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Mobile bag technique for estimation of nutrient digestibility when hay is supplemented with alternative fibrous feedstuffs in horses

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ABSTRACT

To evaluate the effect of substituting hay with alternative fibrous feedstuffs, the total collection of faeces was used to measure the apparent total tract digestibility (ATTD). Nutrient disappearance and digestion kinetics were examined with the mobile bag technique (MBT) and marker passage measurements. Four caecally-cannulated horses (body weight (BW) 558 ± 32 kg) were used in a cross-over design experiment with two periods of 14 days adaptation and four days of faecal collection. Horses were fed three times a day with either a hay-only (HAY) diet or a mixture of hay:supplement (MIX) (15.1 and 8.4:6.7 g dry matter (DM)/kg BW/day, respectively). The hay used in both treatments (HAY and MIX) was mainly of Timothy and first cut. The MIX supplement diet consisted of oat hulls, alfalfa-, sugar beet pulp- (SBP), grass- and soya hull pellets, each given in 0.44 g DM/kg BW/meal. On day 15 in each period, 20 bags of either hay or SBP and 6–12 bags (1×2x12 cm; 37 μm pore size; 0.5 g feed) of each feedstuff and ytterbium (Yb, 3 g) were placed in the stomach or caecum, respectively. Bags were harvested from the caecum every hour and faeces were checked for bags every fourth hour, collection time was noted and data from the bags were used to estimate pre-caecal, hindgut and total tract nutrient disappearance. Further, faecal sub-samples of 300 g were collected, weighed and stored for Yb analysis and further estimation of feed mean retention time. Rate and extent of feed degradation were estimated from the MBT assuming exponential degradation. The ATTD of DM was similar between the two diets ($P > 0.05$), but the HAY diet had higher ATTD of crude protein (CP) ($P = 0.001$), neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash (aNDF) ($P = 0.006$), acid detergent fibre (ADF) ($P = 0.017$), hemicellulose ($P = 0.001$) and cellulose$_{NDF}$ ($P < 0.001$). The hindgut mean retention time (MRT) for Yb was longer for the MIX than the HAY diet ($P < 0.001$). No differences for DM, aNDF or ADF digestibility were measured when comparing the ATTD with nutrient disappearance from bags found in the time interval 20–30 h, indicating the ATTD of these nutrients can be predicted by the MBT. The estimated degradation (D_t), but not effective

Abbreviations: ADF, acid detergent fibre expressed inclusive of residual ash; ADL, acid detergent lignin; aNDF, neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash; ANOVA, analysis of variance; AP, alfalfa pellets; ATTD, apparent total tract digestibility; BW, body weight; CP, crude protein; dD, diet digestibility; DE, digestible energy; DF, dietary fibre; dH, hay digestibility; DM, dry matter; dS, digestibility coefficient of supplement; Dt, degradation after time; ED, effective degradability; GE, gross energy; GP, grass pellets; H, hindgut; I-NSP, insoluble non-starch polysaccharides; LOD, limit of detection; LOQ, limit of quantification; MBT, mobile bag technique; MRT, mean retention time; N, nitrogen; NDF, neutral detergent fibre; NSP, non-starch polysaccharides; OH, oat hulls; OM, organic matter; pc, pre-caecal; SBP, sugar beet pulp pellets; SCFA, short-chain fatty acid; SD, standard deviation of mean; SHP, soya hull pellets; T, total tract; TT, transit time; T-NSP, total non-starch polysaccharides; Yb, ytterbium; WSC, water-soluble carbohydrates.

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degradation (ED), is preferred when the MBT is used to predict the ATTD. It can be concluded that hay can be substituted partly by fibrous feedstuffs and that the MBT can predict the ATTD of DM, aNDF and ADF in a mixed ration based on MBT measures on individual feedstuffs.

1. Introduction

The horse is capable of fermenting fibre-rich feedstuffs in its specialised hindgut with the absorption of short-chained fatty acids (SCFA) as energy substrates (Argenzio et al., 1974; Janis, 1976). Roughage, a fibre-rich feedstuff, provides horses with more than 50% of their daily dry matter (DM) intake when pasture is limited (Saastamoinen and Hellämäki, 2012), and a minimum daily DM requirement of 15 g DM/kg body weight (BW)/day is suggested (Harris et al., 2017). As the forage matures, the apparent total tract digestibility (ATTD) of DM (Müller, 2012) and NDF (neutral detergent fibre) (Ragnarsson and Lindberg, 2008) decreases due to plant lignification, hence the energy value of the plant decreases. To increase the daily energy intake, roughage can be substituted with starch-rich grain to performance horses (Julliand et al., 2006). However, a high starch intake in horses is linked to an increased risk of developing colic (Hudson et al., 2001) and gastric ulcers (Luthersson et al., 2009). As alternatives to starch and low-digestible roughage, highly fermentable fibre sources like soybean hulls (Coverdale et al., 2004) and sugar beet pulp (Karlsson et al., 2002) are suggested. Other fibre-rich feedstuffs might be useful roughage alternatives, for example in situations of drought where the availability of roughage might be limited. Feedstuff evaluation in horses is often based on total faecal collection and thereby ATTD of the total ration (Ragnarsson and Lindberg, 2008; Jensen et al., 2014). To determine the digestion of a diet's individual feedstuffs, the mobile bag technique (MBT) has been used to estimate the small intestinal digestibility of starch in studies with horses (Julliand et al., 2006; Rosenfeld and Austbø, 2009) and furthermore used to estimate the digestibility of fibre-rich feedstuffs (Moore-Colyer et al., 2002; Thorringer and Jensen, 2021). However, the MBT can be minimal invasive if used with cannulated horses. Yet, using the MBT to estimate individual feedstuff digestibility can, if combined with effective degradability calculations (Ørskov and McDonald, 1979), provide essential knowledge on feed degradation kinetics within different segments of the horse's gastrointestinal tract. Information on digestibility and degradation kinetics (degradation of DM after time t of mobile bag administration = Dt and effective degradability (ED) that is based on digesta outflow rates in the chosen segment of the gastrointestinal tract) are useful parameters when combining different dietary ingredients to suit horses doing different activities. Hence, this knowledge is useful for improving feedstuff evaluation accuracy and ration formulation for horses. Therefore, the aim of this study is to evaluate the effect of substituting hay with alternative fibrous feedstuffs on nutrient digestibility and degradation kinetics. It was hypothesised that: (1) fibrous supplements can partly substitute for roughage, and (2) the MBT can be used to predict the digestibility of individual feedstuffs and hence estimate the total ration digestibility fed to horses at maintenance.

2. Materials and methods

2.1. Experimental design

All housing, management and experimental procedures followed the laws and regulations for experimental animals in Norway (Norwegian Government, 2015). The study was designed as a cross-over experiment with four horses and two periods. Each period consisted of 14 days of adaptation followed by four consecutive days of data collection (Fig. 1).

2.2. Animals

Four healthy, caecum-cannulated Norwegian cold-blooded trotter geldings (age 16–26 years) with an average body weight (BW $\pm$ standard deviation of mean (SD)) of 558 $\pm$ 32 kg were used in the experiment. All horses were followed routinely with veterinary check-ups including vaccinations, dental examinations and teeth floating. Horses were housed in individual stalls (3 $\times$ 3 m) with rubber mats and wood shavings as bedding material. During the adaptation periods, the horses were allowed access to a gravel

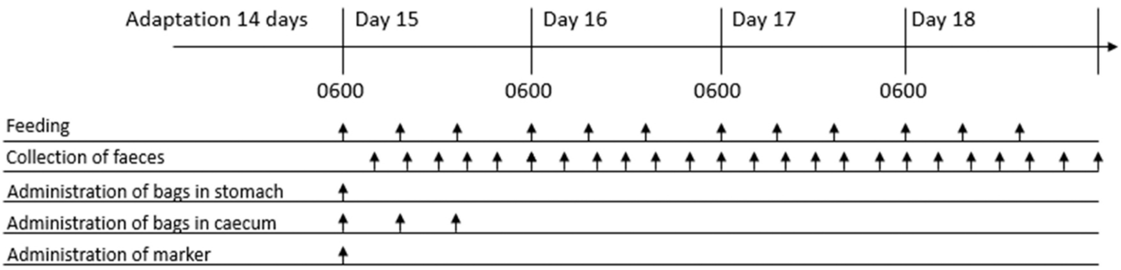

Fig. 1. The experimental setup illustrating feeding times (06.00, 14.00 and 22.00 h), faecal collection times (every 4th h), administration of mobile bags in the stomach and the caecum and administration of marker in caecum during the 4 days of data collection in each of the two experimental periods.

paddock for 6–8 h, divided into two visits. In the collection periods, one outdoor visit for 1 h was allowed daily.

2.3. Diets

All horses were fed three equal meals a day (06.00, 14.00 and 22.00 h) with either a hay-only (HAY)diet (15.1 g DM/kg BW/day) or a mixture of hay:supplement (MIX) (8.4:6.7 g DM/kg BW/day). The MIX diet consisted of alfalfa pellets (AP), grass pellets (GP), oat hulls (OH), soya hull pellets (SHP) and sugar beet pulp pellets (SBP) each given in 0.44 g DM/kg BW/meal. The hay fed in both diets (HAY and MIX) consisted mainly of Timothy from first cut. The experiment was designed as a cross-over experiment with two experimental periods, meaning that two horses were fed the HAY diet and two horses the MIX diet in period 1, and then the horses changed diets for period 2. Each horse then served as their own control in the experiment. Samples of all feedstuffs were collected daily during the 4 days of data collection within the two periods and stored in sealed plastic bags for later analyses. The feedstuffs' chemical composition is presented in Table 1, and the daily nutrient intake for each diet is presented in Table 2. Each meal of the MIX diet was soaked in water (3 L) approximately 1 h before feeding. A commercial supplement of vitamins and minerals (Champion Multitilskudd, Felleskjøpet Forutvikling, Trondheim, Norway) was fed in both diets (80 g/day). Horses fed the HAY diet received 25 g/day of sodium chloride. Water was always available and measured individually by automatic water troughs, and during the adaptation period water was also available from buckets in the gravel paddock.

2.4. Total collection of faeces

Four consecutive days of total faecal collection from each horse was performed using a collection harness (Stablemaid, Melbourne, Australia). Each collection harness was emptied every 4th h, and daily faecal excretion was stored in plastic bins with a lid at 3°C. Procedures for the mobile bags found in the faeces are described below. Each horse's daily faecal excretion was weighed, then mixed thoroughly by hand and an electrical concrete mixer (electric concrete mixer, Atika, 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

Table 1
Dry matter (g/kg), chemical composition and energy content (MJ/kg DM) of the individual feedstuffs (g/kg DM)[a] used for the two diets (HAY: hay-only[b] and MIX: hay[b]+supplement[c]).

Nutrients[d]	Feedstuffs[c]					
	Hay	AP	GP	OH	SBP	SHP
DM	896	923	926	883	887	894
CP	145	123	149	58.7	81.4	112
CF	22.7	21.1	23.7	20.0	49.0	14.2
Starch	–	–	–	181	–	–
WSC	74.2	82.9	49.3	39.2	88.1	14.2
Ash	73.2	81.1	132	32.3	64.0	51.7
aNDF	615	452	524	648	420	671
ADF	313	298	324	315	217	468
ADL	64.8	85.1	74.9	125	55.4	34.2
Hemicellulose	302	154	200	333	203	203
$Cellulose_{NDF}$	248	213	249	190	161	433
Dietary Fibre						
DF	582	532	546	606	669	770
Klason lignin	96.6	131	140	121	36.8	31.2
T-NSP	485	402	406	484	633	739
I-NSP	454	327	346	456	343	587
S-NSP	30.7	74.7	60.0	28.3	290	152
$Cellulose_{DF}$	246	180	191	206	171	358
Arabinose	32.0	25.4	27.8	28.4	170	50.4
Fructose	0.16	0.81	0.78	0.17	1.07	3.01
Galactose	9.72	15.3	15.6	9.63	44.4	26.8
Glucose	21.0	12.6	16.9	15.2	7.0	12.0
Manose	3.16	10.7	9.20	3.04	12.0	62.5
Rhamnose	2.54	4.14	3.13	0.28	10.9	8.27
Uronic acid	28.1	76.6	57.9	13.1	207	132
Xylose	142	76.0	83.4	209	10.2	86.5
GE	19.1	18.6	18.0	19.2	18.3	17.7

[a] Composition of mineral and vitamin supplement: Ca, 100 (g/kg); Mg, 32 (g/kg); Cu, 840 (mg/kg); Zn, 2830 (mg/kg); Fe, 2460 (mg/kg); Mn, 1530 (mg/kg); I, 18 (mg/kg); Co, 6 (mg/kg); Vitamin A, 10,7000 (I.U./kg); Vitamin D, 11,300 (I.U./kg); Vitamin E, 9600 (mg/kg).
[b] Mainly Timothy from first cut.
[c] AP, alfalfa pellets; GP, grass pellets; OH, oat hulls, SHP, soya hull pellets; and SBP, sugar beet pulp pellets.
[d] DM, dry matter; CP, crude protein; CF, crude fat; WSC, water soluble carbohydrates; aNDF, neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; DF, dietary fibre; T-NSP, total non-starch polysaccharides; I-NSP, insoluble non-starch polysaccharides; S-NSP, soluble non-starch polysaccharides and GE, gross energy.

Table 2
Daily nutrient intake (g/kg body weight (BW), unless otherwise stated) for the two diets (HAY: hay-only[a], MIX: hay[a] + supplement[b]).

Nutrient[c]	HAY	MIX	±SD
DM	15.1	15.1	0.02
CP	2.20	1.91	0.14
CF	0.03	0.05	0.01
Starch	–	0.25	–
WSC	1.01	0.90	0.05
Ash	1.10	0.61	0.25
aNDF	9.27	8.76	0.29
ADF	4.71	4.77	0.03
ADL	0.95	1.02	0.04
Hemicellulose	4.56	3.99	0.28
$Cellulose_{NDF}$	3.73	3.73	0.002
Dietary Fibre			
DF	8.76	9.03	0.13
Klason lignin	1.45	1.41	0.02
T-NSP	7.31	7.61	0.15
I-NSP	6.85	6.55	0.15
S-NSP	0.46	1.07	0.30
$Cellulose_{DF}$	3.72	3.54	0.09
Arabinose	0.48	0.67	0.10
Fructose	0.002	0.01	0.003
Galactose	0.15	0.23	0.04
Glucose	0.32	0.26	0.03
Manose	0.15	0.23	0.05
Rhamnose	0.04	0.06	0.01
Uronic acid	0.42	0.88	0.23
Xylose	2.14	1.81	0.16
GE (MJ/kg BW)	0.29	0.28	0.003

[a] Mainly Timothy from first cut.
[b] AP, alfalfa pellets; GP, grass pellets; OH, oat hulls, SHP, soya hull pellets; and SBP, sugar beet pulp pellets.
[c] DM, dry matter; CP, crude protein; CF, crude fat; WSC, water soluble carbohydrates; aNDF, neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; DF, dietary fibre; T-NSP, total non-starch polysaccharides; I-NSP, insoluble non-starch polysaccharides; S-NSP, soluble non-starch polysaccharides and GE, gross energy.

experiment, the daily subsamples were pooled and used to composite a single representative sample for each horse. For further analysis, the daily faecal subsamples were thawed and mixed into two new subsamples (~ 500 g/sample).

2.5. Mobile bag technique

The MBT was used to estimate the individual feedstuffs' digestibility based on nutrient disappearance from the bags after administration and the subsequent recovery of the bags in the caecum or faeces (Macheboeuf et al., 1996; Hyslop, 2006). Bags (1 ×2 × 12 cm) 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 as described by Thorringer and Jensen (2021). For bags placed in the stomach, a steel washer (1 cm external diameter, weight 0.3 g) was sealed into the end of each bag, allowing capture with a magnet in the caecum (Fig. 2). The weight of the marked empty bags and bags filled with individual feedstuffs (500 mg/bag, and a feed to surface area of 20.8 mg/cm^2 according to Thorringer and Jensen, 2021) were recorded. All feedstuffs were milled to pass a 1.5 mm screen. An overview of the mobile bags administered is provided in Table 3. All bags were soaked in cold tap water approximately 20 s pre-administration, and

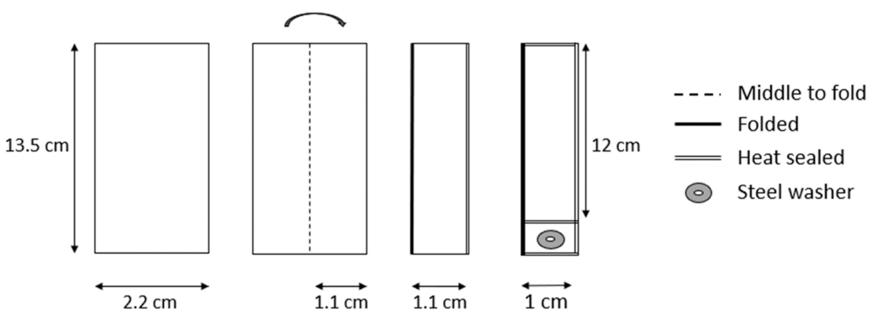

Fig. 2. Illustration of mobile bag construction with example of bags administered in the stomach.

bags were administered to the stomach via a nasogastric tube flushed with approximately 1.5 L of tap water before the morning meal on day 1. A bag (48 ×2 cm, L x W) containing a double-sided magnet was placed in the caecum and attached to the cannula to catch the mobile bags at arrival in the caecum. Every hour the magnet was harvested for bags, starting 1.5 h after administration, and ending 8.5 h after administration. The mobile bags administered in the stomach and not harvested in the caecum were collected in faeces during the following days. Mobile bags containing each of the six individual feedstuffs were administered into the caecum through the cannula during each meal on Day 1 in the collection periods (Table 3) and captured in faeces during the following days.

Faeces were inspected for bags at every collection during the 4 days of faecal collection. The collection time of each bag was noted and, thereafter, hand-rinsed in cold tap water and stored at –20°C. At the end of the experiment all bags (harvested in caecum and collected in faeces) were thawed at room temperature, placed in a washing bag (28 ×37 cm), washed in cold water for 35 min without spinning (Woolprogram, Avantixx 7 Varioperfect, Bosch, Gerlingen-SchillerhÖhe, Germany) and dried at 45°C for 48 h. Bags were left for equilibration at room temperature (approximately 25°C) for 24 h before weighing and calculating DM loss. Control bags (4 bags/feedstuff) were not administered to the horses but soaked for 1 h before washing and drying as described above to determine the disappearance of nutrients from the bags. The in-situ disappearance of DM for each individual bag was determined by weight after drying. To obtain enough residue for chemical analysis, mobile bags administered in the stomach and recovered in the caecum were pooled for each feedstuff, and each of the hourly collection times and bags recovered in faeces were pooled in four time intervals (1: 10–19 h, 2: 20–29 h, 3: 30–39 h and 4: 40–100 h). Mobile bags administered into the caecum and recovered in faeces were pooled for each feedstuff and in four time intervals (1: 10–19 h, 2: 20–29 h, 30–39 h and 4: 40–100 h).

2.6. *Mean retention time*

A marker solution was prepared by mixing 30 g of ytterbium acetate (III) tetrahydrate (Yb, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) with 5 L of demineralised water. Each horse was administered 500 mL of Yb solution (3 g Yb) into the caecum through the cannula with a 500 mL syringe connected to an 18 cm long tube after feeding the morning meal. Each time the collection harness was emptied for faecal output, a subsample of approximately 300 g was weighed, and the subsample was stored at –20°C for later analyses of Yb.

2.7. *Chemical analysis*

All analyses of faeces and feedstuffs used for mobile bags and feeding were performed in duplicates. Faeces samples, feedstuff samples and mobile bag residues were ground to 1 mm (mixer mill MM 301, Retsch GmbH, Haan, Germany) before analysis. Samples of faeces from each horse, feedstuffs fed and bulked residues from collected bags according to feedstuff, collection time interval and place of recovery were analysed for DM by drying to constant weight (24 h at 105 °C ± 2 °C). Ash was determined by incineration at 550 °C for 16 h. All samples were milled to 0.5 mm before analysing for nitrogen according to the Dumas method (Elementar Analysensysteme GmbH, Hanau, Germany), and CP was calculated as N × 6.25. Neutral detergent fibre was assayed with a heat-stable amylase and expressed including residual ash (aNDF), ADF was expressed including residual ash, and ADL for all samples were measured by the filter bag technique described by (ANKOM 2017a, b). Non-starch polysaccharides (NSP) and dietary fibre (DF) in the feedstuffs and faeces were analysed as described by Bach Knudsen (1997). In duplicates, three parallel runs of total NSP (T-NSP) and insoluble NSP (I-NSP) and their constituent sugars were determined as alditol acetates by gas-liquid chromatography for neutral sugars and by a colorimetric method for uronic acids. Soluble NSP (S-NSP) was determined as T-NSP – I-NSP. Klason lignin was measured as the sulphuric acid insoluble residue as described by Theander et al. (1994). From the analyses of T-NSP and Klason lignin, dietary fibre (DF) was calculated as DF = T-NSP + Klason lignin. All feedstuffs' fed were analysed for water-soluble carbohydrates (WSC) according to Randby et al. (2010). Oat hulls were milled to 0.5 mm (mixer mill MM 301, Retsch GmbH, Haan, Germany) and analysed for starch

Table 3
Overview of administrated mobile bags (n) to each horse (stomach and caecum) for the individual feedstuffs when fed two diets at Day 1 of collection in each period.

Place[a]	Diet[b]	Feedstuff	Administration time of mobile bags		
			0600	1400	2200
Stomach[c]	HAY	Hay	20		
	MIX	Hay	6		
		SBP	14		
Caecum	HAY	Hay	2	2	2
	MIX	Hay	2	2	2
		AP	2	2	2
		GP	2	2	2
		OH	4	4	4
		SBP	4	4	4
		SHP	2	2	2

[a] Stomach: morning meal at 0600 h and caecum: each meal at 0600, 1400 and 2200 h.
[b] HAY: hay-only (mainly Timothy from first cut) and MIX: hay (mainly timothy first cut) + supplement (AP, alfalfa pellets; GP, grass pellets; OH, oat hulls, SHP, soya hull pellets; and SBP, sugar beet pulp pellets.).
[c] More bags with SBP than hay was used as SBP was assumed to have a greater nutrient disappearance than hay.

according to the methodology described by the Association of Official Analytical Chemists (AOAC, 1990, 996.11 method) and thereafter read on a chemistry analyser (RX4041 Daytona+, Randox Laboratories, Crumlin, Great Britain). Gross energy (GE) was determined using a bomb calorimeter method (6400 Automatic Isoperibol Calorimeter, Parr Instrument Company, Illinois, USA). Crude fat was analysed according to the accelerated solvent extractor method (Dionex ASE 350, Thermo Fisher Scientific, Waltham, USA).

Faeces were analysed for the Yb concentration, and samples of 0.2–0.3 g faeces were weighed into acid-washed teflon tubes (Agilent, Santa Clara, USA) and 0.25 mL HBF_4 (48%), 5 mL HNO_3 (sub-boiled) and 2 mL H_2O was added. Thereafter, all samples were decomposed in an UltraClave (Milestone Microwave UltraClave III, Milestone S.R.L, Sorisole, Italy) at 260°C for 20 min. To ensure the system was running as it should, both reference material and blanks were included in the UltraClave. Thereafter, all samples were cooled and diluted with 50 mL H_2O, then 5 mL were further diluted with 5 mL H_2O to decrease the fluoride concentration before analysing the Yb concentration by dichroic spectral combiner (5110 ICP-OES, Agilent, Santa Clara, USA). Limit of detection (LOD) and limit of quantification (LOQ) were calculated from 3 and 10 times the SD of the blanks (n = 6), respectively.

3. Calculations

3.1. The ATTD of nutrients

The ATTD of individual nutrients for the two diets was calculated as:

$$\text{ATTD} = ((\text{Intake (g)} - \text{faecal excretion (g)}) / (\text{intake (g)}) \quad (1)$$

Furthermore, the ATTD of the supplements in the MIX diet was calculated from the digestibility coefficients of the HAY according to Martin-Rosset et al. (1984):

$$dS = (dD - (h \times dH)) / s \quad (2)$$

Where dS is the supplement's digestibility (coefficient), dD is the diet digestibility (coefficient), dH is the digestibility of the hay (coefficient), h the fraction of hay in the diet and s the fraction of the supplement in the diet.

3.2. Transit time of the mobile bags and mean retention time for Yb

The characteristics of the mobile bags and Yb transit through the hindgut were assessed by calculating the transit time (TT) for the mobile bags and mean retention time (MRT) for Yb according to Faichney (1975): .

$$\text{TT/MRT} = \sum Bi \times ti \quad (3)$$

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

3.3. DM degradation curves

Individual DM disappearance curves for each feedstuff and mobile bags (stomach and caecum) combined with place of collection (caecum or faeces) were made. These curves were subjected to the Ørskov and McDonald (1979) model for evaluating the degradation profile of the individual feedstuffs:

$$D_t = a + b\,(1 - e^{-ct}) \quad (4)$$

Where D_t is the degradation after time t of administration, b is the potential degradation (insoluble but potentially degradable 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 the asymptote a+b.

3.4. Effective degradability

The effective degradability (ED) was calculated for all bag Eq. 5 at chosen outflow rates (k): 0.05%, 0.033%, 0.025%, 0.020% and 0.017% per h to obtain DM disappearance from the mobile bags to assumed digesta MRT in the hindgut of 20, 30, 40, 50 and 60 h:

$$ED = a + bc / (c + k) \quad (5)$$

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

4. Statistical analysis

All statistical analyses were performed in R studio (Team, 2020). One-way analysis of variance (ANOVA) was performed on water intake with a model comprising intake in litres as the response and diet as the predictor. The ATTD was subjected to a mixed model with individual nutrient as the response, diet as the predictor and horse as the random effect. Two-way ANOVA was done on in-situ nutrient disappearance with a model comprising nutrient disappearance as the response and time or time interval and feedstuff as the predictors. An interaction between feed x time was found for precaecal in-situ disappearance and therefore included. No other interactions were found between predictors and were therefore excluded. To compare ATTD with in-situ nutrient disappearance for the hindgut, a three-way ANOVA was used. Data were compromised to a model with the individual nutrient disappearance or digestion as the response and time, method, and diet as the predictors. No interactions were found and therefore excluded. The effective degradability ED values and the degradation D_t were subjected to two-way ANOVA using time and feedstuff as predictors. No interactions between predictors were found significant and therefore excluded. The TT for the mobile bags with individual feedstuffs were subjected to a mixed model, with TT for the individual gastrointestinal segments used as the response, feedstuff as the predictor and horse as the random effect. The MRT for Yb was subjected to a mixed model with MRT for the hindgut used as the response, diet as the predictor and horse as the random effect. Significant differences of least-square means were analysed by Tukey's Honest Significant Difference test when relevant. All results are presented as least-square means ± *SD*, and effects are considered significantly different if $P < 0.05$.

5. Results

5.1. Chemical composition of the diets

The chemical composition of the individual feedstuffs is given in Table 1. The DM content varied from 883 to 926 g/kg, with OH having the lowest and GP the highest DM content. A larger numerical variation was measured for CP, with OH having the lowest CP content at 58.7 g/kg DM and GP having the highest CP content at 149 g/kg DM. Crude fat content was generally low and varied from 14.2 to 49.0 g/kg DM, with SBP having the highest content, as fat was added in the pelleting process. Starch was determined in OH to 181 g/kg DM and assumed to be zero in the other feedstuffs. The content of WSC varied from 14.2 to 88.1 g/kg DM, with SHP having the lowest and SBP the highest content. Sugar beet pulp pellets had the lowest and SHP the highest content of both aNDF and ADF compared to the other feedstuffs (Table 1). Soya hull pellets had the highest content of fibre expressed as aNDF, ADF, cellulose, T-NSP, I-NSP and DF compared to the other feedstuffs (Table 1). The S-NSP was markedly higher in SBP and SHP compared to the other feedstuffs and highest for SBP. Klason lignin was highest in GP, whereas for ADL the highest content was measured in OH. However, for both Klason lignin and ADL, the lowest content was measured in SHP. The constituent sugars of the feedstuffs varied, with different dominant sugars in each feedstuff. In AP and GP, xylose and uronic acid were the dominating sugars, whereas xylose was supreme in hay and OH. For SBP, arabinose and uronic acid dominated, and only uronic acid dominated in SHP. The GE content varied from 17.7

Table 4
Apparent total tract digestibility (ATTD) of the two diets, hay-only (HAY)[a] and hay[a]+supplement[b] (MIX), and the estimated ATTD of the supplement (dS).

Nutrient[c]	HAY	MIX	±SD	P-value	dS
DM	0.625	0.612	0.006	0.354	0.596
CP	0.754	0.713	0.020	< 0.001	0.641
aNDF	0.593	0.533	0.030	0.006	0.447
ADF	0.553	0.480	0.036	0.017	0.390
Hemicellulose	0.634	0.596	0.019	0.001	0.530
$Cellulose_{NDF}$	0.613	0.503	0.055	< 0.001	0.364
GE	0.589	0.583	0.003	0.549	0.575
Dietary fibre and monomers					
DF	0.471	0.469	0.035	0.913	0.467
T-NSP	0.603	0.589	0.030	0.478	0.573
$Cellulose_{DF}$	0.608	0.548	0.046	0.009	0.511
Arabinose	0.745	0.804	0.031	< 0.001	0.844
Fructose	0.067	0.659	0.295	< 0.001	0.751
Galactose	0.816	0.811	0.020	0.695	0.808
Glucose	0.760	0.666	0.063	< 0.001	0.468
Manose	0.643	0.879	0.165	0.715	0.910
Rhamnose	0.805	0.829	0.020	< 0.001	0.842
Uronic acid	0.815	0.870	0.031	< 0.001	0.890
Xylose	0.479	0.397	0.052	0.002	0.239

[a] Mainly Timothy from first cut.
[b] AP, alfalfa pellets; GP, grass pellets; OH, oat hulls, SHP, soya hull pellets; and SBP, sugar beet pulp pellets.
[c] DM, dry matter; CP crude protein; aNDF, neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash; $Cellulose_{NDF}$, cellulose estimated form aNDF and ADF; ADF, acid detergent fibre; GE, gross energy; DF, dietary fibre; T-NSP, total non-starch polysaccharides, and $Cellulose_{DF}$, cellulose estimated from DF analysis.

to 19.2 MJ/kg DM and was highest for OH and lowest for SHP. The DM and GE intake of the two diets was similar (Table 2). Horses received 91.4 and 94.4 MJ digestible energy (DE) per day with the MIX and HAY diets, respectively. Substituting hay partly with the supplement increased the daily starch intake with 0.25 g/kg BW and decreased CP intake with 0.3 g/kg BW. Neutral detergent fibre intake was slightly higher (0.9 g/kg BW) for the HAY diet compared to the MIX diet. However, the daily ADF and $cellulose_{NDF}$ remained the same. Dietary fibre, T-NSP, S-NSP and $cellulose_{DF}$ were higher in the MIX diet compared to the HAY diet, whereas I-NSP and xylose intake were highest with the HAY diet. Furthermore, the water intake increased ($P < 0.001$) when horses received the HAY diet (38.0 ± 5.3 L) compared to the MIX diet (33.3 ± 4.5 L).

5.2. The apparent total tract digestibility

The ATTD of the individual nutrients for the two diets is presented in Table 4. There was no difference in the ATTD of DM between the two diets. There was an effect of diet on the ATTD of CP ($P < 0.001$), aNDF ($P = 0.006$) and ADF ($P = 0.017$) including hemicellulose ($P = 0.001$) and $cellulose_{NDF}$ ($P < 0.001$), as they were higher in the HAY diet compared to the MIX diet (Table 4). There was no effect of diet on the ATTD of DF, T-NSP, mannose and galactose. The ATTD of xylose ($P = 0.002$), glucose ($P < 0.001$) and $cellulose_{DF}$ ($P = 0.009$) was greater for the HAY diet compared to the MIX diet. However, a greater ATTD ($P < 0.001$) of rhamnose, fructose, arabinose and uronic acid was measured in the MIX diet than the HAY diet (Table 4). The estimated dS is also presented in Table 4, and it provides an indication of the ATTD of the supplement alone. When differences between the two diets were present, as presented above, this was also present in the estimated dS. As for DM, CP, aNDF and ADF, the estimated dS was numerically lower than both the HAY and the MIX diet. For the monomers arabinose, manose, rhamnose and uric acid, the dS was numerically greater compared to the HAY and MIX diets.

5.3. Washing loss of nutrients

Dry matter loss from the washing of the control bags varied from 0.181 for SHP to 0.299 for OH (Table 5). Ash loss was in general high and varied from 0.316 for OH to 0.812 for hay. Loss of aNDF and ADF was generally low, with hay having the lowest (negative values are small and might be due to measurement error) and SBP having the highest loss.

5.4. Nutrient disappearance

Pre-caecal disappearance of DM as well as aNDF for SBP and hay is shown in Fig. 3. An interaction between feed × time ($P = 0.006$) was present for the DM disappearance, with SBP having a greater DM disappearance over time than hay. Neutral detergent fibre disappearance was greater for SBP than hay ($P < 0.001$), and the disappearance increased over time ($P = 0.002$).

A comparison of hindgut nutrient disappearance for bags with the six different feedstuffs is shown in Table 6. The DM disappearance differed between feedstuffs ($P < 0.001$) and increased over time ($P = 0.002$), with the lowest disappearance for OH and the highest disappearance for SBP. Further, OH had the lowest disappearance of aNDF and ADF followed by hay, AP and GP compared to SBP and SHP ($P < 0.001$).

5.5. Comparison of in-vivo ATTD and in-situ disappearance of nutrients

The DM, aNDF and ADF digestibility of the HAY and MIX diets were estimated from the nutrient disappearance of the individual feedstuffs presented in Table 6. These were further compared with the nutrient ATTD presented in Table 7. There was no effect of diet for DM or ADF digestibility estimates, but aNDF digestibility was greater for the HAY diet compared to the MIX diet ($P = 0.021$). Time affected the estimated digestibility of DM ($P = 0.005$), aNDF ($P = 0.011$) and ADF ($P = 0.039$), but there was no difference between the estimated digestibility of DM, aNDF and ADF and the ATTD for time interval 1–3, 2–3 and 1–4, respectively.

Table 5
Washing loss[a] of nutrients from different feedstuffs[b].

Nutrients[c]	Hay[d]	AP	GP	OH	SBP	SHP
DM	0.264	0.267	0.265	0.299	0.195	0.181
Ash	0.812	0.601	0.519	0.316	0.480	0.517
aNDF	-0.008	0.019	0.011	0.029	0.045	0.086
ADF	-0.023	0.016	0.010	0.009	0.064	0.065

[a] Washing loss measured by washing mobile bags for 35 min using a wool program without spinning.
[b] AP, alfalfa pellets; GP, grass pellets; OH, oat hulls, SHP, soya hull pellets; and SBP, sugar beet pulp pellets.
[c] DM, dry matter; aNDF, neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash and ADF, acid detergent fibre.
[d] Mainly Timothy from first cut.

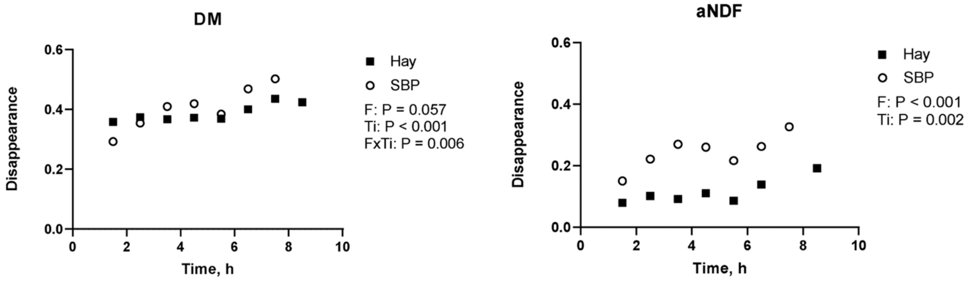

Fig. 3. Pre-caecal disappearance of dry matter (DM) and neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash (aNDF) determined from the mobile bag technique for the two feedstuffs (F): sugar beet pulp pellets (SBP) and hay (mainly Timothy, first cut) over time (Ti; 1.5–8.5 h). P-values are given for feedstuff (F), time (Ti) and the interaction feedstuff x time (FxTi).

Table 6
Hindgut disappearance of DM, aNDF and ADF for the individual feedstuffs to each time interval (1:10–19 h, 2: 20–29 h, 3: 30–39 h and 4: 40–100 h).

		Feedstuffs[1]							P-value	
Nutrients[2]	Time	Hay	AP	GP	OH	SBP	SHP	±SD	Feed	Time
DM	1^{y}	0.604^{bc}	0.585^{bc}	0.587^{c}	0.450^{d}	0.684^{a}	0.648^{b}	0.073	< 0.001	0.002
	2^{y}	0.623^{bc}	0.628^{bc}	0.610^{c}	0.463^{d}	0.810^{a}	0.632^{b}	0.101		
	3^{xy}	0.653^{bc}	0.622^{bc}	0.560^{c}	0.469^{d}	0.844^{a}	0.708^{b}	0.117		
	4^{x}	0.705^{bc}	0.667^{bc}	0.643^{c}	0.486^{d}	0.900^{a}	0.846^{b}	0.136		
aNDF	1^{y}	0.492^{b}	0.352^{b}	0.446^{b}	0.222^{c}	0.595^{a}	0.628^{a}	0.139	< 0.001	0.001
	2^{y}	0.528^{b}	0.407^{b}	0.462^{b}	0.239^{c}	0.732^{a}	0.592^{a}	0.153		
	3^{y}	0.560^{b}	0.407^{b}	0.420^{b}	0.248^{c}	0.791^{a}	0.706^{a}	0.187		
	4^{x}	0.634^{b}	0.486^{b}	0.532^{b}	0.272^{c}	0.876^{a}	0.878^{a}	0.215		
ADF	1^{y}	0.432^{b}	0.326^{b}	0.406^{b}	0.178^{c}	0.501^{a}	0.575^{a}	0.127	< 0.001	0.001
	2^{y}	0.473^{b}	0.371^{b}	0.426^{b}	0.200^{c}	0.660^{a}	0.519^{a}	0.141		
	3^{y}	0.510^{b}	0.379^{b}	0.394^{b}	0.205^{c}	0.745^{a}	0.663^{a}	0.182		
	4^{x}	0.596^{b}	0.465^{b}	0.502^{b}	0.227^{c}	0.857^{a}	0.879^{a}	0.227		

[1] AP, alfalfa pellets; GP, grass pellets; hay mainly Timothy first cut; OH, oat hulls, SHP, soya hull pellets; and SBP, sugar beet pulp pellets.
[2] DM, dry matter; aNDF, neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash and ADF, acid detergent fibre.
[a, b, c, d] Feedstuffs: Values within each feedstuff row per nutrient are different if superscript differs ($P < 0.05$).
[x, y] Time: Values with each time interval column per nutrient (for all feedstuffs) are different if superscript differs ($P < 0.05$).

Table 7
The apparent total tract digestibility (ATTD) of DM, aNDF and ADF for the two diets measured with total faeces collection (in-vivo) and estimated with mobile bags in the hindgut (in-situ) to each time interval (1:10–19 h, 2: 20–29 h, 3: 30–39 h, 4: 40–100 h).

		Method							
		In-vivo	In-situ					P-value	
Nutrient[1]	Diet[2]	ATTD	1	2	3	4	±SD	Diet	Time
DM	HAY	0.625^{b}	0.604^{b}	0.623^{b}	0.653^{ab}	0.705^{a}	0.035	0.489	0.005
	MIX	0.612^{b}	0.593^{b}	0.628^{b}	0.643^{ab}	0.708^{a}	0.039		
aNDF	HAY	0.593^{b}	0.492^{c}	0.528^{bc}	0.560^{abc}	0.634^{a}	0.049	0.021	0.011
	MIX	0.533^{b}	0.456^{c}	0.493^{bc}	0.522^{abc}	0.613^{a}	0.052		
ADF	HAY	0.553^{ab}	0.432^{b}	0.473^{ab}	0.510^{ab}	0.596^{a}	0.058	0.075	0.039
	MIX	0.480^{ab}	0.403^{b}	0.442^{ab}	0.483^{ab}	0.588^{a}	0.062		

[1] DM, dry matter; aNDF, neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash and ADF, acid detergent fibre.
[2] HAY: hay-only (mainly Timothy, first cut) and MIX: hay + supplements (AP, alfalfa pellets; GP, grass pellets; OH, oat hulls, SHP, soya hull pellets; and SBP, sugar beet pulp pellets.).
[a, b, c] Values within a row are different if superscript differs ($P < 0.05$).

5.6. *Transit time of mobile bags and MRT for Yb*

The transit times for pre-caecal, hindgut and total tract of mobile bags with the individual feedstuffs are presented in Table 8. The average pre-caecal TT for bags with hay and SBP was 2.55 h (Table 8). The hindgut TT for bags with the six feedstuffs varied from 26.3 to 41.6 h. Bags with SHP had a significantly longer TT compared with bags containing SBP ($P = 0.035$). The total tract TT for bags with hay and SBP varied from 30.3 to 35.3 h with no difference. The MRT for Yb in the hindgut depended on diet ($P < 0.001$), with 23.6 h

Table 8
Dry matter degradation parameters[1] for the individual feeds[2] and transit time (TT) in h for the different segments. Effective degradability (ED) and degradation (D_t) for mean retention times of 20, 30, 40, 50 and 60 h for all feeds.

								P-values	
Feed	HAY	AP	GP	OH	SBP	SHP	±SD	Feed	Time
a	0.286	0.278	0.273	0.335	0.198	0.248	0.041		
b	0.440	0.403	0.392	0.203	0.756	0.632	0.178		
c	0.073	0.102	0.089	0.104	0.070	0.051	0.019		
a+b	0.726	0.681	0.665	0.539	0.954	0.880	0.139		
TT									
Pre-caecal	2.31				2.79		0.24	0.169	
Hindgut	32.3^{ab}	27.5^{ab}	26.9^{ab}	30.2^{ab}	26.3^{b}	41.6^{a}	5.26	< 0.05	
Total tract	30.3				35.3		2.50	0.204	
ED									
20^{z}	0.547^{c}	0.548^{c}	0.524^{c}	0.473^{d}	0.638^{a}	0.567^{b}	0.049	< 0.001	< 0.001
30^{y}	0.588^{c}	0.582^{c}	0.558^{c}	0.489^{d}	0.709^{a}	0.630^{b}	0.067		
40^{xy}	0.614^{c}	0.602^{c}	0.579^{c}	0.499^{d}	0.754^{a}	0.672^{b}	0.079		
50^{x}	0.631^{c}	0.615^{c}	0.593^{c}	0.506^{d}	0.785^{a}	0.702^{b}	0.087		
60^{x}	0.644^{c}	0.624^{c}	0.603^{c}	0.511^{d}	0.808^{a}	0.724^{b}	0.094		
D_t									
20^{y}	0.624^{c}	0.629^{c}	0.599^{c}	0.513^{d}	0.766^{a}	0.652^{b}	0.075	< 0.001	< 0.001
30^{x}	0.677^{c}	0.662^{c}	0.638^{c}	0.530^{d}	0.860^{a}	0.743^{b}	0.101		
40^{x}	0.702^{c}	0.674^{c}	0.654^{c}	0.536^{d}	0.907^{a}	0.798^{b}	0.117		
50^{x}	0.715^{c}	0.679^{c}	0.660^{c}	0.538^{d}	0.931^{a}	0.831^{b}	0.126		
60^{x}	0.720^{c}	0.680^{c}	0.663^{c}	0.538^{d}	0.942^{a}	0.850^{b}	0.131		

[1] a, soluble part of the feed, b, potential digestible (insoluble part of the feed), c, rate constant for degradation of b per h and a+b is the potential degradable fraction, calculated on the mobile bags administrated to the caecum.
[2] AP, alfalfa pellets; GP, grass pellets; hay (mainly Timothy first cut); OH, oat hulls, SHP, soya hull pellets; and SBP, sugar beet pulp pellets.[a, b, c, d] Feedstuffs: values within each feedstuff row per nutrient are different if superscript differs (P < 0.05).
[x, y, z] Time: values for each mean retention time column per ED and Dt (for all feedstuffs) are different if superscript differs (P < 0.05).

and 25.7 h for the HAY and MIX diets, respectively.

5.7. Dry matter degradation curves

Fitted DM degradation curves from Ørskov and McDonald (1979) for the six different feedstuffs are shown in Fig. 4. The mobile bags collected in faeces from 14 to 80 h after administration in the caecum and the fitted DM degradation agrees with the raw data for each feedstuff (Fig. 4). For the six feedstuffs, the parameter a (the soluble part of the feed) varied from 0.198 to 0.286, with SBP having the lowest and hay the highest values (Table 8), which agrees with the washing loss of DM (Table 5). The potential degradation b (the insoluble part of the feed) varied from 0.203 to 0.756, with SBP having the numerically highest and OH the lowest value (Table 8). Sugar beet pulp pellets had the numerically highest potential degradable fraction a+b with 0.954, whereas OH had the lowest with 0.539 (Table 8). An effect of time was found for the ED and D_t with 20 h having the lowest estimate compared to the rest ($P < 0.001$). Type of feed also affected the ED and D_t ($P < 0.001$), with OH having the lowest values, whereas AP, hay and GP had similar values followed by SHP, and SBP had the highest values of all the feedstuffs. In general, to reflect the average TT for the total tract of 30.3 h for the mobile bags with hay, an ED and D_t of 30 h predicts the DM degradation to be 0.588 and 0.677, respectively (Table 6). Fitted DM degradation curves from Ørskov and McDonald (1979) using the bags placed in the stomach and found in the caecum or in faeces are shown in Fig. 5. The a, b and c values for hay were 0.338, 0.382 and 0.050, and for SBP 0.251, 0.718 and 0.067, respectively. The degradation curves from bags with hay and SBP placed in the stomach followed the degradation curves from bags placed in the caecum (Fig. 5), indicating that the estimates for the six feedstuffs in Table 8 are valid.

6. Discussion

6.1. Composition of feedstuffs and diets

In the present study, the aim was to evaluate the effect of substituting hay partly with other fibrous feedstuffs while still fulfilling the daily feed intake recommendations of 15 g DM/kg BW (Harris et al., 2017). Weather conditions might limit forage supply, and the background for conducting this study was a severe drought in 2018 resulting in a lack of roughage. Alternative fibrous feedstuffs were chosen for their availability and nutrient composition. From the chemical analysis, it was clear that the nutrient and especially the carbohydrate composition varied markedly between the different feedstuffs, and their physiochemical properties might affect their usefulness in diets for horses with different energy requirements. Horses received between 91.4 and 94.4 MJ digestible energy (DE) per day with the two diets, which is above the requirements for horses in maintenance (National Research Council, 2007). In this study, both aNDF and DF were analysed. The DF analysis gives a detailed description of the fibre fraction, including the S-NSP content, compared to the aNDF analysis, where S-NSP is lost (Bach Knudsen, 2001). This was most pronounced for SBP and SH, where aNDF was noticeably lower compared to DF because of a high S-NSP content in these two feedstuffs. Generally, this makes it difficult to compare

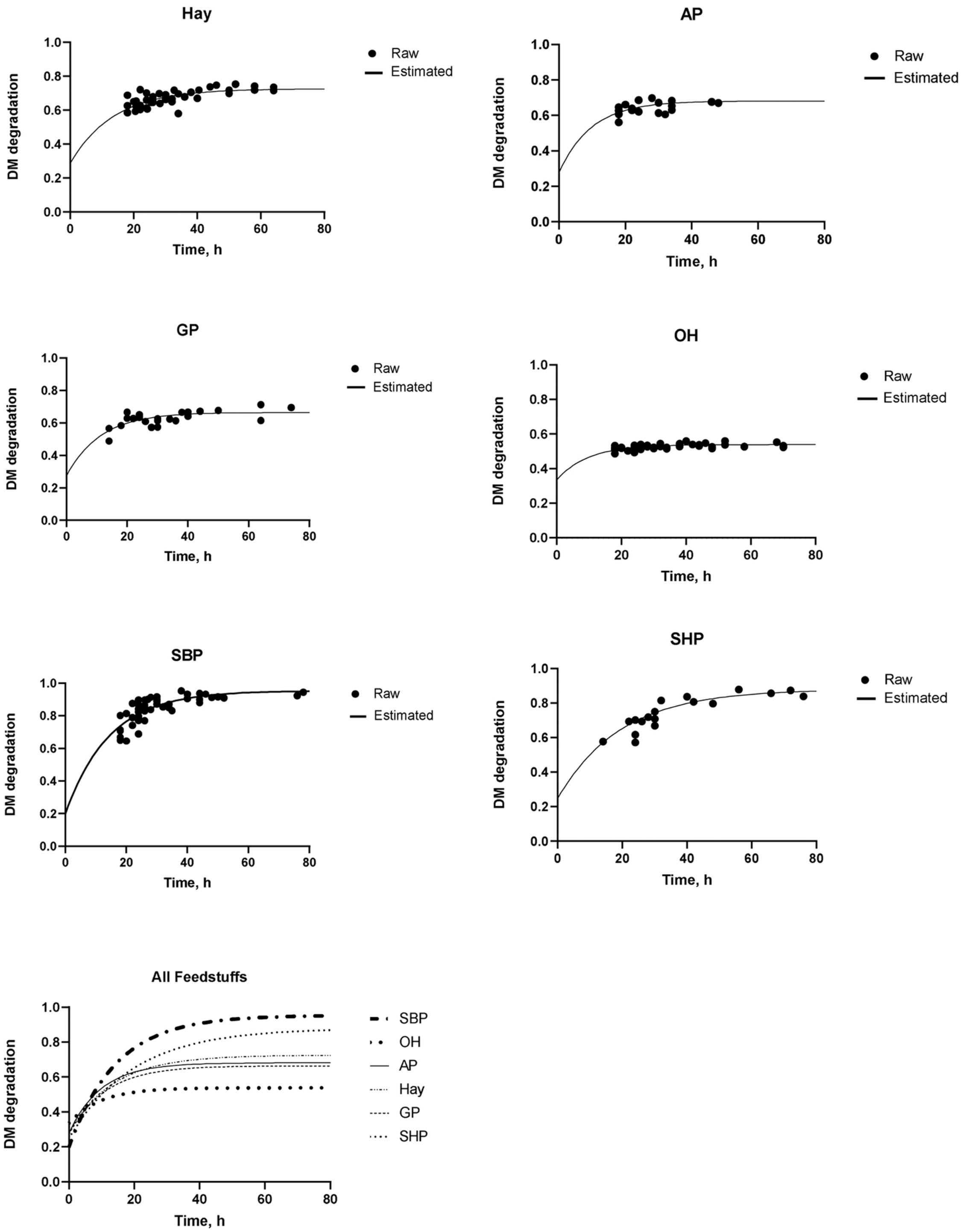

Fig. 4. Ørskov and McDonald (1979) degradation curves and raw data of dry matter (DM) for hay (mainly Timothy, first cut), alfalfa pellets (AP), grass pellets (GP), oat hulls (OH), sugar beet pulp (SBP) and soya hull pellets (SHP) based on mobile bags administrated into the caecum and collected in faeces.

the two analytical methods in a meaningful way, as different fractions are measured. Oat hulls are not only a fibrous feedstuff, as it contains a relatively high content of starch (181 g/kg DM), probably as a result of the dehulling process in which the endosperm may have been disrupted (Doehlert et al., 2010). The CP content varied between the feedstuffs, and depending on their inclusion level in the diet, this could affect the need for protein supplements to fulfil daily protein requirements. However, the nutrient composition of

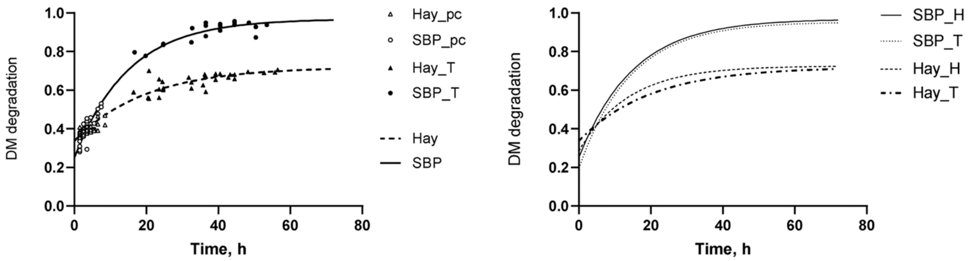

Fig. 5. Ørskov and McDonald (1979) degradation curves of dry matter (DM) for hay (mainly Timothy, first cut) and sugar beet pulp pellets (SBP) fitted to raw data from mobile bags administered in the stomach and found in caecum (pre-caecal, pc) or found in faeces (total tract, T) and administered in caecum and found in faeces (hindgut, H).

feedstuffs and diets cannot stand alone, as their digestibility might vary to a large extent, as discussed below.

6.2. Apparent total tract digestibility of the two diets

The DM ATTDs of the two diets were similar, and there was no difference in the ATTD of DF between the two diets. The daily intake of fibre (aNDF, ADF, hemicellulose and cellulose$_{NDF}$) was also similar for the two diets. However, the ATTD of aNDF, ADF, hemicellulose and cellulose$_{NDF}$ was higher in the HAY diet compared to the MIX diet. From this, the dS has decreased the ATTD of these fibre fractions in the MIX diet markedly. This can be explained by the lack of the S-NSP fraction in the aNDF analysis, as this fibre fraction was higher in the MIX diet compared to the HAY diet, with SBP and SHP especially contributing to this. The ATTD of CP was highest for the HAY compared to the MIX diet. A higher intake of CP in the diet is positively correlated with higher pre-caecal (Farley et al., 1995) and ATTD of CP (Farley et al., 1995; Oliveira et al., 2015). This can explain the higher ATTD of CP in the HAY diet, as the horses had a higher daily intake of CP. As expected, the ATTD of T-NSP was higher than the ATTD of DF for both diets, as T-NSP does not include the indigestible fraction of lignin. For the constituent sugars fructose, galactose, mannose and uronic acid, the ATTD was highest in the MIX diet, with SBP especially contributing with soluble uronic acid, in correspondence with findings by Jensen et al. (2014). The dominating constituent sugar in hay and OH was xylose. However, the daily intake of xylose was highest for the HAY diet, in correspondence with the higher ATTD of xylose in the HAY diet compared to the MIX diet. The considerably low ATTD of xylose for the dS indicates a low ATTD of xylose in some of the MIX diet's fibrous feedstuffs. As OH have a relatively high content of xylose, this might have decreased the ATTD of xylose in the MIX diet. Altogether, this confirms the hypothesis that hay can be substituted with other fibrous feedstuffs for horses at maintenance, but the differences in nutrient composition and ATTD of the two diets indicate differences in the ATTD of the individual feedstuffs, differences which cannot be identified in measuring ration ATTD.

6.3. Nutrient disappearance from control and mobile bags

The washing procedure for the bags has been discussed by several authors but has not yet been standardised (Dhanoa et al., 1999; Moore-Colyer et al., 2002). The procedure can affect the nutrient loss and rinsing of the residue in the mobile bags (Jarosz et al., 1994). In the present study, the DM loss varied from 0.181 to 0.299, which is in correspondence with earlier studies (Moore-Colyer et al., 2002; Thorringer and Jensen, 2021). The pre-caecal DM disappearance was highest in SBP compared to hay over time. This can partly be explained by a higher aNDF loss and, furthermore, a possibly higher loss of WSC and CP (not analysed). This was confirmed by Moore-Colyer et al. (2002) for pre-caecal CP disappearance for SBP and hay, with disappearances of 0.77 and 0.52, respectively. Moreover, the S-NSP fraction is higher in SBP than hay, and it might be easier for the fibre-utilising microbes in the stomach and small intestine to utilize the S-NSP (Bach Knudsen, 2001; de Fombelle et al., 2003). The same may be the case for the hindgut and total tract disappearance, as SBP had a higher DM loss than hay at all timepoints. An in-sacco study by Udèn and Van Soest (1984) found a positive correlation between incubation time in the caecum and DM disappearance for timothy hay. In theory, the increased incubation time or slower TT in the hindgut will allow microbes to penetrate the mesh and thereby have a longer time to degrade the fibre fraction of the feed. This is in correspondence with the increased hindgut disappearance of DM, aNDF and ADF with increased incubation or slower TT for all feedstuffs, despite large differences in overall nutrient disappearance between individual feedstuffs.

6.4. In-vivo ATTD and in-situ disappearance

The MBT has primally been used in horses to investigate the nutrient disappearance of starch-rich cereals (de Fombelle et al., 2004; Rosenfeld and Austbø, 2009; Philippeau et al., 2014). However, studies investigating fibrous feedstuffs by the MBT and, further, in comparison to the ATTD are scarce. An earlier study by Rodrigues et al. (2012) measured similar DM disappearance and ATTD when horses were fed coastcross hay. In the present study it was hypothesised that the MBT can be used to estimate the total ration nutrient digestibility as an alternative to the ATTD of the ration. It was measured that time had an effect when comparing the DM, aNDF and ADF ATTD and disappearance from the mobile bags, confirming an earlier study showing the same effect of time (Thorringer and Jensen, 2021). This time effect is important for future studies aiming to predict the ATTD by use of the MBT. Furthermore, present feed evaluation systems do not take this time effect into account. From the present study, the hypothesis is accepted when using time intervals 2 and 3 to represent the ATTD of DM, aNDF and ADF of the total ration of hay and the other fibrous feedstuffs.

6.5. Transit time of mobile bags and MRT of Yb

The passage rate of digesta is affected by several factors (Van Weyenberg et al., 2006). In the present study, the pre-caecal TT was 2.31 and 2.79 h for hay and SBP, respectively. These are shorter than measured in Moore-Colyer et al. (2002), with 3.27 and 4.22 h for hay cubes and unmolassed SBP, but the processing of the hay cubes (Drogoul et al., 2000), differences in chemical composition (Moore-Colyer et al., 2003) and the larger feed to surface area of the mobile bags used (Hyslop and Cuddeford, 1996) could have prolonged the TT. Further, it is unclear whether the ponies were fed before or after administration of the mobile bags into the stomach. In the present study, horses were fed after the administration of the mobile bags into the stomach, which might have affected the gastric emptying and pre-caecal TT of the bags (Lorenzo-Figueras et al., 2005). Hence, no difference was measured for the hindgut TT between the bags with hay and SBP. Surprisingly, SHP had a longer hindgut TT than all the other feedstuffs. This might be explained by the high aNDF content in SHP (Moore-Colyer et al., 2003) and, further, the possibility of a high water-binding capacity as a result of the high S-NSP content (Bach Knudsen, 2001; Brøkner et al., 2012). This finding contradicts the belief that I-NSP, which is high in SHP, primarily shortens the TT (Bach Knudsen, 2001). However, the water-binding capacity, together with swelling, may outweigh the effect of I-NSP. This can also explain the higher hindgut disappearance for both SHP and SBP, as S-NSP with especially pectin increases both swelling and water-binding capacity, increasing the surface area for microbes to degrade (Bach Knudsen, 2001). Further, the total tract TT of bags agreed with earlier studies (Thorringer and Jensen, 2021). In the present study, Yb was the marker chosen for the determination of the diet's MRT in the hindgut. This was based on earlier studies, and the fact that Yb follows the particle part of digesta (Drogoul et al., 2000; Van Weyenberg et al., 2006) as the objective was to evaluate the passage of the fibrous feedstuffs. The MRT of the HAY diet agrees with earlier studies using Yb as a marker for the total tract MRT (Moore-Colyer et al., 2003; Jensen et al., 2014). However, the MRT for the MIX diet was 2 h longer than for the HAY diet. This can be explained by several factors, but most likely the higher S-NSP content in the MIX diet prolonged the MRT as a result of increased water-binding capacity and swelling of the feedstuffs (Bach Knudsen, 2001; Brøkner et al., 2012). Furthermore, the higher water intake measured with the HAY diet has earlier been associated with a shorter MRT (Pagan et al., 1998). Jensen et al. (2014) substituted hay (18.5 g DM/kg BW) partly with molassed SBP (14.7 and 2.6 g DM/kg BW hay and SBP, respectively) but did not measure any difference in MRT in the total tract. However, the DM intake was higher than in the present study, and furthermore, the horses had a higher DM intake (g DM/kg BW per day) with the hay diet compared to the hay substituted with molassed SBP, which may have outweighed the effect of the swelling and water-binding capacity of the SBP. Finally, the particle size of the feedstuffs might have affected the MRT, as reported by Drogoul et al. (2000), where the MRT was longer on ground-pelleted hay compared to chopped hay.

6.6. Dry matter degradation curves

From the MBT data, both the rate and the extent of feed degradation can be estimated by use of the models provided by Ørskov and McDonald (1979). An advantage is that the ED values can be estimated by taking the passage rate of digesta into account and thereby provide information valid to compare against the ATTD and MRT of the diets. In the present study, the DM degradation curves, and ED values were estimated on data from the control bags and from mobile bags recovered in faeces after administration into the caecum for all six feedstuffs. The fitted DM degradation curves agreed well with the raw data from the mobile bags. However, the estimated potential degradability a+b was higher for hay when comparing with the DM ATTD of the HAY diet, as expected. The parameter a (the soluble part of the feed) was in correspondence with the DM loss from the control bags of hay; hence, the insoluble but potentially degradable part b is higher than that measured with ATTD. This can be related to more bags found after 40 h, representing a higher DM disappearance than the ATTD. The ED fits to the ATTD of the HAY diet when the outflow rate is between 0.025% and 0.020% per h, corresponding to an MRT of 40–50 h. This MRT represents the TT of the solid digesta (Clauss et al., 2014; Jensen et al., 2014; Hummel et al., 2017). However, the predicted MRT for ED does not represent the MRT for the HAY diet, which was 23.6 h. This has been discussed earlier by Thorringer and Jensen (2021), who found that the ED corresponds to the DM ATTD of a hay diet when MRT was 60 h and argue that the MBT only covers a narrow range of the TT, resulting in an underestimation of ED when biologically relevant MRT is used in the calculations. The in-sacco method with fixed incubation times (e.g., in the caecum) could be used to measure the early timepoints lacking, as discussed by Thorringer and Jensen (2021). Therefore, the same conclusion can be drawn, as in Thorringer and Jensen (2021), that the ED is not an appropriate measure of feed degradation when using mobile bags. However, the DM ATTD of the HAY diet agreed well with the D_t of 20 h, which furthermore fits better with the MRT of the HAY diet. Therefore, a more appropriate estimate is given when using D_t than ED for calculation of degradability based on the MBT. Besides the DM degradation curves fitted to mobile bags administrated in the hindgut and recovered in faeces, DM degradation curves were also fitted to the data from the control bags and bags recovered in the faeces after administration into the stomach for hay and SBP. The estimated potential degradability a+b agrees with the a+b estimated from the bags placed in the caecum and recovered in faeces. Furthermore, the DM curves followed the DM curves from bags placed in the caecum and recovered in faeces. This indicates not only that the degradation parameters are valid but also suggests the use of the mobile bags in intact horses for more detailed evaluation of individual feedstuffs.

7. Conclusion

From this study, it can be concluded that hay can be substituted with other fibrous feedstuffs to fulfil the daily minimum dry matter recommendations. The mobile bag technique can be used to directly predict the total ration digestibility and apparent total tract digestibility of dry matter, neutral detergent fibre and acid detergent fibre when using bags found between 20 and 39 h after administration, as the disappearance from the mobile bags otherwise will either be lower or higher than the apparent total tract digestibility. Furthermore, the degradation (D_t) is useful to estimate the apparent total tract digestibility of dry matter with biologically relevant mean retention times. In general, the dietary fibre analysis provided a comprehensive description of the fibrous feedstuffs used. Combining dietary fibre analysis, physiochemical properties and the apparent total tract digestibility of feedstuffs provides important information when planning diets for horses with different energy requirements. Overall, this study demonstrated that the mobile bag technique potentially can be used in intact horses for estimating the apparent total tract digestibility of individual feedstuffs of a mixed diet, and the method allows for more detailed feedstuff evaluation in horses than total collection measurements.

Ethics statement

The experimental design and procedures in this study were in accordance with Norwegian legislation and ethical guidelines.

Software and data repository resources

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

CRediT authorship contribution statement

Nana Wentzel Thorringer: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Project administration. **Martin Riis Weisbjerg:** Writing – review & editing, Supervision. **Rasmus Bovbjerg Jensen:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of interest

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

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