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Comparison of gastrointestinal transit times in stabled Thoroughbred horses fed freshly cut pasture and three conserved forage-based diets

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Abstract

Context. The type of forage offered to horses varies in physical form, moisture content and nutrient quality, and these variables could affect the intake, passage rate and digestibility of the forage consumed.

Aims. To investigate the changes in passage rate of digesta through the gastrointestinal tract in horses fed four different forage-based diets (diet effect).

Methods. Thoroughbred mares (n = 6) were stabled in loose boxes for 6 weeks. During Weeks 1, 3 and 5 (washout periods), all horses were fed freshly cut pasture, either in restricted quantities (Week 1) or *ad libitum* (Weeks 3 and 5). Using a 3 × 3 Latin square design during Weeks 2, 4 and 6, each pair of horses was abruptly transitioned to one of three conserved forage-based diets (chopped ensiled forage fed exclusively or with oats, or perennial ryegrass hay with oats) fed *ad libitum*. At the beginning of each week, indigestible polyethylene markers (n = 200) were administered to the horses via a nasogastric tube, followed immediately by transition to the new diet.

Key results. There was a significant diet effect on the daily dry-matter intake of feed (P < 0.0001), percentage of time spent eating (P < 0.001), frequency of voiding faeces (P < 0.05) and quantity of faeces voided (P < 0.0001). There was a significant horse effect on the daily dry-matter intake of feed (P < 0.0001) and quantity of faeces voided (P < 0.0001), but no differences in the percentage of time spent eating or the frequency of voiding faeces. There were significant diet and horse effects on the time to recovery of the first marker in the faeces (P < 0.01 and P < 0.01 respectively) and the mean retention time of markers in the gastrointestinal tract (P < 0.05 and P < 0.001 respectively). Mean retention time was negatively correlated with feed intake and quantity of faeces voided ($r^2 = -0.51$ and $r^2 = -0.64$ respectively).

Conclusions. Longer mean retention time was associated with a greater fibre content in the diet and a restricted feed supply, thus supporting the hypothesis that horses alter mean retention time on the basis of a nutrient absorption optimisation model.

Implications. Feed composition, but also the quantities offered, may alter measurement of apparent feed digestibility in horses.

Keywords: conserved forage, faeces, feed intake, gastrointestinal transit time, mean retention time, pasture, passage rate of digesta, Thoroughbred horses.

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Introduction

Horses are hindgut fermenters adapted to eating a high-forage diet (Janis 1976). When kept on pasture, horses spend most (~70%) of their time grazing a variety of forage species (Crowell-Davis *et al*. 1985; Randall *et al*. 2014). In New Zealand, most horses are kept on pasture all year round (Fernandes *et al*. 2014*b*; Verhaar *et al*. 2014; Rogers *et al*. 2020), but it is common practice to intensively manage racehorses and some competition horses in stables for parts of the day, with variable turnout periods for grazing (Rogers *et al*. 2007; Stowers *et al*. 2009; Verhaar *et al*. 2014; Wood *et al*. 2019). A variety of conserved forages, grain and grain by-products are frequently incorporated into the diet of many horses, especially when pasture is unavailable or inadequate in quantity and nutritive value, or to provide additional dietary energy for performance (Goodwin *et al*. 2002; Harris 2009;

Hoffman *et al*. 2009). Gastrointestinal disorders may occur in horses and ponies, with some animals being more prone than others (Clarke *et al*. 1990; Hudson *et al*. 2001; Julliand 2005). A common recommendation to prevent gastrointestinal disturbances in horses is to avoid abrupt dietary changes and to maintain high proportions of forage in the diet (Julliand *et al*. 2001). Although several studies have investigated aspects of conserved forages fed to horses (Drogoul *et al*. 2001; Müller *et al*. 2008; Muhonen *et al*. 2009, 2010), the effect of different conserved forages on the passage rate of digesta and the microbial population in the hindgut is still poorly understood (Fernandes *et al*. 2014*a*; Garber *et al*. 2020).

Many types of conserved forages are fed to horses, and vary in physical form, such as, for example, long-stemmed, chaffed or pelleted forages, the nutrient quality of which can vary depending on several factors (Müller and Uden 2007). For example, conserved forages may consist of variable grass and legume species such as ryegrass (*Lolium perenne*), clover (*Trifolium repens*), timothy (*Phleum pratense*), lucerne (*Medicago sativa*) or a blend of multiple forage species, and these may be harvested at different stages of maturity (i.e. early, middle or late stages), resulting in a variable nutrient quality of the forage (Lewis *et al*. 1995; Hoffman *et al*. 2001; Müller and Uden 2007).

Traditionally, the most common method for conservation of forage was in the form of hay. More recently, feeding horses ensiled forages (such as haylage) is becoming increasingly popular (Müller and Uden 2007). Several authors have reported potential benefits associated with ensiled forages, including higher voluntary feed intake, palatability and digestibility (Müller and Uden 2007; Ragnarsson and Lindberg 2010), reduced dust particles due to the higher moisture content (Clements and Pirie 2007), and easier transportation and convenient storage due to polyethylene-wrapped packaging (Patel *et al*. 2014; Verhaar *et al*. 2014), than with hay.

Passage rate of digesta (feed) through the gastrointestinal tract has been previously investigated, and is best described by measuring the mean retention time (MRT) of indigestible markers that travel through the gastrointestinal tract with the feed consumed (Pearson and Merritt 1991; Cuddeford *et al*. 1995; Pearson *et al*. 2001). The transit time (T_1) is described as the time when the first marker is recovered in the faeces, and is an indication of the minimum time between ingestion of the feed and voiding of faeces (Udén *et al*. 1982).

Differences in the passage rate of digesta through the gastrointestinal tract have been reported in equids (e.g. horses, ponies and donkeys) fed different diets, i.e. 24–26 h when grazing or fed cut pasture (Grace *et al*. 2003) versus 26–77 h when fed different types of conserved forages (Cuddeford *et al*. 1995; Pearson *et al*. 2001; Moore-Colyer *et al*. 2003), and 43–52 h when fed ground-pelleted hay versus 27–46 h when fed chopped hay (Drogoul *et al*. 2000). The physical form of the forage and the particle size of digesta were identified as dominant effects (Drogoul *et al*. 2000; Moore-Colyer *et al*. 2003). Furthermore, increasing the proportion of forage in the diet at the expense of grain reduced the passage rate of digesta through the gastrointestinal tract, from 42 h for a 50:50 hay and barley diet, to 30 h for a 100% hay diet (Drogoul *et al*. 2001). In ponies fed *ad libitum* versus restricted quantities, the passage rate increased from 21 to 31 h for chopped alfalfa, and from 32 to 36 h for chopped oat straw (Pearson *et al*. 2001). However, while the passage rate of different types of forage and mixed forage–grain diets has been investigated in horses, following an adaption period after dietary change, the passage rate (MRT and T_1) immediately following abrupt transition between forage-based diets has not been investigated. It is of importance to know that this as passage rate will affect the composition of the hindgut microbiota and this may contribute to the manifestation of gastrointestinal disorders with acute changes in diet.

As part of a larger study to examine the effect of an abrupt change of forage diets on the hindgut microbiota, the current study described here aimed to investigate the changes in T_1 and MRT when horses were fed four different forage-based diets (diet effect), and to examine variation among horses (horse effect) on the passage rate by using indigestible markers.

Materials and methods

Animals and experimental design

The use of animals, experimental procedures and collection of the faecal samples for the study were approved by the Massey University Animal Ethics Committee (MUAEC), Massey University, Palmerston North, New Zealand (Protocol number 14/35). The management of the horses used in the study (including feeding, housing, husbandry and welfare) were in accordance with the guidelines set within the code of ethical conduct for the use of live animals for research, testing and teaching. A veterinarian examined the horses on a weekly basis to ensure that all horses remained clinically normal during the study period.

Using a 3 × 3 replicated Latin square design, stabled horses were abruptly transitioned to three randomly allocated conserved forage-based diet treatments. Between dietary-treatment periods, each of 7 days in duration, the horses were provided with a washout period of 7 days, during which freshly cut pasture was fed *ad libitum* to each horse. Feed and faecal samples were collected at regular intervals following each dietary transition, and data were recorded to investigate the differences in gastrointestinal transit times between horses and diets.

Six (non-pregnant) Thoroughbred mares were enrolled in the study (mean age ± standard deviation (s.d.), 13.5 ± 3.7 years, and mean bodyweight (BW) ± s.d., 528 ± 26 kg, weighed at the beginning of the study). Prior to the study, the horses were maintained on a commercial Thoroughbred stud farm (Palmerston North, Manawatu, New Zealand), and managed as a cohort on predominantly perennial ryegrass–clover pasture (typically ~80–95% perennial ryegrass (*Lolium perenne*) and ~5–20% white clover (*Trifolium repens*)). The horses had received an annual vaccination (Equivac 2 in 1, Pfizer Animal Health, Australia) and the most recent anthelmintic treatment had been administered 1 week before the commencement of the study. At the start of the study, all horses were examined by a veterinarian and were of similar body condition (body condition score (BCS) = 5, on a 9-point scale; Henneke *et al.* 1983), as assessed by the author (K. A. Fernandes). The horses were reported to have been in good health during the 6 months preceding the study.

Three days before the start of the study, the horses were transported from the stud farm to the trial site. On arrival, the horses were transferred into individual, adjacent, outdoor holding paddocks (15 × 15 m, containing ryegrass–clover pasture, ~1000 kg dry matter (DM)/ha) to facilitate adaptation to the new environment. A fresh faecal sample was collected (from a recently voided faecal mass) from each horse within 2 h of arrival, and examined for faecal egg count. During the 3-day adaptation period, each horse was offered ~12 kg DM of ryegrass–clover hay per day, fed twice daily, in addition to the limited amount of grazing available in the paddocks.

Housing and stable management during the trial

On Day 1 of the trial, the horses were individually stabled in 3 × 3.5 m loose boxes, with sawdust bedding to a depth of 8–10 cm. The loose boxes were adjacent to each other and the top half of the stable door was kept open at all times, allowing visual contact among all horses. The horses were turned out individually each day during the trial into two 6 × 8 m concrete yards, for 30 min in the morning and afternoon. The general health and appetite of the horses were observed daily. BCS (1–9; Henneke *et al.* 1983) and BW (measured using walk-on scales, TruTest economy plus-700, Auckland, New Zealand, accuracy 0.5 kg) of the horses were recorded on the 1st, 4th and 7th day of each treatment block.

The horses were provided *ad libitum* access to water and a 500 g trace-mineral salt block (Summit Littlix multi-mineral salt block, Dominion Salt Ltd, Mount Maunganui, New Zealand). Cut grass and hay was provided in hay nets (large size nylon-rope hay net, 107 cm in length, with 15 × 15 cm mesh openings) and the chopped ensiled forage diet, oats and some cut pasture were provided in a feed bin.

Diets and feeding management

The horses were offered three forage-based diets, with freshly cut pasture being fed to the horses as a washout diet between the dietary treatment blocks (Diets P1, P2 and P3 were fed during Weeks 1, 3 and 5 of the trial respectively) over the 6-week period. The three treatment diets were a commercial chopped ensiled forage (Diet FE), the same commercial chopped ensiled forage mixed with whole oats (Diet FE +O), and a perennial ryegrass hay fed with whole oats (Diet H+O). The nutrient composition of the dietary components is provided in Table 1.

During Weeks 1, 3 and 5 of the trial, the horses were fed freshly cut grass obtained from ryegrass–clover pasture (typically comprising of ~80–95% perennial ryegrass (*Lolium perenne*) and ~5–20% white clover (*Trifolium repens*), sourced from two 2 ha paddocks on an adjacent dairy farm (No. 4 Dairy Farm, Massey University, Palmerston North, New Zealand). Prior to Day 1 of the trial, the pasture had not been grazed for a 6–8-week period (previously grazed by cattle and never by horses) and had an average sward height of 15–20 cm (~3000–3500 kg DM/ha). The grass was cut using a sickle bar mower to a height of 3–5 cm above the ground, between 0800 hours and 0900 hours (AM pasture cut) and between 1600 hours and 1700 hours (PM pasture cut) each day. The cut grass was immediately transported to the trial site and stored in a feed room for less than 12 h before feeding.

The horses were provided with two hay nets containing ~10–15 kg and one bucket containing ~5–8 kg of cut pasture (fresh weight). As per individual horse requirements, the hay nets and feed buckets were refilled (either when empty or at ~4-h intervals at 0800 hours, 1200 hours, 1600 hours, 2000 hours and 2400 hours) to provide 1.3–2.3% BW of feed (DM basis). Due to logistic difficulties, the quantity of cut pasture offered during Diet Period P1 was restricted compared with *ad libitum* access to feed in Diet Periods P2 and P3.

Table 1. Nutrient analysis (on a DM basis) of dietary components used in the study on passage rate of digesta in horses

Total digestible nutrients (TDN) = crude protein (CP) + (fat × 2.25) + neutral detergent fibre (NDF) + NSC. DM, dry matter; ADF, acid detergent fibre; WSC, water-soluble carbohydrates; ESC, ethanol-soluble carbohydrates; RFV, relative feed value; NA, not applicable

Parameter	Pasture 1	Pasture 2	Pasture 3	FE (ensiled lucerne and timothy)	Hay	Whole oats
DE MJ/kg	10.8 ± 0.4	11.0 ± 0.4	10.7 ± 0.3	10.7 ± 0.3	9.3 ± 0.3	15.1 ± 0.2
% DM	15.6 ± 1.7	16.4 ± 2.5	15.8 ± 2.4	39.5 ± 1.4	80.5 ± 1.3	83.4 ± 1.1
% Ash	13.2 ± 1.0	11.8 ± 0.8	12.2 ± 1.3	7.8 ± 0.5	7.6 ± 0.6	3.0 ± 0.0
% Crude protein	25.0 ± 1.6	22.9 ± 1.7	19.2 ± 2.7	17.3 ± 1.3	11.7 ± 0.7	10.9 ± 0.2
% Crude fat	4.9 ± 0.2	4.7 ± 0.4	4.4 ± 0.6	3.9 ± 0.3	1.9 ± 0.4	5.9 ± 0.2
% CHO	56.9 ± 2.2	60.7 ± 2.2	64.3 ± 4.1	71.0 ± 1.6	78.7 ± 1.3	80.3 ± 0.3
% NDF	45.7 ± 2.3	46.6 ± 2.4	49.3 ± 1.8	58.1 ± 3.1	62.9 ± 2.2	24.4 ± 2.1
% ADF	26.6 ± 1.6	28.2 ± 1.3	29.8 ± 1.9	39.1 ± 1.3	43.7 ± 2.2	12.1 ± 1.0
% Lignin	3.7 ± 0.7	4.8 ± 1.1	4.9 ± 1.0	6.4 ± 0.3	7.5 ± 0.6	2.5 ± 0.3
% Starch	1.0 ± 1.0	1.4 ± 0.9	0.5 ± 0.5	2.6 ± 0.6	1.3 ± 0.4	50.3 ± 3.2
% WSC	20.6 ± 1.5	21.8 ± 2.9	23.4 ± 2.4	10 ± 1.6	9.0 ± 1.2	NA
% ESC	13.9 ± 2.9	13.9 ± 3.9	17.7 ± 1.7	5.4 ± 1.2	6.6 ± 1.3	NA
% NFC	30.3 ± 3.0	32.5 ± 2.2	34.5 ± 4.7	31.9 ± 1.6	35.0 ± 1.3	68.2 ± 1.3
% TDN	70.8 ± 2.2	70.1 ± 2.4	68.3 ± 2.2	68.1 ± 1.5	59.1 ± 1.0	86.6 ± 1.3
RFV	169 ± 11	163 ± 10	151 ± 7	119 ± 8	94 ± 3	NA

During Weeks 2, 4 and 6, one pair of horses was randomly allocated to one of the following diets: FE, FE+O or H+O according to a 3 × 3 Latin-square design. Diet FE was a commercially available chopped ensiled forage diet (FibreEzy® (FE), Fibre Fresh Feeds Ltd, Reporoa, New Zealand), and comprised lucerne (*Medicago sativa*; alfalfa; 50%) and timothy (*Phleum pratense*; 50%) grass, chopped into 1–5 cm stubbles, and ensiled with molasses (1%). Diet FE was offered at a minimum of ~2.5–3.0% BW (DM basis), as two feeds at 0800 hours and 2000 hours, (~20 kg Diet FE/horse. day, on as-fed basis). To ensure *ad libitum* feeding, additional quantities of Diet FE were provided when less than 25% of the feed offered was remaining in the bucket.

Diet FE+O comprised the same chopped ensiled forage provided in Diet FE, mixed with whole oats (*Avena sativa*), and Diet H+O comprised perennial ryegrass hay fed with whole oats. The perennial ryegrass hay typically contained ~80% perennial ryegrass (*Lolium perenne*), and small proportions of white (*Trifolium repens*) and red (*Trifolium pratense*) clovers, herbs and weeds. The hay was harvested and processed as one batch at the same location (Manawatu, New Zealand), and was stored in a dry covered shed for ~6 months before the study period. Both forage components of the diets FE+O and H+O were fed *ad libitum* at ~2% BW (DM basis)/ horse.day.

The quantity of oats offered was calculated on the basis of 50% of the minimum daily digestible energy requirements for maintenance for a 500 kg horse (~35 MJ/horse.day; National Research Council 2007), equivalent to 2.5 kg DM/horse.day, divided into two feeds. Diet FE+O was provided in a feed bin twice daily at 0800 hours and 2000 hours, and to ensure *ad libitum* feeding, additional quantities of the chopped ensiled forage were provided when less than 25% of feed was remaining in the bucket. Hay was offered in two hay nets (weighing ~5–8 kg, as-fed basis) at 0800 hours each day (similar to the procedure described for Diet P). The hay nets were replenished at 2000 hours with additional quantities of hay, so as to provide *ad libitum* access to feed.

Refusals were collected twice daily (morning between 0700 hours and 0800 hours and evening between 1900 hours and 2000 hours) and weighed (TruTest-703 Electronic Scales, Auckland, New Zealand) to determine the amount of feed consumed (Glunk *et al*. 2013*b*). DM intake (DMI, kg/day) of each diet was calculated as the difference between the weight of feed consumed and the weight of the refusals (including spillage) measured on an as-is basis, and the values were corrected to a DM basis for each feed.

Feed samples

Representative feed samples of each diet fed to the horses were collected to determine the nutrient content. One pooled sample (~1 kg wet weight) of Diet P1 (*n* = 7), and two samples (representing the AM and PM cuts) for Diets P2 (*n* = 14) and P3 (*n* = 14) were collected each day. The pasture samples consisted of ~10 smaller subsamples from different locations within the freshly cut pasture. One sample (~200 g) was collected on alternate days for the chopped ensiled forage used in Diets FE and FE+O (*n* = 9) and hay (~500 g) used in Diet H+O (*n* = 9), and one sample of whole oats (200 g) was collected per week (*n* = 3). The feed samples were weighed and stored at –20°C until analysis.

At the end of the trial, the feed samples (*n* = 56) were lyophilised (FD18, Cuddon Freeze Dry, Blenheim, New Zealand) and ground to pass through a 1 mm screen (Cyclotec 1093 Sample Mill, Foss, Hillerod, Denmark). The processed samples were analysed for nutrient content by a commercial forage testing laboratory (Equi-Analytical, Ithaca, NY, USA). The method of analysis used was a combination of near-infrared and plasma spectrophotometer techniques to analyse DM, crude protein, neutral and acid detergent fibres, lignin, water-soluble and ethanol-soluble carbohydrates, starch, fat, ash and minerals.

Indigestible markers

The passage rate of a diet (time taken for a diet to transit through the gastrointestinal tract) was estimated using solid-phase indigestible markers according to previously described methods (Blaxter *et al*. 1956; McGreevy *et al*. 2001; Pearson *et al*. 2006; Rosenfeld *et al*. 2006). The solid-phase markers used in the present trial were hollow cylindrical pieces (4–5 mm length, 5 mm outer diameter, ~40 mg weight) prepared from polyethylene tubing (Ledathene, Leda, Wellington, New Zealand). At 0800 hours on the first day of each treatment block (Days 1, 8, 15, 22, 29 and 36), the horses were intubated and 200 polyethylene markers were administered via a nasogastric tube with 1–2 L of water. Green- and blue-coloured polyethylene markers were used on alternate 7-day treatment blocks.

Faecal samples

Faecal matter was collected from all horses at hourly intervals from 6 h after nasogastric administration of the markers, until 96 h post-administration of markers. The total wet weight of the faeces voided (kg FW/day) by each horse was recorded at the end of each day. The markers were retrieved manually by sifting through the faecal matter collected during the first 4 days of each week, and the number of markers recovered was recorded to estimate gastrointestinal transit time.

Determination of gastrointestinal transit time

The percentage of markers recovered in the faeces of each horse was calculated by dividing the number of markers recovered by the number of markers administered. The transit time delay of the markers was measured as the time (h) post-administration of markers to the first appearance of a marker in the faeces (T_1; Moore-Colyer *et al*. 2003; Rosenfeld *et al*. 2006). The MRT (h) of the markers in the gastrointestinal tract for each diet was calculated by an equation previously described for particulate-phase MRT in ruminants and horses (Faichney 1975; Rodrigues *et al*. 2012).

Behaviour monitoring

During the first 4 days (Days 1–4, 8–12, 15–18, 22–25, 29–32 and 36–39) of each treatment block, the frequency of voiding faeces was recorded and the behaviour of horses was monitored by using an instantaneous-scan sampling technique (Bateson 1991), by using an ethogram to describe eating/drinking, standing alert, resting, abnormal behaviours, and other behaviours. Every hour, the behaviour of the horses was observed from a distance of 10–20 m outside the loose box and one scan sample per horse was recorded (n = 96 per horse per week, total n = 3456 observations). If an hourly observation was not recorded, it was considered as a missing value for all six horses at that time point.

Statistical analyses

The distribution of variables in the dataset was tested for normality using the Shapiro–Wilk test, and the data are presented as mean ± s.d. for parametric data or median ± interquartile range for non-parametric data.

Significant differences among groups (diets and horses) were determined using an ANOVA for parametric data, followed by a *post hoc* Bonferroni test (DMI, quantity of faeces voided, T_1, and MRT), and the Kruskal–Wallis test for non-parametric data (BW and percentage of time spent eating). Count-time data (cumulative recovery of markers and frequency of voiding faeces) were tested for significant effects of diet and horse by using Kaplan–Meier survival functions and the Log-rank test (Mantel–Cox test).

The DMI (g DM/kg $BW^{0.75}$) and the quantity of faeces voided (FW) by each horse on each diet were calculated as average of the first 4 days in each dietary treatment block (n = 36 observations), and the relationships of DMI and FW with MRT (h) were compared using the Spearman's rank correlation. The variables g DM/kg $BW^{0.75}$, kg FW and percentage of time spent eating (of 6 horses on 6 diets) were ranked as low or high, and MRT was ranked as short or long. The ranked variables were compared using multiple correspondence analysis in two dimensions, with normalisation on principal coordinates. The data were analysed in STATA version 12.1 (Stata Corporation, College Station, TX, USA) with significance set at $P < 0.05$.

Results

The horses remained clinically normal during the 6-week study period. The faecal egg counts at the start of the study were between 0 and 50 eggs per gram of faeces. There was no change in body condition (BCS 5 (range 5–6)) or bodyweight (528 ± 10 vs 548 ± 12) throughout the trial.

Overall, 75–95% of the markers administered to the horses were recovered from the faeces collected during the first 96 h. The log-rank test indicated a significant effect of diet on the cumulative percentage of markers recovered in the faeces within the 4-day collection interval ($P < 0.001$; Fig. 1). Diet P1 (restricted cut pasture) differed from Diet Periods P2 and P3 (*ad libitum* cut pasture; $P < 0.001$), but there was no difference between Diet Periods P2 and P3 ($P = 0.168$). There was a trend for a difference among Diets FE, FE+O and H+O ($P = 0.061$). There was a significant horse effect on the cumulative percentage of markers recovered in the faeces ($P < 0.001$). There appeared to be greater variation in marker recovery curves for some diets (Diets P2 and FE+O vs Diet P3) and horses (H4, H5 and H6 vs H2) ($P < 0.001$ for each comparison; Figs S1 and S2, available as Supplementary material to this paper).

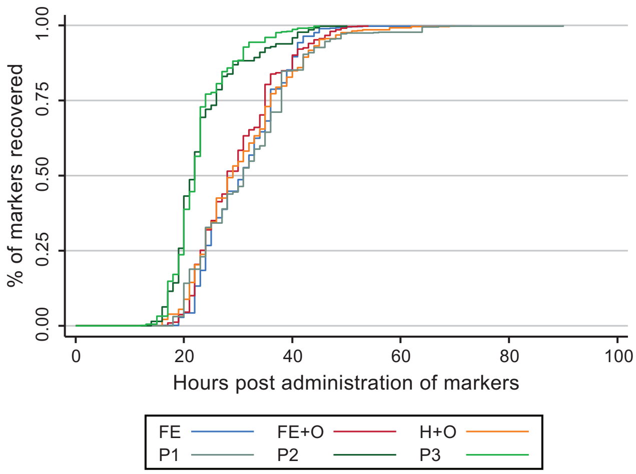

Fig. 1. Comparison of the Kaplan–Meier survival functions for the cumulative percentage of polyethylene markers recovered in the faeces of six Thoroughbred horses fed six diets during the 6-week study period. The diets comprised cut pasture (Diets P1, P2 and P3 fed in Weeks 1, 3 and 5 respectively), an ensiled timothy–lucerne forage (Diet FE), ensiled timothy–lucerne forage fed with oats (Diet FE+O) and perennial ryegrass hay fed with oats (Diet H+O).

The mean time in hours (h) ± s.d. for recovery of the first marker for each diet across all horses was 19 ± 4 (P1), 14 ± 3 (P2), 15 ± 3 (P3), 21 ± 5 (FE), 19 ± 4 (FE+O) and 17 ± 2 (H+O), and ranged for horses from 15 ± 3 (Horse 6) to 22 ± 5 (Horse 3). Both diet ($P < 0.01$) and horse ($P < 0.01$) had a significant effect on the time at which the first marker was recovered. Retention time (h) of the markers in the gastrointestinal tract for each horse, stratified by diet, is presented in Table 2. There was a significant effect of diet ($P < 0.05$) and horse ($P < 0.001$) on the MRT.

The horses voided between 8 and 21 kg/day of faeces (FW) at the rate of one faecal output every 2 h. The quantity of faeces voided per day differed significantly among diets ($P < 0.0001$) and among horses ($P < 0.0001$). Horses 1 and 2 voided a greater weight of faeces (12–21 kg/day) when compared with the other horses (8–16 kg/day; Table 3). The Kaplan–Meier survival functions indicated a diet effect ($P = 0.046$) on the cumulative frequency of faeces voided, with no difference observed among horses.

All horses consumed between 1.1% and 1.5% BW of a feed (DM basis), equivalent to 64–91 MJ of digestible energy per day. The quantity of feed offered to, and consumed by, the horses is presented in Table 4. The DMI (kg/day) differed significantly among horses ($P < 0.0001$) and diets ($P < 0.0001$), with a significant ($P < 0.0001$) diet × horse interaction. The among-horse effect remained significant when DMI was expressed as %BW and $BW^{0.75}$ ($P < 0.0001$ for each comparison). Within each dietary treatment week, the horses consumed ~1–5% less feed on the first day than on the subsequent days in the week, but this reduction in feed intake was not significant. The quantity of feed (g DM/kg $BW^{0.75}$.day) consumed was highest for the diets containing chopped

Table 2. Mean retention time (h) of markers recovered in the faeces obtained from horses during the study
H1–6, Horses 1–6; P1, P2 and P3, cut pasture fed to the horses in Weeks 1, 3 and 5 respectively; FE, chopped ensiled forage; FE+O, chopped ensiled forage mixed with whole oats; H+O, perennial ryegrass hay fed with whole oats. s.d., standard deviation; $P < 0.05$ and $P < 0.001$, significant diet and horse effects respectively (R^2 = 0.87). Different letters within a row or column indicate significant differences (*post hoc* Bonferroni test)

Horse	Dietary treatment						
	P1	P2	P3	FE	FE+O	H+O	Mean ± s.d.
H1	28	20	22	31	30	24	26 ± 4a
H2	26	21	24	28	28	26	26 ± 2a
H3	38	31	26	38	38	35	34 ± 4b
H4	30	24	22	28	25	36	28 ± 5ab
H5	38	25	23	36	33	32	31 ± 5ab
H6	34	23	23	28	29	35	29 ± 5ab
Mean ± s.d.	32 ± 5a	24 ± 4b	23 ± 2b	32 ± 4a	31 ± 5a	31 ± 5a	

Table 3. Wet weight of faeces voided by horses (kg/day) when fed each diet during the study period
H1–6, horse numbers; P1, P2 and P3, cut pasture fed to the horses in Weeks 1, 3 and 5 respectively; FE, chopped ensiled forage; FE+O, chopped ensiled forage mixed with whole oats; H+O, perennial ryegrass hay fed with whole oats. s.d., standard deviation. $P < 0.0001$ and $P < 0.0001$, significant diet and horse effects respectively (R^2 = 0.88). Different letters within a row or column indicate significant differences (*post hoc* Bonferroni test)

Horse	Dietary treatment						Mean ± s.d.
	P1	P2	P3	FE	FE+O	H+O	
H1	12 ± 1	18 ± 5	19 ± 3	16 ± 4	19 ± 3	16 ± 3	17 ± 4a
H2	14 ± 3	20 ± 5	19 ± 3	19 ± 5	21 ± 4	14 ± 4	18 ± 5a
H3	8 ± 2	9 ± 2	13 ± 3	13 ± 3	9 ± 2	11 ± 3	11 ± 3bc
H4	8 ± 2	14 ± 3	14 ± 3	10 ± 2	10 ± 3	11 ± 2	11 ± 3cd
H5	9 ± 3	14 ± 3	16 ± 2	16 ± 4	12 ± 2	12 ± 2	13 ± 4d
H6	10 ± 1	14 ± 4	16 ± 3	16 ± 4	13 ± 4	9 ± 1	13 ± 4d
Mean ± s.d.	10 ± 3a	15 ± 5b	16 ± 3b	15 ± 5b	14 ± 5bc	12 ± 3ac	

Table 4. Feed offered and consumed by the horses during the study period
P1, P2 and P3, cut pasture fed to the horses in Weeks 1, 3 and 5 respectively. FE, chopped ensiled forage; FE+O, chopped ensiled forage mixed with whole oats; H+O, perennial ryegrass hay fed with whole oats

Parameter	Unit	Dietary treatment					
		P1	P2	P3	FE	FE+O	H+O
Feed offered	kg/day (as-fed basis)	46 ± 10	57 ± 8	60 ± 9	24 ± 4	27 ± 5	15 ± 3
Feed consumed	kg/day (as-fed basis)	38 ± 8	44 ± 8	46 ± 9	19 ± 4	19 ± 5	9 ± 2
Feed consumed	kg/day (DM basis)	6.1 ± 1.3	7.0 ± 1.3	7.3 ± 1.4	7.4 ± 1.5	7.6 ± 2.1	6.9 ± 1.5

ensiled forage (Diets FE and FE+O), followed by the Diets P2, P3 and H+O, and lowest for Diet P1. Across all diets, Horses 1 and 2 consumed higher quantities of feed than the other horses (Table 5). MRT was negatively correlated with kg DMI/day (r = –0.45), g DM/kg $BW^{0.75}$ (r = –0.51) and kg FW (r = –0.64). Multiple correspondence analysis showed that horses that consumed more feed and voided more faeces had a shorter MRT than did horses that consumed less feed and voided less faeces, with 80% of the variation being explained on one principal coordinate.

When observed at hourly-intervals over the first four days of each treatment block, horses were most often found to be resting (43–56% of total observations), eating (24–39%) or standing alert (12–24%), with a similar pattern observed across all diets (Fig. S3). There was a significant difference in the percentage of time spent eating among diets ($P < 0.001$), with no significant differences among horses (P = 0.96). Horses were observed to be eating more often during Diet Periods P2 and P3, when compared with Diet Period P1 ($P < 0.01$) and this finding was consistent with the amount of feed offered during the diet periods. Horses spent more time eating Diet H+O than Diets FE and FE+O ($P < 0.01$). Some diets (Diets P1, FE and FE+O) appeared to show more variation among horses in time budgets.

Discussion

The present study investigated the passage rate of digesta through the gastrointestinal tract after dietary transition. The results obtained supported the hypothesis that diet quantity and

Table 5. Feed (g DM/kg BW$^{0.75}$.day) consumed by horses during the study period

H1–6, Horses 1–6; P1, P2 and P3, cut pasture fed to the horses in Weeks 1, 3 and 5 respectively; FE, chopped ensiled forage; FE+O, chopped ensiled forage mixed with whole oats; H+O, perennial ryegrass hay fed with whole oats. s.d., standard deviation. The kilogram of DM consumed differed significantly among horses ($P < 0.0001$) and diets ($P < 0.0001$), with diet × horse interaction, $R^2 = 0.95$. Different letters within a row or column indicate significant differences (Bonferroni test)

Horse	Dietary treatment						
	P1	P2	P3	FE	FE+O	H+O	Mean ± s.d.
H1	62 ± 8	71 ± 10	78 ± 10	64 ± 8	91 ± 12	73 ± 19	73 ± 15a
H2	63 ± 11	70 ± 8	72 ± 8	68 ± 4	87 ± 11	65 ± 15	70 ± 12a
H3	50 ± 12	50 ± 7	56 ± 10	61 ± 10	45 ± 4	56 ± 10	53 ± 10c
H4	47 ± 9	54 ± 7	60 ± 11	58 ± 10	54 ± 5	53 ± 5	54 ± 9cd
H5	49 ± 10	62 ± 6	64 ± 7	64 ± 13	61 ± 3	63 ± 7	61 ± 10bd
H6	54 ± 9	62 ± 7	64 ± 12	80 ± 15	74 ± 3	61 ± 5	66 ± 12ab
Mean ± s.d.	54 ± 11e	62 ± 11f	65 ± 12f	66 ± 12f	69 ± 19f	62 ± 13f	

composition, and horse, have significant effects on the passage rate of digesta. The proportion of polyethylene markers recovered in the faeces was equal to, or higher than, the recovery rates reported in other studies (McGreevy *et al*. 2001; Boscan *et al*. 2006). This high percentage of marker recovery and the frequency of faecal collection (hourly intervals) indicated that a high precision could be attained when estimating the MRT of the diets used in the present study (Rosenfeld *et al*. 2006).

The dietary transitions may have affected the feed intake of the horses in the present study, as small decreases in DMI were observed on the days the diets were changed; however, these were limited to within 1–5% of subsequent days. Ideally, to quantify the effect of diet and horse, transit time would have been monitored once the horses were habituated to the diet. However, the objective of the larger study was to quantify the effect of diet change (pasture to forage to pasture) on the faecal microbiota. Thus, we were interested in the transit time of digesta during the period of first introducing the new diet, so we could relate these data to any changes in microbiota.

Dry-matter intake for these horses at ~1.3% BW was lower than that reported for similar cohorts of horses, ~2% BW (Grace *et al*. 2002*a*, 2002*b*, 2003). The DMI may have been reduced as an indirect effect of keeping the mares in a loose box/stable, as opposed to the pasture environment, within which they were usually managed. There are indications within the literature that for horses usually managed with a pasture-based system, there is a moderate reduction in DMI with the confinement to a loose box/stable environment (Grace *et al*. 2003). Irrespective of the diet offered, the average DMI did not decrease below 1.3% BW, indicating that the horses consumed sufficient DM (~7 kg DM/horse.day; DE ~64–91 MJ/day) to meet or exceed NRC recommended maintenance requirements (National Research Council 2007). The constant BW and BCS of all horses throughout the study period supported the calculation that even with these lower DMI, maintenance or greater digestible energy was being consumed.

Only two horses (H1 and H5) exhibited abnormal behaviours (box walking and weaving respectively), which were observed rarely, irrespective of the diet. The weaving and box walking in these horses are typically expressed in anticipation of more (new) food, or less frequently, due to confinement in stables (Cooper *et al*. 2005). Despite the occasional recording of abnormal behaviours, the time spent eating for Horses 1 and 5 did not differ from that of the other horses, and their BWs remained constant during the study.

Shorter MRTs have been reported after feeding *ad libitum* than restricted quantities of alfalfa and oat straw (Pearson *et al*. 2001). Similar reductions in T_1 and MRT were observed in the present study when horses were fed *ad libitum* (P2 and P3) compared with restricted (P1) quantities of cut pasture. The MRT increased from 23–24 h to 32 h when the level of cut pasture was inadvertently restricted and became similar to the MRTs observed when the horses were fed *ad libitum* conserved forage (31–32 h). The adaptation of horses to the confinement of the stable environment during Week 1 may have confounded the results of the study, but the clustering of marker–recovery curves confirmed that the level of feeding (*ad libitum* vs restricted), and hence feed intake, had a significant effect on, and perhaps drives the passage rate of digesta through the gastrointestinal tract.

The MRT were consistent among the periods when the horses were fed *ad libitum* cut pasture (P2 and P3), and these values were similar to a previous report of weanling Thoroughbred horses fed cut pasture in stables or when grazing *ad libitum* in paddocks (Grace *et al*. 2003). The comparable MRTs reported between the studies is noteworthy, considering the differences in the age of horses (adult vs weanling) and the type of particulate phase markers (polyethylene markers vs mobile nylon-bag technique) used. There may also be a breed-effect difference in MRT. When compared on a per kilogram metabolic BW, Welsh-cross pony geldings that were fed at restricted levels consumed similar quantities of ensiled forage (62 vs 66 g DM/kg BW$^{0.75}$.day) as the Thoroughbred mares fed *ad libitum* in the present study, indicating that the relative rate of feed intake may have been slower in our horses than in the ponies. Therefore, given the variability in the feed intake of different equid types (e.g. horses, ponies and donkeys; Cuddeford *et al*. 1995; Drogoul *et al*. 2001; Pearson *et al*. 2001), some caution is warranted when comparing the results obtained in the present study with previous investigations, the majority of which were conducted on ponies (Cuddeford *et al*. 1995; Pearson *et al*. 2001; Moore-Colyer *et al*. 2003).

The inconsistencies in the feed intake and passage rate of digesta reported in previous work may have been due to differences in the feeding and management of the animals used in the studies (de Fombelle *et al*. 2004; Rodrigues *et al*. 2012; Jensen *et al*. 2014). A large variation in voluntary feed intake and passage rate of digesta was also observed among the horses in the present study, even though the experimental design controlled for breed, diet and management of the horses. The MRT varied by up to 10 h among the horses, with the longest MRT being observed for Horse 3, and this slower passage rate appeared to be linearly related to the DM of feed consumed (Horse 3 consistently consumed less DM than did other horses in the study). Perhaps other horse-specific factors, such as the rate of DMI, individual metabolic rates and reproductive status, also influence the feeding behaviour of horses, but the effects of these factors on the DMI and passage rate of digesta are poorly understood, and may require further investigation.

The model for DMI (g DM/kg $BW^{0.75}$.day) showed a significant diet × horse interaction, such that some horses were observed eating Diets FE and FE+O more frequently than Diet H+O. Horses are reported to prefer forages harvested at early maturities (Staniar *et al*. 2010; Särkijärvi *et al*. 2012), and preferentially consume greater quantities of lucerne forage than other forage species (LaCasha *et al*. 1999; Rodiek and Jones 2012), and ensiled forage when compared with hay prepared from the same forage species (Müller and Uden 2007). The preference of some horses for diets containing ensiled forage (45% moisture) when compared with hay (10% moisture) may be due to the greater palatability of the chopped ensiled forage diet and the shorter time required to consume it (chopped forage of low DM content takes less time to chew and mix with saliva than does long-stem forage of a higher DM content). Differences in the fibre length of the forages (cut pasture, chopped ensiled forage and long-stem hay) may have had some influence on the MRTs of digesta (Moore-Colyer *et al*. 2003), but the effects of diet on the passage rate of digesta observed in the present study appeared to be more likely due to differences in other horse-specific factors that may have influenced the feed-intake levels, such as individual preferences or palatability of a diet. Nonetheless, the rate of adaptability to a diet, its palatability and individual preferences of horses are poorly understood and merit further investigation.

Some horses in the present study had higher DMIs and shorter MRTs than did others. The underlying reason for the inter-horse variation may be similar to that observed for an inter-species variation, where donkeys were found to consume less DM than were ponies, and, consequently, had longer retention times and higher apparent digestibility of the feed consumed (Cuddeford *et al*. 1995; Pearson *et al*. 2001; Boscan *et al*. 2006). DMI is negatively correlated with the apparent digestibility of feed (Morel 2010), and this relationship supports the argument that longer retention time allows more time for microbial fermentation in the hindgut and increases the apparent digestibility of the feed consumed (Cuddeford *et al*. 1995). This physiological strategy may be beneficial to the survival of horses under feral conditions, where decreases in pasture growth rates, and thus pasture availability in summer and winter, may reduce the quantity of feed consumed; however, a consequent increase in the MRT of digesta may allow better digestibility of the feed consumed, which may then meet the daily energy requirements for maintaining BW (Kuntz *et al*. 2006).

However, this potential survival strategy could be a problem when managing obese domestic horses and ponies (easy-keepers), when kept on restricted access to pasture (shorter grazing turnout periods or limited sward height) designed to reduce their caloric intake (Geor 2010). Horses and ponies are capable of increasing their forage consumption during restricted grazing periods by increasing the rate of feed intake, with reports of ~40% of daily DMI consumed by ponies during a 3-h restricted grazing turnout (Ince *et al*. 2005), and greater mean rates of DMI observed in horses during 3- and 6-h restricted grazing turnouts than during 24-h access to grazing (Glunk *et al*. 2013*a*). Although restricting pasture access decreases the total intake of pasture, the compensatory increase in the rate of feed intake (during the turnout period) and the longer MRT of digesta (due to the restricted feeding time) may enable horses and ponies to maintain BW even when the quantity of feed is restricted (Dugdale *et al*. 2011; Argo *et al*. 2012; Glunk *et al*. 2013*a*).

Variation in feed intake affects the passage rate of digesta and the apparent digestibility; therefore, these factors should be taken into consideration when formulating specific feeding regimens for horses and ponies, and when comparing the rate of change in microbiota populations when horses transition between diets. The differences observed in the feed intake levels and MRTs may explain some of the previously reported variation in the population of hindgut or faecal microbiota (Dougal *et al*. 2014; Fernandes *et al*. 2014*a*), and perhaps explain the increased susceptibility of some horses and ponies to gastrointestinal disturbances. This hypothesis requires further investigation.

Conclusions

The results of the present study highlighted that diet-specific factors (such as feed type, composition and intake level) influence the MRT of digesta in the gastrointestinal tract. As expected, there was among-horse variation due to horse-specific factors (such as eating behaviour, voluntary DMI and quantity of faeces voided). The level of feed intake appears to drive the passage rate of digesta, and thus, reduced intake may potentially increase the apparent digestibility of the feed consumed, due to the greater time available for microbial fermentation.

Data availability

The data that support this study will be shared on reasonable request to the corresponding author.

Conflict of interest

Chris Rogers is an Associate Editor of *Animal Production Science* but was blinded from the peer-review process for this paper.

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