Animal 15 (2021) 100050

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Methodical considerations when estimating nutrient digestibility in horses using the mobile bag technique

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ARTICLE INFO

Article history:
Received 3 February 2020
Received in revised form 12 August 2020
Accepted 13 August 2020
Available online 10 December 2020

Keywords:
Degradation profiles
Effective degradability
Hindgut
In-situ
Total faeces collection

ABSTRACT

Total collection of faeces is considered the golden standard for estimating apparent total tract digestibility (**ATTD**) in horses. However, the evaluation of individual feedstuffs is limited and determination of nutrient digestibility in different segments of the gastrointestinal tract (**GIT**) is excluded. The rationale for performing this study was that the mobile bag technique (**MBT**) can provide information on individual feedstuffs' degradation, and the use of fistulated animals does provide additionally information regarding degradation in individual segments of the GIT. Recommendations for using the MBT in ruminants are well established, but limited methodical studies have been published with horses. The objective of this study was to evaluate the MBT by comparing the ATTD with the nutrient disappearance and degradation kinetics of hay in horses. It was hypothesised that DM degradation as estimated by the MBT is equal to the ATTD of the DM. Furthermore, we hypothesised that bag size has no effect on nutrient disappearance but increasing the feed to surface area (**FSA**) decreases the DM disappearance. Five caecum cannulated horses were fed a hay-only diet (6.7 kg DM/day) with 14 days of adaptation followed by four consecutive days of total faeces collection. Three bag sizes (height × length × side, cm; 1.2 × 10 × 2, 3 × 4 × 2, 1 × 6 × 2) and three FSAs (10.4, 20.8 and 41.7 mg/cm^2) were administrated at each meal (3 meals/day) on days 1 and 2 of the collection. Faeces were checked for bags every 6th h, the collection time was noted and the DM disappearance together with the transit time (**TT**) for each bag type was estimated. Dry matter disappearance from the individual bags was fitted to degradation profiles, and the effective degradability (**ED**) and degradation (**D$_t$**) were determined. The results of the study showed that the ATTD of DM, organic matter (**OM**), NDF and ADF can be predicted based on their disappearance from the mobile bags, but that ash and CP are overestimated in comparison to the ATTD. The TT for the bags was 29.2 h, and when using a mean retention time of 30 h to predict ED and D$_t$, it was clear that ED was underestimated, whereas D$_t$ reflected the ATTD of DM. In conclusion, the MBT can be used to estimate the degradability of DM, OM and fibre as these nutrients resemble the ATTD. The bag size did not affect the DM disappearance, but the FSA should be kept below 20 mg/cm^2 as higher levels might limit the degradation kinetics.

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Implications

Appropriate feedstuff evaluation is important for accurate ration formulation for horses. Total tract digestibility provides valuable information on the nutrient and energy digestibility of the total diet but provides limited information about individual feedstuffs. This study contributes to the methodical development of the mobile bag technique and makes some recommendations for its use in future equine studies in intact and cannulated horses when studying degradation of individual feedstuffs.

Introduction

Feedstuff evaluation is important for optimising nutrient supply and for accuracy in ration formulation for horses (Hyslop, 2006). To optimise this, the apparent total tract digestibility (**ATTD**) can be measured using different methods, such as the total collection of faeces or the mobile bag technique (**MBT**). The ATTD provides information about the digestibility of a diet or individual feedstuffs, but it gives no information as to where in the gastrointestinal tract (**GIT**) or at what rate the different feedstuffs are degraded. However, a combination of the MBT with effective degradability (**ED**) calculations (Ørskov and McDonald, 1979) can provide essential knowledge on feed degradation kinetics in different segments of the equine GIT (Hyslop, 2006). This has been studied widely in ruminants (Hvelplund et al., 1992; Volden and Harstad,

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https://doi.org/10.1016/j.animal.2020.100050
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1995) and has been used to determine the degradation kinetics of four botanically diverse fibrous feeds in the small intestine and total tract of ponies (Moore-Colyer et al., 2002).

In the Nordic feed evaluation system for ruminants (Åkerlind et al., 2011) in-sacco bags are recommended with a pore size of 38 µm and a feed to surface area (**FSA**) of 10 mg/cm^2 for feedstuffs when studying digestion in the rumen (Åkerlind et al., 2011). However, recommendations for the technique are unclear when applied in horses because the MBT has been adjusted in relation to knowledge obtained from pigs and ruminants (Hyslop, 2006; Åkerlind et al., 2011). In equine studies, Macheboeuf et al. (1996) are often interpreted as a recommendation for the MBT (bag diameter 1 cm, length 6 cm and porosity 48 µm). A study with ponies showed that the dimensions of the mobile bag affect transit time (**TT**) and DM disappearance from the bags (Hyslop and Cuddeford, 1996). Methodical studies investigating the possible effects of bag size and FSA on nutrient disappearance in horses are scarce, and further studies are needed to standardise the method. The objective of this study was, therefore, to evaluate the MBT in horses by use of nutrient disappearance and degradation kinetics for hay in comparison to the ATTD. We hypothesise that the degradable DM as estimated by the MBT is equal to the ATTD of DM. Furthermore, we hypothesise that bag size has no effect on the estimated DM disappearance, but that increasing the FSA will decrease DM disappearance.

Material and methods

Experimental design

All housing, management and experimental procedures followed the laws and regulations for experimental animals in Norway (i.e. Regulations on the use of animals in experiments of July 2015). The entire experiment lasted for 18 days with 14 days of diet adaptation followed by four consecutive days of data collection (Fig. 1).

Animals

Five healthy caecum cannulated Norwegian cold-blooded trotter geldings (age 14–26 years) with an average BW (±SD) of 547 ± 27 kg were used in the experiment. All horses were followed routinely with veterinarian check-ups including vaccinations, dental examinations and teeth floating. The horses were housed in individual stalls (3 × 3 m) containing rubber mats and wood shavings as bedding. During the diet adaptation period, the horses were allowed access to a gravel paddock for 7–8 h per day, divided into two visits, and during data collection once a day for 1 h.

Diet

The horses were fed three times a day (0600, 1400 and 2000 h) with a hay-only diet. The total DM intake of hay was 6.7 kg/day, divided into three equal meals. The hay meals were fed from hay cribs attached to the front of the individual stalls 62 cm above the floor. A commercial vitamin and mineral supplement with the composition: Ca, 100 (g/kg); Mg, 32 (g/kg); Cu, 840 (mg/kg); Zn, 2 830 (mg/kg); Fe, 2 460 (mg/kg); Mn, 1 530 (mg/kg); I, 18 (mg/kg); Co, 6 (mg/kg); Vitamin A, 107 000 (I.U./kg); Vitamin D, 11 300 (I.U./kg); Vitamin E, 9 600 (mg/kg) (Champion Multitiskud, Felleskjøpet Forutvikling, Trondheim, Norway, 80 g/day) and sodium chloride (25 g/day) was added to the crib when feeding the morning meal. The chemical composition of the hay was DM: 898 g/kg, ash: 56.7 g/kg DM, NDF: 574 g/kg DM, ADF: 333 g/kg DM, CP: 136 g/kg DM, water-soluble carbohydrates (**WSC**): 114 g/kg DM and gross energy (**GE**) 19.1 MJ/kg DM. Horses were fed to fulfil their maintenance energy and nutrient requirements according to Nordic standards. Water was available in the individual stalls through automatic troughs at all times but was only available in the gravel paddock during diet adaptation.

Total collection of faeces

Four consecutive days of total faecal collection from each horse was obtained by use of collection harnesses (Stablemaid, Melbourne, Australia). Collection harnesses were emptied every 6th h (0600, 1200, 1800 and 0000 h) and immediately before the horses were allowed access to the gravel paddock (1000 h). Procedures for mobile bags found in the faeces are described below. The faeces collected daily were stored in plastic bins, with lids, at 3 °C. They were weighed and mixed thoroughly by hand and with an electric concrete mixer (Atika, electric concrete mixer, Germany). Daily faecal output was measured, DM determined and a daily subsample of 10% of the collected faeces (fresh weight) was stored at −20 °C for further analysis. After the experiment, the daily subsamples were pooled and used to create a single representative sample for each horse. For further analysis, the daily pooled subsamples were thawed and then mixed into two new samples (approximately 500 g/sample).

Mobile bag technique

The mobile bags were made from precision woven open mesh fabric with 36 µm porosity (Sefar Nitex, 03–36/28; Sefar AG, Heiden, Switzerland). The bags were prepared by cutting a suitable size piece of mesh (large enough to heat-seal the edges) and folding it in the middle (Fig. 2). The mesh was heat-sealed along one side and one end; it was then turned inside-out to avoid sharp edges and marked with a permanent marker for identification. Three bag sizes were prepared with different proportions (height × length × side) and three or four FSA (Table 1). The weight of the empty bags and of the bags filled with hay (milled to pass a 1.5 mm screen) was recorded (Table 1), and the bags were closed by heat-sealing the end. One bag of each combination of size and FSA was soaked in cold tap water and placed in the caecum through the cannula before each feeding on collection days 1 and 2 (Fig. 1), resulting in seven bags per horse per administration and 42 bags per horse in total.

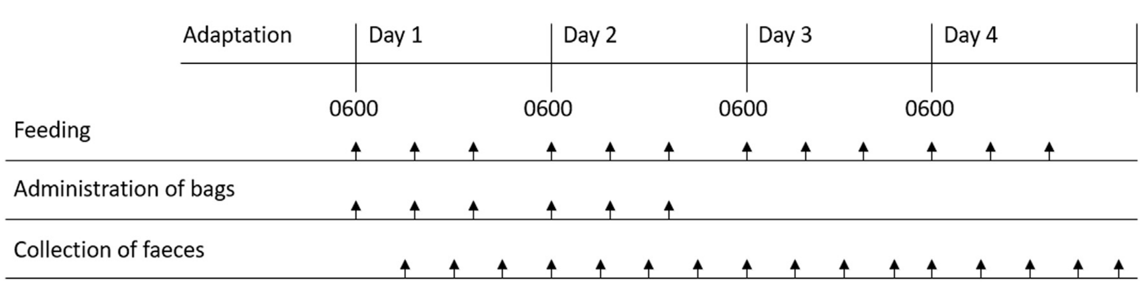

Fig. 1. The experimental set-up illustrating feeding times (0600, 1400 and 2000 h), faecal collection times (every 6 h) and times mobile bags were administered (at every meal on days 1 and 2).

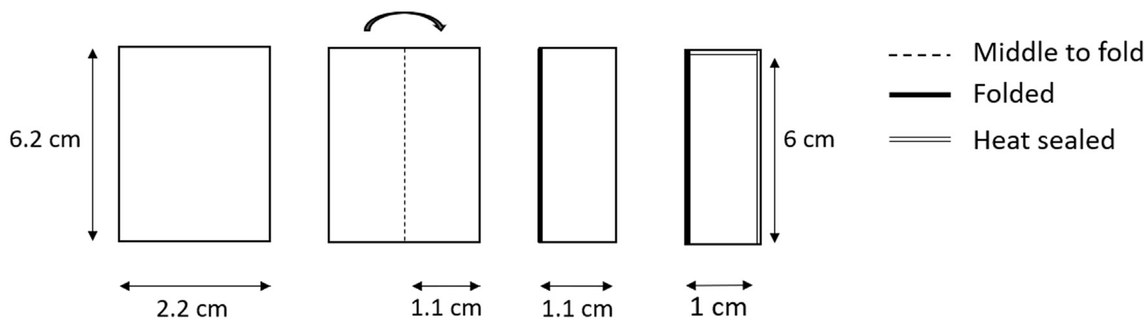

Fig. 2. Illustration of mobile bag construction with example of bag size 1 × 6 × 2 cm (height × length × side).

Faeces were inspected for bags at every collection during the 4 days of faecal collection (bags found before the horses were allowed access to the gravel paddock were included in the 1200 h collection). Each bag's collection time was noted; it was hand-rinsed in cold tap water and stored at −20 °C. At the end of the experiment, all bags were thawed at room temperature, placed in a washing bag (28 × 37 cm) and washed in cold water for 35 min, without spinning (Woolprogram, Avantixx 7 Varioperfect; Bosch, Gerlingen-SchillerhÖhe, Germany) and then dried for 48 h at 45 °C. Bags were left at room temperature (approximately 25 °C) for equilibration for 24 h before weighing. Control bags (4 bags/combination) were not administered to the horses but were soaked in tap water for 1 h before washing and drying as described above to determine the disappearance of nutrients from the bags. The DM of each individual bag was determined by the weight after drying. To obtain sufficient residue for chemical analysis, all mobile bags collected were pooled for each bag combination (except bag type E) for a specific collection time interval (15–30, 31–50 and 51–115 h).

Chemical analysis

All analyses were performed in duplicate except for the mobile bag residue. A sample of the hay fed and of the bulk residues from the collected bags, according to the collection time interval, was analysed for DM by drying to a constant weight (24 h at 105 °C ± 2 °C). Samples were then incinerated at 550 °C for 16 h for ash determination. Neutral detergent fibre, ADF and ADL were measured by the filter bag technique described by ANKOM (2017a and 2017b). Water-soluble carbohydrates were determined as described by Randby et al. (2010). Nitrogen was measured according to the Kjeldahl method (Kjeltec™ 8400 analyzer; Foss, Hillerød, Denmark) and CP was calculated as N × 6.25. Gross energy was determined using the bomb calorimeter method (6400 Automatic Isoperibol Calorimeter; Parr Instrument Company, Moline, IL, USA).

Calculations

The apparent total tract digestibility of nutrients

The ATTD of individual nutrients and energy was calculated as:

$$\mathrm{ATTD} = \frac{\mathrm{Intake\ (g)} - \mathrm{faecal\ excretion\ (g)}}{\mathrm{intake\ (g)}} \times 100\% \quad (1)$$

Transit time of the mobile bags

The characteristics of the mobile bags' transit through the hindgut were assessed by calculating the TT as described by Faichney (1975):

$$\mathrm{TT} = \sum \mathrm{B_i} \times \mathrm{t_i} \quad (2)$$

where B_i is the number of bags collected at time t_i as a proportion of the total number of bags collected, and t_i is the time elapsed between administration of the bags and the midpoint of the ith collection interval.

Dry matter degradation curves

The DM disappearance curves from the seven combinations of mobile bag size and FSA were subjected to the Ørskov and McDonald (1979) model (Eq. (3)) for evaluating the degradation profile of hay:

$$\mathrm{D_t} = \mathrm{a} + \mathrm{b}\left(1 - e^{-ct}\right) \quad (3)$$

where D_t is the degradation after time t of administration, b is the potential degradation (insoluble part of feed) of the component which will in time be degraded, c is the rate constant for degradation of b per h, a is the intercept (soluble part of feed) of the degradation curve when $t = 0$ and e is the exponential. The potentially degradable fraction of the feed can then be expressed as a + b.

Effective degradability

The ED was calculated for all the bag types using Eq. (4) at chosen outflow rates (k): 0.05, 0.033, 0.025 and 0.017% per h to obtain DM disappearance from the mobile bags to assumed digesta mean retention time (**MRT**) in the hindgut at 20, 30, 40 and 60 h:

$$ED = a + \frac{bc}{c + k} \quad (4)$$

where a, b and c are as described above, and k is the chosen outflow rate.

Statistical analysis

All statistical analyses were performed in Rstudio (version 1.1.456; Rstudio Inc., Boston, MA, USA). Linear regression was done on nutrient disappearance with a model comprising nutrient disappearance as response, and time, bag size and FSA as predictors.

The degradation at $t = 0$ (a), the potential degradation (b), the rate constant (c), the potentially degradable fraction (a + b), the TT for the mobile bags, the ED values and the D_t for bag types were subjected to linear regression using bag size and FSA as predictors.

Bag type E was excluded from all statistical analyses as only one FSA of 41.7 mg/cm^2 was included in the study. No interactions were found between the predictors and they were therefore excluded. Significant differences of least-square means were analysed by Tukey's honest significant difference test. All results are presented as least-square means ± SD. Effects are considered significantly different if $P < 0.05$.

Table 1
Seven different combinations of mobile bag size (height × length × side, cm), feed to surface area (FSA, mg/cm^2) and number of bags per horse.

Bag type	Surface area (cm)	FSA (mg/cm^2)	Number of bags per horse
A	1.2 × 10 × 2	10.4	n = 6
B	1.2×10 × 2	20.8	n = 6
C	6 × 1 × 2	10.4	n = 6
D	6 × 1 × 2	20.8	n = 6
E	6 × 1 × 2	41.7	n = 6
F	3 × 4 × 2	10.4	n = 6
G	3 × 4 × 2	20.8	n = 6

Results

In-vivo nutrient digestibility

From the total collection of faeces, the ATTD of nutrients and energy was calculated (Fig. 3). The ATTD of the following nutrients and energy was DM: 55.9 ± 0.8%, OM: 56.7 ± 0.9%, ash: 42.8 ± 2.6%, CP: 52.8 ± 4.1%, NDF: 53.8 ± 1.8%, ADF: 44.8 ± 2.2% and GE: 53.5 ± 0.8%.

Recovery of mobile bags

A total of 30 bags of each type was placed in the caecum of the five horses, but some bags were either not found or discarded (e.g. bags were excluded if a hole was detected after recovery). The total number of recovered bags was 29 for type A, 30 for type B, 28 for type C, 26 for type D, 24 for type E, 28 for type F and 28 for type G. The heat-sealing of bag type E tended to open more often than the other bag types.

Washing loss and nutrient disappearance from the mobile bags

The small amount of residue derived from the mobile bags limited the possibilities for performing all chemical analyses on all control bags and bag types for each time interval.

Bag type E had the lowest numerical DM loss of 11.5% compared to the other bag types that varied from 21.7 to 24.2% (Fig. 3). Loss of ash varied from 70.7 to 80.4% and OM from 19.1 to 21.5% for all bag types except bag type E. The loss of CP was determined only for bag type B, and it

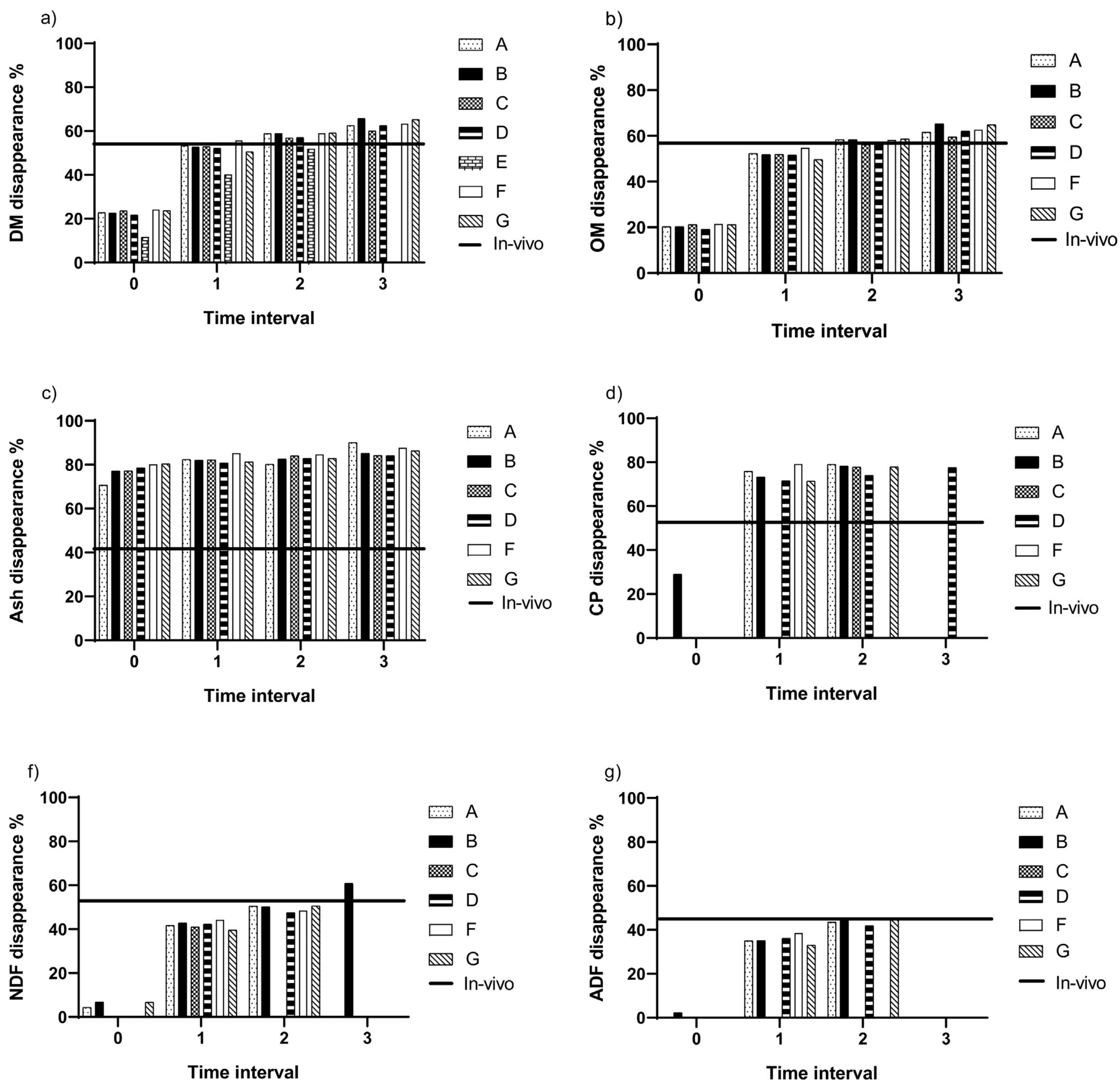

Fig. 3. *In-vivo* apparent total tract digestibility of nutrients and *in-situ* nutrient disappearance from different mobile bag types and time intervals (0 = control bags; 1 = 15–30 h; 2 = 31–50 h; 3 = 51–115 h).

was 29.0%. Neutral detergent fibre was determined for bag types A and B with losses of 4.4 and 6.8%, respectively.

In-situ nutrient disappearance for the seven bag types at the three time intervals is shown in Fig. 3. A time effect ($P < 0.05$) was found for the disappearance from the mobile bags of DM, OM, ash, CP, NDF and ADF with the disappearance increasing with time. There was no effect of bag size or FSA on DM, ash or ADF disappearance from the bags. Furthermore, no effect of bag size was found for CP, but disappearance was lower ($P < 0.05$) from bags with an FSA of 20.8 mg/cm^2 compared to those with an FSA of 10.4 mg/cm^2 (Fig. 3d). Disappearance of NDF from the mobile bags was affected by both FSA ($P < 0.05$) and bag size ($P < 0.05$) with a higher disappearance from bags with an FSA of 20.8 mg/cm^2 compared to 10.4 mg/cm^2 and from bags of size 1.2 × 10 × 2 cm compared to those of 1 × 6 × 2 cm (Fig. 3f). Visual inspection of the results in Fig. 3 indicates that the ATTD of DM, OM, NDF and ADF can be predicted based on disappearance from mobile bags, whereas ash and CP disappearance from the bags are overestimated compared to the ATTD.

Dry matter degradation curves

Fitted DM degradation curves from Ørskov and McDonald (1979) for the seven different bags types are shown in Fig. 4. The mobile bags were found in faeces from 16 to 113 h after they were administrated in the caecum, and the fitted DM degradation is in correspondence with the raw data for each bag type (Fig. 4). There were no effects of FSA and bag size on parameters a and c, but the potential degradable b increased with increasing FSA ($P < 0.001$), and bag size 3 × 4 × 2 resulted in higher degradation than the other sizes ($P = 0.02$) (Table 2). The potentially degradable fraction a + b of the hay was higher ($P = 0.02$) with an FSA of 20.8 mg/cm^2 than with an FSA of 10.4 mg/cm^2 (Table 2). The TT of the mobile bags varied from 26.1 to 32.3 h (Table 2), and bag size and FSA had no effect on the TT of the mobile bags. In general, to reflect the average TT of 29.2 h for the mobile bags, an ED and D_t of 30 h predicts the DM degradation to be 49.0 and 56.4%, respectively (Table 2). Hence, the D_t of 30 h reflects the ATTD of DM (55.9%), whereas for the ED an MRT of 60 h is needed.

Discussion

In-vivo apparent total tract digestibility and in-situ disappearance

Studies using total collection of faeces are considered the golden standard for measuring the ATTD of a diet or of individual feedstuffs, whereas few studies have used the MBT as an alternative or in combination with the total collection of faeces in cannulated or even intact horses. Therefore, the main objective of the present study was to evaluate the use of MBT in horses using nutrient disappearance and by modelling degradation kinetics for hay in comparison to the ATTD. However, several factors should be considered when comparing the two methods. In this study, mobile bags were administrated in the caecum, thereby omitting enzymatic degradation of the feedstuff and instead aiming at microbial degradation. It was assumed that the fraction of hay that would potentially be digested enzymatically by the host enzymes also was fermented, and hence, the estimates from the MBT would reflect the ATTD. However, this needs to be validated further in a future study. The soluble part of the feedstuff that disappears from the mobile bag is expected to be easily digested in the small intestine. In the present study, the soluble part of the feedstuff was estimated to be approximately 23.1% for bags with an FSA of 10.4–20.8 mg/cm^2. This is in correspondence with Moore-Colyer et al. (2002), where the washing loss from bags containing hay cubes was found to be 24%. Furthermore, the DM disappearance from bags containing hay cubes captured in the caecum after passing the stomach and small intestine in cannulated ponies was 32% (Moore-Colyer et al., 2002). This difference between washing loss and nutrient disappearance when captured in the caecum indicates pre-caecal nutrient digestibility of, for example, protein which was found to be 52% for the hay cubes (Moore-Colyer et al., 2002). In this study, pre-caecal digestion was omitted by administering bags directly into the caecum, but it is assumed that the nutrient fractions that would have been digested pre-caecal were fermented in the hindgut; hence, it is expected that the nutrient disappearances presented here reflect the ATTD.

The disappearance of DM, OM, NDF and ADF was in line with the ATTD for these nutrient fractions. Ash disappearance from the mobile bags was approximately twice as high as the ATTD of ash, and nitrogen disappearance and therefore CP from the bags was higher relative to the ATTD of CP, despite that the enzymatic degradation in the stomach and the small intestine was omitted. The *in-situ* disappearance may be a better reflection of true CP digestibility as the ATTD of CP is affected by N from microbes, ammonia and endogenous losses (Hvelplund et al., 2003). Moreover, feed residue in the mobile bags might be contaminated by microbial N (Varvikko and Lindberg, 1985), but this is considered to be low as the washing procedure decreases this contamination (Hvelplund et al., 2003).

Washing of the mobile bags

The washing procedure for the collected bags is done to rinse off mucous, endogenous enzymes and microbial biomass from the feed residue (Van Straalen et al., 1993), but there will also be a loss of particles including nutrients (Moore-Colyer et al., 2002). In the present study, the average DM loss from control bags with an FSA of either 10.4 or 20.8 mg/cm^2 was twice as high as the loss from bags having an FSA of 41.7 mg/cm^2, indicating that soluble particles are withheld in bags with an FSA of 41.7 mg/cm^2. The washing loss consists mainly of ash and CP (probably also WSC, but this was not analysed), whereas the fibre fractions NDF and ADF are mainly withheld in the bags. This is in accordance with the findings of Moore-Colyer et al. (2002), where the DM loss from control bags containing hay cubes was 24%. The washing procedure has been highlighted by several authors as it has not been standardised and can affect the loss from the control bags and the rinsing of the residue in the mobile bags (Dhanoa et al., 1999; Moore-Colyer et al., 2002).

Nutrient disappearance from the mobile bags

In the present study, the inclusion of different bag sizes of varying FSA was investigated as no clear recommendations for the use of MBT have yet been established for equine studies. An earlier abstract by Macheboeuf et al. (1996) is often annotated for its recommendations for the dimensions: diameter 1 cm, length 6 cm and porosity of 48 μm. However, these dimensions limit the use of feed material as a high FSA affects the disappearance of nutrients from the bags negatively, as shown with bag type E in the present study. Hyslop and Cuddeford (1996) found that increasing the surface area of the mobile bag prolonged the TT and additionally increased the disappearance of DM and NDF from the bags. However, the amount of feed used in the study by Hyslop and Cuddeford (1996) is unclear, and the results may be affected by the FSA. In contrast, in the present study, no effect of the bag dimensions on TT was found, but DM disappearance was lower with the FSA of 41.7 mg/cm^2 than with those of 10.4 or 20.8 mg/cm^2. Udén and Van Soest (1984) found a corresponding decrease in “cell wall” disappearance in ruminants and ponies when FSA was increased markedly (6.5 vs 50 mg/cm^2).

In practice, in the present study, the bags with an FSA of 41.7 mg/cm^2 tended to open as a result of the volume of hay in the bag. This may not be the case when grains are used and thereby concentrates may allow a higher FSA compared to roughage without affecting the DM disappearance. For example, studies have used an FSA varying from 21.5 to 83.3 mg/cm^2 with concentrate feeds such as barley, maize and oats (Rosenfeld and Austbø, 2009; Philippeau et al., 2014). Furthermore, no effect of bag size or FSA was measured for ash, CP and ADF, but nutrient

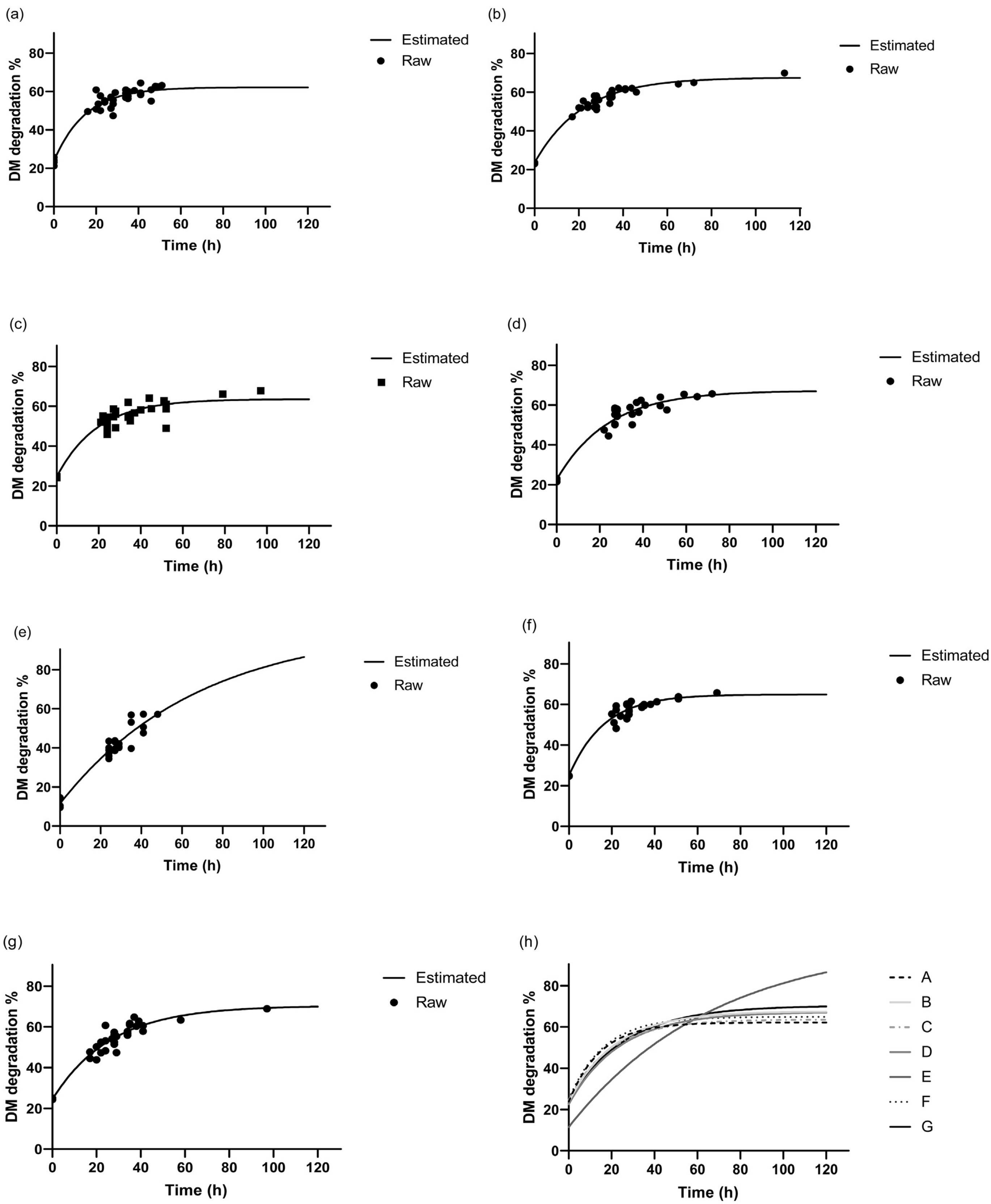

Fig. 4. Ørskov and McDonald (1979) degradation curves of DM from hay, based on mobile bags with different feed to surface area (FSA) and sizes. (a) bag size 1.2 × 10 × 2 with FSA 10.4 mg/cm^2; (b) bag size 1.2 × 10 × 2 with FSA 20.8 mg/cm^2; (c) bag size 1 × 6 × 2 with FSA 10.4 mg/cm^2; (d) bag size 1 × 6 × 2 with FSA 20.8 mg/cm^2; (e) bag size 1 × 6 × 2 with FSA 41.7 mg/cm^2; (f) bag size 3 × 4 × 2 with FSA 10.4 mg/cm^2; (g) bag size 3 × 4 × 2 with FSA 20.8 mg/cm^2; (h) DM degradation for all bag types.

Table 2

Dry matter degradation parameters and transit time (TT) in h for the different mobile bag types (A–G) with different sizes (height × length × side, cm) and feed to surface areas (FSA, mg/cm²). Effective degradation (ED) and degradation (D_t) in percent are presented for mean retention times of 20, 30, 40 and 60 h for hay.

Bag	A	B	C	D	E	F	G	±SD	*P*-values	
Size	1.2×10×2	1.2×10×2	1×6×2	1×6×2	1×6×2	3×4×2	3×4×2		Size	FSA
FSA	10.4	20.8	10.4	20.8	41.7	10.4	20.8			
a	23.7	23.6	24.6	22.5	11.5	24.9	24.3	4.42	≥0.05	≥0.05
b	38.5	44.1	39.0	44.6	90.6	40.0	46.1	17.2	0.02	<0.001
c	0.067	0.046	0.051	0.042	0.014	0.061	0.038	0.016	≥0.05	≥0.05
a + b	62.2	67.6	63.6	67.2	102.0	64.9	70.4	12.9	≥0.05	0.02
TT	27.8	32.2	32.3	30.8	28.9	26.1	27.1	2.31	≥0.05	≥0.05
ED										
20 h	45.8	44.8	44.5	42.9	32.0	47.0	44.3	4.65	≥0.05	≥0.05
30 h	49.5	49.2	48.3	47.5	39.2	50.9	48.9	3.59	≥0.05	≥0.05
40 h	51.8	52.2	50.9	50.6	45.0	53.4	52.2	2.54	≥0.05	≥0.05
60 h	54.6	56.0	54.1	54.5	53.9	56.4	56.4	1.01	≥0.05	≥0.05
D_t										
20 h	52.3	50.3	49.8	48.0	34.5	53.2	48.9	5.81	≥0.05	≥0.05
30 h	57.1	56.7	55.4	54.6	43.8	58.6	55.8	4.58	≥0.05	≥0.05
40 h	59.6	60.8	58.7	58.9	51.7	61.5	60.4	3.04	≥0.05	≥0.05
60 h	61.5	64.9	61.9	63.7	64.5	63.9	65.7	1.44	≥0.05	0.04

disappearance increased with time interval, in correspondence with other studies (Moore-Colyer et al., 2002; Hymøller et al., 2012).

Studies have shown that feed disappearance is affected positively by pore size both in ponies, when pore size was increased from 5 to 37 μm (Udén and Van Soest, 1984), and in ruminants, when it was increased from 20 to 40 μm (Varvikko and Lindberg, 1985). However, the pore size of the material should allow microbes to enter the bag and fermentation end-products to pass out of the bag. The pore size of the bags might depend on the nutrient of interest and its digestion as smaller pore sizes might limit microbial access to the bags. According to the Nordic feed evaluation system for ruminants, the recommendation is that bags with a pore size of 11–15 μm should be used for evaluating digestion in the small intestine and with a pore size of 38 μm when evaluating fibre degradation in the rumen of cows (Åkerlind et al., 2011). A pore size of 36 μm was used in the present study as the microbial degradation of a fibrous feedstuff was the focus of interest. The effect of different pore sizes has received little attention in equine studies.

Dry matter degradation curves

An advantage of the MBT is the possibility of obtaining knowledge about individual feedstuffs compared to the total collection of faeces. Furthermore, both rate and extent of feed degradation can be estimated from the models of Ørskov and McDonald (1979), which allow ED values to be calculated taking the passage rate of digesta through the GIT into account. In the present study, the DM degradation curves and the ED were estimated on data from the control bags and from mobile bags recovered between 16 and 113 h after administration in the caecum. The fitted DM degradation curves correspond well with the raw data from the mobile bags. However, the insoluble but potential degradable part b was overestimated for bag type E, and the potential degradability a + b of the DM in the hay was estimated to be 102%. This can be explained by a lack of data from time 0–16 h and from the time interval 3 for bag type E and furthermore, by an underestimated particle loss from the control bags. Despite this, all other bag types correspond well with the model parameters with the potential DM degradability a + b ranging from 62.2 to 70.4% in comparison to the DM ATTD of 55.9%.

The insoluble but potential degradable part b was affected by both the FSA and the bag size, with increasing DM degradation for mobile bags with an FSA of 20.8 mg/cm² and for a bag size of 3 × 4 × 2 cm, indicating the possibility of overestimating the DM degradation. The potentially degradable DM, a + b, was affected by the FSA as it was higher for bags with an FSA of 20 mg/cm² than of 10.4 mg/cm². The higher the digestibility of a feed, the less material is available for analysis; hence, a balance is needed where the FSA is as high as possible without affecting the degradability of the feed.

The recommendations in the Nordic feed evaluation system for ruminants are to use an FSA of 5–15 mg/cm² when evaluating digestion in the small intestine and of 10 mg/cm² when evaluating fibre degradation in the rumen (Åkerlind et al., 2011). In this study, an FSA slightly above these recommendations was used to increase the amount of residue available for analysis, and based on our results, care should be taken when using an FSA of more than 20 mg/cm² as the bags with an FSA close to 40 mg/cm² clearly affected the degradability negatively. This was not surprising as earlier studies have found a similar effect when the FSA was increased (Cherian et al., 1989; Vanzant et al., 1998).

The ED corresponds to the ATTD when the outflow rate is 0.017% per h, corresponding to an MRT of 60 h. However, this MRT does not represent the *in-vivo* MRT for hay. The MRT depends on the DM intake as increasing intake decreases MRT (Clauss et al., 2014; Miyaji et al., 2014) and the MRT has been estimated to be approximately 24–30 h for the liquid phase and 21–48 h for the solid phase in horses fed hay (Clauss et al., 2014; Jensen et al., 2014; Hummel et al., 2017). The degradation parameter estimates may be less precise as the MBT results in a narrow range of TTs, and therefore observations cover only a narrow time range, resulting in an underestimation of ED when biologically relevant MRT is used in the calculations. The same interpretations can be drawn from the results presented by Moore-Colyer et al. (2002). ED is therefore not an appropriate measure of feed degradation when using mobile bags; however, using D_t seems to be more appropriate as the estimates of degradation at biologically relevant MRT are more in line with the ATTD.

The in-sacco technique with the fixed placement of bags in a specific compartment of the GIT, for example, the caecum, followed by the recovery of the bags at specific time points could be an alternative to the MBT and would provide information on feed degradation kinetics in the early stages of degradation. This is standard procedure in the Nordic feed evaluation system for ruminants (Åkerlind et al., 2011), but only a few studies have tried to adapt this technique for use with horses (Drogoul et al., 2000; Hyslop, 2006). However, it may be an alternative to the total collection of faeces and the MBT.

Conclusion

In conclusion, this study showed that the MBT can be used to estimate degradability of DM, OM and fibre from hay, which resemble the

ATTD of these nutrient fractions in horses. The bag sizes used in the present study did not have any major effects on the results, including DM disappearance, but it is suggested that the FSA should be kept below 20 mg/cm^2 as higher levels might limit particle loss from control bags and degradation kinetics. Degradation (D_t), but not ED, might be useful for estimating the ATTD with biologically relevant MRT. The MBT has the potential to be a useful technique for evaluating more complex diets, including more feedstuffs, and the modelling of degradation kinetics may give a better understanding of nutrient digestion in horses.

Ethics approval

Not applicable. Experimental design and procedures in this study were in accordance with Norwegian legislation and ethical guidelines.

Data and model availability statement

Data involved in the present study are not deposited in any official archive.

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Nana Wentzel Thorringer: conceptualisation, formal analysis, investigation, data curation, writing – original draft. Rasmus Bovbjerg Jensen: conceptualisation, methodology, investigation, resources, writing – review and editing, supervision, funding acquisition.

Declaration of interest

The authors have no interest to declare associated with this publication.

Acknowledgements

The authors gratefully acknowledge Mette Henne for technical assistance during the animal trial as well as Martin Weisbjerg for thoroughly reviewing this paper.

Financial support statement

Felleskjøpet Fôrutvikling (Trondheim, Norway) financed the experiment.

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