821 | 823 | 834 | 835 |
| CP | 62 | 123 | 122 | 92 | 92 |
| Starch | 36 | 186 | 512 | 111 | 274 |
| WSC | 186 | 94 | 58 | 140 | 122 |
| ADF | 356 | 197 | 83 | 277 | 220 |
| NDF | 615 | 351 | 196 | 483 | 405 |
| GE (MJ/kg) | 18.3 | 19.2 | 18.1 | 18.7 | 18.2 |
| Sodium | 1.3 | 3.1 | 0.6 | 2.2 | 0.9 |
| Potassium | 6.8 | 8.3 | 5.5 | 7.5 | 6.1 |
| Calcium | 4.1 | 12.3 | 12.1 | 8.2 | 8.1 |
| Phosphorus | 1.3 | 3.7 | 3.0 | 2.5 | 2.2 |
| Magnesium | 1.6 | 3.6 | 1.7 | 2.6 | 1.6 |
(Table 3). No D-lactate was detected and so total lactate consisted of L-lactate only.

4. Discussion

The aim of this study was to examine the effects of feeding a low- or high-starch diet on faecal parameters as a model of changes that may arise within the hindgut as a consequence of feeding such diets. Whilst the amount of starch present in the HS diet was at levels that have been reported to elicit unfavourable changes in the hindgut environment of ponies (McLean et al., 2000), faecal cellulolytic and amylolytic bacterial counts did not show any differences between the two diets fed. In situ data from ponies cannulated at the caecum and ventral colon has shown a significant decrease in the amount of cellulolytic and an increase in the number of amylolytic bacteria present in the caecum and colon of ponies changed from a diet of grass hay to that containing a 50:50 ratio of hay and barley (de Fombelle et al., 2001; Julliand et al., 2001). Therefore, it may be the case that faeces are not a good indicator of the microbial populations present in the hindgut of the horse and thus the results of this study do not reflect the changes that may have occurred within the hindgut environment. Conversely, it may be that the culture-based technique employed in this study to quantify bacterial numbers may not have been sensitive enough to detect changes within the bacterial population. For example, Muhonen et al. (2010) reported no difference in the number of cellulolytic or amylolytic bacteria in the equine caecum, colon or faeces, using culture-based methods suggesting that faeces are a reasonable model for estimating changes in the bacterial populations present in the various segments of the hindgut. Conversely, Hastie et al. (2008) detected lower levels of candidate fibrolytic and amylolytic in the caecum compared to faeces of healthy horses using molecular-based techniques (real-time PCR). Consequently, it is possible that in the current study there was no difference in the total number of cellulolytic and amylolytic bacteria present in the faeces, but there may have been a shift in the number of individual amylolytic bacteria, for example Streptococcus bovis, which are known to proliferate as a result of high-levels of starch entering the hindgut (Julliand et al., 2001). Studies in humans have shown that almost 80% of microbes in faecal material cannot be cultured (Suau et al., 1999). Indeed, whilst Muhonen et al. (2010) also reported no differences in the number of cellulolytic or amylolytic bacteria in the faeces of horses fed diets containing different amounts of starch content using a culture-based technique, Willing et al. (2009) measured higher levels of amylolytic bacteria, specifically members of the S. bovis/equinus complex, in faeces collected from horses that were fed a high-starch diet compared to a forage-only diet using a molecular-based methodology.

Diet did not affect faecal total VFA or lactate concentrations in the current study, which contradicts other studies (McLean et al., 2000; de Fombelle et al., 2001). However, Campbell et al. (1997) found that caecal VFA concentrations in rats were not correlated with faecal VFA concentration. Conversely, Meylan et al. (2002) reported faecal VFA concentrations to be a good indicator of fermentation patterns in the large intestine of dairy cows and Berg et al. (2005) reported a similar situation for horses. However, VFA and lactate concentrations are known to fluctuate over the course of the day (Goodson et al., 1988); therefore, repeated measurements or a different sampling time may have revealed difference between diets in the current study. Moreover, inter-animal variability in faecal VFA concentration was significant, which may have affected the ability to detect dietary differences.

Table 3
The effect of donor animal on the cellulolytic and amylolytic bacteria (log_{10} g/kg DM), VFA and lactate content (g/kg DM) of the faeces of individual ponies fed a low- (LS) or high-starch (HS) diet.

<table>
<thead>
<tr>
<th>Pony</th>
<th>LS</th>
<th>HS</th>
<th>s.e.d</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pony 1</td>
<td>11.42</td>
<td>12.35</td>
<td>10.92</td>
<td>12.00</td>
</tr>
<tr>
<td>Pony 2</td>
<td>10.84</td>
<td>10.76</td>
<td>10.58</td>
<td>11.33</td>
</tr>
<tr>
<td>Pony 3</td>
<td>6.19a</td>
<td>7.66ab</td>
<td>7.38ab</td>
<td>7.47ab</td>
</tr>
<tr>
<td>Pony 4</td>
<td>4.49a</td>
<td>5.39ab</td>
<td>5.04ab</td>
<td>5.30ab</td>
</tr>
<tr>
<td>Pony 5</td>
<td>0.29a</td>
<td>0.41ab</td>
<td>0.39ab</td>
<td>0.40ab</td>
</tr>
<tr>
<td>Pony 6</td>
<td>1.43</td>
<td>1.86</td>
<td>1.96</td>
<td>1.78</td>
</tr>
<tr>
<td>Pony 7</td>
<td>0.53</td>
<td>0.34</td>
<td>0.60</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Values in rows not sharing common superscripts differ significantly (P < 0.05).
5. Conclusion

It would appear that the effect of feeding LS or HS on faecal parameters is minimal; however, further work is required to establish if a truly conclusive link can be made using faecal material to provide information on bacterial community structure within the hindgut.

Acknowledgements

The authors would like to acknowledge M.S. Dhanoa for expert statistical advice.

References


