Mathematical modelling of digesta passage rate, mean retention time and *in vivo* apparent digestibility of two different lengths of hay and big-bale grass silage in ponies

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Welsh-cross pony geldings (about 300 kg live weight) were used in a 4×4 Latin square experiment to determine the rate of passage and apparent digestibility of unchopped big-bale grass silage (BBL), chopped big-bale grass silage (BBS), unchopped grass hay (HL) and chopped grass hay (HS) offered at approximately 15 g/kg live weight per d. On day 1 of collection weeks, ponies were fed 85 g ytterbium chloride hexahydrate-marked feed 1·5 h after the morning meal. Total faecal collections commenced 8 h later and continued for 168 h. Apparent digestibilities of feed DM, organic matter (OM), crude protein (CP, N×6·25), acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) were also determined. Faecal excretion data were subjected to the models of Pond *et al.* (1988) and digesta mean retention time (MRT) calculated from these models and using the algebraic method of Thielmans *et al.* (1978). Silage had significantly ($P<0.05$) higher digestibilities of DM, OM, CP, ADF and NDF than hay; however, chop length had no effect. All the models of Pond *et al.* (1988) accurately described ($R^2>0.8$) the pattern of faecal marker excretion. MRT of BBL (29·0 h) > BBS (27 h) > HS and HL (26 h). Compartmental analysis using the G3 model of Pond *et al.* (1988) showed BBL and HS diets had longer MRT in the time-dependent compartment, whereas BBS and HL had longer MRT in the time-independent compartment. Results from this experiment indicate that BBL and BBS are readily accepted and digested by ponies. While Yb is a successful external marker for determining total tract MRT and for modelling faecal excretion curves in horses, the results did not allow any definite conclusions to be drawn on digesta MRT within the different compartments of the equid gut.

Digesta passage rate: Mean retention time: Apparent digestibility: Horse

Mathematical modelling of faecal excretion data using indigestible external markers is a non-invasive method that can be used to obtain digesta passage rate and mean retention time (MRT) in animals. Such models simulate aspects of the digestive system and so allow a greater understanding of digesta kinetics. Although mathematical models have been applied to ruminant animal data (Uden *et al.* 1982; Mertens, 1989; Lalles *et al.* 1991; Ellis *et al.* 1994), the process of using these models to compartmentalise the digestive tract is far from established and rarely have these models been used to describe the passage of digesta through the equid gastrointestinal tract.

Mathematical models are generally categorised into two types: the first are time-independent models (Grovum & Williams, 1973; Dhanoa *et al.* 1985), which are deterministic in nature and therefore assume that digesta flows irreversibly through a fixed number of sequential compartments, according to first order kinetics (Lalles *et al.* 1991). The second are termed time-dependent models. These are based on assumptions of probability (stochastic models) and use γ-functions (non-exponential residence time distributions) to describe the time-dependent passage of digesta through different segments of the gastrointestinal tract. The inherent flexibility of time-dependent models has allowed them to be successfully fitted to ruminant animal faecal excretion data (Pond *et al.* 1988) where the more rigid time-independent models have failed (Milne *et al.* 1978; Ellis *et al.* 1979; Uden *et al.* 1982). The failure of time-independent models may be partly attributed to the fact that they assume constant volume and instantaneous mixing of digesta within a gut compartment, along with equal opportunity for particle escape irrespective of digesta residence time (Ellis *et al.* 1994). Digesta passing through the

fermentation section of the equid gut, which consists of the caecum and the four distinct regions of the large colon (Jackson, 1998), has to pass through narrow flexures as it flows through right and left ventral, to left and right dorsal chambers. While mixing within each of these chambers may occur quickly, it is unlikely that the passage of digesta through the entire large intestine would follow first order kinetics. Therefore, the physiological arrangement of the equid digestive tract will cause digesta passage to be a time-dependent process, whereby the probability of particle escape is increased with time. This assumption is supported by a previous preliminary study in ponies of Moore-Colyer (2000), which found time-dependent models to be more successful at describing the pattern of faecal excretion than time-independent models.

Published accounts on the process of mathematically modelling faecal excretion data in horses are strictly limited (Corino *et al.* 1992), with most workers confining their calculations to a determination of digesta MRT using a variety of algebraic equations (Uden *et al.* 1982; Pearson & Merritt, 1991; Nyberg *et al.* 1993). Such information has indicated that the lower degradability of certain forage foodstuffs in equids compared with ruminant animals (Vander Noot & Gilbreath, 1970) is due, at least in part, to the shorter MRT within the equid gastrointestinal tract (Uden *et al.* 1982). However, to date no attempt has been made at using time-dependent models to track the passage of digesta through the individual gut compartments, and therefore to try to explain why the degradability of certain foodstuffs is more influenced by MRT than others.

Information on the behaviour of digesta within the different segments of the gut would allow a clearer understanding of the dynamic interactions between feed, enzymes and gut microflora and would promote better ration formulation for horses engaged in a wide variety of activities.

The objectives of the following experiment were to assess the suitability of Yb as an indigestible marker for measuring digesta passage rate in ponies and to examine the accuracy with which three time-dependent and two time-independent (Dhanoa *et al.* 1985; Pond *et al.* 1988) models described the pattern of faecal excretion in ponies. On the basis of these models, biological interpretation was attempted, whereby the individual compartments identified by the model were designated as representing certain regions within the equid gastrointestinal tract. In addition, the digestibility of two different chop-lengths of hay and silage were measured, to determine if particle length had any influence on the degradability and MRT of the feeds within the gastrointestinal tract of ponies.

Materials and methods

Animal management

Four mature Welsh-cross pony geldings (about 300 kg live weight) were used in a 4×4 Latin square change-over design experiment consisting of four 21 d periods, each split into a 14 d adaptation phase and a 7 d faecal collection period. Ponies received a similar amount of feed for adaptation and collection periods and were individually loose-housed in 3·6×3·6 m loose boxes; the floors were covered with rubber matting (Davies and Co, Kettering, Northants, UK) and water was available *ad libitum*. During adaptation weeks the ponies were allowed 30 min free exercise in an outdoor sand arena, while in collection weeks, ponies were walked in hand, covering a distance of 2·25 km (1·4 miles)/d.

The ponies were offered one of four diets at a level equivalent to 15 g feedstuff DM/kg live weight per d. The diets comprised unchopped big-bale grass silage (BBL), chopped big-bale grass silage (BBS), unchopped grass hay (HL) and chopped grass hay (HS). Precise details on the cutting date of the timothy (*Phleum pratense*)– perennial rye grass (*Lolium perenne*) hay were not available, but the number of flowering heads in the bales was indicative of a crop cut in mid-late summer. The big-bale silage was conserved on the University of Wales Lluest farm from a perennial rye grass sward, which received 0·5 kg N/km^2 on 1st March and 1·5 kg/km^2 as 20:10:10 N:P:K on 9th April. The grass was mowed on 8th June and left to wilt for 48 h before being made into big round bales and wrapped in four layers of heavy-gauge plastic wrap. The diets were fed in two equal meals per d at 08.00 and 20.00 hours. Equivite mineral and vitamin supplement (60 g; Equivite; Spillers Speciality Feeds, Milton Keynes, UK) was mixed with 20 g soaked sugarbeet pulp and offered after the morning feed had been eaten. In addition, each pony had free access to a salt lick, located in the corner of each stable. Pony live weight was recorded at 10.30 hours every Monday throughout the trial period using a weighbridge (Basic Weigher; Tyler Farm Equipment, Grantham, Lincs, UK).

Food preparation

The chopped hay and silage rations were prepared from the same bales that were used for the corresponding long forage samples. Each morning, the daily silage ration was removed from the bale with a fork and placed into 40 litre plastic tubs. The proportion to be chopped was then fed into a straw chopper, which consisted of a set of rotating knives fitted to a Honda G3000, 5·22 kW petrol engine and a WIC 8705 drum (Fosse, York, UK). The chopped forage was then stored in covered 40 litre plastic tubs until it was fed to the ponies. The hay was chopped in the same manner as the silage except that sufficient hay to last for 3 weeks was chopped at one time.

Particle size determination

A sample of fresh forage (25 g) was taken from each batch of food to determine the particle size. The length of each individual particle was measured and put into one of the following size categories (mm): 0–25, 26–50, 51–75, 76–100, 101–150, >150. Each group of particles were then placed into foil trays and weighed to determine the percentage of particles within each length category.

Preparation of ytterbium-marked food

Yb-marked food was prepared using ytterbium (III) chloride hexahydrate (99 %; Aldrich Chemical Company,

Gillingham, Dorset, UK), by the immersion method of Teeter *et al.* (1984). At 09.30 hours on the Monday of each collection week 85 g Yb-marked food mixed in with 100 g pre-soaked sugarbeet pulp was fed to the ponies.

Apparent digestibility and nutritive value measurements

During the 7 d faecal collection periods *in vivo* apparent digestibilities of DM organic matter (OM), crude protein (CP, N×6·25), acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) were determined by total faecal collections. Daily faecal samples for each pony were separately stored in covered plastic bins for 24 h, whereupon faeces were thoroughly mixed and a sub-sample of 800 g was taken and stored at −20°C prior to lyophilisation. At the end of each collection period, a bulk faecal sample was compiled from the 7 d outputs, according to the proportion of the daily faecal DM output for each pony (Cochran & Gaylean, 1994).

Composite samples of the diets and the dried, bulked faecal samples, milled to pass a 1 mm screen, were subsequently analysed for DM, OM, CP, ADF and NDF contents according to the methods of the Association of Official Analytical Chemists (1990).

Digesta rate of passage measurements

Faecal collections commenced 8 h after the administration of the Yb; this involved collecting all defecations up to 48 h, followed by 2 h collections up to 72 h, 4 h collections up to 96 h, 8 h collections from 96–120 h and 12 h collections up to 168 h. Time of faecal voiding and subsequent faecal sample weight were recorded for every sample. A 200 g sub-sample from each collection was then placed into a pre-weighed foil tray, weighed and dried in a force-draught oven at 60°C for 48 h and stored at room temperature, prior to chemical analysis.

Preparation of feed and faecal samples for ytterbium analysis

The concentrations of Yb in feed and faecal samples were determined as follows: 2 g dried, ground sample (1 mm mesh screen) were weighed into a numbered crucible and placed into a furnace and ashed at 550°C for 2 h. After cooling, several drops of HNO_3 (50 ml/l) were added to the crucible to dissolve the ash; this was then transferred to a 20 ml volumetric flask and made up to volume using additional HNO_3 (50 ml/l) solution. After thorough mixing, the solution was transferred to a plastic bottle and left for 12 h to settle, whereupon the supernatant fraction was decanted into a clean numbered medical bottle. A sample of this solution (0·1 ml) plus 9·6 ml milli-Q water (Fisher Scientific Ltd, Loughborough, Leics., UK), 0·2 ml concentrated HNO_3, and 0·1 ml Rh (10 μg/ml HNO_3 (50 ml/l)) solution were placed into sample tubes, thoroughly shaken and fed through an induction-coupled plasma MS. The pick-up time for the samples was set at 35 s with a scan time of 35 s. Between samples the probe was placed in HNO_3 (50 ml/l) to minimise cross-contamination of samples.

Data analysis

Faecal excretion data were fitted to the four models of Pond *et al.* (1988) and the multi-compartmental model of Dhanoa *et al.* (1985). The equations used to fit the G1, G2, G3 and G4 models are detailed and these were solved using a SAS computer program (1985; SAS Inst. Inc, Cary, NC, USA) with the procedure recommended by Moore *et al.* (1992).

$$F = C_2 k_1 \left(e^{-k_2 TD} - e^{-k_1 TD}\right)/(k_1 - k_2), \quad \text{(G1 model)}$$

$$F = C_2 \left(\delta^2 e^{-k_2 TD} - e^{-\lambda_1 TD}\left(\delta^2 + \delta \lambda_1 TD\right)\right), \quad \text{(G2 model)}$$

$$F = C_2 \left(\delta^3 e^{-k_2 TD} - e^{-\lambda_1 TD}\left(\delta^3 + \delta^2 \lambda_1 TD + \delta^2 \lambda_1 TD^2/2\right)\right) \quad \text{(G3 model)}$$

and

$$F = C_2 \left(\delta^4 e^{-k_2 TD} - e^{-\lambda_1 TD}\left(\delta^4 + \delta^3 + \delta^2 \lambda_1 TD + \delta^3 \lambda_1 TD^3/3\right)\right), \quad \text{(G4 model)}$$

where F is the fractional concentration of marker, C_2 is the initial concentration in second compartment, C_2 is dose (D)/volume at second compartment (V), k_1 and k_2 are exponentially distributed rate parameters, λ is the rate parameter for γ-distributed residence times, $\delta = \lambda_1/(\lambda_1 - k_2)$ and TD=(t − λ) time delay (time post – dose to first appearance of marker in the faeces) (Pond *et al.* 1988).

All models were tested for accuracy of fit using linear regression, to determine R^2 values for each pony on each diet. The R^2 values were then subjected to a two-way ANOVA using Genstat 5 (1993; Lawes Agricultural Trust, Rothamsted Experimental Station, Harpendon, Herts., UK) and least significant difference test to determine if significant differences existed between the 'goodness of fit' of the models when fitting different diets, and if a difference existed between models within a diet.

The results obtained from the four compartmental models of Pond *et al.* (1988) were then used to calculate total tract MRT.

The MRT for the G1, G2, G3 and G4 models of Pond *et al.* (1988) were obtained from:

$$1/\lambda + 1/k_2 + TD = TMRT, \quad \text{(G1 model)}$$

$$2/\lambda + 1/k_2 + TD = TMRT, \quad \text{(G2 model)}$$

$$3/\lambda + 1/k_2 + TD = TMRT, \quad \text{(G3 model)}$$

and

$$4/\lambda + 1/k_2 + TD = TMRT, \quad \text{(G4 model)}$$

where TMRT is the total tract MRT. The algebraic equation of Thielmans *et al.* (1978) was used to determine MRT from the following equation:

$$MRT = \Sigma ti \; Ci \; \Delta ti right/\Sigma Ci \; \Delta ti,$$

where ti is the time post dose, taken as the mid-point between successive samples, Ci is the concentration of marker in faeces and Δti is the difference in time between successive samples.

The MRT from the one algebraic and four compartmental models were then subjected to a two-way ANOVA (Genstat 5, 1993; Lawes Agricultural Trust) and a least significant difference test, to determine if significant differences existed in MRT between BBL, BBS, HL and HS and if differences existed between the MRT obtained from different models within diets.

Compartmental analysis

The four models were tested for goodness of fit to observed data using R^2 analysis. The model of best fit, determined from the R^2 data, was then used for further compartmental analysis. The mean digesta passage rates and retention times for each diet through each of the gastrointestinal tract compartments of the four ponies were then calculated and these values subjected to an analysis of variance (Genstat 5, 1993; Lawes Agricultural Trust). Thus, mean values were obtained for a time-dependent compartment λ, a time-independent compartment k_2 and a TD factor τ for each diet.

Results

Animal management

All four ponies remained healthy during the 12-week experimental period and readily consumed the forage offered to them each day. Feed refusals were negligible, ranging from 44 g DM for HS to 123 g DM for BBS/d. All the ponies readily accepted the oral pulse dose of 85 g Yb-marked food at the start of each collection period.

Food composition

The chemical compositions of each of the four experimental forages are detailed in Table 1. The DM content of BBL was slightly lower than for BBS (353 *v.* 371 g/kg respectively): this difference is likely to be due to the additional handling and the chopping of the BBS forage. All other variables were similar between the chopped and long silage. Similarly, no differences in chemical compositions were noted between HS and HL. The two silages contained more that fourfold as much moisture as the hay, 190 and 195 g less ADF and NDF respectively and 60 g more CP (N×6·25) than the hay/kg DM. The average particle size was greatest for the BBL at 293 mm, followed by the HL at 180 mm, with the chopped BBS and HS being only 68 and 53 mm respectively.

Food intake and apparent digestibility measurements

Table 2 details the average DM intake of the ponies when offered the four forages at the rate of 15 g/kg live weight per d. The DM of the silage varied slightly from bale to bale throughout the 12-week experiment. Consequently, the amount of DM offered per d varied, resulting in significantly ($P<0.05$) higher intakes of BBS (0·58 kg/d) compared with intakes of the two hay diets. No difference in intake was noted between the BBL and any of the other diets. Furthermore, no significant differences in the

Table 1. Composition and particle size of the four experimental feeds offered to the four ponies (g/kg DM)*

(Mean values)

	Diet†			
	BBL	BBS	HL	HS
DM	353	371	861	860
ADF	307	305	397	395
NDF	505	503	695	706
CP	166	166	102	105
Particle size (mm)	293	68	180	53

BBL, unchopped big-bale grass silage; BBS, chopped big-bale grass silage; HL, unchopped hay; HS, chopped hay; ADF, acid-detergent fibre; NDF, neutral-detergent fibre; CP, crude protein (N×6·25).
* For details of preparation of diets, see p. 110.
† Feeds were supplemented with 60 g mineral and vitamin supplement (Equivite,) (per kg): Ca 100 g, P 40 g, Na 20 g, Mg 20 g, lysine 10 g, Fe 300 mg, Zn 1000 mg, Mn 800 mg, Cu 800 mg, I 20 mg, Se 10 mg, Co 10 mg, choline chloride 1000 mg, biotin 20 mg, pyridoxine 100 mg, thiamin 240 mg, cyanocobalamin 2000 μg, riboflavin 240 mg, pantothenic acid 480 mg, nicotinic acid 100 mg, folic acid 70 mg, α-tocopherol 10 g, cholecalciferol 0·05 mg, retinol 4·35 mg.

digestibilites of DM, ADF or CP were noted between the normal or chopped form of either forage. However, significantly ($P<0.01$) higher digestibilites of DM, ADF, NDF and CP were recorded for both the silages compared with both hays, reflecting the higher nutritive value of the silage compared with the hay.

Digesta passage rate studies

Recovery of marker in the faeces was $>95\%$ for all feeds, and although differences existed between marker concentration on each forage ranging from 12·922 for HS to 29·178 mg/g for BBS, the concentration of Yb in the faeces was easily detectable.

Description of faecal excretion data using mathematical models

The multi-compartmental time-independent model of Dhanoa *et al.* (1985) failed to converge with some of the faecal excretion data collected; thus, a workable solution was not attained and so the model was subsequently rejected from further analysis. The other compartmental models tested were the G1 time-independent model of Pond *et al.* (1988) and the three time-dependent models G2, G3, G4 of Pond *et al.* (1988).

Table 3 shows the R^2 data obtained when the four models of Pond *et al.* (1988) were fitted to the faecal excretion data. All models fitted the data with reasonable accuracy, with values ranging from 0·862 for HS G2 to 0·955 for HL G4. When tested on the HS and BBL diets the G2 model was significantly ($P<0.05$) less accurate than the G3 (0·950) and G4 (0·955) models when fitted to HL data. BBS G2 was also significantly less ($P<0.05$) accurate than the HL G4 model. For any given model there were no significant differences across or within diets. Overall, the G1 and G2 models described the data significantly less well than the G3 and G4 models. Although there was no significant difference between the G3 and G4 models, the G3 model was marginally more

Table 2. DM intakes (kg/d) and *in vivo* apparent digestibility coefficients for the four experimental feeds offered to the four ponies at 15 g/kg live weight†

(Mean values)

	Diet					
	BBL	BBS	HL	HS	SED	Statistical significance of effect
LW (kg)	292[b]	294[b]	280[a]	282[a]	2·51	*
DM intake (kg/d)	4·27[ab]	4·46[b]	3·83[a]	3·88[a]	0·247	*
DM intake[0·75]	60·5	62·3	55·9	56·3	3·380	NS
Digestibility						
DM	0·67[a]	0·67[a]	0·48[b]	0·50[b]	0·015	**
ADF	0·64[a]	0·63[a]	0·39[b]	0·42[b]	0·019	**
NDF	0·62[a]	0·61[a]	0·43[b]	0·46[b]	0·014	**
CP	0·74[a]	0·74[a]	0·58[b]	0·58[b]	0·021	**

BBL, unchopped big-bale grass silage; BBS, chopped big-bale grass silage; HL, unchopped hay; HS, chopped hay; LW, live weight; ADF, acid-detergent fibre; NDF, neutral-detergent fibre; CP, crude protein (N×6·25).
* $P < 0.05$, ** $P < 0.01$.
[a,b]Mean values within a row with unlike superscript letters were significantly different; *($P < 0.05$), **($P < 0.01$).
† For details of diets and procedures, see Table 1 and p. 110.

accurate than the G4 and was subsequently used for further compartmental analysis.

Fig. 1 depicts the faecal excretion data from each pony and the average G3 (Pond *et al.* 1988) -fitted curves for all four forage diets. The pattern of marker excretion was similar for each diet, although in all cases the fitted curve failed to describe the early phase of marker excretion.

Mean retention time of digesta within the total tract determined using ytterbium-marked food

The average MRT for the four forages are detailed in Table 4. The average MRT across all models for BBL was significantly ($P < 0.05$) longer at 29 h compared with the other three forages, which ranged from 26 h for HL and HS to 27 h for BBS. The average values for the models indicated that the G2 model produced a significantly ($P < 0.05$) longer MRT value of 29 h than the G3, G4 and Thielmans *et al.* (1978) equation of 26·0 h. The

time-independent G1 model did not differ significantly from any of the other models when predicting MRT.

When considering the individual differences between the five models and the four forages, the G4 model for HL produced the shortest MRT of 23·8 h, which differed significantly ($P < 0.05$) from all but the BBS value of 27·7 h for the values predicted by the G2 model, and all but the Thielmans *et al.* (1978) model when applied to the BBL diet.

The algebraic model of Thielmans *et al.* (1978) uses actual concentration of marker recovered in the faeces, and was therefore used as the standard method for comparing the accuracy of the MRT values obtained from the compartmental models

Compartmental analysis

The G3 model of Pond *et al.* (1988) best described the faecal excretion patterns obtained when Yb marker was offered as an oral pulse dose to the four ponies.

Table 3. Accuracy of fit, as determined by R^2, of the four mathematical models of Pond *et al.* (1988), for describing faecal excretion data using ytterbium-marked food in four ponies consuming four experimental feeds†

(Mean values)

	Diet				
Model‡	BBL	BBS	HL	HS	Mean of models
G1	0·855[abc]	0·888[abc]	0·910[abc]	0·880[abc]	0·883[x]
G2	0·865[a]	0·872[ab]	0·875[abc]	0·862[a]	0·868[x]
G3	0·935[abc]	0·905[abc]	0·950[bc]	0·923[abc]	0·928[y]
G4	0·937[abc]	0·902[abc]	0·955[c]	0·917[abc]	0·927[y]
SED		0·0413§			
Mean of diets	0·898[p]	0·891[p]	0·922[w]	0·895[p]	
SED		0·0206‖			0·0206¶
Statistical significance of effect		*	*		*

BBL, unchopped big-bale grass silage; BBS, chopped big-bale grass silage; HL, unchopped hay; HS, chopped hay.
* $P < 0.05$.
[a,b,c]Mean values in a column or row (model and diet) with unlike superscript letters were significantly different ($P < 0.05$).
[x,y]Mean values in a column (model) with unlike superscript letters were significantly different ($P < 0.05$).
[p,w]Mean values in a row (diet) with unlike superscript letters were significantly different.
† For details of diets and procedures, see Table and p. 110.
‡ G1 to G4 time-dependent models of Pond *et al.* (1988).
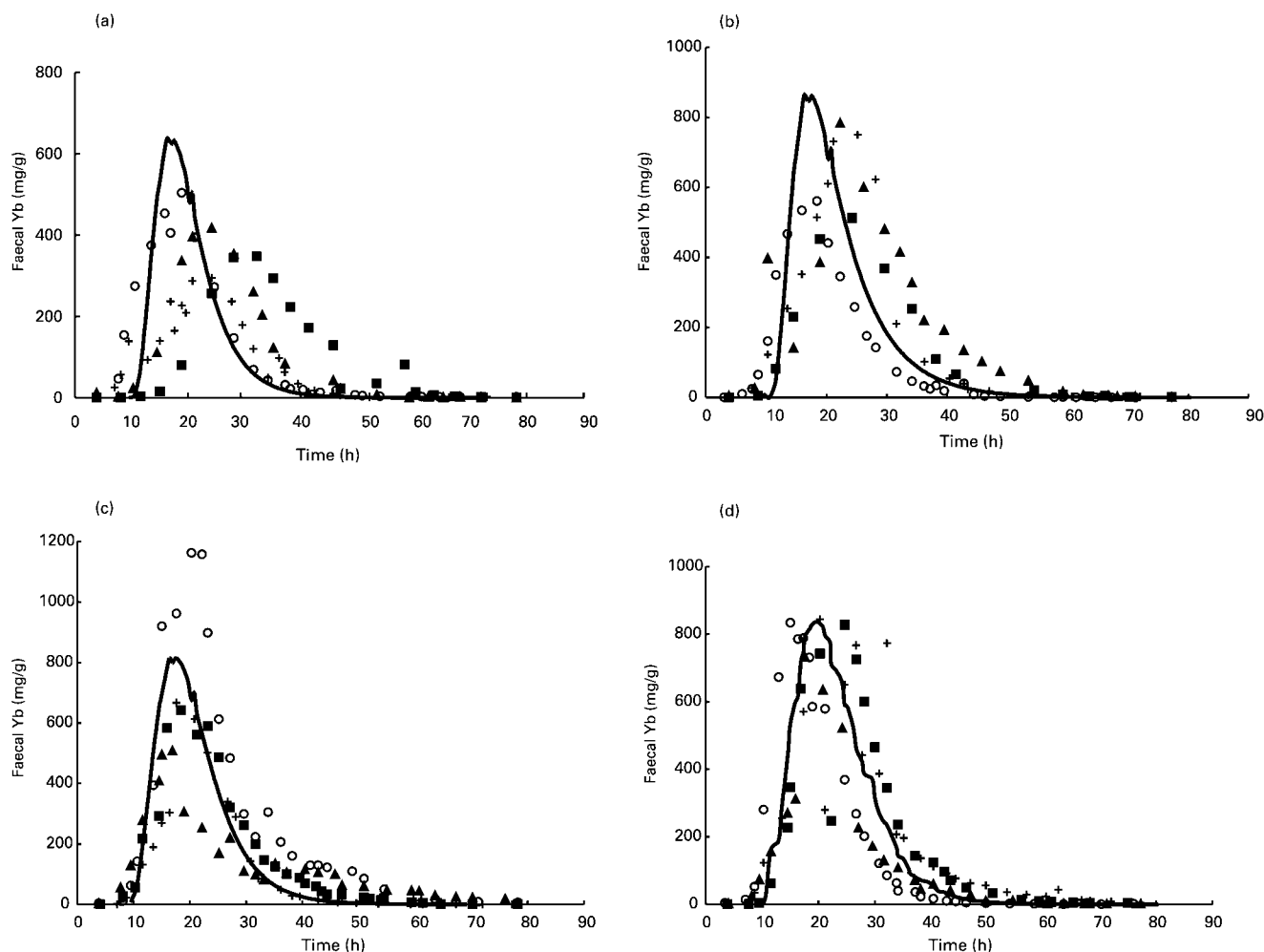§ Model and diet SED.
‖ Diet SED.
¶ Model SED.

Fig. 1. Faecal excretion curves obtained using ytterbium-marked feed from ponies consuming unchopped big-bale grass silage (BBL, (a)), chopped big-bale grass silage (BBS, (b)), unchopped hay (HL, (c)) and chopped hay (HS, (d)) in four ponies. For details of diets and procedures, see Table 1 and p. 110. Individual points represent results from individual ponies. —, Average fitted curves using the G3 model of Pond *et al.* (1988). R^2 values: BBL 0·94, BBS 0·90, HL 0·95, HS, 0·93.

Table 5 details the mean passage rate and retention time parameters obtained when using the G3 time-dependent compartmental model. The BBL and HS appear to remain slightly longer in the λ compartment, whereas the BBS and HL remain longer in the k_2 compartment. The TD for both chop lengths of silage was slightly longer than was noted for the hay feed, but these differences were not significant. The significant ($P<0.05$) difference in MRT between BBL and HL shows the BBL to be retained for 4·2 h longer than the HL diet.

Discussion

Food composition

The DM contents of the forages offered to the ponies in this experiment were similar to those recorded by the Ministry of Agriculture Fisheries and Food (1992) for samples of typical UK hay and big-bale grass silage.

While the CP (N×6·25) contents for both forages are also similar to those quoted by the Ministry of Agriculture Fisheries and Food (1992), they are markedly higher than

the values recorded in a previous experiment by Moore-Colyer (2000) of 44 (hay) and 111 (big-bale grass silage) g/kg DM. ADF and NDF contents were within the range recorded by the Ministry of Agriculture Fisheries and Food (1992) for these forages.

In vivo *apparent digestibility*

The apparent digestibilities of DM, ADF, NDF and CP (N × 6·25) for both HS and HL were significantly lower than for BBS and BBL and reflect the stage of growth of the grass at harvest, and thus the higher nutritive value of the silage compared with the hay. The apparent digestibility values for the silage agree with those of Keating (1998) and Moore-Colyer (2000), although the hay in this experiment was 10−30 % more digestible than recorded in previous studies. These comparisons clearly demonstrate the variability of UK hay, and thus indicate that analysis of hay prior to ration formulation is advisable.

The CP (N×6·25) content of 103 g/kg DM and DM intake of 3·8 kg hay diet/d in the present experiment meant that the

Table 4. Retention times of digesta (h), calculated from four mathematical models and one algebraic equation fitted to faecal excretion data obtained from four ponies given an oral pulse dose of ytterbium-marked food while consuming four experimental diets†

(Mean values)

	Diet				
Model‡	BBL	BBS	HL	HS	Mean of models
G1	30·6[de]	27·5[abcde]	25·1[abc]	25·6[abc]	27·2[xy]
G2	31·0[e]	27·7[bcde]	28·4[cde]	28·1[cde]	28·8[y]
G3	28·2[cde]	27·0[abcd]	24·0[ab]	24·6[abc]	25·9[x]
G4	27·9[abcd]	26·4[abc]	23·8[ab]	25·0[abc]	25·8[x]
Thielmans' equation§	27·2[abcdef]	25·3[abcd]	26·1[abcd]	24·9[abcd]	25·9[x]
SED		1·920‖			
Mean of diets	28·9[p]	26·8[w]	25·5[w]	25·6[w]	
SED		0·859¶	*	*	0·960††
Statistical significance of effect					*

BBL, unchopped big-bale grass silage; BBS, chopped big-bale grass silage; HL, unchopped hay; HS, chopped hay.
* $P < 0.05$.
[a,b,c,d,e,f]Mean values in a column or row (model or diet) with unlike superscript letters were significantly different ($P < 0.05$).
[x,y]Mean values in a column (model) with unlike superscript letters were significantly different ($P < 0.05$).
[p,w]Mean values in a row (diet) with unlike superscript letters were significantly different ($P < 0.05$).
† For details of diets and procedures, see Table 1 and p. 110.
‡ G1 to G4 time-dependent models of Pond *et al.* (1988).
§ Thielmans *et al.* (1978).
‖ Model and diet SED.
¶ Diet SED.
†† Model SED.

Table 5. Rate parameters λ and k_2 and calculated mean retention time (MRT) for the λ compartment (LC), k_2 compartment (kC), time delay (TD) and total tract MRT obtained from the Pond *et al.* (1988) G3 model applied to faecal excretion data collected from ponies given an oral dose of ytterbium-marked food while consuming four experimental feeds†

	Diet					
	BBL	BBS	HL	HS	SED	Statistical significance of effect
λ	0·67	0·81	0·61	0·34	0·381	NS
LC	10·64	7·03	6·65	10·61	2·376	NS
k_2	0·186	0·151	0·176	0·223	0·0556	NS
kC	6·55	8·73	7·58	4·85	2·751	NS
TD	11·05	11·26	9·79	9·05	1·068	NS
MRT	28·24[b]	27·02[a]	24·02[a]	24·51[a]	1·769[a]	*

BBL, unchopped big-bale grass silage; BBS, chopped big-bale grass silage; HL, unchopped hay; HS, chopped hay.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
* $P < 0.05$.
† For details of diets and procedures, see Table 1 and p. 110.

ponies exceeded their theoretical daily digestible CP requirement of 176 g/d (National Research Council, 1989). This is in contrast to an earlier study of Moore-Colyer & Longland (2000), where, despite a good DM intake of 4·95 kg/d, the low CP content of only 44 g/kg DM, coupled with the low CP digestibility coefficient of 0·2 meant that the ponies' intakes were below the National Research Council (1989) recommendations for daily CP intake. However, despite the positive relationship between CP content and digestibility noted in the present study, a total collection apparent digestibility experiment cannot determine the site where the CP is digested. Thus, the availability of CP in forage diets may be low, as fibrous feedstuffs are largely degraded by microbial action in the large intestine. Therefore, studies that examine CP digestibility within the different segments of the equid gastrointestinal tract are required if the bioavailability of CP in forages is to be determined.

In agreement with Cymbaluk (1990) and Cuddeford *et al.* (1995), the present experiment indicated that a negative correlation exists between DM digestibility and the ADF and NDF contents of forages. This relationship requires further study, as it could prove a useful tool in predicting the apparent digestibility of forage from laboratory analysis alone.

Digesta passage rate studies

Mathematical modelling of faecal excretion data. Successful mathematical modelling of faecal excretion data has the potential to yield useful information to horse-owners on the flow of digesta through the individual gut compartments, as well as giving a value for MRT for both the individual compartments and for the total tract. However, in order to make full use of this process, selection of an appropriate model that accurately describes the pattern of faecal excretion must be done.

The method employed in the present experiment for assessing the 'goodness of fit' of the models tested was by examining the R^2 values. On this basis, it was found that the time-dependent compartmental models of Pond

et al. (1988) fitted the faecal excretion data much better than the time-independent models. Moreover, the fact that the model of Dhanoa *et al.* (1985) did not fit all the data collected further indicated that the digesta flow in the ponies was a time-dependent process. Mertens (1989) suggested that when equal outflow rates exist for the ascending (λ) and descending (k_2) phases of faecal excretion curves it is an indication that digesta passage is in fact a time-dependent process. The pattern of marker excretion in the present experiment showed similar rates for the ascending and descending phases of the curves, a trend also noted in an preliminary study in ponies fed hay cubes and a oat hull–naked oat (66:33, w/w) diet by Moore-Colyer (2000). Thus, all the evidence to date would seem to suggest that digesta flow in ponies fed fibrous diets is a time-dependent process and is therefore best described using time-dependent models.

Although the models of Pond *et al.* (1988) were originally developed for ruminant animals, their form allows them to describe accurately the passage of marked particles through a tubular gut, because the initial rate function (i.e. the rate of appearance of marker in the faeces) is zero and increases with time (Pond *et al.* 1988). This is a key factor of time-dependent models that allows them to deal with the pattern of digesta passage through the gastro-intestinal tract of the horse.

Moore *et al.* (1992) found the best fits for ram faecal excretion data were achieved using the higher order of γ-functions, i.e. the G3 and G4 models, with poorer fits obtained from the G1 and G2 models. This was also noted by Moore-Colyer (2000) and in the present experiment, as the best-fit model proved to be the higher γ order of G3.

Pond *et al.* (1988) suggested that the use of time-dependent models produced better agreement between actual and fitted data in the early stages of marker excretion. However, as can be seen in Fig. 1, the models did not fit the early phase of marker excretion in all four diets examined in the present experiment and tended to overestimate the time before actual appearance of marker in the faeces. Moore-Colyer (2000) also noted this when feeding an oat hull–naked oats mixture to ponies. Failure to collect sufficient numbers of samples during the early stages of marker excretion is frequently blamed for poor agreement between predicted and observed results (Dhanoa *et al.* 1985; Matis *et al.* 1989). However, in the current experiment, faecal collections started 8 h post-dosing, which resulted in about seven data points compiling the ascending phase of the graphs. Moreover, the best fit was achieved using the G3 model, which generally requires good quality data in order to model a faecal excretion curve (Pond *et al.* 1988); thus, lack of data in the early phases of marker excretion was clearly not the problem here. The lack of accuracy in describing the early expulsion of marker is more likely to be explained by the fact that the Pond *et al.* (1989) model was produced to describe digesta passage through a ruminant animal gut and that the considerably shorter MRT noted in equids, and thus the faster appearance of marker in the faeces compared with ruminant animals, cannot be adequately described using a ruminant animal model.

Total tract mean retention time. MRT can be derived algebraically using the Thielmans *et al.* (1978) equation, which because it uses the collected faecal marker data rather than assuming a continuous rate of faecal output, is regarded as the most accurate method of achieving total tract residence time (Warner, 1981). Uden *et al.* (1982), Pearson & Merritt (1991) and Moore-Colyer (2000) have all used variations of this equation to establish MRT of hay in equids. These workers have reported MRT ranging from 23 to 29 h, which are similar to the average values obtained for all the models in this experiment of 25 to 29 h, but are slightly less than the 42 and 32 h recorded by Moore-Colyer (2000), when ponies were offered oat hulls–naked oats and hay cube diets. The average MRT of the HL and HS (26 h) were significantly shorter than the 29 h recorded for the BBL diet. Pearson & Merritt (1991) also reported a longer (5 h) MRT when ponies were fed a poorly degradable straw diet than when they consumed a more degradable hay diet. In the present experiment the hay diets were 18 % less degradable than the silage diets and may have contributed to the shorter MRT. However, the BBS diet also had a significantly shorter MRT of 27 h than the BBL, but was equally well degraded. This anomaly is curious, because the shorter MRT of the BBS cannot be attributed to a shorter particle size as the HS has a retention time equal to that of the HL; thus, some other and as yet unexplained factor must have contributed to the shorter MRT.

Good agreement was seen for the MRT of all four forages between the Thielmans *et al.* (1978) G3 and G4 models, whereas the G2 model consistently over-estimated MRT. Although no significant difference existed between the G1 model and any of the other values, it did produce a 1·3 h longer retention time than the G3, G4 and Thielmans *et al.* (1978) models and may reflect the fact that the G1 model is a time-independent model. Lalles *et al.* (1991) and Gomez *et al.* (1992) also reported good agreement between both algebraic and compartmental models in early weaned dairy calves and sheep fed hay or concentrate diets, and this appears to be the case here, indicating that both the models of Pond *et al.* (1988) and Thielmans *et al.* (1978) can be used to determine MRT in equids.

However, no specific model is as yet recommended for estimating compartmental MRT in ruminant animals, and as the work done to date with equids is extremely limited, the choice of the G3 model for compartmental analysis was based on the R^2 data obtained in this experiment and on previous work by Moore-Colyer (2000).

Compartmental analysis. The general assumption when dealing with compartmental models is that the model consists of compartments where particle mixing may occur, e.g. rumen and abomasum, while the TD, where mixing is minimal, represents a minimum fixed time that is required for particles to pass through the gut and appear in the faeces (Matis *et al.* 1989). Most ruminant-animal researchers believe that the slower time-independent (k_1) compartment is the rumen, the faster time-dependent λ compartment is a combination of the caecum and proximal colon, while the TD represents the passage of digesta through the omasum and the intestines, and is closely correlated with the first appearance of

marker in the faeces (Grovum & Williams, 1973; Pond et al. 1988). Theoretical consideration of the anatomy of the equid gastrointestinal tract suggests that the size, positioning and movement of the caecum would result in a short digesta retention time (i.e. the fast compartment), while its sac-like anatomy suggests that instantaneous mixing may occur and that outflow could be independent of residence time, i.e. a time-independent compartment. In contrast, the anatomy of the large colon, with its four sacculated sections divided by narrow flexures (Frandson, 1981), would pre-dispose this part of the gut to be the time-dependent section. Although it is possible that particle mixing within each of the distinct regions of the colon occurs rapidly, implying time-independency, the passage through the complete organ would be time-dependent. The rate constant for the time-dependent section of the faecal excretion curve, λ, is a sum of two or more time-independent rates, thus passage of digesta through the large colon could easily fit such a mathematical equation. Argenzio et al. (1974) found the passage of digesta was particularly slow from the ventral to the dorsal colon (through the pelvic flexure) and from the dorsal to the small colon. In addition, fluid marker was retained for prolonged periods in the large colon, having passed from the stomach through the caecum into the proximal colon relatively quickly; thus, passage through the large intestine could be a time-dependent process.

However, such clear apportioning of the different equid gut compartments according to the rate at which digesta passes through the tract cannot be conclusively achieved from the data collected in the present experiment. The values presented in Table 5 show the BBL and HS diets to have longer retention times for the time-dependent λ compartment, while BBS and HL had longer retention times for the time-independent k compartment. Thus, unlike the conclusions of the ruminant-animal researchers Matis (1972) and Ellis et al. (1994), time-dependency cannot be consistently associated with the faster turnover compartment.

Faichney & Boston (1983) found that in ruminant animals under certain dietary circumstances outflow from the caecum could be slower than from the rumen. This suggests that the dietary ingredients, or the manner in which the food is presented to the animal, can influence the rate at which digesta passes through the different chambers of the gut. However, the results of the present experiment show no clear influence of forage type or particle size on the passage rate of digesta through the different gut compartments, therefore, no informed conclusion as to caecal or colonic digesta passage rates can be formed.

The TD is thought to represent the narrow tubular sections of the gut, where food has little opportunity to mix, thus a time-delay factor is introduced to account for this (Ellis et al. 1994; Matis et al. 1989). Moore-Colyer (2000) found that a TD compartment was clearly identified when Cr-marked food was placed into the caecum of the ponies and thus passed through the hindgut only, and that the value for the TD was similar for large intestine passage rate and total tract passage rate, indicating that it may relate to the small colon, where faeces collects before excretion. This suggests that the time digesta remains

within the stomach and small intestine are included in the λ and k_2 compartment retention times and not in the TD. While no clear differences exist between the two compartments between the hay and silage diets offered here, there is a tendency for the TD to be 2 h shorter for the hay than for the silage. This may have been the major contributing factor to the difference in total tract MRT between the hay and BBL diets.

The lack of a definite conclusion on compartmentalising the gut is not a problem unique to the present experiment. Having developed a multi-compartment model, which accurately described eighty sets of ruminant animal faecal excretion data, Dhanoa et al. (1985) could not say with certainty which of the two main rate constants represented the rumen and which represented the caecum. Results from their first study indicated that k_1 was the rumen, while k_2 was the caecum. However, their second study did not produce similar conclusions. It seems that clear biological interpretation of modelled faecal excretion data is extremely difficult. In the present study, the lack of clear compartment identification could be a result of the use of ruminant animal models for fitting pony faecal excretion data. However, as the R^2 values indicate, the models did accurately describe the marked faecal excretion patterns of the ponies. Alternatively, the modelling process itself, which relies on end-point excretion data, could lack sufficient sensitivity to allow the prediction of digesta flow through the different compartments of the gut. Furthermore, if faecal excretion data can be successfully modelled using a wide variety of models (time-independent G1 and time-dependent two compartment models G2, G3 and G4) that make different assumptions regarding digesta flow, the ability of the modelling procedure to partition flow through the various segments of the equid digestive tract may be limited.

Considerably more work is required in this area before definitive conclusions can be drawn regarding digesta passage rate through the different compartments of the equid gastrointestinal tract. The limitations illustrated here may be overcome if a model were derived specifically for equids based on the rate of passage of digesta through a horse's gut rather than using ruminant animal models that are based on much slower rates of passage. Moreover, rate of passage data is known to be very variable, so the limitations in the quantity of data produced from this 4×4 Latin square experiment means that the conclusions that can be drawn on gut compartmentalisation are minimal. More animals could be used, although power calculations based on current variability levels suggest that four times the number of ponies would be required if significant differences were to be detected.

Conclusions

Results from the present experiment indicate that big-bale grass silage in both long and chopped form is readily accepted and digested by ponies and can be used as a replacement for hay in diets for horses. Moreover, the higher nutrient content and digestibility of silage makes it an ideal forage for performance horses as the nutrient content of silage is significantly greater than hay on a per kg basis.

Yb is a successful external marker for determining total tract MRT of fibre-based diets in ponies. Chop length of forages appears not to influence digesta MRT and, in addition, marker excretion patterns allowed existing time-dependent compartmental mathematical models to be successfully applied to faecal excretion data. Total tract MRT was successfully calculated from model rate parameters, and although compartmental retention times could be obtained, the variability of the data hampered the production of a conclusive biological interpretation on the compartmentalisation of the different segments of the equid gastrointestinal tract.

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