Norwegian University of Life Sciences
Faculty of Biosciences
Department of Animal and Aquacultural Sciences

Philosophiae Doctor (PhD)
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Carbohydrate digestion in horses

Methods, processing, and future perspectives

Karbohydratfordøyelse hos hester

Metoder, bearbeiding og fremtidsperspektiver

Nana Helena Wentzel Thorringer

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List of papers

This thesis is based on the following papers:

Paper I.

N.W. Thorringer and R.B. Jensen. 2021.
Methodical considerations when estimating nutrient digestibility in horses using the mobile bag technique. Animal, 15(1), 100050. DOI: https://doi.org/10.1016/j.animal.2020.100050, **Published.**

Paper II.

N.W. Thorringer, M.R. Weisbjerg, and R.B. Jensen. 2022.
Mobile bag technique for estimation of nutrient digestibility when hay is supplemented with alternative fibrous feedstuffs in horses. Animal Feed Science and Technology, Available online 27 November 2021, 115168. DOI: https://doi.org/10.1016/j.anifeedsci.2021.115168, **Published.**

Paper III.

N.W. Thorringer, M.R. Weisbjerg, and R.B. Jensen. 2020.
The effects of processing barley and maize on metabolic and digestive responses in horses. Journal of Animal Science, 98(12), 1-11. DOI: https://doi.org/10.1093/jas/skaa353, **Published.**

Summary

Understanding the digestion of nutrients in different segments of the gastro-intestinal tract of horses is important for correct feed evaluation. This thesis aimed to examine starch and fibre digestion and fermentation in various segments of the equine gastrointestinal tract using different methods. First, it was verified whether the disappearance of nutrients obtained by the mobile bag technique (MBT) could predict the apparent total tract digestibility (ATTD) from the total faeces collection (TFC) method when horses were fed hay-only or hay and fibrous feedstuffs. Results on dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) showed that disappearances from the MBT can be used to predict the ATTD. Second, digestion kinetics were modelled using degradation data from individual mobile bags and advanced degradation models. No differences were measured between the ATTD of DM and degradation to time t (Dt) from mobile bags placed in the stomach or hindgut and recovered in faeces when biological relevant mean retention times (MRT) were used in the model. Finally, metabolic, and digestive responses to a meal of processed (toasting or micronizing) barley or maize fed at 1 g starch/kg body weight (BW) showed no consistency in either plasma glucose or insulin responses. This was despite greater pre-caecal starch digestion measured with the MBT of micronized maize (MM) compared to micronized barley (MB), toasted maize (TM), or barley (TB). Changes in digestive responses in the caecum were measured with increased total short-chain fatty acid (SCFA) concentration with a corresponding decrease in pH in the caecum 3 h after feeding the processed grains. This indicated that some starch by-passed the enzymatical digestion in the small intestine and was instead fermented in the hindgut. Overall, this thesis shows that the MBT can predict the ATTD of individual feedstuffs and of the total ration. However, it can be challenging to study starch digestion using only one method, thus a comprehensive approach is required when investigating starch digestion in horses. Further studies using the in-sacco method to predict early fibre degradation would therefore be of interest. Moreover, studies are required to establish a comprehensive overview of different processing methods, including in-depth processing details and their effects on enzymatical starch digestion of various grains. The new knowledge generated in this thesis is important for further development and standardization of feed evaluation systems for horses.

Keywords: degradation kinetics; equine; fibre; in-situ; pH; plasma glucose; plasma insulin, short-chain fatty acids; starch; total faeces collection.

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Sammendrag

Å forstå fordøyelsen av næringsstoffer i ulike segmenter av mage-tarmkanalen til hester er viktig for riktig fôrvurdering. Denne avhandlingen undersøkte stivelse- og fiberfordøyelse og fermentasjon i ulike segmenter av hestens mage-tarmkanal ved hjelp av forskjellige metoder. Først, ved å verifisere om forsvinningen av næringsstoff oppnådd ved mobil-pose-teknikken (MBT), kan forutsi den tilsynelatende totale fordøyeligheten i fordøyelsessystemet (ATTD) fra metoden total oppsamling av avføring (TFC) når hester fôres med høy eller fibrøst fôr. Resultater for tørrstoff (DM), organisk materiale (OM), nøytral løseligfiber (NDF) og syreløselig fiber (ADF) viste at forsvinning fra MBT kan forutsi ATTD. Videre ble fordøyelseskinetikk modellert ved å bruke nedbrytningsdata fra individuelle mobile poser og avanserte nedbrytningsmodeller. Ingen forskjeller ble målt mellom ATTD for DM og degradering til tid t (Dt) fra mobile poser plassert i magen eller blindtarmen og samlet opp i avføringen når biologisk relevante gjennomsnittlige retensjonstider (MRT) ble brukt i modellen. Til slutt viste responsene i metabolisme og fordøyelighet i et måltid med bearbeidet (ristet og mikronisert) bygg og mais med 1 g stivelse/kg kroppsvekt (BW) ingen sammenhengende effekt verken i plasmaglukose- eller plasmainsulinrespons, til tross for høyere stivelsesfordøyelighet i mage- og tynntarm for mikronisert mais (MM) sammenlignet med mikronisert bygg (MB), og ristet mais (TM) eller bygg (TB). I tillegg ble endringer i fordøyelsesresponser i blindtarmen målt ved økt total kortkjedet fettsyrekonsentrasjon med tilsvarende reduksjon i pH i blindtarmen 3 timer etter fôring av de bearbeidede kornene. Dette indikerer at noe stivelse passerte den enzymatiske fordøyelsen i tynntarmen og i stedet ble fermentert i tykktarmen. Samlet sett viser denne avhandlingen at MBT kan forutsi ATTD fra individuelle fôrmidler til en totalrasjon. Dessuten kan det være vanskelig at studere stivelsesfordøyelighet ved hjelp av kun en metode. Derfor kreves en omfattende undersøkelse for at evaluere stivelsesfordøyelighet hos hester. Ytterligere studier som bruker in-sacco metoden for at forutsi tidlig fibernedbrytning er av interesse, dessuten er ytterligere studier nødvendig for å etablere en omfattende oversikt over ulike prosesseringsmetoder med dyptgående prosessdetaljer og deres effekt på ulike korns enzymatiske stivelsesnedbrytning. Den nye kunnskapen skapt i avhandlingen er viktig for videreutvikling og standardisering av fôrvurderingssystem for hester.

Søkeord: nedbrytningskinetikk; Hest; fiber; i-pose; pH; plasma glukose; plasma insulin; kortkjedede fettsyrer; stivelse; total avførings oppsamling.

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Abbreviations

a	Soluble fraction of a feed
ADF	Acid detergent fibre
ADL	Acid detergent lignin
ATTD	Apparent total tract digestibility
AUC	Area under the curve
b	Potential degradable fraction
BW	Body weight
c	Rate constant for degradation of *b*
CFU	Colony-forming units
dD	Diet digestibility
DE	Digestible energy
DF	Dietary fibre
dH	Digestibility of forage
DG	Degree of gelatinization
DM	Dry matter
dOM_{horse}	Digestible organic matter for horses
dS	Digestibility coefficient of a supplement
Dt	Degradation after time
e	Exponential
ED	Effective degradability
EDTA	Ethylenediaminetetraacetic acid
EMS	Equine metabolic syndrome
FSA	Feed to surface area
FU	Feed units
GI	Glycaemic index
GIT	Gastrointestinal tract
h	Fraction of forage in a diet
IR	Infrared radiation
I-NSP	Insoluble non-starch polysaccharides
k	Outflow rate of digesta
MB	Micronized barley
MBT	Mobile bag technique
ME	Metabolizable energy

Mi	Concentration of marker excreted at time *Mi* as a proportion of total marker excreted
MM	Micronized maize
MRT	Mean retention time
MSBP	Molassed sugar beet pulp
N	Nitrogen
NDF	Neutral detergent fibre
NE	Net energy
NE_m	Net energy at maintenance
NSC	Non-structural carbohydrates
NSP	Non-starch polysaccharides
OM	Organic matter
s	Fraction of supplement in a diet
SBP	Sugar beet pulp
SCFA	Short-chain fatty acid
SD	Standard deviation of mean
SEM	Standard error of mean
S-NSP	Soluble non-starch polysaccharides
t	Time
ti	Time elapse between administration of marker and midpoint of marker excretion at *i*th time interval
TB	Toasted barley
TFC	Total faeces collection
TM	Toasted maize
TMRT	Total tract mean retention time
T-NSP	Total non-starch polysaccharides
TT	Transit time
WSC	Water-soluble carbohydrates
Yb	Ytterbium

1 Introduction

Horses are herbivores eating plants consisting of primarily carbohydrates (~80% of DM) as their main energy source (Bach Knudsen, 2001; Hoffman, 2013). In the following section carbohydrates will be classified.

1.1 Carbohydrate classification

Depending on perspective, carbohydrates can be classified in different ways. From an equine digestive point of view, carbohydrates are divided into hydrolysable and fermentable carbohydrates (Hoffman, 2013, Figure 1). Hydrolysable carbohydrates are hydrolysed to simple sugars by enzymes in the small intestine. Fermentable carbohydrates cannot be enzymatically digested and are instead fermented to short-chain fatty acids (SCFA) by microorganisms, a process that takes place primarily in the hindgut. Hydrolysable carbohydrates, also called non-structural carbohydrates (NSC), can be divided into three sub-groups: resistant starch (resistant to small intestine enzyme hydrolysis due to the physical or chemical structure), starch, and water-soluble carbohydrates (WSC). While fructans are part of the WSC, they are polymers of oligo- and polyfructosyl sucrose linked by β-2.1 or β-2.6 glycosidic bonds resistant toward enzymatical digestion, and are therefore classified as fermentable carbohydrates (Hoffman, 2013). Fermentable carbohydrates, also called non-starch polysaccharides (NSP), can be divided into soluble and insoluble NSP (S-NSP and I-NSP, respectively). In the following section the primary focus will be on starch and NSP.

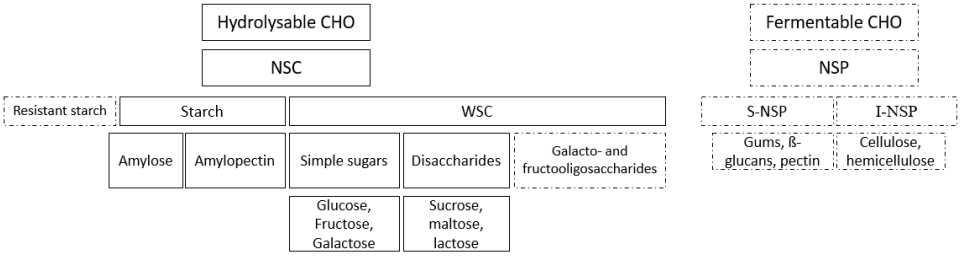

Figure 1. Classification of hydrolysable (solid line) and fermentable carbohydrates (dashed line) (CHO) modified from Hoffman et al. (2001). Non-structural carbohydrates (NSC) are divided into three subgroups: starch, resistant starch, and water-soluble carbohydrates (WSC). Non-starch polysaccharides (NSP) are divided into soluble and insoluble NSP (S-NSP and I-NSP, respectively).

1.2 Starch

Starch is a polysaccharide containing glucose units linked by glycosidic bonds (Hoffman, 2013). It consists of linear α-1.4 amylose and the more branched molecule α-1.6

amylopectin (Figure 2). Starch is the main storage carbohydrate in grains, and it is accumulated in granules of the endosperm deposited in layers with various amylose and amylopectin content (Svihus et al., 2005; Hoffman, 2013). The ratio between amylose and amylopectin defines whether grain starch is categorized as "normal," "waxy," or "high amylose." The "normal" grain starch consist of approximately 25% amylose, whereas "waxy" starch may have little or no amylose (<10%) and "high" amylose starch may contain up to 70% (Svihus et al., 2005; Cowieson et al., 2019). The "waxy" gene is mainly found in barley and maize, which contain approximately 75% and 99% amylopectin, respectively (Svihus et al., 2005; Cowieson et al., 2019).

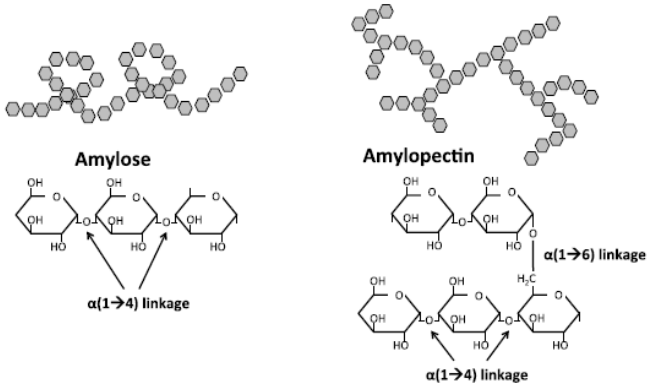

Figure 2. Illustration of the chemical structure of amylose and amylopectin obtained from Romano and Kumar (2019).

1.2.1 Starch digestion

In horses, starch is already exposed to α-amylase in the mouth (Hoffman, 2013). However, the amount of α-amylase in saliva is limited in horses (8-108 IU/L) (Fuentes-Rubio et al., 2015) in comparison to the saliva of pigs (265-7,060 IU/L) (Fuentes et al., 2011), and the retention of feed in the mouth is short, hence negligible when estimating starch digestibility. Studies have found indications of starch degradation in the stomach assuming microbial fermentation in the non-glandular part of the stomach (de Fombelle et al., 2003; Varloud et al., 2004, 2007; Coenen et al., 2006). This assumption is built on the lack of α-amylase in the stomach, and the only way for α-amylase to enter the stomach is by duodenal reflux when horses are fasted (Murray and Schusser, 1993). Varloud et al. (2004) measured the apparent digestibility of "starch and sugar" by use of indigestible markers in the stomach with a digestibility varying from 41-76%. However, the gastric degradation of starch and sugar is still a less investigated area of starch digestion.

The major enzymatic digestion of starch occurs in the small intestine. Here, the enzymes α-amylase and amylopectinase are released from the pancreas into the duodenum via the

pancreatic duct cleaving the α-1.4 and α-1.6 linkages (Merritt and Julliand, 2013). The free glucose units are transported into the bloodstream (Argenzio, 1993; Dyer et al., 2002). Meyer et al. (1995) found the enzymatical starch digestion in the small intestine to vary to a large extent when horses were fed different grains with approximately 2 g starch/kg BW/meal (Table 1). Due to low α-amylase secretion in horses (0-7 U/g mucosa/min vs. 0-200 U/g mucosa/min in pigs, Roberts, 1974) only limited amounts of starch can be digested in the small intestine, with remaining undigested parts passing to the hindgut.

1.2.2 Starch fermentation

Starch that escapes digestion in the small intestine is fermented to lactate and SCFA in the hindgut by starch-utilizing microbes (Argenzio et al., 1974; Hintz et al., 1971a; Julliand et al., 2001; Medina et al., 2002). This results in a greater ratio of propionate at the expense of acetate compared to a forage diet (Hintz et al., 1971a; Julliand et al., 2001; Medina et al., 2002). This should be avoided as disturbance of the microbiota can lead to colic (Hudson et al., 2001), colitis (Costa et al., 2012), laminitis (Garner et al., 1975) and in severe cases inflammatory bowel disease (Kalck, 2009). Further, the altered microbiota adapted for starch degradation can potentially impair fibre fermentation (Julliand et al., 2006).

Starch-utilizing bacteria (*Lactobacilli and Streptococci spp*.) and lactate-utilizing bacteria are present in larger concentrations in the stomach (8.1, 7.4, and 7.2 log10 CFU/ml, respectively) than cellulolytic bacteria (1.2 log10 CFU/ml) (de Fombelle et al., 2003). Similarly, presence of *Lactobacilli* (6.5 log10 CFU/ml), and concentrations of lactate (1 mmol/l) and total SCFA (7.3 mmol/l) in the stomach 2 h after feeding a morning meal of pelleted grains (1 g starch + sugar/kg BW) were observed (Varloud et al., 2007). Additionally, Varloud et al. (2004) reported more than 40% starch and sugar digestibility in the stomach independent of diet. Furthermore, horses fed a starch-rich diet (4.5 g/kg BW/day) had greater concentrations of SCFA and propionate in the stomach (9.4 and 1.4 mmol/l higher, respectively) and small intestine (average 4 mmol/l greater) than horses fed a diet of 2.2 g starch/kg BW/day (de Fombelle et al., 2003). Altogether, this reveals that some starch and sugars are fermented pre-caecally, but the extent of this fermentation still needs investigation. Therefore, fermentation of starch is considered more important compared to fibre fermentation pre-caecally (see 1.4.2 Fibre fermentation). Collectively, the hydrolysis and fermentation in the stomach, the enzymatic digestion in the small intestine, and the fermentation in the hindgut, cause starch (and WSC) to be almost completely digested when estimated by apparent total tract digestibility (ATTD) (Wolter et al., 1982; McLean et al., 1999a; Jensen et al., 2014).

1.2.3 Factors affecting pre-caecal starch digestion

As earlier reviewed by Kienzle (1994) and Julliand et al. (2006), several factors affect pre-caecal starch digestibility in horses, as illustrated in Figure 3. These will be covered individually through the section; however, additive effects and interactions are present between factors.

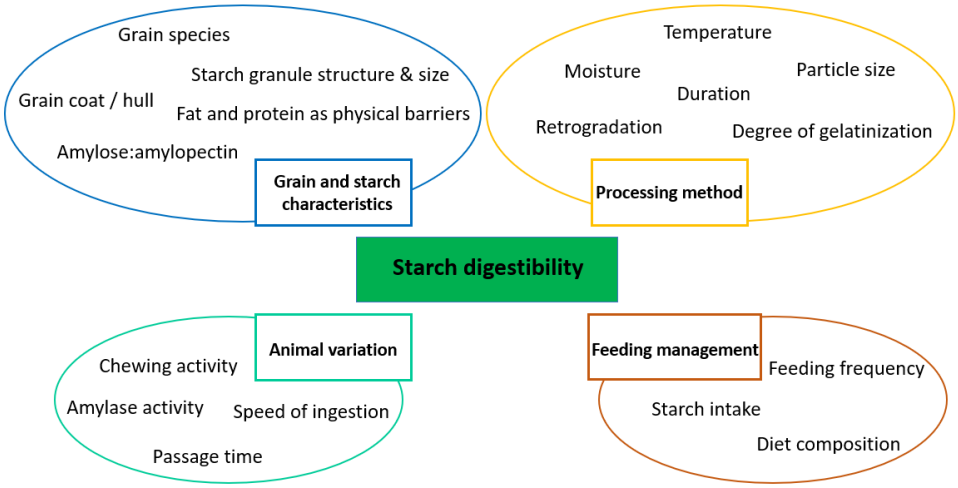

Figure 3. Factors affecting pre-caecal starch digestibility in horses.

1.2.3.1 Feeding management

Feeding management, with a focus on starch intake (g/kg BW/meal) and its effect on pre-caecal starch digestion, has been investigated over the last 50 years (Hintz et al., 1971a; Householder et al., 1979). In general, the greater starch intake the lower pre-caecal starch digestibility (Figure 4). To avoid starch by-passing to the hindgut, an early study by Potter et al. (1992) suggested a maximum of 3.5-4 g starch/kg BW/meal. Reviewing the literature, Kienzle (1994) suggested a maximum of 2 g starch/kg BW/meal could be fed to avoid by-pass starch. This was supported by Julliand et al. (2006).

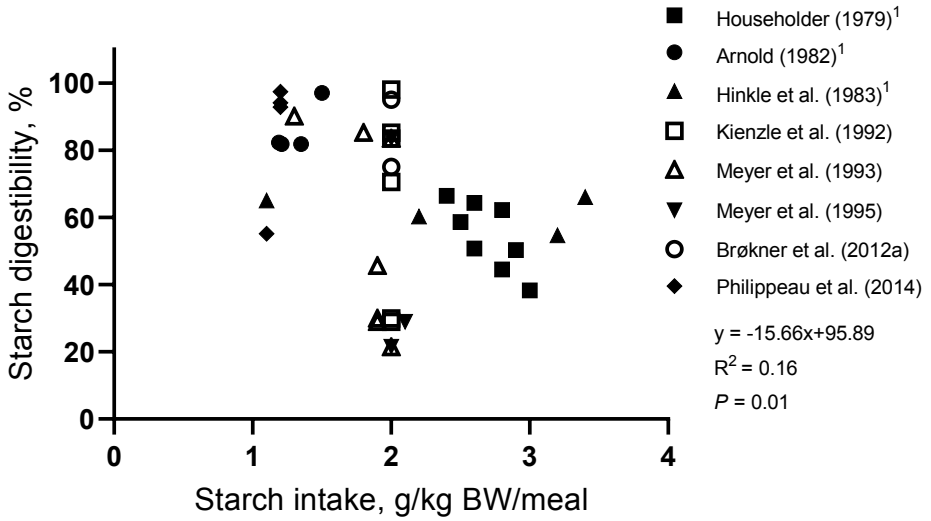

Figure 4. Pre-caecal starch digestibility (%) as a response to starch intake (g/kg body weight (BW)/meal). [1]Cited in Potter et al. (1992).

A high starch intake is related to prolonged gastric emptying (~1 h longer, Métayer et al., 2004) and, longer pre-caecal (~1 h longer) (de Fombelle et al., 2004) and total tract mean retention time (TMRT, up to 12 h longer) (Drogoul et al., 2001; Jensen et al., 2014) in relation to a high forage intake. In contrast, increased feeding frequency shortens the pre-caecal MRT (de Fombelle et al., 2004). Longer transit time (TT) for mobile bags is reported to increase pre-caecal starch disappearance (Hymøller et al., 2012) and starch disappearance in the caecum (McLean et al., 1999bc). In theory, a longer MRT can be associated with greater starch digestibility, as digesta are exposed to enzymes for a longer time.

1.2.3.2 Animal variation

Variation between individual animals is often recorded in digestibility experiments with horses (Kienzle et al., 1997; Ragnarsson and Lindberg, 2010; Jensen et al., 2012). The variation may be because of anatomic differences in gastro-intestinal size and divergences in eating behaviour (Meyer et al., 1995). These factors may interfere with the digestibility of starch. Eating behaviour involves both the chewing activity and the speed of ingestion. This influences the particle size in the jejunal chyme (Kienzle et al., 1997), as a hasty eater may not chew sufficiently. Further, amylase activity in the jejunum chyme was greater when horses were fed grains (barley, maize, or oats) compared to a hay-only diet (31 and 15 U/g chyme, respectively; Kienzle et al., 1994). Additionally, amylase activity in the chyme was reported to vary between animals (Kienzle et al., 1994).

1.2.3.3 Grain characteristics

Studies have compared the pre-caecal starch digestibility of barley, maize, and oats. Among these, oat starch was found to have greater digestibility than barley and maize (Table 1). Svihus et al. (2005) concluded that a high ratio of amylose to amylopectin is negatively correlated with starch digestibility. This may be explained by the crystalline regions being easier to solubilize than the amorphous regions (mainly amylose). The size of the starch granules may also affect digestibility, as small granules have a larger surface area in relation to volume than large ones, and thus a larger area for enzymes to approach. This is the case for oat starch, which has small granules compared to maize starch (Svihus et al., 2005).

Table 1. Pre-caecal starch digestibility (%) of barley, maize, and oats determined by the marker method or the mobile bag technique (MBT).

Reference	Starch intake[1]	Method	Place of digestion	Digestibility		
				Barley	Maize	Oats
Arnold et al. (1981)[2]	?	Marker[3]	Pre-caecal		80	95
Meyer et al. (1995)[4]	2	Marker[3]	Pre-ileal	22	29	84
Moore-Colyer et al. (2006)[5]	?[6]	MBT	Pre-caecal	82	73	99
de Fombelle et al. (2004)[5]	0.8-1.5	MBT	Pre-caecal	86	88	100
	1.5-2.8	MBT		87	91	100
Brøkner et al. (2012a)[7]	2[8]	MBT	Pre-caecal	75		98
Rosenfeld and Austbø (2009)[9]	0.5-1	MBT	Pre-caecal	71	66	95

[1] g/kg body weight/meal.

[2] Cracked grains.

[3] Chromium oxide.

[4] Crushed barley, whole maize, and oats.

[5] Grains ground to 3 mm screen size when used in mobile bags.

[6] 350 g pelleted feed/day (unknown starch intake).

[7] Grains ground to 1 mm screen size when used in mobile bags.

[8] Morning meal for a hay+barley+sugar beet pulp (SBP) or hay+oats+SBP diet.

[9] Digestibility of the grains are provided in averages of different processing methods (ground, pelleted, extruded, and micronized) and moreover ground to 1 mm screen size when used in mobile bags.

Starch can interact with both lipids and protein in the grain matrix. This may impair starch digestibility directly and indirectly by reducing the contact between starch and enzymes and reducing swelling of the starch granule (McAllister et al., 1993; Svihus et al., 2005). Lipids are more associated with amylose than amylopectin (Morrison et al., 1984; Baldwin et al., 1997) and this can lead to less starch being gelatinized during feed processing.

1.2.3.4 Processing of grains

Grains used for horses can be subjected to mechanical, thermal, or thermo-mechanical processing methods in either wet or dry conditions (Julliand et al., 2006) (Table 2). The main aims for processing grains are to increase the nutritional value by increasing nutrient digestibility, and to improve the hygienic quality (Kienzle, 1994; Hill, 2007). Some common processing methods for equine feedstuffs are thermal micronizing, flaking, or pelleting (Table 2) and mechanical rolling or grinding. Mechanical processing methods can break the connections between starch granules, which decreases the particle size of the grain. This increases the surface area in relation to the volume of particles and thereby renders them easier for enzymes to digest (Kienzle, 1994; Meyer et al., 1995). This corroborates with results reported by Philippeau et al. (2014), who measured a greater pre-caecal starch digestibility with ground barley compared to untreated barley (Table 3). Similar effects were found for untreated and ground maize (Meyer et al., 1995).

Table 2. Characteristics (temperature (temp., ˚C), duration (seconds, sec) and moisture content (%)) of different processing methods (Van der Poel, 1990; Svihus et al., 2005; Julliand et al., 2006; Newton, 2020).

Process method	Type of processing	Processing details			Heat source
		Temp.	Duration	Moisture	
Thermal	Roasting	90-190	40-60	-/+	IR[1]
	Micronizing	80-130	40-60	18-21	IR
	Extrusion	80-200	30-150	+	Steam
Thermo-mechanical	Expander	80-140	1-15	10-20	Steam
	Toasting[2]	100-140	60-300	+	Steam
	Toasting[3]	90-105	1800-2700	+	Steam
	Flaking	90-95	600-1200	?	Steam
	Pelleting	60-95	70-250/25-35	+	Steam

[1] IR, infrared radiation.

[2] Pressure toasting.

[3] Conventional toasting.

Thermal processing methods can include both heat and pressure, and grains are often pre-treated with moisture to increase the effect. Thermal-mechanical processing methods can include mechanical treatment, moisture, heat, and pressure either all together or separately, depending on processing method. Thermal and thermal-mechanical processing methods increase the degree of gelatinization (DG) of starch. Gelatinization is a process by which the internal structure of the starch granule is broken (Figure 5, Donald, 2001). It is an initial swelling process that increases the interface between crystalline regions (mainly amylopectin) and amorphous regions (mainly amylose). During gelatinisation, viscosity increases as starch granules swell and additionally gels of solubilized amylose are produced (Hermansson and Kidman, 1995; Svihus et al., 2005; del Carmen Robles-Ramirez et al., 2012). This can increase the

susceptibility of amylose and increase starch digestibility. The DG depends on moisture content, temperature (starts around 50-70 ˚C), and duration (Julliand et al., 2006; Liu et al., 2019). Further, the size of the starch granules affects the DG. Large granules have a greater gelatinization enthalpy (sum of energy in J/g starch) than small granules (Svihus et al., 2005; Zhu, 2017), indicating that large granules are easier to gelatinise than small. As mentioned (in 1.2.3.3 Grain characteristics), amylose-lipid complexes can possibly lead to less starch gelatinized during processing (Svihus et al., 2005) and the possibility of increased resistant starch formation (Russell et al., 1989; Sievert and Pomeranz, 1989) both reducing pre-caecal starch digestibility (Asp and Björck, 1989; Åkerberg et al., 1998). Therefore, a linear relationship between DG and pre-caecal starch digestibility might not be unambiguous.

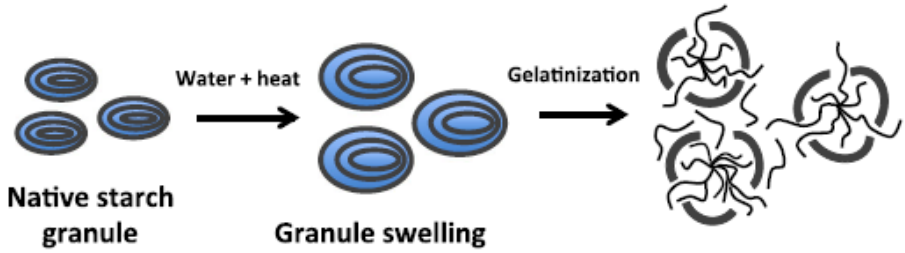

Figure 5. Illustration of structural modifications of starch granules during the gelatinization process obtained from Romano and Kumar (2019).

Table 3. The effect of different processing methods on starch digestibility (%) in various segments of the equine gastrointestinal tract (GIT) measured by in-vivo (marker and total faeces collection, TFC) and in-situ (the mobile bag technique, MBT) techniques.

Reference	Starch intake[1]	Feed	Processing	Temp. (˚C)	Duration (sec)	Moisture (%)	Screen size (mm)	Method	GIT	Digestibility
Meyer et al. (1995)	2	Barley	Crushed		?		?	Marker[2]	Pre-ileal	22
	2.1	Maize	Whole							29
	2.2		Crushed		?		≥1.4[3]			30
	2.2		Ground		?		≤1			47
	2	Oat	Whole							84
	1.8		Rolled		?		?			85
Rosenfeld and Austbø (2009)	0.3[4]	Barley, maize, and oats	Ground		?		1	MBT	Pre-caecal	72
			Micronized	100	45	?	?			85
			Extruded	130	?	?	?			70
			Pelleted	82-85	?	?	?			82
Philippeau et al. (2014)	2.7[5]	Barley	Whole				2.5[6]	MBT	Pre-caecal	55
			Ground		?		4			97
			Steam flaked	?	?	?	4			94
			Pelleted	?	?	?	4			93
McLean et al. (1999a)	2.1	Barley	Rolled		?		?	TFC	Total tract	97
	2.1		Micronized	?	?	?	?			97
	2.1		Extruded	?	?	?	?			97

[1] g/kg body weight (BW)/meal, unless otherwise stated.

[2] Chromic oxide.

[3] Approximately 87% ≥1.4 mm and approximately 98% ≤1 mm.

[4] Calculated average starch intakes (g/kg BW/meal) based on average starch content of processed barley, maize, and oats and average BW of 509 kg.

[5] Morning meal.

[6] Screen size of the feedstuff in the mobile bag.

Literature is, however, inconclusive in terms of the impact that individual processing methods have on DG. Vervuert al. (2007) and Philippeau et al. (2014) measured no difference in DG for ground and whole barley (Figure 6). However, thermo-mechanical processing methods resulted in greater DG (Vervuert et al., 2007; Philippeau et al., 2014), with the greatest DG reported for popped barley (Vervuert et al., 2007). Similar was reported for thermo-mechanically processed maize (Vervuert et al., 2004) and oats (Vervuert et al., 2003). However, steaming oats did not increase the DG compared to whole oats (Vervuert et al., 2003). At unfavourable processing conditions for gelatinization (e.g., temperature >100˚C at an insufficient water-condition) the content of resistant starch increases (Russell et al., 1989; Sievert and Pomeranz, 1989). Thus, resistant starch escapes to the hindgut where it has dietary fibre-like functions resulting in production of SCFA (Åkerberg et al., 1998). This indicates that the individual processing conditions (temperature, duration, and moisture content) can differ thus affecting DG and site of digestion in the gastrointestinal tract (GIT).

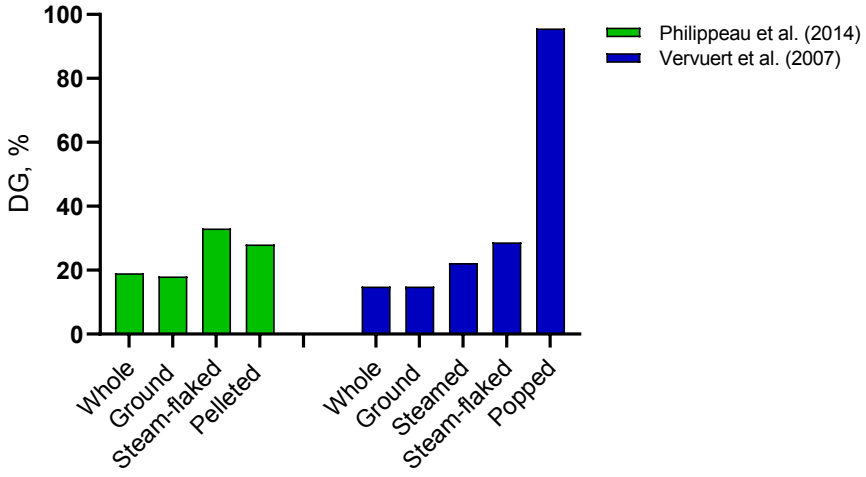

Figure 6. The degree of gelatinization (DG, %) for whole barley and barley having undergone different processing methods.

1.3 Glucose and insulin regulation

Insulin is the main regulator for blood glucose concentration in horses (Geor, 2013). The primary stimulus for insulin secretion is glucose, however it can also be induced by the amino acids' arginine and leucine (Geor, 2013). Insulin is first secreted in an initial rapid phase and thereafter released less intensely when a stimulus is given (Wilcox, 2005). In horses, basal serum glucose is measured to approximately 5 mmol/l (Vervuert et al., 2008, 2009ab; Lindåse et al., 2018) and approximately 5 µU/ml for insulin (Vervuert et al., 2009ab).

1.3.1 Glycaemic and insulinaemic response

After a meal both plasma glucose and insulin will increase (Healy et al., 1995; Vervuert et al., 2004, 2007, 2008). Therefore, glycaemic and insulinaemic responses are measures of changes in plasma glucose and insulin to a given feedstuff or meal. Both can be measured by glucose and insulin peaks, time to reach peaks, and area under the curve (AUC) for plasma glucose and insulin concentrations over time (Harris and Geor, 2009). These measures can be used to evaluate if starch is rapidly digested (high and fast peaks) and to what extent, as a large AUC indicates higher digestibility than a small AUC. Another approach to measure the glycaemic response after a meal is the glycaemic index (GI). Originally it was developed for human foods to determine the potential of a given carbohydrate source to increase plasma glucose concentrations (Granfeldt et al., 2006; Harris and Geor, 2009). Mainly, it was used to formulate diets with low glycaemic impact for humans with diabetes (Wolever and Mehling, 2002). In horses, several studies have estimated the GI for grains (Vervuert et al., 2003, 2004; Rodiek and Skull, 2007; Nielsen et al., 2010). However, the procedure (starch intake and reference feed used to calculate GI) is inconsistent and hence difficult to compare results across studies.

1.3.2 Factors affecting glucose and insulin responses

1.3.2.1 Processing

Processing has been studied for its ability to affect both glucose and insulin responses in horses (Vervuert et al., 2003, 2004, 2007, 2008; Philippeau et al., 2014) and for having a positive effect on pre-caecal starch digestibility, and thereby glucose absorption, with a corresponding increase in plasma glucose and insulin responses.

The effects of different feedstuffs on plasma glucose and insulin responses are presented in Table 4. However, results are conflicting. Vervuert et al. (2007) reported a greater plasma glucose peak when horses were fed steam-flaked barley compared to ground or steamed barley (Table 4). Despite this observation, there was no difference in glucose response between whole and steam-flaked barley. Further, steamed barley resulted in the lowest overall glucose response than the other processing methods. Thus, results were inconsistent when barley was processed. Similar processing methods were used for oats and maize, but no effect was measured on either plasma glucose or insulin responses (Table 4). Another study (Vervuert et al., 2008) reported that micronized and extruded barley resulted in greater plasma glucose (1.7 and 3 mmol/l larger, respectively) and insulin peaks (39 and 85 µU/ml larger, respectively) than rolled barley. However, results among processing methods were inconsistent, as only extruded barley resulted in a greater AUC for plasma glucose compared to rolled barley (731 vs. 275 mmol/l/min). Based on results from Vervuert et al. (2007, 2008), barley starch may be more enzymatically digestible when thermal processed than unprocessed.

Only one study has combined glucose responses with pre-caecal starch digestion (Philippeau et al., 2014). In this study, horses were fed 2.7 g barley starch/kg BW before sampling. However, the processing method did not affect mean whole blood glucose, peak, or AUC, despite processed barley's greater pre-caecal starch digestibility in relation to whole barley. Details of the processing methods were lacking but a numerical difference in DG was reported (Figure 6). Generally, oats have the greatest glucose and insulin response independent of processing method compared to maize and barley (Table 4). This indicates that oats are more digestible in the small intestine, and that possibly less starch by-passes to the hindgut compared to maize and barley. Therefore, oats are more appropriate to use in horse feed than maize and barley. Furthermore, the processing methods of steam-flaking, steaming, extrusion, and micronizing may have some advantages for grain starch digestion compared to untreated grains.

Table 4. Glycaemic and insulinaemic (mean ± SD) responses in equine blood plasma when fed processed maize, oats, or barley (1.2-1.5 g starch/kg body weight/day) obtained from Vervuert et al. (2003, 2004, 2007).

Feedstuff	Processing method	Peak		Area under the curve (AUC)	
		Glucose (mmol/l)	Insulin (µU/ml)	Glucose (mmol × min/l)	Insulin (µU × min/ml)
Maize	Whole	6.6 ± 0.8	23.6 ± 12.9	1630 ± 170	4334 ± 2129
	Ground	6.2 ± 1.2	30.4 ± 22.9	1527 ± 175	4539 ± 2456
	Steamed	6.1 ± 1.1	26.4 ± 14.6	1480 ± 111	4291 ± 2271
	Micronized	6 ± 0.5	25.4 ± 8.1	1505 ± 101	5129 ± 2415
	SF[1]	5.9 ± 0.3	23.1 ± 9.4	1513 ± 48	4373 ± 1796
	Popped	6.3 ± 1.2	18.8 ± 10.8	1691 ± 283	3511 ± 1929
Mean		**6.2 ± 0.9**	**24.6 ± 13.1**	**1558 ± 148**	**4363 ± 2166**
Oats	Whole	6.4 ± 0.9	31.9 ± 23	1659 ± 254	6052 ± 4623
	Ground	6.6 ± 0.9	49.3 ± 54	1697 ± 318	9946 ± 11,415
	Steamed	6.8 ± 1.4	41.9 ± 38	1638 ± 253	7641 ± 5930
	SF	6 ± 0.2	22.9 ± 6.8	1549 ± 67	4662 ± 1351
	Popped	5.9 ± 1.2	27.2 ± 21.6	1576 ± 186	4998 ± 3166
Mean		**6.3 ± 0.9**	**34.6 ± 28.7**	**1624 ± 216**	**6660 ± 5297**
Barley	Whole	6.1 ± 0.5^{ab}	19.1 ± 6.5^{a}	161 ± 87.2^{ab}	2041 ± 614^{a}
	Ground	5.7 ± 0.7^{a}	19.9 ± 8.2^{a}	127 ± 58.5^{ab}	2923 ± 1532^{ab}
	Steamed	5.8 ± 0.3^{a}	23.1±11.9^{ab}	103 ± 61^{a}	2440 ± 1338^{ab}
	SF	6.5 ± 0.6^{b}	29.5 ± 11.9^{b}	205 ± 62.9^{b}	3837 ± 1440^{b}
	Popped	6.1 ± 0.4^{ab}	21.5 ± 7.6^{a}	242 ± 53.5^{b}	2173 ± 1194^{ab}
Mean		**6 ± 0.5**	**22.6 ± 9.2**	**168 ± 64.6**	**2683 ± 1224**

[1] SF, Steam-flaked.

[a, b, c] Values within a row for barley are different if superscript differs (P<0.05).

1.3.2.2 Inclusion level of non-structural carbohydrates

A few studies have investigated the inclusion level of starch and WSC's effect on plasma glucose and insulin (Vervuert et al., 2009a; Lindåse et al., 2018). Vervuert et al. (2009a) investigated the effect of increasing the starch intake from 0.3 to 2 g/kg BW/meal. An increase in both plasma glucose and insulin responses (AUC) was reported when the meal exceeded 1.1 g starch/kg BW. To avoid development of insulin dysregulation, the authors suggested that starch intake should not exceed 1.1 g/kg BW/meal. Lindåse et al. (2018) evaluated the plasma glucose and insulin response when two breeds (Icelandic and Standardbred) were fed haylage with varying NSC content (low: 42, medium: 136, and high: 182 g/kg dry matter (DM)) resulting in different NSC intakes (low: 0.3, medium: 0.8, and high: 1.1 g NSC/kg BW/meal). A greater glucose peak was reported with increased NSC intake (6.5, 7.1, and 7.7 mmol/l, respectively). Moreover, the early glucose response (first 60 min of AUC) was greater with the high compared to low NSC intake (21 and 40 mmol/l×min, respectively). Similar reports emerged for the Icelandic horses for the overall AUC glucose (low: 248 and high 460 mmol/l×min) and corresponding insulin response (low: 9782 and high 14420 µIU/ml×min, respectively).

1.3.2.3 Feeding management

Feeding management such as the first or second meal and restricting forage before and/or after the test meal is reported to affect plasma glucose and insulin responses. Karasu et al. (2015) found lower plasma glucose and insulin responses after feeding the second meal rather than the first meal with starch (0.9 g/kg BW). Vervuert et al. (2009c) measured an effect of fasting before and/or after feeding cracked maize on both plasma glucose and insulin responses. The authors (Vervuert et al., 2009c) suggested that horses should be fasted 12 h before and after a test meal, as fermentation of forage generates propionate, which has glycogenic properties (Ford and Simmons, 1985).

1.4 Non-starch polysaccharides (fibre)

Fermentable fibrous carbohydrates, also called NSP, can be divided into soluble- and insoluble carbohydrates (S-NSP and I-NSP, respectively, Figure 1). These are both resistant to enzymatic digestion in the small intestine but degradable by microbial fermentation primarily in the large intestine. The S-NSP consists of gums (e.g., ß-glucans), mucilage, and pectin, whereas I-NSP consist of cellulose and hemicellulose (Hoffman, 2013). Gums and mucilage are galactopolysaccharides whereas pectin is a structural polysaccharide containing 1.4-linked α-D-galactosyluronic acid residues, all rapidly fermented in the hindgut (Hoffman, 2013; McDonald et al., 2011). Cellulose is polymers of glucose bound by ß1-4 linkages (glycosidic bonds) whereas hemicellulose includes several polymers mostly xylose, glucose, mannose, and arabinose (Figure 7) and both are found in the plant cell wall (Hoffman, 2013; Varloud et al. 2004). These are slowly fermentable and often

referred to as fibre. Lignin is also considered as fibre; though, it is not a carbohydrate but a non-degradable complex phenolic polymer (Hoffman, 2013; McDonald et al., 2011). However, it is still an important compound, as it can reduce the digestibility of other nutrients (Ragnarsson and Lindberg, 2008).

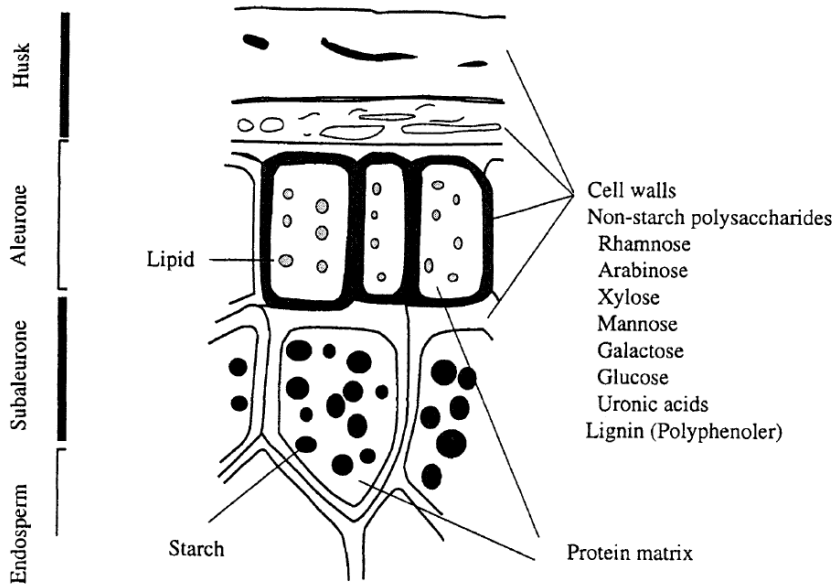

Figure 7. Illustration of cell material in oats from Bach Knudsen (2001).

Figure 7 illustrates the cell materials in oats. The husk of a grain is mainly composed of I-NSP and lignin, whereas the endosperm is composed of starch embedded in a matrix of protein, ß-glucans, and soluble arabinoxylans (Bach Knudsen, 2001). The cell wall consists of NSP and lignin, where pectin in particular is found in the middle lamella (Bach Knudsen, 2001; Hoffman, 2013). Polysaccharide and lignin content varies among plant species (Table 5). Total NSPs constitute approximately 60% of forage (hay) and in grains it constitute 7-36%, dependent on species (Table 5). Oats have the greatest content of T-NSP, followed by barley and then maize, as the oat hull is rich in I-NSP (Table 5).

Table 5. Nutrient composition of various feedstuffs (g/kg dry matter (DM)).

Ref.[1]	Feed	Nutrients[2]							
		Starch	WSC	NDF	ADF	DF	T-NSP	I-NSP	S-NSP
Bach Knudsen (1997)	Barley	587	21	-	-	221	189	131	55
	Maize	690	20	-	-	108	97	88	9
	Oats	468	17	-	-	298	232	192	40
	Oats[3]	557	-	-	-	148	116	63	53
Brøkner et al. (2012b)	Barley	575	40	130	-	192	170	125	45
	Maize	701	35	90	-	80	76	69	7
	Oats	343	18	338	-	439	360	269	91
	Hay	1	100	577	-	699	600	586	14
Jensen et al. (2014)	Barley	583	35	143	50	200	175	133	42
	MSBP[4]	1	230	331	153	499	488	248	240
	Hay	1	84	685	376	683	569	528	41

[1] Ref., reference.

[2] WSC, water soluble carbohydrates; NDF, neutral detergent fibre; ADF, acid detergent fibre; T-NSP, total non-starch polysaccharides; I-NSP, insoluble non-starch polysaccharides; S-NSP, soluble non-starch polysaccharides.

[3] Dehulled oats.

[4] MSBP, molassed sugar-beet pulp.

1.4.1 Fibre analyses

One common method of evaluating fibre content of feedstuffs is described by Van Soest et al. (1991). This method quantifies neutral detergent fibre (NDF: cellulose, hemicellulose, and lignin), acid detergent fibre (ADF: cellulose and lignin) and acid detergent lignin (ADL: lignin), by extraction with detergents (Van Soest et al., 1991). Another method is dietary fibre (DF) analysis. It quantifies T-NSP, I-NSP, and their constituent sugars (Bach Knudsen, 1997). From this, total DF (T-NSP + Klason lignin) and S-NSP (T-NSP – I-NSP) can be calculated. This method covers more fibre components than the NDF method, as NDF does not include pectin, gums (ß-glucans), mucilage or any hemicellulose soluble in neutral detergent. Additionally, the insoluble residue lignin can be quantified by an enzymatic-chemical procedure that removes sugars, starch, and NSP by sulfuric acid (Theander et al., 1994). This method is called Klason lignin (Theander et al., 1994).

1.4.2 Fibre fermentation

Carbohydrates that are not enzymatically digested in the small intestine can instead be fermented by microbes in the hindgut. The microbiota constitutes bacteria, protozoa, anaerobic fungi, bacteriophages, and archaea (Julliand and Grimm, 2016). Some have been isolated and described; however, most microbes have not yet been identified (Julliand and Grimm, 2016). Furthermore, most studies conducted on the microbiota in

horses have focused on bacteria, therefore the following section will focus on bacteria in general.

1.4.2.1 Microbiota along the equine gastrointestinal tract

In horses, bacteria have been identified along the entire GIT. The total anaerobic bacterial concentration in the stomach is high, varying from 5.5 to 9 log10 colony-forming units (CFU)/mL gastric fluid (de Fombelle et al., 2003; Varloud et al., 2007; Julliand et al., 2018). The small intestine contributes with a total anaerobic bacteria concentration between 7.7 and 8.8 log10 CFU/mL digesta (de Fombelle et al., 2003). The bacterial community present in the hindgut has a total anaerobic bacteria concentration varying from 7.1 to 9.1 log10 CFU/mL digesta (Julliand et al., 2001; de Fombelle et al., 2003; Muhonen et al., 2009). The bacteria count in the colon is slightly higher than in the caecum (8.6 vs. 7.7 log10 CFU/mL digesta, respectively) (de Fombelle et al., 2003). Despite a similar concentration of total anaerobic bacteria through the GIT, the volume (stomach 8-15 l, small intestine 60 l, caecum 32 l and colon 91 l) (Merritt and Julliand, 2013) and retention time of the digesta in the different segments (see 1.5 Passage rate) are diverse. Thus, the greatest fermentation and thereby production of SCFA occurs in the hindgut.

1.4.2.2 Production of short-chain fatty acids

The microbiota's primary function is to provide the horse with energy through carbohydrate fermentation. Fermentation includes several steps to produce the end-products SCFA—mainly acetate, propionate, and butyrate (Figure 8). The first step involves hydrolysis of the carbohydrates to their constituent monosaccharides. For example, cellulose is degraded by ß-D-glucosidase, whereas hemicellulose is degraded by α-L-arabinosidase (Merritt and Julliand, 2013). The monomeric sugars are then hydrolysed in the bacterial cells through the Embden-Meyerhoff pathway to form pyruvate, SCFAs, and gasses (Leek, 1993; Merritt and Julliand, 2013).

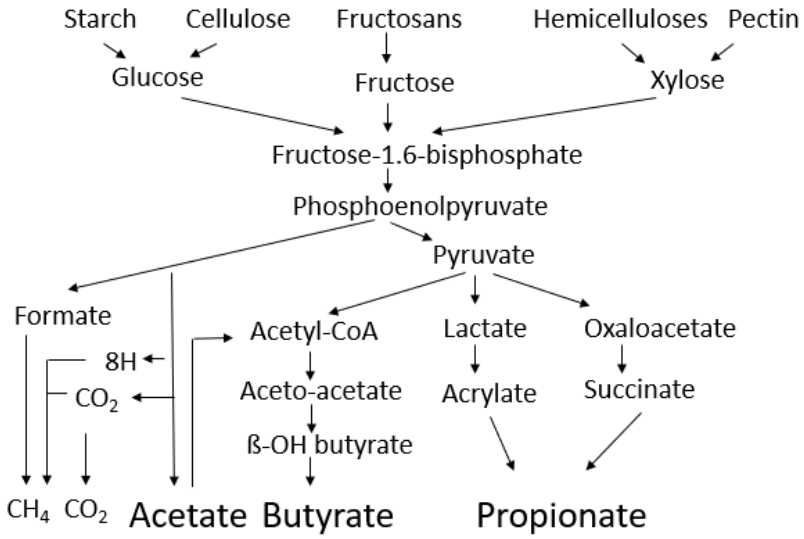

Figure 8. Fermentation pathways of carbohydrates (obtained from Leek (1993)).

Cellulolytic bacteria have optimal growth conditions at pH 6.7 (Van Soest, 1994). Fibre fermentation is possible pre-caecally where pH is between 6-7 and 6-7.4 in the non-glandular part of the stomach and small intestine, respectively (de Fombelle et al., 2003; Merritt and Julliand, 2013). As mentioned (1.4.2.1 Microbiota along the equine gastrointestinal tract), total anaerobic bacteria are present in both the stomach and small intestine (de Fombelle et al., 2003). Additionally, cellulolytic bacteria are present in the stomach and small intestine (de Fombelle et al., 2003; Varloud et al., 2007), but the concentration is small (~1.3 log10 CFU/ml), and fibre fermentation is assumed to be negligible (de Fombelle et al., 2003). The pre-caecal apparent NDF digestibility is reported to be 12-14 % (Hintz et al., 1971b; Moore-Colyer et al., 2002; Varloud et al., 2004). This suggest a minor pre-caecal cellulose (NDF) fermentation.

1.4.3 Factors affecting fibre fermentation

As earlier reviewed by Harris et al. (2017) several factors affect fibre digestibility in horses, as illustrated in Figure 9. These will be covered individually through the section; however, additive effects and interactions are present between factors.

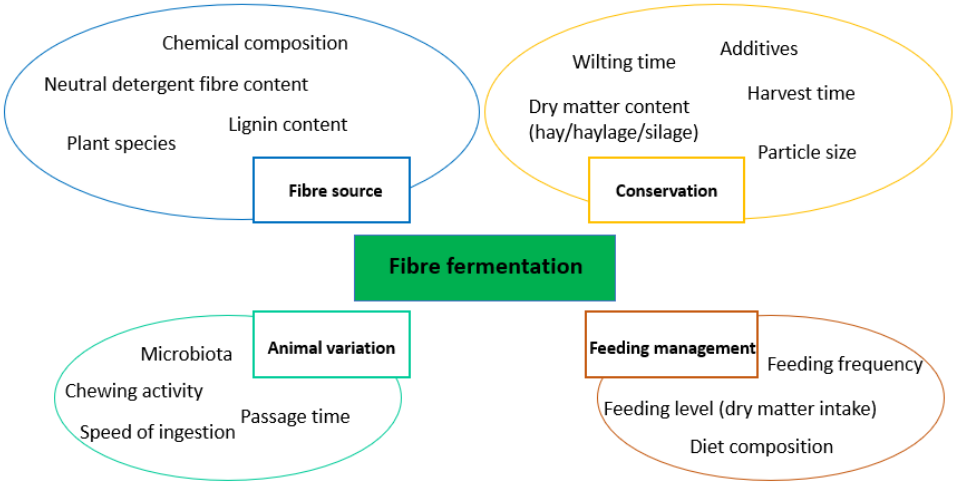

Figure 9. Factors affecting fibre fermentation in horses.

1.4.3.1 Chemical composition

Regardless of production site, the concentration and proportions of the fermentation end-products depend on the chemical composition of a feedstuff (Table 6). A diet rich in starch results in a greater total SCFA concentration compared to a diet rich in fibre in the hindgut (Medina et al., 2002; de Fombelle et al., 2003; Jensen et al., 2016; Warzecha et al., 2017). Additionally, fermentation of starch can alter the proportions of the individual SCFAs and further decrease pH, altogether altering microbial composition (McLean et al., 2000; Julliand et al., 2001; Medina et al., 2002; Jensen et al., 2016; Warzecha et al., 2017). This may lead to a further decrease in pH which favours lactate-utilizing bacteria in the hindgut. Overall, this may impair fibre fermentation (Julliand et al., 2006).

Table 6. Daily intake (g/kg body weight (BW)) of starch and neutral detergent fibre (NDF) and its effect on lactate concentration (mmol/l, unless otherwise stated), total short-chain fatty acids concentration (SCFA, mmol/l), proportions of acetate, propionate, and butyrate (mol %), and the pH in the equine caecum.

Reference	Type of diet	Starch	NDF	Lactate	SCFA	Acetate	Propionate	Butyrate	pH
McLean et al. (2000)	NDF	0^{b}	10.2	0.1^{a}	49	77^{ab}	17^{c}	6	6.5^{a}
	Starch	4.2^{a}	6.4	1^{b}	54	63^{c}	30^{a}	7	6.3^{b}
	Starch	4.3^{a}	6.1	0.2^{ab}	49	72^{b}	22^{bc}	6	6.3^{ab}
	Starch	4.2^{a}	5.7	0.3^{ab}	53	68^{bc}	25^{ab}	7	6.4^{ab}
Moore-Colyer et al. (2000)	NDF	0.2	8.9	0.3^{b}	56^{a}	76^{ab}	17^{b}	6^{b}	6.5
	Starch	3	10.8	3.7^{a}	45^{ab}	74^{b}	23^{a}	3^{a}	6.7
	NDF	0.2	5.6	0.9^{b}	35^{b}	79^{a}	16^{b}	5^{b}	6.6
Julliand et al. (2001)[1]	NDF	0[2]	20.3	53^{a}	85	72^{a}	19^{a}	-	6.7^{a}
	Starch	3.1[3]	9.4	227^{a}	87	68^{b}	24^{b}	-	6.4^{ab}
	Starch	4.3[4]	6.3	436^{a}	93	67^{b}	26^{b}	-	6.3^{b}
Medina et al. (2002)[1]	Starch	2.5	8.8	168^{b}	68	74^{a}	19^{b}	5^{b}	7.2^{a}
	Starch	6.7	6.6	408^{a}	66	66^{b}	26^{a}	6^{a}	6.9^{b}
de Fombelle et al. (2003)	Starch	2.2	4	0.3	83^{b}	54^{b}	20^{b}	7^{b}	6.2^{b}
	Starch	4.5	3.5	0.2	122^{a}	81^{a}	28^{a}	9^{a}	6.4^{a}
						MEAN			
Starch-rich diets (>1 g/kg BW/day)				1	72	69	24	6	6.5
NDF-rich diets (>5 and starch <1 g/kg BW/day)				0.4	56	76	17	6	6.6

[1] Lactate concentration (mg/l) not included in mean calculations.

[2] 100% hay diet (assuming 0 g starch/kg dry matter (DM)).

[3] 70% hay and 30% barley, (assuming 580 g starch/kg DM).

[4] 50% hay and 50% barley (assuming 580 g starch/kg DM).

[a, b, c] Values within a column for each reference are different if superscript differs ($P<0.05$).

Fermentation of fibre and production of SCFA is important for a normal GIT function and energy supply to the horse. Therefore, a daily minimum forage provision of 15 g DM/kg BW/day is recommended (Harris et al., 2017). However, no further description of the forage's chemical composition has been established. The chemical composition of grass and forage is dependent on plant species (Brøkner et al., 2012b; Longland, 2013), environmental conditions (soil, weather) (Longland, 2013), management factors (fertilizer, harvest techniques, and storage conditions) (Müller, 2012a; Harris et al., 2017; Loaiza et al., 2017), and most importantly, stage of maturity at harvest (Ragnarsson and Lindberg, 2008; Müller, 2012b). As the plant matures, the lignin content increases, resulting in a decreased ATTD of DM, NDF and ADF (Darlington and Hershberger, 1968; Ragnarsson and Lindberg, 2008; Ragnarsson and Jansson, 2011; Müller, 2012b) assuming a corresponding decrease in SCFA concentration in the hindgut. The ATTD of T-NSP increases when forage is substituted with sugar beet pulp (SBP) (Murray et al., 2008; Jensen et al., 2014), as the S-NSP fraction is greater in SBP than forage (Brøkner et al., 2012b; Jensen et al., 2014; Murray et al., 2008). The higher digestibility of SBP than forage (e.g., lucerne hay and silage) is explained by its higher content of hemicellulose and pectin (Murray et al., 2008; Santos et al., 2011; Brøkner et al., 2012a). When SBP substituted hay (85:15% hay:SBP vs. 100% hay) Jensen et al. (2016) did not measure any difference in pH and SCFA concentration in the hindgut. However, literature is scarce on the effects of S-NSP and I-SNP on the microbial composition, production of SCFA, and pH in the hindgut. Therefore, more knowledge on the effect of fibre composition and other factors (Figure 9) on the digestive processes in the hindgut is needed additionally to the established recommendation for daily forage intake for horses.

1.4.3.2 Feeding level

An increased feeding level results in a shorter TMRT (Clauss et al., 2014), and it may impair fibre fermentation as the exposure time to microbiota is shortened. The ATTD of DM and NDF decreased when feeding level increased from 10.7 to 18.1 g DM/kg BW/day (Ragnarsson and Lindberg, 2010), while feeding ad libitum compared to 75% of ad libitum feeding did not affect the ATTD of NDF but shortened the MRT in ponies (Pearson et al., 2006). Similar findings were reported by Martin-Rosset and Dulphy (1987) and Martin-Rosset et al. (1990). However, the disagreement between studies may be a result of differences in the chemical composition of the diets provided.

1.5 Passage rate

Several expressions are used when describing the passage of digesta through the GIT (Table 7). Typically, these include minimum-, mean- and maximum retention time. However, in this section the focus will be on MRT of digesta (or expressed as TT for mobile bags).

Table 7. Terminology of passage rate (Van Weyenberg et al., 2006).

Term	Definition
Passage rate	The flow of material within or through the entire tract per unit of time.
Gastric emptying	Measurement of how fast the feed travels through the stomach after a meal.
Mean retention time[1]	The integrated average time between a marker administration and excretion.

[1] Expressed as transit time (TT) for mobile bags.

1.5.1 Indigestible markers

Indigestible markers are used to determine the MRT of solids and liquids through the GIT (Argenzio et al., 1974; Drogoul et al., 2000; Miyaji et al., 2014). In the following section, markers for determination MRT of digesta will be presented.

The passage rate can be calculated by use of an indigestible marker. The characteristics of the marker's transit through the GIT can be assessed by calculating the MRT as described by Faichney (1975) for a single dose of a marker with time-sequence sampling:

$$\mathrm{MRT} = \sum_{i=1}^{n} t_i \times M_i \qquad Equation\ 1$$

Where *Mi* is the concentration of marker at time *ti* as a proportion of the total marker excreted and *ti* is the time elapsed between the administration of marker and the midpoint of the *i*th collection interval.

1.5.1.1 Marker procedure

Indigestible markers can occur naturally in the feed or be added as external markers (Sales, 2012). In horse feed, naturally occurring markers are acid insoluble ash, ADL, and n-alkanes (Van Weyenberg et al., 2006; Sales, 2012). External markers can be titanium dioxide, chromic oxide, ytterbium (Yb), chromium-ethylenediaminetetraacetic acid (EDTA), cobalt-EDTA, thulium (Drogoul et al., 2000; Moore-Colyer et al., 2003; Sales, 2012; Schaafstra et al., 2018). When choosing a marker, several criteria should be considered. Faichney (1975) summarized the criteria for an ideal marker:

- Strictly non-absorbable.
- Must not affect or be affected by the GIT and/or its microbial population.
- Must be physically equal to or closely associated with the material it is to mark.
- Its method of estimation in digesta samples must be specific and sensitive and it must not interfere with other analyses.

Yet Faichney (1975) stated that no available marker satisfy all of the above-mentioned criteria. Studies that use markers for nutrient digestibility or passage rate can be difficult to compare, as different markers, feedstuffs, and procedures are used (Van Weyenberg et al., 2006). Feeding level can also affect the passage rate of digesta through the GIT and additionally complicate the comparison between studies.

1.5.2 Factors affecting digesta passage rate

1.5.2.1 Dry matter intake

Increasing the feed intake (g DM/kg BW) influences passage rate by shortening the MRT (Figure 10). Clauss et al. (2014) measured shorter MRT for the solid and the liquid phase when horses were fed ad libitum (average 27 and 22 h, respectively) compared to restricted hay feeding (average 43 and 34 h, respectively). This agrees with earlier studies in which horses were fed ad libitum or restricted forage (Pearson et al., 2006; Miyaji et al., 2011).

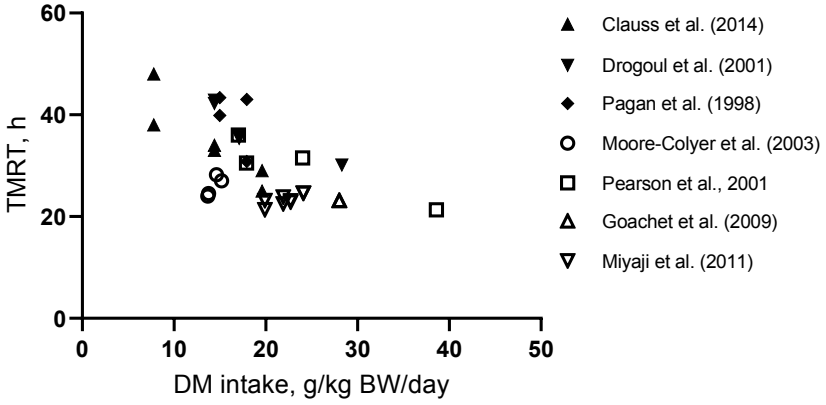

Figure 10. Comparison of dry matter (DM) intake (g/kg body weight (BW)/day) on total tract mean retention time (TMRT, h) of digesta from different studies.

1.5.2.2 Particle size

Several authors have reported that particle size has an effect on passage rate (Argenzio et al., 1974; Drogoul et al., 2000; Miyaji et al., 2011). A marker moved at a faster rate through the entire GIT when particle size increased (2 mm, 1 cm, and 2 cm; Argenzio et

al., 1974). This aligns with findings by Drogoul et al. (2000), who determined that smaller particles remained longer in the hindgut and the total tract than did larger particles. The TMRT was 8 h shorter for chopped hay (78% particles >0.8 mm) than ground-pelleted hay (84% particles <0.8 mm). Also, Miyaji et al. (2011) reported a longer TMRT for smaller (5 mm: ~28 h) hay particles than longer particles (10 mm: ~26 h).

1.5.2.3 Composition of the diet

Gastric emptying takes approximately 1-6 h (Van Weyenberg et al., 2006). The passage rate, including gastric emptying rate, is different for the solid and liquid phases, with the liquid phase flowing faster through the GIT than the solid phase. After 0.5 h, 75 % of the liquid phase marker left the stomach, whereas 75 % of the solid marker remained (Argenzio et al., 1974). Métayer et al. (2004) reported slower gastric emptying of the solid phase when horses were fed a starch-rich meal (1.3 g/kg BW; 2.4 h) compared to a meal low in starch (0.7 g/kg BW; 1.5 h). The digesta passage rate is more rapid through the duodenum than the ileum (14-15 vs. 11 movements/min, respectively) (Van Weyenberg et al., 2006), with an MRT of approximately 3-6 h through the small intestine for the solid phase (Moore-Colyer et al., 2002; de Fombelle et al., 2004). Most likely, a major part of digesta have reached the caecum after approximately 3 h (Van Weyenberg et al., 2006). For mobile bags the average pre-caecal TT was 3.3 h in caecum cannulated ponies (Moore-Colyer et al., 2002). Similarly, Drogoul et al. (2000) reported longer pre-caecal MRT for chopped hay (3.6 h) than ground pelleted hay (2 h) when calculated by difference of MRT for total tract and hindgut by use of markers. Moreover, for starch-rich feedstuffs, pre-caecal TT, estimated by the mobile bag technique (MBT), was longer when horses were fed three meals (6.6 h) rather than five meals a day (5.9 h) (de Fombelle et al., 2004). However, studies on pre-caecal MRT are scarce, as it requires physical modification or euthanisation of horses. The TMRT is estimated to approximately 24-30 h for the liquid phase and 21-48 h for the solid phase when horses are fed hay (Clauss et al., 2014; Jensen et al., 2014; Hummel et al., 2017). Substituting forage with grain prolongs the TMRT (Pagan et al., 1998; Drogoul et al., 2001; Jensen et al., 2014).

1.5.2.4 Cannulation

The MRT may be affected by cannulation (Austbø and Volden, 2006), but results are inconsistent (Drogoul et al., 2000; Austbø and Volden, 2006). Drogoul et al. (2000) measured a longer TMRT of Yb-labelled hay in intact ponies than in cannulated ponies (caecum and right-ventral colon) (49 h and 42 h, respectively). Prolonged TMRT after cannulation (caecum) has been reported by Pulse et al. (1973) and Austbø and Volden (2006) (23, 27 and 31 h before, 1 and 15 months after caecum cannulation). Further studies are required to determine the effect on TMRT years after cannulation.

1.6 Methods to measure carbohydrate digestion

Feedstuff evaluation is important for optimizing nutrient supply and ration formulation for horses (Hyslop, 2006). To achieve this, both in-vivo and in-situ methods have been developed. For in-vivo techniques, total faeces collection (TFC) will be covered here, and for in-situ techniques, the focus will be on the MBT. To distinguish between estimation of nutrient digestibility in these different methods, various terms will be used. For the TFC, *digestibility* will be used, whereas for the MBT, two terms, *disappearance*, and *degradations*, will be used.

1.6.1 In-vivo techniques

1.6.1.1 Total faeces collection

The total faeces collection is the "gold standard" method for determination of digestibility. This is a common method used for feedstuff evaluation in horses (Goachet et al., 2009). The total collection usually has an adaptation period of 14 days followed by 5-6 consecutive days of total faeces collection (Table 8). In horses, a collection harness can be used which allows for quantitative collection of faeces. The ATTD of a diet can thereby be determined according to equation:

$$\text{ATTD} = \frac{Nutrient\ intake - Nutrient\ excretion}{Nutrient\ intake} \times 100 \qquad Equation\ 2$$

According to Martin-Rosset et al. (1984), the digestibility of a supplement is equivalent to the weighted sum of the nutrient supplied from forage and the additional amount of supplement. The ATTD can thereby be calculated for the supplement added to the basal diet of, for example, forage with a known digestibility:

$$\text{dS} = \frac{dD - (h \text{ x } dH)}{s} \qquad Equation\ 3$$

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

The TFC method is time consuming and expensive in terms of feedstuff and labour time compared to other methods (e.g., MBT) (Goachet et al., 2009). To decrease labour time and expenses, the adaptation period can be shortened. The adaptation period varies from 7 to 28 days (Table 8) and is included to ensure feed residues from the previous diet are excreted and that the microbiota has adapted to the new diet. The maximum retention time of the GIT must be considered when number of adaptation days is determined. Furthermore, the new diet's chemical composition in relation to the previous diet can

affect the numbers of days needed for adaptation (Muhonen et al., 2009; Warzacha et al. 2017; Garber et al., 2020). The length of the adaptation period can vary depending on the digestive responses of interest. For digestibility trials, effects can be detected 1-2 days after an abrupt feed change from hay to silage or visa-versa, but microbiota might need a longer adaptation period (Muhonen et al., 2009). However, an abrupt change from a forage-only diet to a diet rich in starch (>1 g/kg BW/meal) should be done with caution (Garner et al., 1975; Hudson et al., 2001; Luthersson et al., 2009; Warzacha et al., 2017).

Table 8. Number (*n*) of horses included, days of adaptation, and consecutive collection days when using the total faeces collection (TFC) method.

Reference	*n*	Adaptation	Collection	Feed
Vander Noot et al. (1967)	4	14	6	Hay + grain
Hintz et al. (1971a)	3	21	7	Hay + grain
Palmgren Karlsson et al. (2000)	4	9	2 × 2[1]	Hay + grain
Palmgren Karlsson et al. (2002)	4	16	2	Hay + concentrate
Bergero et al. (2005)	4	14	6	Hay
Jansson et al. (2006)	7	28	3	Hay + concentrate
Van Weyenberg et al. (2007)	4	21	5	Hay + grain
Murray et al. (2008)	4	10	7	Hay + SBP[2]
	4	16	5	Silage + SBP
Goachet et al. (2009)	6	7	5	Hay + concentrate
De Marco et al. (2012)	6	14	6	Hay/hay + grain
Schaafstra et al. (2015)	4	14	10	Hay
Schaafstra et al. (2017)	4	14	10	Haylage+ concentrate
Longland et al. (2018)	4	14	5	Hay + concentrate
		Mean		
	4.4	15.1	5.6	

[1] One day of rest in between four days of collection.

[2] SBP, sugar beet pulp.

The number of consecutive collection days varies from 2 to 10 days (Table 8). This is done to reduce the possible variation occurring between animals and collection days and to obtain enough data to have a valid mean of digestibility. There are disparities between the recommended number of collection days in existing literature. Hintz and Loy (1966) suggested 4 days of collection when horses were fed a hay-grain diet to determine digestibility. This is consistent with Vander Noot et al. (1967), who suggested that 4 days of TFC would be sufficient, but to reduce variation between animals (when few animals are used), 5 consecutive days of collection would be optimal. However, Goachet et al. (2009) did not find that a decrease in collection days from 5 to 4 or 3 days had any effect on DM, organic matter (OM), or NDF digestibility. Schaafstra et al. (2015, 2017) and Martin-Rosset et al. (1984) recommend 14 days adaptation and 5-6 consecutive days of TFC. Conclusively, the recommended number of days for TFC is between 3 and 6.

1.6.2 In-situ techniques

The in-situ techniques cover the in-sacco and MBT. These techniques have been used widely in both pigs and ruminants to determine the apparent digestibility of nutrients (Sauer et al., 1983; Udén and Van Soest, 1984; Vanzant et al., 1998). Yet there are some differences between the two techniques, as the in-sacco requires physical modification of animals. The MBT can be used in both physically modified and intact animals, as the bags can be administered naso-gastrically and recovered in faeces (Macheboeuf et al., 1996; Hyslop et al., 1998).

1.6.2.1 The mobile bag technique

de Reaumur (in Sauer et al., 1983) first recorded the MBT in 1756. Since then, the technique has evolved from employing metal tubes to the use of linen bags (Spallanzani, 1782 cited in Sauer et al., 1983), which are replaced by nylon and polyester bags today (Macheboeuf et al., 1996; Hyslop et al., 1998; McLean et al., 1999b; Brøkner et al., 2012a). The method was originally developed for sheep (de Reaumur, 1756, cited in Sauer et al., 1983) and thereafter adapted for humans (Spallanzani, 1782, cited in Sauer et al., 1983) and later for pigs and cattle (Petry and Handlos, 1978; de Boer et al., 1987). Udén and Van Soest (1984) compared the MBT in ponies, heifers, sheep, and rabbits. Today, studies using the MBT in horses are still limited and are mostly published as abstracts (Macheboeuf et al., 1996; Hyslop et al., 1999; McLean et al., 1999b), leaving out important information and making them difficult to repeat. However, these abstracts are the foundation of the technique when used in horses.

The MBT, if combined with effective degradation calculations, can provide additional knowledge on degradation kinetics in different segments of the GIT (Ørskov and McDonald, 1979). The MBT has been used to evaluate pre-caecal starch digestion in horses (de Fombelle et al., 2004; Brøkner et al., 2012a; Hymøller et al., 2012; Philippeau et al., 2014; Rosenfeld and Austbø, 2009) and further, the extent of degradation of fibrous feeds in the small intestine and total tract of ponies (Moore-Colyer et al., 2002).

1.6.2.2 The mobile bag technique procedure

Descriptions of the mobile bag procedure differ among literature (Table 9). To flush the mobile bags into the stomach, between 500 and 5000 ml of water is used. After the administration into the stomach or caecum, the mobile bags can either be harvested in the caecum or collected in faeces. Mobile bags harvested in the caecum are equipped with one or two steel washers and collected with a magnet (Moore-Colyer et al., 2002; Brøkner et al., 2012a; Hymøller et al., 2012). To determine the DM disappearance, the individual bags are oven-dried (40-60˚C, 48-72 h or to constant weight) (Moore-Colyer et al., 2002; Rodrigues et al., 2012; Philippeau et al., 2014). For further analysis, handling of

the feed residue varies between studies. While one study analysed the individual bag for its nutrient disappearance (Hymøller et al., 2012), most studies pool the bag residues to gain enough feed residue. The mobile bags can then be pooled by time or by horse (ignoring the time effect) (Moore-Colyer et al., 2002; Philippeau et al., 2014; Rodrigues et al., 2012). Hyslop et al. (1998) pooled the bags' residue by horse and then grouped the bags according to their collection time.

Table 9. Overview of the mobile bag technique (MBT) with various feedstuffs used in horses harvested from the caecum (pre-caecal) or faeces (total tract).

Reference	Bag material	Bag size (cm)	Pore size (µm)	Feed amount (mg)	FSA[1] (mg/cm²)	Feed	Screen size (mm)	Segment
McLean et al. (1999b)	Polyester	1×6 1×4	7	165 300	13.8- 37.5	Starch	?	Pre-caecal
de Fombelle et al. (2004)	Nylon	1×6	46	400	21.5	Grains	3	Pre-caecal
Brøkner et al. (2012a)	Nylon	1.5×15	11	500- 2000	11-44	Forage +grains	1	Pre-caecal
Hymøller et al. (2012)	Nylon	1.5×15	11	1500	33.3	Grains	1 and ?[2]	Pre-caecal
Philippeau et al. (2014)	Nylon	1×6	50	400	21.5	Grains	< 4	Pre-caecal
Jensen and Prestløkken (2018)	?	1.5×15	15 37	1000	22.2	Grains	1.5	Pre-caecal
Macheboeuf et al. (1996)	Polyester	1×6	?	200 400	10.6 21.3	Forage +grains	?	Pre-caecal Total tract
Moore-Colyer et al. (2002)	Polyester	1×6	41	350	29	Forage	1	Pre-caecal Total tract
Rosenfeld and Austbø (2009)	Nylon	1×6	37	500- 1000	42-83	Grains	1	Pre-caecal Total tract
Hyslop et al. (1998)	Polyester	1×4 1×6	41	130 200	32.5 33.3	Forage	?	Total tract
Rodrigues et al. (2012)	Nylon	3×6.5	45	633	16.2	Forage	1	Total tract
				Mean				
Pre-caecal		1.2×8.5	28.3	523.8[3]	22.8[4]		1.8	
Total tract		1.7×5.5	41	318.8[3]	23.8[4]		1	

[1] FSA, feed to surface area.

[2] Blended, unknown screen size.

[3] Brøkner et al. (2012a) and Rosenfeld and Austbø (2009) not included.

[4] McLean et al. (1999a), Brøkner et al. (2012a) and Rosenfeld and Austbø (2009) not included.

1.6.2.3 Recommendations for the mobile bag technique

In the Nordic feed evaluation system, NorFor for ruminants, mobile bags are used to determine digestion in the small intestine (Åkerlind et al., 2011). It is recommended that

mobile bags have, a pore size of 11-15 µm and a feed to surface area (FSA) of 5-7 mg/cm^2 for forage and 10-15 mg/cm^2 for concentrate (Åkerlind et al., 2011). For the in-sacco technique used in the rumen, recommendations are for a pore size of 38 µm and an FSA of 10 mg/cm^2 (Åkerlind et al., 2011). While the in-sacco and MBT are two different in-situ procedures, these recommendations can still be applied to the MBT when studying microbial degradation in horses. Despite that, studies have used the MBT in the equine hindgut and/or the total tract, no recommendations have yet been established. Although Macheboeuf et al. (1996) are often cited for recommendations of the MBT for horses described in a study aiming to investigate pre-caecal and total tract DM and nitrogen (N) digestibility (Table 9). The authors stated that the DM and N disappearance were close to the in-vivo total tract digestibility (results not presented). Therefore, based on the aim of the study, assigning Macheboeuf et al. (1996) for recommendations of the MBT should be done with caution.

1.6.2.3.1 Pore size

The pore size of the mobile bags is important, as it should allow enzymes and microbes to enter the bag and at the same time prevent the entry of digesta particles, retain undigested particles, and allow fermentation end-products to exit. A small pore size may restrict the entry of enzymes and microbes (e.g., protozoa range 5-250 µm) (Stewart et al., 1988), whereas a large pore size may increase the loss of undigested particles. Pore size affects the nutrient disappearance in pigs and ruminants (Varvikko and Lindberg, 1985; Varvikko and Vanhatalo 1989; Cherian et al., 1989). Varvikko and Vanhatalo (1989) found that increasing the pore size increased the DM disappearance for all feedstuffs tested (ryegrass, barley, and barley straw) in the duodenum of cows. Udén and Van Soest (1984) measured an effect of porosity on “cell wall” disappearance in the rumen when the porosity increased from 5 to 37 µm (54 and 63%, respectively). Jensen and Prestløkken (2018) reported increased DM and starch pre-caecal disappearance of pelleted barley (1.5 mm) in horses as the pore size increased (DM 15 µm: 79% and 37 µm: 82%; starch 15 µm: 69% and 37 µm: 81%). Starch-rich feedstuffs may require a smaller pore size than fibrous feedstuffs.

1.6.2.3.2 Particle size

Particle size should accommodate the pore size to avoid undigested particles from exiting the bags. In pigs, increasing the feedstuff’s particle size (from 0.5, 1, 1.5 to 2 mm) used in mobile bags decreased the N disappearance (91, 90, 90 and 88%, respectively) (Cherian et al., 1989). Similar findings could be expected in horses, as smaller particles have a larger surface area in relation to volume. Feedstuffs, used in mobile bags, are ground, or milled to obtain a homogenous feed sample and to imitate feed mastication in the mouth. Most studies in horses have used a screen size of 1 mm independent of feedstuff (Table 9).

1.6.2.3.3 Size of the mobile bags

For mobile bags flushed into the stomach, sizes vary from 1×4 cm to 1.5×15 cm (Table 9). Increasing the size of the bag allows for more feedstuff used and thereby a possible increase in the remaining residue. However, the bag size has limits, as it must travel along the GIT. In pigs, no differences are measured for N disappearance (~91% for soyabean meal) between two bag sizes (2×5 cm and 2.5×4 cm), yet the 2×5 cm bag travelled faster than the 2.5×4 cm (80 and 60% recovered 48 h after administration, respectively) (Cherian et al., 1989). In ponies, a preliminary study (Hyslop and Cuddeford, 1996), indicated that bag size affected TT and hence nutrient disappearance. Moreover, further research is needed to investigate the effect of bag size on nutrient disappearance in horses.

1.6.2.3.4 Feed to surface area

A way to compare the MBT between studies is to use the FSA. Udén and Van Soest (1984) found an effect of FSA on "cell wall" disappearance (16% difference) when the FSA increased from 6.5 to 50 mg/cm^2. This aligns with observations by Hyslop and Cuddeford (1996), who found indications of decreased DM and NDF disappearance in horses when FSA increased from ≤10.5 to ≥20.3 cm^2. In addition, an FSA of ≤10.5 cm^2 fitted the in-vivo DM and NDF ATTD. Increasing the sample size (500 and 1000 mg), and thereby the FSA (not stated), decreased N disappearance of soyabean meal (93 vs. 90%) and meat and bone meal (83 vs. 81%) in pigs (Cherian et al., 1989). The recommended FSA may differ depending on the type of feedstuff, as forages can be greater in terms of volume compared to grains. Therefore, a large FSA for forage may prevent enzymes and microbes from penetrating the bag and end-products from exiting the bag compared to grains. To establish recommendations for the FSA, further studies are needed for individual feedstuffs (forage vs. grains) and to further validate it against the in-vivo methods.

1.6.2.3.5 Washing procedure

For the washing of mobile bags, *loss* is used to describe nutrients exiting the bag. Bags administered into the GIT must be washed after collection to rinse off mucous, endogenous enzymes, and microbial biomass from the feed residue (Van Straalen et al., 1993), all to avoid interference when determining nutrient disappearance. While several studies have discussed the washing procedure (Cherney et al., 1990; Dhanoa et al., 1999; Moore-Colyer et al., 2002; de Fombelle et al., 2004; Hyslop, 2006), no standard procedure has yet been developed. Hand-rinsing would in theory be the mildest form of washing in relation to machine washing. However, Cherney et al. (1990) reported similar DM loss in both hand-rinsing and washing by machine (20 vs. ~23% for maize). In hand washing, mucous, endogenous enzymes, and microbial biomass may not be rinsed off the remaining residue. Therefore, in combination with standardisation, machine washing has been implemented. Studies have implemented programs without spinning (Cherney et

al., 1990; de Fombelle et al., 2004; Rodrigues et al., 2012), and others with (Moore-Colyer et al., 2002), with the latter leading to the possibility of excessive loss. In theory, pore size, FSA, particle size of the feed, and type of feedstuff can affect nutrient loss at washing. However, methodology studies investigating these factors' effect on nutrient loss are lacking.

To determine the nutrient loss at time 0, bags can be washed exclusively (without entering the GIT). This nutrient loss is primarily the soluble and rapidly degradable fraction of the feed, and this is needed when determining degradation kinetics.

1.6.2.3.6 Degradation kinetics

One main advantage of the MBT is that it facilitates the study of degradation kinetics. Originally the method was developed for the in-sacco technique in ruminants (Ørskov and McDonald, 1979), but has since been adapted for the MBT in horses to study degradation kinetics in the small intestine (McLean et al., 1999b) and the total tract (Hyslop et al., 1998; Moore-Colyer et al., 2002). These studies reported that the MBT can be successfully used to determine the DM degradation kinetics in horses. Using this method and based on the assumption that a constant part of the potential degradable fraction is degraded per time unit (1st order kinetics), the DM degradability can be described by a non-linear model fitted to the degradation curve:

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

Where D_t = the potential degradability after time *t*, *a* = the intercept of degradation at Y-axis at time 0 (soluble and completely degradable fraction, washed out of the bag), *b* = the potential degradation of a component (the insoluble but potentially degradable), *c* = the rate constant for the degradation of *b* (% per h), and *e* = exponential (Figure 11). Further, the potential degradable fraction of a feed is expressed as *a*+*b*. Other methods have been developed taking the lag phase into the account (Dhanoa, 1988) and the non-linear models (Vieira et al., 1997; López et al., 1999). However, the model by Ørskov and McDonald (1979) is commonly used and the one this thesis will focus on.

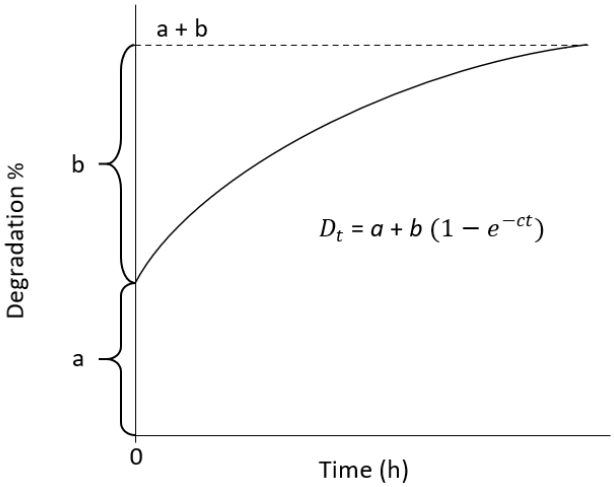

Figure 11. Illustration of a dry matter degradation curve adapted from Weisbjerg et al. (1990).

The soluble fraction (*a*) of a feedstuff can be determined by bag washing. Yet some small insoluble particles may be lost at time 0. However, the potential degradable fraction (*a*+*b*) is built on the assumption that, all particles washed out, is rumen degradable (Weisbjerg et al., 1990), thus in horses, hindgut degradable. The potential error due to losses of particle mass can be estimated by measuring true water solubility (e.g., over filter paper), after which particle loss can be estimated as the difference between *a* and true solubility. Further, this could allow for corrections based on particle losses (Hvelplund and Weisbjerg, 2000).

Despite the possibility of estimating the potential degradability of feedstuffs, Dt does not consider the fractional rate of digesta passage through the GIT. However, the effective degradability (ED) includes an additional outflow rate (*k*):

$$\mathrm{ED} = a + \left(\frac{bc}{c + k}\right) \qquad Equation\ 5$$

By including the outflow rate, several feedstuffs can be compared as it allows for a common time scale. In horses, studies often use the outflow rates 0.10, 0.05, and 0.025% per h to obtain DM degradation equal to MRTs of 10, 20, and 40 h (Hyslop et al., 1999; McLean et al., 1999c; Moore-Colyer et al., 2002). The ED depends on the outflow rate, and the lower outflow rate (longer retention) of digesta in the GIT the greater ED.

1.6.3 Comparison of the mobile bag technique and total faeces collection

The MBT aims to determine individual feedstuffs' digestibility and be an alternative to ATTD determined by quantitative collection. However, studies validating the MBT against the TFC in horses are scarce. Rodrigues et al. (2012) reported similar total tract DM disappearance and ATTD for coastcross hay (53 and 51%, respectively). Their findings indicate that the MBT underestimates the NDF and ADF ATTD (Table 10). However, the ATTD of NDF (72%) and ADF (69%) were probably inaccurate, as the ATTD of DM was 51%.

Table 10. The apparent total tract digestibility (ATTD, %) and mobile bag disappearance (%) of dry matter (DM), neutral detergent fibre (NDF), and acid detergent fibre (ADF) of coastcross hay.

	Araujo et al. (2000)		Rodrigues et al. (2012)	
Nutrient	ATTD	Disappearance	ATTD	Disappearance
DM	44[a]	45[a]	51[a]	53[a]
NDF	46[a]	37[b]	72[a]	43[b]
ADF	35[a]	17[b]	69[a]	42[b]

[a, b] Values within a row for each reference are different if superscript differs ($P<0.05$).

Araujo et al. (2000) reported greater ATTD of NDF and ADF than disappearances (Table 10). Horses were fed 22 g DM/kg BW/day of coastcross hay, and the mobile bags allocated in the stomach contained 20 mg/cm^2 coastcross hay (1 mm) with a porosity of 60.3 µm. A possible explanation for the difference in digestibility between methods may be that the mobile bags' feed residue was pooled after each horse, independent of collection time, thereby leaving out a possible time effect. Based on the studies presented above, further research is needed to validate the MBT against the TFC in horses.

2 Background

The equine gastrointestinal tract (GIT) has acclimated to continuous consumption of pasture and fibrous plants (Janis, 1976). This starts in the mouth where saliva production is activated by chewing and continues to the stomach where gastric acid is continuously produced. The small intestine has a short mean retention time (MRT, 3 h) with limited amylase concentration and the absence of a gall bladder, resulting in continuous secretion of bile acid and enzymes into the duodenum (Van Weyenberg et al., 2007; Merritt and Julliand, 2013). Finally, the horse has a large hindgut with a highly specialised microbiota for fibre fermentation, resulting in short-chain fatty acids (SCFA) as an end-product (Julliand and Grimm, 2016). Yet, the hindgut microbiota is sensitive towards the diet's nutritional composition (Julliand et al., 2006). Fermentation of starch is associated with increased lactate and total SCFA concentration, and additionally, with alterations in the proportion of the individual SCFA with increased propionate at the expense of acetate (Julliand et al., 2001; Medina et al., 2002). This results in a decreased pH and changes in the microbial composition with an increase in the starch-utilizing bacteria compared to fibre fermenting bacteria (Julliand et al., 2001; Medina et al., 2002). Additionally, starch intake is associated with gastrointestinal disorders such as gastric ulcers (Luthersson et al., 2009), colic (Hudson et al., 2001), insulin dysregulation (Durham et al., 2019), and laminitis (Garner et al., 1975). Thus, the way we keep horses today challenges the GIT. Therefore, to maintain a healthy microbiota and thereby a healthy horse, a maximum of 2 g starch/kg body weight (BW)/meal is suggested (Julliand et al., 2006) with further minimum recommendation of 15 g dry matter (DM)/kg BW/day derived from forage (Harris et al., 2017). However, for easy keepers and horses at maintenance, it can be difficult to meet the DM recommendation without exceeding the horse's energy demand. For high-performing horses (growth, gestation, lactation, and hard work), though, it can be difficult to fulfil their energy demand without exceeding the suggested starch recommendation. Therefore, feedstuff evaluation addressing both chemical composition and digestion through the GIT is important.

The in-vivo method total faeces collection (TFC) is interpreted as the "gold standard" in equine digestibility trials. This method provides information on a nutrient's apparent total tract digestibility (ATTD); however, it is limited to a total ration and concerns the whole digestive tract. Another method to determine nutrient digestibility is the mobile bag technique (MBT). This method allows for determination of individual feedstuffs' nutrient digestibility and, additionally, with use of cannulated horses, digestibility in various segments of the GIT (Hyslop, 2006). If the MBT and cannulated horses are combined with feed degradation kinetics, it can provide essential knowledge on degradation rate within different segments of the GIT (Hyslop et al., 1998; McLean et al., 1999b; Moore-Colyer et al., 2002). However, some European countries like France, have banned cannulation of

horses. Thus, alternative methods, validated against the TFC method, are needed for feedstuff evaluation in horses.

2.1 Objective and hypothesis

The main objective of the thesis was to investigate starch and fibre digestion in various segments of the equine gastrointestinal tract using different methods. Three experiments were conducted, and the following hypotheses were tested:

- The mobile bag technique can predict the digestibility of individual feedstuffs in different segments of the gastrointestinal tract, and hence estimate the total ration digestibility.
- Digestibility data from mobile bags can be used to model digestibility kinetics of individual feedstuffs in different segments of the gastrointestinal tract.
- Processing of grains affects the site of starch digestion in the gastrointestinal tract, thereby affecting the metabolic and digestive responses in the plasma and caecum, respectively.

3 Material and Methods

Table 11. Summarization of the experimental design with objective and methods for the three **papers** (**I**, **II**, and **III**).

Experimental design	Objective	Horses (*n*)	Adaptation (days)	Data collection (days)	Diet	Methods	Measurements	**Paper**
Longitude	To evaluate the MBT[1] in horses by use of nutrient disappearance and degradation kinetics for hay in comparison to the ATTD[2].	5	14	4	Hay	TFC[3], MBT.	ATTD, nutrient disappearance, transit time.	**I**
Cross-over	To evaluate the effect of substituting hay with alternative fibrous feedstuffs on nutrient digestibility and degradation kinetics.	4	14	4	Hay Hay + fibrous supplement[4]	TFC, MBT, External marker.	ATTD, nutrient disappearance, transit time, and mean retention time.	**II**
4x4 Latin square	To compare the effects of micronizing and toasting on starch digestion of barley and maize.	4	8	2	Hay + MM[5] Hay + MB Hay + TM Hay + TB	MBT, blood, caecal fluid, pH, processing.	Nutrient disappearance, glucose and insulin responses, SCFA[6] concentration, pH over time.	**III**

[1] MBT, mobile bag technique.
[2] ATTD, apparent total tract digestibility.
[3] TCF, total faeces collection.
[4] Alfalfa pellets, grass pellets, oat hulls, soya hull pellets, and sugar beet pulp pellets.
[6] MM, micronized maize; MB, micronized barley; TM, toasted maize; TB, toasted barley.
[7] SCFA, short-chain fatty acids.

Table 12. Overview of the statistical analyses executed in the three **papers** (**I**, **II**, and **III**).

Model	Response	Predictors	Means and variation	**Paper**
Linear regression	Degradation parameters.	Feed to surface area and size.	Least square means ± standard deviation.	**I**
3-way ANOVA[1]	Disappearance or ATTD[2].	Time, method, and diet.	Least square means ± standard deviation.	**II**
2-way ANOVA	Effective degradability and degradation to time *t*.	Time and feedstuff.	Least square means ± standard deviation.	
ANOVA	Disappearance.	Feed, treatment, time (DM[3]), or time-interval (starch) and interactions.	Least square means ± standard error of mean.	**III**
ANOVA	Glucose or insulin.	Feed, treatment, and interactions.	Least square means ± standard error of mean.	
Mixed models for repeated measurements	SCFA[4] or pH.	Fixed effect of feed, treatment, time and their interaction and random effect of horse.	Least square means ± standard error of mean.	

[1] ANOVA, analysis of variance.
[2] ATTD, apparent total tract digestibility.
[3] DM, dry matter.
[4] SCFA, short-chain fatty acids.

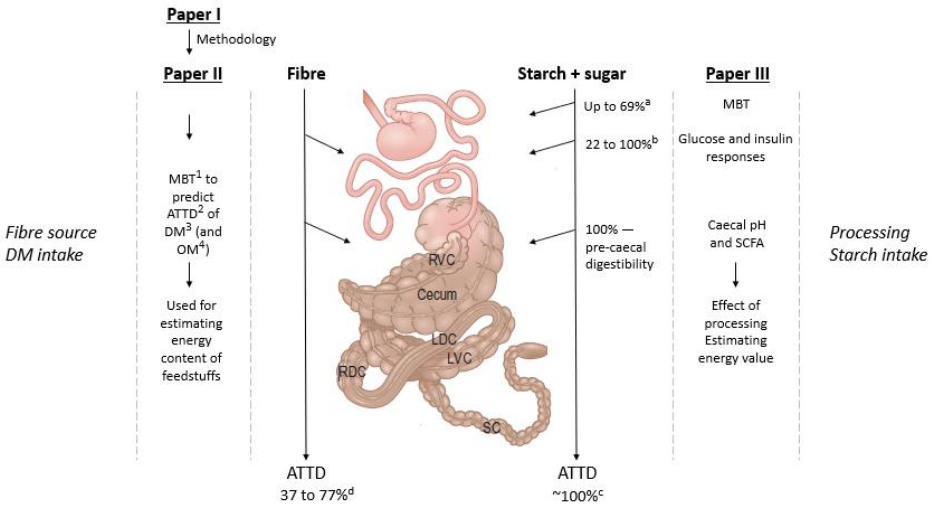

Figure 12. Overview of fibre and starch digestibility through the gastrointestinal tract illustrated by solid arrows. Within each paper the aim and methods are presented between the dashed lines. The largest impactors on fibre (left) and starch (right) digestibility listed in italics. The gastrointestinal tract figure is modified from Merritt and Julliand (2013). [1]MBT, mobile bag technique; [2]ATTD, apparent total tract digestibility; [3]DM, dry matter; [4]OM, organic matter. [a]Varloud et al. (2004); [b]Meyer et al. (1995); de Fombelle et al. (2004); Brøkner et al. (2012a); Moore-Colyer et al. (2006); [c]McLean et al. 1999a; Jensen et al. (2014); [d]Palmgren Karlsson et al. (2002); Ragnarsson and Lindberg (2008).

4 Summary of papers

4.1 Paper I

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

Total faeces collection is considered the "gold standard" for estimating apparent total tract digestibility (ATTD) in horses. However, the evaluation is limited to a total ration and the whole gastrointestinal tract (GIT). The rationale for performing this study was that the mobile bag technique (MBT) can provide information on individual feedstuffs' degradation, and the use of cannulate animals provides additional information on degradation in individual segments of the GIT. The MBT is well-established in ruminants, but methodical studies for using the MBT in horses are limited. 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 dry matter (DM) degradation as estimated by the MBT is equal to the ATTD of DM. Furthermore, we hypothesised that bag size has no effect on nutrient disappearance, but that 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 (Dt) were determined. The results of the study showed that the ATTD of DM, organic matter (OM), neutral detergent fibre (NDF), and acid detergent (ADF) can be predicted based on their disappearance from the mobile bags. The TT for the bags was 29.2 h, and when using a mean retention time of 30 h to predict ED and Dt, it was clear that ED was underestimated, whereas Dt 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 DM disappearance, but the FSA should be kept below 20 mg/cm^2 as higher levels might limit degradation.

4.2 Paper II

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

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 using 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 adaptation days 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 pellets and 6–12 bags (height × length × side; 1 × 12 × 2 cm; 37 µm pore size; 0.5 g feed) of each feedstuff and ytterbium (Yb) were placed in the stomach or caecum, respectively. Bags were harvested from the caecum every hour and faeces were checked for bags every 4^{th} h, collection time was noted, and data from the bags were used to estimate pre-caecal, hindgut, and total tract nutrient disappearance. Further, faecal subsamples were collected, weighed, and stored for Yb analysis and further estimation of feed mean retention time (MRT). Rate and extent of feed degradation were estimated from the MBT. The ATTD of DM was similar between the two diets, but the HAY diet had higher ATTD of neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash (aNDF), and acid detergent fibre (ADF). The hindgut MRT for Yb was longer for the MIX than the HAY diet. No differences for DM, aNDF, or ADF digestibility were measured when comparing the ATTD with nutrient disappearance using bags found in the time interval of 20–30 h, indicating that the ATTD of these nutrients can be predicted by the MBT. The estimated degradation (Dt), but not effective degradation (ED), is preferred when the MBT is used to predict the ATTD. In conclusion, hay can be substituted partly by fibrous feedstuffs and the MBT can predict the ATTD of DM, aNDF, and ADF in a mixed ration based on MBT measures on individual feedstuffs.

4.3 Paper III

The effects of processing barley and maize on metabolic and digestive responses in horses

The competition for customers increases the search for new grain processing methods for equine feeds, but the effect on starch digestibility and metabolic responses varies. Therefore, to evaluate the effect of the processing methods, toasting and micronizing, on starch digestion and the effect on metabolic responses, the mobile bag technique (MBT) and plasma glucose and insulin concentrations in the blood were used to estimate nutrient disappearance and metabolic responses pre-caecally. The pH in caecum and short-chain fatty acid (SCFA) concentrations were used to estimate the metabolic response in the caecum. Four caecally cannulated horses (body weight (BW) 565 ± 35 kg) were used in a 4 × 4 Latin square design with four periods of 8 days of diet adaptation and 2 days of data collection. Diets were formulated using hay and processed grains: micronized barley (MB), toasted barley (TB), micronized maize (MM), and toasted maize (TM), and balanced to provide 1 g starch/kg BW in the morning meal. On day 9 in each period, blood and caecal fluid samples were taken before the morning meal and hourly thereafter for 8 h. On day 10 in each period, 15 bags of either MB, TB, MM, or TM (height × length × side; 1 × 12 × 2 cm; 15 µm pore size; 1 g feed) were placed in the stomach. The dry matter disappearance from the mobile bags was highest for the MM at all time points compared with the other feedstuffs. Maize and micronizing had the highest starch disappearance compared with barley and toasting. No treatment effect was measured for any of the glucose and insulin parameters. No feed effect was measured for the insulin parameters. Plasma glucose peaked later for maize than for barley, and TB had a larger area under the curve for glucose than MB, MM, and TM. The concentration of total SCFA increased after feeding, with a higher concentration for barley than for maize. No treatment or feed effects were measured for pH, but time affected pH that decreased after feeding. In conclusion, toasting was not as efficient as micronizing to improve pre-caecal starch digestibility; therefore, the preferred processing method for both barley and maize is micronizing. Further, the amount of starch escaping enzymatical digestion in the small intestine was higher than expected.

5 Discussion

The present study aimed to investigate fibre and starch digestibility determined using different methods originally developed for ruminants but adapted to horses. Other experiments have often used the "gold standard" method of TFC. However, to investigate several feedstuffs simultaneously and in different segments of the GIT, the MBT is of great importance. This method, however, needs to be further developed for horses and hence validated against the TFC. The experiments in this thesis were conducted on caecal cannulated horses, and even though differences between them and intact horses may occur, these horses were of great importance, as they provided the opportunity to gain in-depth knowledge on digestive parameters in various segments of the GIT. This discussion will focus on the main results from the three **papers** (**I, II,** and **III**).

5.1 Experimental diets

For the daily forage intake, the recommendation is 15 g DM/kg BW/day with a minimum of 12.5 g/kg BW/day (Harris et al., 2017). The diet provided in **paper I**, 12.3 g/kg BW/day, was slightly below the minimum recommendation whereas in the forage:grain diet in **paper III**, hay provided 14.2 g/kg BW/day of the total DM intake closer to the recommendations (Table 13). The DM intake can influence the MRT (Clauss et al., 2014; Miyaji et al., 2014) and possibly digestibility (Pagan et al., 1998). Thus, Clauss et al. (2014) reported a longer MRT with a very low DM intake (7.3 g/kg BW/day) than at ad libitum intake (MRT of 38 and 23 h) and a lower ATTD of DM (34 and 48%). However, when DM intake was just below or above the recommendations (13.4 and 18.3 g/kg BW/day, respectively), no difference in forage DM ATTD or MRT was recorded (Clauss et al., 2014). For **paper II**, the daily DM intake of hay for the mixed diet was low (8.4 g/kg BW/day), but the diet was supplemented with other fibrous feedstuffs (6.7 g/kg BW/day). The DM recommendation does not account for the diet's chemical composition; hence it is independent of the fibre (e.g., NDF) and WSC contents for the forage. Therefore, the chosen DM intakes in **papers I**, **II** and **III** were considered reasonable. The WSC content was greatest for the forage used in **paper I** (114 g/kg DM) compared to the forages in **papers II** and **III** (74 and 85 g/kg DM, respectively) (Table 13). For the daily WSC intake, no recommendations are established, however, forage WSC content is suggested to be less than 6-10% of DM for horses suffering from equine metabolic syndrome (EMS) and insulin dysregulation (Frank et al., 2010; Ringmark and Jansson, 2013). Hence, integrating the DM recommendations of 15 g/kg BW/day for forage and the suggested forage WSC content, the resulting daily WSC intake will be 1-1.5 g/kg BW. The diets fed in **paper III** contributed with less (Table 13) than the tentative maximum recommendation. Harris et al. (2013) questioned whether the maximum starch recommendation should include the WSC and thereby recommend a daily NSC intake. Supposing that the daily NSC maximum recommendation is 1-1.5 g/kg BW the diets provided in **paper III** (~2.6 g NSC/kg BW/day)

exceeded this tentative maximum recommendation of daily NSC intake. In practise this may be difficult to implement, as it requires a chemical analysis of the forage, and that is not common among horse owners.

Table 13. Daily nutrient[1] intake (g/kg body weight (BW)) for the diets applied in **papers I**, **II**, and **III** given in means[2].

	Paper						
Nutrient	**I**	**II**		**III**[3]			
	Hay	Hay	MIX[4]	MM	MB	TM	TB
DM	12.3	15.1	15.1	15.6	15.6	15.7	15.7
Starch	-	-	0.25	1.39	1.39	1.37	1.34
WSC	1.4	1.01	0.90	1.25	1.25	1.27	1.26
NSC	1.4	1.01	1.15	2.64	2.64	2.64	2.60
aNDF	7.87	9.27	8.76	8.91	8.97	8.97	9.01
ADF	4.57	4.71	4.77	4.92	4.89	4.94	4.91
DF	-	8.76	9.03	-	-	-	-
T-NSP	-	7.31	7.61	-	-	-	-
I-NSP	-	6.85	6.55	-	-	-	-
S-NSP	-	0.46	1.07	-	-	-	-

[1] DM, dry matter; WSC, water soluble carbohydrates; NSC, non-structural carbohydrates; aNDF, neutral detergent fibre assayed with heat-stable amylase and expressed including residual ash; ADF, acid detergent fibre; DF, dietary fibre; T-NSP, total non-starch polysaccharides; I-NSP, insoluble non-starch polysaccharides; S-NSP, soluble non-starch polysaccharides.

[2] Standard deviation of mean (SD) is presented in **papers I** and **II** and standard error of mean (SEM) in **paper III**.

[3] Hay plus micronized maize (MM) or micronized barley (MB) or toasted maize (TM) or toasted barley (TB).

[4] MIX = 8.4:6.7 g DM/kg BW/day of hay and supplement (alfalfa pellets, grass pellets, oat hulls, soya hull pellets and sugar beet pulp pellets), respectively.

5.2 Analytic methods for classifying fibre

The chemical fractionation of the fibre content in feedstuffs can be analysed using several methods (Table 13). The detergent fibre method by Van Soest et al. (1991) determines the fibre fraction as NDF, ADF and ADL. However, it excludes the soluble fibre fraction (Bach Knudsen, 2001) and thereby underestimates the total NSP content. The importance of a broad NSP approach when evaluating fibrous feedstuffs was clear in **paper II** where the fibre content, analysed using the Van Soest (1991) method, was underestimated for SBP pellets and soya hull pellets, as these feedstuffs had a large S-NSP fraction. This aligns with Brøkner et al. (2012b) who reported an underestimation of the fibre fraction for feedstuffs with a high content of S-NSP as SBP (DF 728 g/kg DM and NDF 347 g/kg DM). On the other hand, differences between analyses were less noticeable for feedstuffs with little or no S-NSP e.g., straw (DF 944 and NDF 815 g/kg DM) (Brøkner et al., 2012b). Similarly, Jensen et al. (2014) measured comparable fibre fractions for hay with 683 g DF/kg DM and 685 g NDF/kg DM and likewise higher DF than NDF for molassed sugar beet pulp (MSBP, 499 and 331 g/kg DM, respectively). The importance of the DF analysis has

previously been stressed by Bach Knudsen (2001). These findings imply that the fibre content of novel fibrous feedstuffs or in a scientific matter ought to be analysed by the DF method and include both the I-NSP and S-NSP fraction.

5.3 Factors affecting starch digestion

5.3.1 Starch intake

The starch intake has been discussed previously for its effect on pre-caecal starch digestibility and for its potential to cause health issues like gastric ulcers (Luthersson et al., 2009), laminitis (Garner et al., 1975), and colic (Hudson et al., 2001). The recommendations for maximum starch intake in horse diets have developed from 3.5-4 g/kg BW/meal (Potter et al., 1992) and then decreased to 2 g/kg BW/meal (Kienzle, 1994; Julliand et al., 2006) to avoid by-pass starch to the hindgut. Moreover, Coenen et al. (2011) preliminarily suggested 1 g starch/kg BW/meal, which is consistent with recommendations by Harris et al. (2013). In **paper III**, starch intake was reduced to 1 g starch/kg BW/meal, but despite this, up to 0.3 g/kg BW/meal starch by-pass was measured by the MBT. To reduce the odds ratio of getting gastric ulcers, a maximum of 1 g/kg BW/meal was suggested (Luthersson et al., 2009). Similarly, Vervuert et al. (2009a) recommended a maximum of 1.1 g/kg BW/meal to horses with insulin dysregulation. The recommendation for the maximum starch intake should acknowledge all health aspects including gastric ulcers, glucose and insulin responses, and limit by-pass starch to the hindgut. Hence, based on existing literature, it can be concluded that a maximum of 1 g starch/kg BW/meal should be recommended to avoid the above-mentioned health issues, and **paper III** supports this recommendation in relation to the metabolic and digestive responses measured.

5.3.2 Processing of grains

The main aim when processing grains for horses is to increase pre-caecal starch digestion. When comparing the pre-caecal starch digestion of whole untreated grains with processed grains, effects are consistent (Meyer et al., 1995; Philippeau et al. 2014). On the other hand, the findings of studies comparing different thermo-mechanical processing methods are inconsistent (Rosenfeld and Austbø, 2009; Philippeau et al., 2014). Therefore, whole barley and maize were not included in **paper III**. The aim was instead to study differences on pre-caecal starch digestibility between the processing methods of micronizing and toasting. Although toasting has not previously been investigated in horses, micronizing has. Rosenfeld and Austbø (2009) found higher pre-caecal starch digestibility of micronized grains than extruded (85 and 70%, respectively). In pigs, Canibe and Knudsen (1997) reported lower pre-caecal starch digestibility of peas when toasted than dried. This corroborates **paper III**, where micronizing resulted in a greater pre-caecal starch digestibility compared to toasting. Conversely, the content of

rapidly digestible starch is reported to increase with higher processing temperatures (83 to ~137˚C) for barley and maize (increased 18 and 11% of DM) (Murray et al., 2001). In the present study grains, when toasted, were subjected to a surface temperature of 150˚C, approximately 53˚C higher than micronizing (Table 14). Moreover, increased moisture content increases DG (Liu et al., 2019). The DG for the grains when toasted were lower than when micronized despite having a greater moisture content prior to toasting (22%) than micronizing (13%). This indicates that the processing conditions were not optimal for gelatinization when toasting. Additionally, maize had a greater DG than barley (**paper III**). Similar results are reported by Vervuert et al. (2004, 2007) for whole and processed maize and barley (except popped: 88 and 96%, respectively). Maize had a higher pre-caecal starch digestibility than barley (**paper III**), which aligns with findings by Hymøller et al. (2012). Likewise, Meyer et al. (1995) reported numerically higher pre-caecal digestibility for crushed maize than barley (30 and 22%, respectively). Conversely, Rosenfeld and Austbø (2009) measured similar pre-caecal starch digestibility between barley and maize (71 and 66%, respectively). Overall, this indicates that processing details are important when comparing studies, and moreover that different grains may respond differently to diverse processing methods. There is a need for comprehensive studies including processing details (temperature, moisture, and duration) and DG when starch digestibility is investigated in horses.

Table 14. Processing details (temperature (temp., ˚C), duration (seconds, sec) and moisture content (%)) of micronizing and toasting used in **paper III** and earlier studies[1].

Reference	Type of processing	Processing details			Heat source	Particle size, mm
		Temp.	Duration	Moisture		
Paper III	Micronizing	90-105	45	13.4[2]	IR[3]	0.15
Studies[1]		80-130	40-60	18-21	IR	
Paper III	Toasting	150	1800	21.6[2]	Steam	0.35-1
Studies[1]	Toasting[4]	100-140	60-300	+	Steam	
	Toasting[5]	90-105	1800-2700	+	Steam	

[1] Van der Poel (1990), Svihus et al. (2005), and Newton (2020).

[2] Average moisture content between maize and barley within each processing method.

[3] IR, infrared radiation.

[4] Pressure toasting.

[5] Conventional toasting.

5.4 Glucose and insulin responses

One hypothesis in this thesis was, that processing grains affect the site of starch digestion in the GIT, thus affecting the metabolic responses in plasma. Increasing the pre-caecal starch digestibility in horses should be reflected in increased plasma glucose and insulin responses (Svihus et al., 2005; Julliand et al., 2006). An effect of time on glycaemic and insulinaemic responses in plasma was measured in **paper III**, but the effect of processing was not clear. The glycaemic responses have been used as an indirect measure of the pre-caecal starch digestibility (Hoekstra et al., 1999; Vervuert et al., 2004, 2008). However, a disjoint between pre-caecal starch digestion and glucose responses has been reported (Hintz et al., 1971ab; Philippeau et al., 2014), which is consistent with the results from **paper III**. Philippeau et al. (2014) reported no connection between glucose responses, and greater DG or pre-caecal starch digestibility of processed barley, and Vervuert et al. (2003, 2004) reported no differences in plasma glucose and insulin responses between whole and processed oats, maize, and barley, regardless of a numerically greater DG for processed grains. Vervuert et al. (2007) reported that popped barley had higher numerical DG (96%) than steam-flaked (29%) and whole (15%) barley, however, this was not reflected in plasma glucose AUC. In **paper III**, an interaction between feedstuff and processing was measured for the glucose response with toasted barley (TB) having a greater AUC than toasted maize (TM). Factors like DG, gastric emptying and/or meal size could have caused this effect as discussed in **paper III**. These results highlight the difficulties of using plasma glucose and insulin responses solely for evaluating the effect of processing grains.

The results presented in **paper III** are novel as this is the first paper to include both feed characteristics, starch disappearance measured with the MBT, plasma responses and caecal pH and SCFA concentrations. In theory, increasing starch digestion in the small intestine will increase plasma glucose and insulin responses, thus, less starch should reach the caecum and be fermented. Data from **paper III** support this, as there tended to be a positive correlation between $AUC_{glucose}$ and minimum pH in the caecum (Figure 13, a). Furthermore, there tended to be a positive correlation between DM disappearance from mobile bags and caecal pH when correlating bags found at specific time point with the corresponding pH values at that time (Figure 13, b). Fermentation of starch in the stomach is another factor that might affect the measured responses in plasma and caecum. It is unknown to what extent starch is fermented in the stomach (**paper III**), but gastric fermentation of starch and sugar has been reported up to 76% (Varloud et al., 2004). If starch is fermented in the stomach less starch will be available for digestion in the small intestine resulting in a lower glycaemic response than expected.

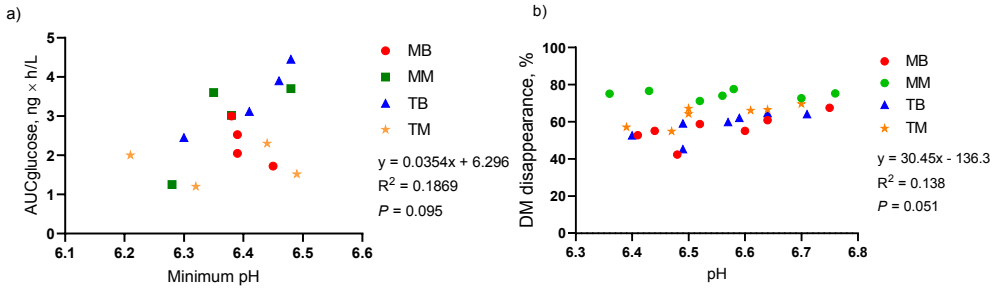

Figure 13. **a)** Linear correlation between minimum pH in caecum and area under the curve (AUC) for plasma glucose. **b)** Linear correlation between hourly pre-caecal dry matter (DM) disappearance (%) (measured the second day of data collection) and corresponding average pH for individual feedstuffs (TM, toasted maize; MM, micronized maize; TB, toasted barley; MB, micronized barley, measured at the first day of data collection).

Plasma glucose and insulin responses were not investigated in **papers I** and **II**, although forage NSC affects the plasma glucose and insulin concentrations (Borgia et al., 2011; Lindåse et al., 2018). Besides the glucose absorbed in the small intestine, the fermentation end-products propionate and to a lesser extent valerate, are important in regulating the plasma glucose concentration through hepatic gluconeogenesis (Ford and Simmons, 1985). Propionate produced in the hindgut contributes with approximately 50% blood glucose when horses are fed hay (Simmons and Ford, 1991). Therefore, forage results in a more stable plasma glucose response than diets rich in starch (Brøkner et al., 2016; Jensen et al., 2016). Out of the diets used in **papers I**, **II** and **III**, that of **paper III** (2.6 g/kg BW/day) provided higher NSC intake than that of **papers I** and **II** (1.4 and ~1 g/kg BW/day NSC, starch is assumed to be 0), thus assuming a greater plasma glucose and insulin response in **paper III**, than **paper I** and **II**.

5.5 Digestive response in caecum

As described above, responses in plasma glucose and insulin of starch digestion are difficult to interpret. Therefore, combining plasma parameters with digestive responses such as pH, the concentration of SCFA, and their individual proportions can provide a more comprehensive approach to the interpretation.

5.5.1 Caecal pH

If starch by-passes the enzymatic digestion, alterations in SCFA and pH can be detected in the hindgut (Hintz et al., 1971a; Julliand et al., 2001; Medina et al., 2002; de Fombelle et al., 2003). The importance of starch intake (g/kg BW/meal) on hindgut pH is stressed by Julliand et al. (2006). Increasing the starch intake decreases caecal pH, as illustrated in Figure 14. Different methodologies for measuring caecal pH can affect the results. In

paper III the caecal pH was higher when measured in-vivo by a pH-electrode than in collected caecal samples (Figure 15). Næsset et al. (2018) suggested that the alkaline digesta from the ileal influx increased the pH in the caecal top layer when measuring caecal pH at two locations in the caecum (unweighted pH-electrode vs. a weighted pH-electrode). Sampling site may be the explanation for the high pH values measured in the caecum regardless of a high starch intake (3.4 g/kg BW/meal) by Medina et al. (2002) (Figure 14).

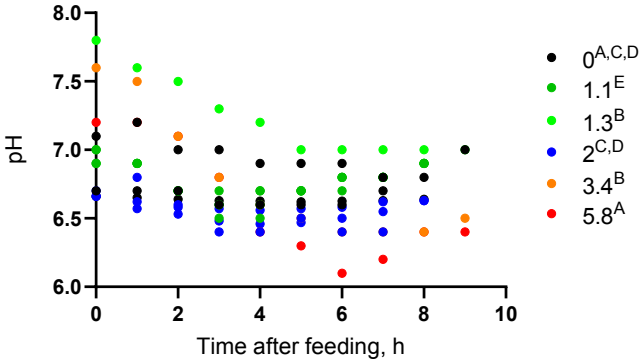

Figure 14. Caecal pH after feeding different starch intakes (g/kg body weight (BW)/meal) obtained by data from [A]Willard et al. (1977), [B]Medina et al. (2002), [C]Brøkner et al. (2012a), [D]Jensen et al. (2016), and [E]**paper III**.

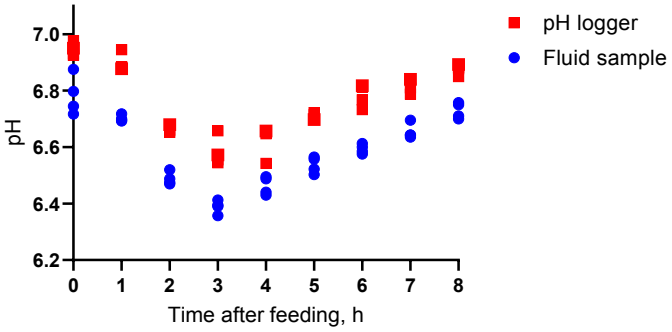

Figure 15. Comparison between pH measured with a pH-electrode in caecum (pH logger) and caecum fluid samples when feeding four diets (1.1 g starch/kg body weight (BW)/meal, **paper III**).

Forage results in a higher caecal pH compared to starch-rich meals (Willard et al., 1977; Julliand et al., 2001; Brøkner et al., 2012a; Jensen et al., 2016). Although no pH measurements were performed in **papers I** and **II**, when horses were fed forage, the pH in caecum is assumed to have been higher than measured in **paper III**. Furthermore, a more stable pH is assumed for forage diets (**papers I** and **II**) compared with the barley and

maize diets provided in **paper III**, as reported in earlier studies (Willard et al., 1977; Brøkner et al., 2012a; Jensen et al., 2016).

In the present study, the minimum pH (~6.4) in **paper III** was reached approximately 3 h after feeding (Figure 15), independent of diet. This aligns with McLean et al. (2000), Brøkner et al. (2012a), and Jensen et al. (2016) when horses are fed barley at 2 g starch/kg BW/meal. Further, a preliminary study reported lower minimum pH in the caecum when horses were fed 2 g than 1 g starch/kg BW/meal (6.2 and 6.4, respectively) (Jensen et al., 2012). In the present study, the minimum pH was below 6.7 for all diets and possibly impaired growth of cellulolytic bacteria with negative impact on fibre digestibility (Van Soest, 1994). However, the caecal pH was at all times greater than 6, which is stated as a critical threshold for normal function of the hindgut (Philippeau et al., 2009).

5.5.2 Caecal pH and blood parameters

Brøkner et al. (2012a) reported an effect of diet on both mean and minimum pH when feeding forage or starch-rich diets, however plasma glucose and insulin (AUC and peak) was not different when similar diets were tested in Brøkner et al. (2016). The diets provided in **paper III** did not evoke any differences between diets on caecal pH and only to a limited extent on plasma glucose-insulin responses and as discussed (5.4 Glucose and insulin responses), alterations in glycaemic responses can be difficult to detect despite differences in pre-caecal starch digestibility and hindgut pH. Inconsistency between plasma glucose and insulin responses and other parameters as pH (**paper III**), pre-caecal starch digestibility (Philippeau et al., 2014; **paper III**), DG (Vervuert et al., 2007; Philippeau et al., 2014; **paper III**), and SCFA concentration in the hindgut (Jensen et al., 2016; **paper III**) confirms that plasma glucose and insulin responses should not be used solely to evaluate feedstuffs digestible potential.

5.5.3 Concentration of short-chain fatty acids in caecum

Changes in the caecal SCFA concentration have either been analysed hourly (Willard et al., 1977; Jensen et al., 2016; Warzecha et al., 2017), as average concentration in time intervals (Moore-Colyer et al., 2000), or as single measurements of concentration (Argenzio et al., 1974; Medina et al., 2002; de Fombelle et al., 2003). The latter discounts possible changes in the fermentation pattern over time. In **paper III**, samples of SCFA were collected before the morning meal (time 0) and then hourly after, but only analysed at 0, 1, 3, 5 and 7 h. This was done to decrease expenses, yet the data was sufficient to detect changes over time, which correspond with Moore-Colyer et al. (2000). In the present study (**paper III**), the total SCFA increased after the meal of processed grains and decreased again over time (Figure 16), which aligns with literature (Jensen et al., 2012; Jensen et al., 2016; Warzecha et al., 2017). Although no differences were found for pH,

barley resulted in higher total SCFA concentration than maize, reflecting pre-caecal starch digestibility in addition to the greater pre-caecal starch digestibility for maize than barley measured by the MBT (**paper III**). Lactate concentration was not measured, but this could have supported the measurements of starch fermentation. However, Julliand et al. (2001) did not measure increased lactate concentration in the caecum when barley proportions increased in the diet. Moreover, McLean et al. (2000) only measured increased lactate in caecum when horses were fed rolled barley compared to micronized and extruded barley. Still, the lactate concentration, together with the pH, SCFA concentration and individual SCFA proportions, could have contributed to the description of starch fermentation.

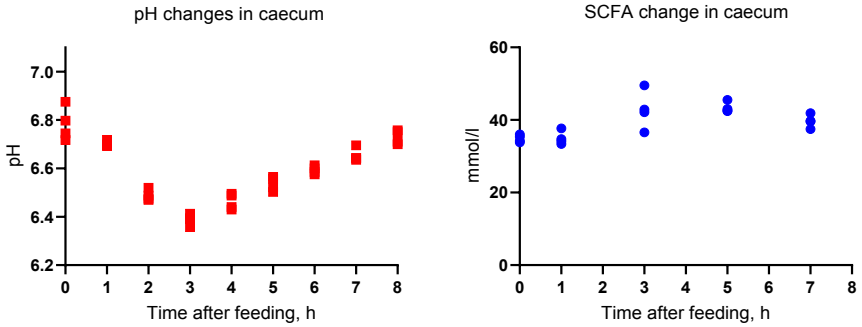

Figure 16. Changes in pH and total short-chain fatty acid (SCFA) concentration in caecum after feeding (modified from **paper III**).

5.6 Digesta passage rate

The digesta passage rate can be measured directly by a marker or indirectly by mobile bags or digestive responses, time to reach minimum pH and maximum SCFA concentration in the caecum. In **paper II**, the pre-caecal TT for mobile bags containing either hay or SBP pellets was on average 2.6 h when horses were fed a diet of 15.1 g DM/kg BW. This is shorter than reported by Moore-Colyer et al. (2002) despite a higher DM intake (>16 g DM/kg BW/day). However, as discussed in **paper II** and in this thesis several factors (processing, differences in chemical composition and larger FSA) could have caused the faster TT of the mobile bags (Hyslop and Cuddeford, 1996; Drogoul et al., 2000; Moore-Colyer et al., 2003). The SCFA concentration was not measured in **paper II**. However, in **paper III** the minimum pH was reached after approximately 3 h with a corresponding maximum SCFA concentration (Figure 16) at comparable DM intake as in **paper II** (15.6-15.7 g DM/kg BW/day). This corresponds to findings by Jensen et al. (2016) at similar DM intake (15.7 g/kg BW/day).

The hindgut TT is an important measure for evaluating feedstuff degradation kinetics and fermentation patterns. Comparing different fibrous feedstuffs' TT through the hindgut in

paper II provided information on the retention of individual feedstuffs. In this **paper** (**II**) soya hull pellets had a longer TT through the hindgut than SBP pellets. This may be explained by the physiochemical properties of the feeds (Bach Knudsen, 2001; Brøkner et al., 2012b). Nevertheless, in **paper I** the hindgut TT of mobile bags with hay was on average 29 h, comparable to the hindgut TT of mobile bags with hay in **paper II** (32 h) (Table 15). However, when measured by the indigestible marker Yb the MRT in the hindgut was faster (23 h) (**paper II**). This indicates the possibility of prolonged retention of mobile bags in the hindgut. However, the hindgut MRT of 23 h measured by Yb (**paper II**) aligns with earlier studies using Yb to determine the TMRT for horses fed solely hay (15 and 18.5 g DM/kg BW/day) (Moore-Colyer et al., 2003; Jensen et al., 2014). In this thesis, horses were kept at maintenance, whereas ponies and horses were exercised in Moore-Colyer et al. (2002) and Jensen et al. (2014), and exercise can decrease TMRT (Orton et al., 1985; Pagan et al., 1998).

Table 15. Transit time (hours, h) for mobile bags used in different segments of the gastrointestinal tract.

Reference	Feedstuff	Transit time for mobile bags		
		Pre-caecal	Hindgut	Total tract
Paper I	Hay		29.4	
Paper II	Hay	2.3	32.3	30.3
	Hay + fibrous feedstuffs[1]	2.8 (SBP)	30.8[1]	32.8[1]
Moore-Colyer et al. (2002)	Fibrous feedstuffs[2]	3.0-4.3		54.8-65.5
Hymøller et al. (2012)	Grains[3]	2.4-4.3		

[1] Alfalfa pellets, grass pellets, hay, oat hulls, soya hull pellets, and sugar beet pulp pellets.

[2] Hay cubes, oat hulls:naked oats, sugar beet pulp, and soya hulls.

[3] Flaked maize, soaked maize, cracked maize, oats, black oats, barley, and soaked barley.

5.6.1 Effect of digesta passage rate on nutrient digestibility

A prolonged MRT of digesta is hypothesized to affect the nutrient digestibility positively both pre-caecal and along the GIT, as digesta is exposed to enzymes and microbiota for a longer time. In **papers I**, **II**, and **III** there was a positive correlation between TT of mobile bags and DM disappearance. A similar effect of time was measured for starch disappearance in **paper III**. This corroborates with earlier in-situ studies (Hyslop et al., 1998, Moore-Colyer et al., 2002, Hymøller et al., 2012). However, correlations between nutrient digestion and MRT are inconsistent when measured in-vivo (Pagan et al., 1998; Drogoul et al., 2000; Miyaji et al., 2011; Clauss et al., 2014), which suggests that parameters other than MRT may affect digestibility.

5.6.2 Cannulated versus intact horses

Austbø and Volden (2006) reported an effect of cannulation on MRT, and some of those horses were included in **papers I**, **II**, and **III**. The TMRT was longer 3 and 15 months after cannulation (4.5 and 7.8 h longer, respectively) compared to pre-surgery measures (Austbø and Volden, 2006). This is consistent with Pulse et al. (1973), who reported that 50% of chromium oxide remained approximately 6 h longer 3 months post cannulation. Conversely, Drogoul et al. (2000) measured longer MRT in intact ponies compared to cannulated (7 h longer). Despite a possible effect of cannulation on MRT in the present study, the MRT values measured are in the range of non-cannulated horses (Pagan et al., 1998; Pearson et al., 2001; Moore-Colyer et al., 2003).

5.7 Methods to estimate nutrient digestibility

The TFC method is often used in digestibility trials for horses, and was the chosen method for validating the MBT in **paper I**. Although, no clear protocol is developed for TFC, recommendations are 14 days of diet adaptation followed by 3-6 consecutive collection days. In **papers I** and **II**, 14 days of adaptation followed by 4 consecutive days of TFC were instituted. The adaptation period of 14 days was chosen as the previous diet consisted of low energy hay compared to the ones provided in **papers I** and **II**. In **papers I** and **II**, the DM digestibility was stable after the second day of collection (Figure 17). This is consistent with findings by Vander Noot et al. (1967), Palmgren Karlsson et al. (2000, 2002), Goachet et al. (2009), and Jansson et al. (2006) and therefore it was concluded that 4 consecutive days of total collection was sufficient to estimate the ATTD when horses are fed a forage diet.

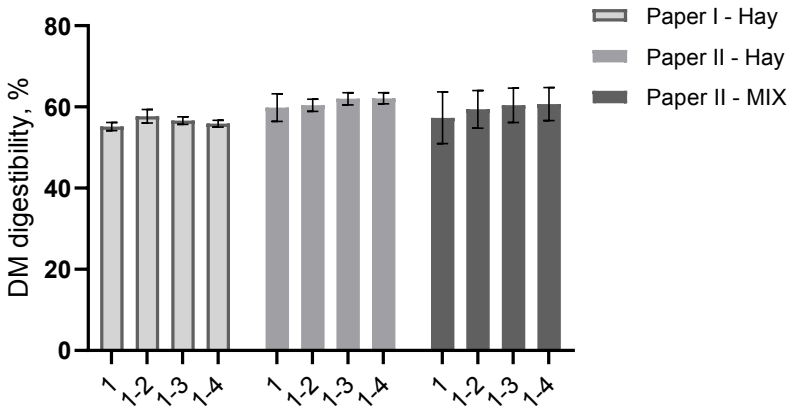

Figure 17. Mean (± SD) dry matter (DM) apparent total trat digestibility (%) for the diets provided in **paper I** (hay-only) and **paper II** (MIX: hay and supplement (alfalfa pellets, grass pellets, oat hulls, soya hull pellets and sugar beet pulp pellets)) determined by quantitative collection of daily faeces from day 1, 1 to 2, 1 to 3, and 1 to 4.

The MBT allows for determining individual feedstuffs' digestibility, it was the chosen method in **papers I**, **II** and **III**. It was necessary to validate it against the TFC (**paper I**) because earlier studies have reported inconsistent effects of bag size (Cherian et al., 1989; Hyslop and Cuddeford, 1996), pore size (Varvikko and Lindberg, 1985; Varvikko and Vanhatalo, 1989; Cherian et al., 1989; Jensen and Prestløkken, 2018), and FSA (Udén and Van Soest, 1984) on nutrient disappearance and TT. Therefore, three bag sizes and four FSA were chosen (**paper I**). A high FSA (>20.8 mg/cm^2) restricted the DM disappearance and underestimated the DM ATTD (**paper I**). Similarly, a higher FSA has been proven to affect nutrient disappearance negatively in heifers (Udén and Van Soest, 1984) and pigs (Cherian et al. 1989). The effect of different pore sizes was not tested in **paper I**, as the recommendations from NorFor (Åkerlind et al., 2011) were considered to be representative of the microbial degradation of fibrous feedstuff in the hindgut. When comparing the DM ATTD of hay with hindgut DM disappearance, similar results were obtained (**papers I** and **II**, Figure 18). Although enzymatical digestion was omitted for mobile bags, the nutrient fraction of hay that is potentially enzymatically degraded pre-caecal is assumed to be soluble and rapidly degradable in the hindgut, and therefore representative for the ATTD. Furthermore, in **paper I** the OM, NDF, and ADF disappearance resembled the ATTD of these nutrients. Similar results were obtained in **paper II** for hay, with the NDF and ADF disappearance for bags collected from 20-39 h after administration in the hindgut. This contradicts with findings by Araujo et al. (2000) and Rodrigues et al. (2012), who pooled bags from each individual horse. The collection time has previously been reported to affect the disappearance of nutrients (Hyslop et al., 1999; Moore-Colyer et al., 2002; Hymøller et al., 2012), and this was also reported in **paper I**. Moreover, Macheboeuf et al. (1996) stressed the importance of using physiological relevant TT for the mobile bags when predicting the in-vivo digestibility. Therefore, based on the conclusions in **paper I** and earlier studies, the feed residue from mobile bags was pooled according to collection time in **papers II** and **III**. In **paper II**, the NDF and ADF disappearances were underestimated with bags retrieved from 10-19 h and overestimated when collected 40-100 h after administration in the caecum, supporting the conclusion that time affects nutrient disappearance and thereby the representativeness of the ATTD. This thesis recommends to pool mobile bags according to their collection time instead of by horse for predicting the ATTD.

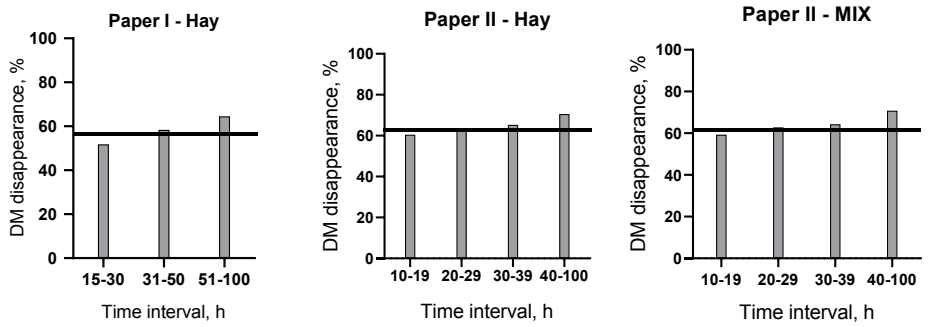

Figure 18. Comparison between the apparent total tract digestibility (ATTD, illustrated by solid line —) of dry matter (DM) and mobile bag DM disappearance to different time intervals (hours, h) obtained from **papers I** (hay) and **II** (hay, and MIX: hay + fibrous feedstuffs (alfalfa pellets, grass pellets, oat hulls, soya hull pellets and sugar beet pulp pellets)).

One major advantage that the MBT has over the TFC is its potential to yield information on rate and extent of feed degradation in different segments of the GIT. Despite this advantage, it is rarely used for feed evaluation in horses (Hyslop et al., 1998; McLean et al., 1999b; Moore-Colyer et al., 2002), and validation against the TFC is needed. Therefore, one aim of this thesis was to validate the use of degradation kinetics by Ørskov and McDonald (1979) (**papers I** and **II**) and to evaluate degradation of several fibrous feedstuffs (**paper II**) as it is commonly used and fitted the raw data. It was concluded that D_t at a biologically relevant MRT can successfully be used to predict the ATTD of DM for hay (**papers I** and **II**). Further, it was concluded that the ED was insufficient to predict the ATTD and the disappearance of DM (**papers I** and **II**). For **papers I** and **II**, the ED fitted the ATTD of DM when the outflow rates were 0.017 and 0.020% corresponding to a MRT for 60 and 50 h, respectively. This does not reflect the in-vivo MRT (Clauss et al., 2014; Jensen et al., 2014; Hummel et al., 2017). This might be a result of a limiting time range (**paper I**: 0 and 16-113 h, and **paper II**: 0 and 10-100 h, after administration) leaving out the DM degradation at the early timepoints (0 to 10-16 h). Similarly, Moore-Colyer et al. (2002) reported collection times for total tract mobile bags varying from 16-158 h after administration. As stressed by Hyslop (2006) a broad range of TT for the mobile bags are needed when calculating degradation kinetics. This is supported by findings in **papers I** and **II**. In **paper III**, the DM disappearance from the mobiles bags was not subjected to degradation kinetics by Ørskov and McDonald (1979). A preliminary study by McLean et al. (1999a) suggested that the MBT could predict the pre-caecal DM degradation in horses. The lack of data from early degradation can result in deceptive results as reported by Moore-Colyer et al. (2002) where the potential degradable fraction *a+b* was estimated

to 100% pre-caecally for oat hull:naked oats and soya hulls despite their high NDF content (~579 g/kg DM) and incomplete degradation (97%) in the total tract.

A novel finding from this thesis is presented in **paper II**, where the degradation kinetics obtained from total tract mobile bags are equal to the ATTD of DM. Therefore, it was concluded that the MBT could potentially be used in intact horses for ATTD assessment of individual feedstuffs in a mixed diet. Similarly, was concluded in a preliminary study and presented in an abstract by Hyslop et al. (1998) where the MBT was successfully used in intact horses to determine fibre degradation. Macheboeuf et al. (1996) likewise stated in their abstract that the DM disappearance in the total tract were consistent with the in-vivo digestibility of diverse feedstuffs, however, data was not presented in the abstract. By use of the MBT in intact horses the ATTD and energy value of individual feedstuffs can be quantified (5.8 Practical considerations).

5.8 Practical considerations

This thesis provides important knowledge on the possibility of replacing forage with alternative fibrous feedstuffs (**paper II**) and the effect that processing grains has on metabolic and digestive responses when feeding 1 g starch/kg BW/meal (**paper III**). Overall, this thesis (**papers I**, **II** and **III**) provides important new insights on nutrient digestibility of different feedstuffs using the MBT, knowledge that can be used to improve feedstuff evaluation in the future. The French energy evaluation system is based on OM digestibility obtained by the indigestible markers or TFC in-vivo methods, to predict the digestible energy (DE) and lastly the net energy (NE) of various feedstuffs (Martin-Rosset, 2015). Furthermore, data from ruminant studies are extrapolated to horses when in-vivo data from horses are absent. This thesis focused on digestibility of DM, NDF, and starch measured by the MBT, as limited residue was available for analysis of other nutrients (**papers II** and **III**). Dry matter digestibility is reported to be 1.6 ± 1.5 lower than OM digestibility (Drogoul et al., 2000; Palmgren Karlsson et al., 2000; Bergero et al., 2002; Ragnarsson and Lindberg 2008, 2010; Goachet et al., 2009; Jensen et al., 2010; De Marco et al., 2012; Schaafstra et al., 2018; Vasco et al., 2021; **paper I**) (9 Appendix), which is consistent with the ATTD of DM and OM in **paper I**. Moreover, as concluded in **papers I** and **II**, the MBT can predict the ATTD of DM (**papers I** and **II**) and OM (**paper I**), thus the MBT is a valuable method for screening feedstuffs for digestibility of OM and to estimate energy value. This is a novel finding, and the results from this thesis demonstrate that the MBT in combination with intact horses has the potential to improve feedstuff evaluation. This can be of great interest for the feed industry measuring energy value of individual feedstuffs or products containing various feedstuffs.

In Table 16 the D_t obtained from mobile bag data (**papers I** and **II**) were used to calculate digestible OM for horses (dOM_{horse}) (9 Appendix). This provide the basis for converting GE with use of dOM_{horse} to DE, metabolizable energy (ME), NE at maintenance (NE_m) and feed units (FU) for the individual feedstuffs used in **papers I** and **II** according to the equations described by Centraal Veevoeder Bureau (CVB, 2021). As discussed previously (**papers I** and **II**) DM degradation increased with time, hence as expected greater dOM_{horse} for all feedstuffs were estimated for a MRT of 30 h compared to 20 h (Table 16). Thus, increasing time for D_t affects a feedstuff's FU (Table 16) and hence the daily feed supply (kg DM). As an example, providing a horse with an energy requirement of 6 FU/day with the hay from **paper I**, it can result in two outcomes dependent on the MRT of the hay:

$$\frac{6\ \text{FU}}{0.61\ \text{FU/kg DM}} = 9.8\ \text{kg DM} \quad \text{or} \quad \frac{6\ \text{FU}}{0.68\ \text{FU/kg DM}} = 8.8\ \text{kg DM}$$

Consequently, to fulfil the energy requirement the daily feed supply differed with 1 kg DM. As discussed earlier (5.6 Digesta passage rate) the MRT depends on DM intake (g/kg

BW). Ragnarsson and Lindberg (2010) found approximately 3% lower ATTD of DM when horses were fed a high DM intake (18 g DM/kg BW/day) than low (10.7 g DM/kg BW/day). Therefore, the MRT of a given feedstuff is pertinence and should ideally be included in a feed evaluation system providing a comprehensive approach to predict the energy value. This allows a given feedstuff to have different energy values dependent on the MRT and chemical composition.

Table 16. Estimated digestible (DE), metabolizable (ME), net energy at maintenance (NE_m, MJ/kg dry matter), and feed units (FU) in the feedstuffs used in **papers I** and **II** obtained from digestible organic matter (dOM_{horse}, %) estimated from degradation to time (D_t) 20 and 30 hours.

Feed[1]	D_t	Time	GE[2]	dOM_{horse}[3]	DE	ME	NE_m	FU[4]
Paper I								
Hay	50.4	20	19.1	52.0	9.2	7.7	5.7	0.61
Hay	56.4	30	19.1	58.0	10.3	8.6	6.4	0.68
Paper II								
Hay	62.4	20	19.1	64.0	11.4	9.5	6.9	0.74
Hay	67.7	30	19.1	69.3	12.3	10.3	7.5	0.80
AP	62.9	20	18.6	64.5	11.2	9.4	6.7	0.71
AP	66.2	30	18.6	67.8	11.8	9.9	7.1	0.75
GP	59.9	20	18.0	61.5	10.3	8.8	6.2	0.66
GP	63.8	30	18.0	65.4	11.0	9.4	6.6	0.70
OH	51.3	20	19.2	52.9	9.4	8.1	6.2	0.65
OH	53.0	30	19.2	54.6	9.7	8.4	6.4	0.68
SBP	76.6	20	18.3	78.2	13.4	11.7	8.3	0.88
SBP	86.0	30	18.3	87.6	15.0	13.1	9.3	0.99
SHP	65.2	20	17.7	66.8	11.0	9.1	6.4	0.69
SHP	74.3	30	17.7	75.9	12.5	10.3	7.3	0.78

[1] AP, alfalfa pellets; GP, grass pellets; OH, oat hulls; SHP, soya hull pellets, and SBP, sugar beet pulp pellets.
[2] GE, gross energy (MJ/kg DM).
[3] dOM_{horse}, digestible organic matter for horses: degradation to time t (D_t) + 1.3 %.
[4] FU, feed units: net energy (NE) feed (MJ/kg DM)/NE barley (9.424 MJ/kg DM).

In this thesis, the MBT was used to measure pre-caecal starch digestion (**paper III**) and the technique could potentially also be used to measure dOM_{horse} for determining the energy value of grains and concentrates. Starch fermented to SCFA in the hindgut yields less energy than if enzymatically digested to glucose in the foregut (Harmon and McLeod, 2001), thus affecting the energy values of the grain. Site of digestion is not incorporated into the evaluation of grains in present feed evaluation systems. The most important

factors affecting site of digestion are starch intake (g/kg BW/meal) (Julliand et al., 2006; Harries et al., 2013), and processing (Meyer et al., 1995; Julliand et al., 2006; Philippeau et al. 2014). The MBT, if used pre-caecally, can quantify the amount of starch digested in the foregut (fermented in stomach plus enzymatical digested in small intestine) and fermented in the hindgut by difference calculation (**paper III**) and thus screen various feedstuffs at different starch intake (g/kg BW/meal) and processing methods. This data should then be included in the feed evaluation system. Therefore, this thesis is one out of several studies required to approach a more accurate energy evaluation system for all types of feedstuffs.

6 Conclusion

It is concluded that the MBT can predict the ATTD of DM and fibre from hay and other fibrous feedstuffs when a proper bag FSA is used. Further, the time interval for mobile bags should resemble a biologically relevant MRT to predict digestibility correctly. This also applies when modelling digestibility kinetics using the MBT with Dt, resembling the DM degradation compared to the ED that underestimates the DM degradation with biologically relevant MRT. Altogether, the MBT allows for a more detailed feedstuff evaluation in both intact and modified horses than the total collection method.

Processed grains can increase pre-caecal starch digestibility and thereby affect the site of digestion more significantly than unprocessed grains. Micronizing resulted in the greatest pre-caecal starch digestion, and thereby less starch by-passed to the hindgut when compared to toasted barley and maize. Metabolic responses of plasma glucose and insulin after feeding reflected pre-caecal starch digestion. Despite this, differences in the plasma glucose and insulin responses between processing methods and feedstuff were inconsistent. In the caecum, the total SCFA concentration increased with a corresponding decrease in pH after feeding. However, processing did not affect the total SCFA concentration and the caecal pH. Therefore, the metabolic and digestive responses measured in this thesis cannot be used as sole parameters for evaluating pre-caecal starch digestibility. On the other hand, when combined with the MBT, these techniques provide a comprehensive description of starch digestion in horses.

Overall, this thesis provided important information on feedstuff evaluation that is of great importance for feed companies, advisors, and maintaining gastrointestinal health of horses.

7 Knowledge gaps for future study

This thesis revealed that the MBT is a promising method for estimating DM and fibre digestibility in horses. However, the technique needs to be improved both in terms of the FSA for grains and optimal pore size for fibre- and starch-rich feedstuffs. This can be investigated by use of different FSA and pore sizes for mobile bags, with validation against indigestible markers for determination of pre-caecal starch digestion and the TFC for fibre fermentation.

The ED underestimated the DM degradation when biologically relevant MRT were used. Therefore, a future study of interest would be to investigate initial feedstuff degradation using the in-sacco method. Furthermore, this would require validation against the total collection method with different DM intakes and forage types affecting the MRT and ATTD.

One major question that this thesis raises is why there are differences between different processing treatments and grains for pre-caecal starch digestibility. One reason may be that grain starch varies in the amylose:amylopectin ratio affecting the DG and enzymatic digestion. An in-vitro study simulating the foregut digestion including different grains and their ratio of amylose:amylopectin and the possible correlation with different processing methods, may answer this question. However, this requires further development of reliable in-vitro models resembling the pre-caecal (and hindgut) digestion of horses.

Moisture content, temperature, and duration are highlighted as important variables for the optimal processing of grains. Changing any of these variables can result in a product that is more or less digestible. However, no study has, to my knowledge, investigated all these variables or the possible interactions on different grains and the effect on pre-caecal starch digestion in horses. These variables need to be investigated further to determine the optimal conditions for micronizing, toasting and other processing methods used for equine feedstuffs. Such investigations will contribute to our understanding of how to optimize enzymatic starch digestion in the small intestine and thereby avoid the starch by-passing to the hindgut. Moreover, while this thesis focuses mainly on carbohydrates, protein can also be affected by processing, and its effect on digestibility in horses are scarce. Therefore, a focus on protein in future studies would be of significant interest and use.

8 References

Åkerberg, A., H. Liljeberg, and I. Björck. 1998. Effects of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. Journal of Cereal Science, 28(1), 71-80.

Åkerlind, M., M., Weisbjerg, T., Eriksson, R., Tøgersen, P., Udén, B.L., Olafsson, O.M., Harstad, and H. Volden. 2011. Feed analyses and digestion methods. In: Volden, H. (Ed.), NorFor: the Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, The Netherlands. pp. 41-54.

Araujo K.V., J.A.F. Lima, E.T. FialhoI, and J.C. Teixeira. 2000. Comparação da técnica do saco de náilon móvel com o método de coleta total para determinar a digestibilidade dos nutrientes de alimentos volumosos em equinos. Revista Brasileira de Zootecnia, 29(3), 752-761. (In Portuguese).

Argenzio, R.A., J.E. Lowe, D.W. Pickard, and C.E. Stevens. 1974. Digesta passage and water exchange in the equine large intestine. American Journal of Physiology-Legacy Content, 226(5), 1035-1042.

Argenzio, R.A. 1993. Digestion and Absorption of Carbohydrate, Fat and Protein. In: Swenson, M.J., and W.O. Reece (Eds.), Dukes Physiology of Domestic Animals. Comstock Publishing Associates, London, United Kingdom. pp. 362-375.

Arnold, F.F., G.D. Potter, J.L. Kreider, and G.T. Schelling. 1981. Carbohydrate digestion in the small and large intestine of the equine. Proceeding 7th Equine Nutrition and physiology symposium, Apr.-May. Airlie house, Warrenton, Virginia, USA. pp. 19-22.

Asp, N.G., and I. Björck. 1989. Nutritional properties of extruded foods. In: Mercier, C., Linko, P., and J.M., Harper (Eds.), Extrusion Cooking. American Association of Cereal Chemists, Minnesota, USA, pp. 399–434.

Austbø, D., and H. Volden. 2006. Influence of passage model and caecal cannulation on estimated passage kinetics of roughage and concentrate in the gastrointestinal tract of horses. Livestock Science, 100(1), 33-43.

Bach Knudsen, K.E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology, 67, 319–338.

Bach Knudsen, K.E. 2001. The nutritional significance of "dietary fibre" analysis. Animal Feed Science and Technology, 90, 3–20.

Baldwin, P.M., C.D. Melia, and M.C. Davies. 1997. The surface chemistry of starch granules studied by time-of-flight secondary ion mass spectrometry. Journal of Cereal Science, 26, 329–346.

Bergero, D., G. Meineri, N. Miraglia, and P.G. Peiretti. 2005. Apparent digestibility of hays in horses determined by total collection of faeces and using internal marker methods. Journal of Food, agriculture and environment, 3(1), 199-202.

Borgia, L., S. Valberg, M. McCue, K. Watts, and J. Pagan. 2011. Glycaemic and insulinaemic responses to feeding hay with different non-structural carbohydrate content in control and polysaccharide storage myopathy-affected horses. Journal of Animal Physiology and Animal Nutrition, 95(6), 798-807.

Brøkner, C., D. Austbø, J.A. Næsset, K.E.B. Knudsen, and A.H. Tauson. 2012a. Equine pre-caecal and total tract digestibility of individual carbohydrate fractions and their effect on caecal pH response. Archives of Animal Nutrition, 66(6), 490-506.

Brøkner, C., K.B. Knudsen, I. Karaman, K.L. Eybye, and A.H. Tauson. 2012b. Chemical and physicochemical characterisation of various horse feed ingredients. Animal Feed Science and Technology, 177, 86–97.

Brøkner, C., D. Austbø, J.A. Næsset, D. Blache, K.E.B. Knudsen, H.H. Hansen, and A.H. Tauson. 2016. Glycaemic and insulinemic response to dietary carbohydrates in horses. Acta Veterinaria Scandinavica, 58(1), 43-48.

Canibe, N., and K.B. Knudsen. 1997. Digestibility of dried and toasted peas in pigs. 1. Ileal and total tract digestibilities of carbohydrates. Animal Feed Science and Technology, 64(2-4), 293-310.

CBV, Centraal Veevoeder Bureau. 2021. CVB Feed table. Feed evaluation system for horses. In: Chemical composition and nutritional values of feedstuff. Obtained from www.cvbdiervoeding.nl, Accessed 07/02/2022.

Cherian, G., W.C. Sauer, and P.A. Thacker. 1989. Factors affecting the apparent digestibility of protein for swine when determined by the mobile nylon bag technique. Animal Feed Science and Technology, 27, 137–146.

Cherney, D.J.R., J.A. Patterson, and R.P. Lemenager. 1990. Influence of in situ bag rinsing technique on determination of dry matter disappearance. Journal of Dairy Science, 73(2), 391-397.

Clauss, M., K. Schiele, S. Ortmann, J. Fritz, D. Codron, J. Hummel, and E. Kienzle. 2014. The effect of very low food intake on digestive physiology and forage digestibility in horses. Journal of Animal Physiology and Animal Nutrition, 98, 107–118.

Coenen, M., A. Mosseler, and I. Vervuert. 2006. Fermentative gases in breath indicate that inulin and starch start to be degraded by microbial fermentation in the stomach and small intestine of the horse in contrast to pectin and cellulose. American Society for Nutrition, 136, 2108S–2110S.

Coenen, M., E. Kienzle, I. Vervuert, and A. Zeyner. 2011. Recent German developments in the formulation of energy and nutrient requirements in horses and the resulting feeding recommendations. Journal of Equine Veterinary Science, 31(5-6), 219-229.

Costa, M.C., L.G. Arroyo, E. Allen-Vercoe, H.R. Stämpfli, P.T. Kim, A. Sturgeon and J.S. Weese. 2012. Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the V3-V5 region of the 16S rRNA gene. PloS one, 7(7), e41484.

Cowieson, A.J. S.L. Vieira, and C. Stefanello. 2019. Exogenous microbial amylase in the diets of poultry: what do we know?. Journal of Applied Poultry Research, 28(3), 556-565.

Darlington, J.M., and T.V. Hershberger. 1968. Effect of forage maturity on digestibility, intake and nutritive value of alfalfa, timothy and orchardgrass by equine. Journal of animal science, 27(6), 1572-1576.

de Boer, G., J.J. Murphy, and J.J. Kennelly. 1987. Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. Journal of dairy science, 70(5), 977-982.

de Fombelle, A., M. Varloud, A.G. Goachet, E. Jacotot, C. Philippeau, C. Drogoul, and V. Julliand. 2003. Characterization of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets. Journal of Animal Science, 77, 293-304.

de Fombelle, A., L. Veiga, C. Drogoul, and V. Julliand. 2004. Effect of diet composition and feeding pattern on the prececal digestibility of starches from diverse botanical origins measured with the mobile nylon bag technique in horses. Journal of Animal Science, 82(12), 3625–3634.

De Marco, M., N. Miraglia, P.G. Peiretti, and D. Bergero. 2012. Apparent digestibility of wheat bran and extruded flax in horses determined from the total collection of feces and acid-insoluble ash as an internal marker. Animal, 6(2), 227-231.

del Carmen Robles-Ramirez, M., A. Flores-Morales, and R. Mora-Escobedo. 2012. Corn tortillas: Physiochemical, structural and functional changes. Cultivation, uses and health benefits, 89-106.

Dhanoa, M.S. 1988. On the analysis of dacron bag data for low degradability feeds. Grass and Forage Science, 43(4), 441-444.

Dhanoa, M.S., J. France, S. Lopez, J. Dijkstra, S.J. Lister, D.R. Davies, and A. Bannink. 1999. Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. Journal of Animal Science, 77, 3385–3391.

Donald, A.M. 2001. Plasticization and self assembly in the starch granule. Cereal Chemistry, 78(3), 307-314.

Drogoul, C., C. Poncet, and J.L. Tisserand. 2000. Feeding ground and pelleted hay rather than chopped hay to ponies: 1. Consequences for in vivo digestibility and rate of passage of digesta. Animal Feed Science and Technology, 87, 117–130.

Drogoul, C., A. de Fombelle, and V. Julliand. 2001. Feeding and microbial disorders in horses: 2: Effect of three hay: grain ratios on digesta passage rate and digestibility in ponies. Journal of Equine Veterinary Science, 21(10), 487-491.

Dugdale, A.H.A., G.C. Curtis, P. Cripps, P.A. Harris, and C.M. Argo. 2010. Effect of dietary restriction on body condition, composition and welfare of overweight and obese pony mares. Equine Veterinary Journal, 42(7), 600-610.

Durham, A.E., N. Frank, C.M. McGowan, N.J. Menzies-Gow, E. Roelfsema, I. Vervuert, K. Feige, and K. Fey. 2019. ECEIM consensus statement on equine metabolic syndrome. Journal of Veterinary Internal Medicine, 33(2), 335-349.

Dyer, J., E.F.C. Merediz, K.S.H. Salmon, C.J. Proudman, G.B. Edwards, and S.P. Shirazi -Beechey. 2002. Molecular characterisation of carbohydrate digestion and absorption in equine small intestine. Equine Veterinary Journal, 34(4), 349-358.

Faichney, G.J. 1975. The use of markers to partition digestion within the gastro-intestinal tract of ruminants. Digestion and metabolism in the ruminant. In: Proceedings of the 4th International Symposium on Ruminant Physiology, University of New England Publishing Unit, Aug., Armidale, NSW, Australia. pp. 277–291.

Ford, E.J.H., and H.A. Simmons. 1985. Gluconeogenesis from caecal propionate in the horse. British Journal of Nutrition, 53(1), 55-60.

Frank, N., R.J. Geor, S.R. Bailey, A.E. Durham, and P.J. Johnson. 2010. Equine metabolic syndrome. Journal of Veterinary Internal Medicine, 24(3), 467-475.

Fuentes, M., F. Tecles, A. Gutiérrez, J. Otal, S. Martínez-Subiela, and J.J. Cerón. 2011. Validation of an automated method for salivary alpha-amylase measurements in pigs (Sus scrofa domesticus) and its application as a stress biomarker. Journal of Veterinary Diagnostic Investigation, 23(2), 282-287.

Fuentes-Rubio, M., F. Fuentes, J. Otal, A. Quiles, F. Tecles, J.J. Cerón, and M.L. Hevia. 2015. Measurements of salivary alpha-amylase in horse: Comparison of 2 different assays. Journal of Veterinary Behavior, 10(2), 122-127.

Garber, A., P. Hastie, and J.A. Murray. 2020. Factors influencing equine gut microbiota: Current knowledge. Journal of Equine Veterinary Science, 88, 102943.

Garner, H.E., J.R. Coffman, A.W. Hahn, D.P. Hutcheson, and M.E. Tumbleson. 1975. Equine laminitis of alimentary origin: an experimental model. American Journal of Veterinary Research, 36, 441-444.

Geor, R.J. 2013. Endocrne and merabolic physiology. In: Geor, R.J., P.A. Harris, and M. Coenen (Eds.), Equine applied and Clinical Nutrition, Health, welfare and performance. Elsevier Health Science, United Kingdom. pp. 33-58.

Goachet, A.G., C. Philippeau, M. Varloud, and V. Julliand. 2009. Adaptations to standard approaches for measuring total tract apparent digestibility and gastro-intestinal retention time in horses in training. Animal Feed Science and Technology, 152(1-2), 141-151.

Granfeldt, Y., X. Wu, and I. Björck. 2006. Determination of glycaemic index; some methodological aspects related to the analysis of carbohydrate load and characteristics of the previous evening meal. European Journal of Clinical Nutrition, 60(1), 104-112.

Harmon, D.L., and K.R. McLeod. 2001. Glucose uptake and regulation by intestinal tissues: Implications and whole-body energetics. Journal of Animal Science, 79(suppl. E), E59-E72.

Harris, P.A., and R.J. Geor. 2009. Primer on dietary carbohydrates and utility of the glycemic index in equine nutrition. Veterinary Clinics of North America: Equine Practice, 26 (1), 23-37.

Harris, P.A., M. Coenen, and R.J. Geor. 2013. Controversial areas in equine nutrient and feeding management. In: Geor, R.J., P.A. Harris, and M. Coenen (Eds.), Equine applied and clinical nutrition. Elsevier Health Science, United Kingdom. pp. 462-463.

Harris, P.A., A.D. Ellis, M.J. Fradinho, A. Jansson, V. Julliand, N. Luthersson, A.S. Santos, and I. Vervuert. 2017. Review: Feeding conserved forage to horses: Recent advances and recommendations. Animal, 11(6), 958-967.

Healy, H.P., P.D. Siciliano, and L.M. Lawrencez. 1995. Effect of concentrate form on blood and gastric fluid variables in ponies. Journal of Equine Veterinary Science, 15(10), 423-428.

Hermansson, A.-M., and S. Kidman. 1995. Starch – a phase-separated biopolymer system. In: Harding, S.E., S.E. Hill, and J.R. Mitchell (Eds.), Biopolymer Mixtures. Nottingham University Press, United Kingdom. pp. 225–245.

Hill, J. 2007. Impacts of nutritional technology on feeds offered to horses: A review of effects of processing on voluntary intake, digesta characteristics and feed utilisation. Animal Feed Science and Technology, 138(2), 92-117.

Hintz, H.F., and R.G. Loy. 1966. Effects of pelleting on nutritive value of horse rations. Journal of Animal Science, 25(4), 1059-1062.

Hintz, H.F., D.E. Hogue, E.F. Walker Jr., J.E. Lowe, and H.F. Schryver. 1971a. Apparent digestion in various segments of the digestive tract of ponies fed diets with varying roughage-grain ratios. Journal of Animal Science, 32(2), 245-248.

Hintz, H.F., R.A. Argenzio, and H.F. Schryver. 1971b. Digestion coefficients, blood glucose levels, and molar percentage of volatile fatty acids in intestinal fluid of ponies fed varying forage-grain ratios. Journal of Animal Science, 33(5), 992-995.

Hoffman, R.M., J.A. Wilson, D.S. Kronfeld, W.L. Cooper, L.A. Lawrence, D. Sklan, and P.A. Harris. 2001. Hydrolyzable carbohydrates in pasture, hay, and horse feeds: direct assay and seasonal variation. Journal of Animal Science, 79(2), 500-506.

Hoffman, R.M. 2013. Carbohydrates. In: Geor, R.J., P.A. Harris, and M. Coenen (Eds.), Equine applied and clinical nutrition. Elsevier Health Science, United Kingdom. pp. 156-167.

Hudson, J.M., N.D. Cohen, P.G. Gibbs, and J.A. Thompson. 2001. Feeding practices associated with colic in horses. Journal of the American Veterinary Medical Association, 219, 1419–1425.

Hummel, J., F. Scheurich, S. Ortmann, L.A. Crompton, M. Gerken, and M. Clauss. 2017. Comparative selective retention of particle size classes in the gastrointestinal tract of ponies and goats. Journal of Animal Physiology and Animal Nutrition, 102, 429–439.

Hymøller, L., M.S. Dickow, C. Brøkner, D. Austbø, and S.K. Jensen. 2012. Cereal starch, protein, and fatty acid pre caecal disappearance is affected by both feed technological treatment and efficiency of the chewing action in horses. Livestock Science, 150, 159–169.

Hyslop, J.J., and D. Cuddeford. 1996. Investigations on the use of the mobile bag technique in ponies. In: Proceedings of the British Society of Animal Science, Cambridge University Press. Mar., Cambridge, United Kingdom. p. 99.

Hyslop, J.J., A.L. Tomlinson, A. Bayley, and D. Cuddeford. 1998. Development of the mobile bag technique to study the degradation dynamics of forage feed constituents in the whole digestive tract of equids. In: Proceedings of the British Society of Animal Science, Cambridge University Press, United Kingdom. p. 129.

Hyslop, J.J., G.J. Stefansdottir, B.M.L. McLean, A.C. Longland, and D. Cuddeford. 1999. In situ incubation sequence and its effect on degradation of food components when measured in the caecum of ponies. Animal Science, 69(1), 147-156.

Hyslop, J.J. 2006. In situ and mobile bag methodology to measure the degradation profile of processed feeds in different segments of the equine digestive tract. Livestock Science, 100(1), 18-32.

Hvelplund, T., and M.R. Weisbjerg. 2000. In situ techniques for the estimation of protein degradability and postrumen availability. In: D.I. Givens, E. Oven, R.F.E. Axford, and H.M. Omed (Eds.), Forage evaluation in ruminant nutrition. CAB International. Oxon, United Kingdom. pp. 233-258.

Janis, C. 1976. The evolutionary strategy of the Equidae and the origins of rumen and cecal digestion. Evolution, 30(4), 757-774.

Jansson, A., A. Sandin, and J.-E. Lindberg. 2006. Digestive and metabolic effects of altering feeding frequency in athletic horses. Equine and Comparative Exercise Physiology, 3(2), 83-91.

Jensen, R.B., D. Austbø, and A.H. Tauson. 2012. Feeding forage before or after oats affects caecum pH profiles of the horse. In: Forages and grazing in horse nutrition. Wageningen Academic Publishers, Wageningen. pp. 327-330.

Jensen, R.B., D. Austbo, K.E. Bach Knudsen, and A.H. Tauson. 2014. The effect of dietary carbohydrate composition on apparent total tract digestibility, feed mean retention time, nitrogen and water balance in horses. Animal, 8, 1788–1796.

Jensen, R.B., D. Austbo, D. Blache, K.E. Bach Knudsen, and A.H. Tauson. 2016. The effect of feeding barley or hay alone or in combination with molassed sugar beet pulp on the metabolic responses in plasma and caecum of horses. Animal Feed Science and Technology, 214, 53–65.

Jensen, R.B., and E. Prestløkken. 2018. The effect of pore size of bags when estimating starch digestion in horses using the mobile bag technique. In: Proceedings of the 9th European Workshop on Equine Nutrition. Aug., Uppsala, Sweden. p. 100050.

Julliand, V., A. de Fombelle, C. Drogoul, and E. Jacotot. 2001. Feeding and microbial disorders in horses: part 3—effects of three hay: grain ratios on microbial profile and activities. Journal of Equine Veterinary Science, 21, 543–546.

Julliand V, A. de Fombelle, and M. Varloud. 2006. Starch digestion in horses: The impact of feed processing. Livestock Science, 100, 44–52.

Julliand, V., and P. Grimm. 2016. HORSE SPECIES SYMPOSIUM: The microbiome of the horse hindgut: History and current knowledge. Journal of Animal Science, 94(6), 2262-2274.

Julliand, S., A. Martin, and V. Julliand. 2018. Effect of dehydrated alfalfa on equine gastric and faecal microbial ecosystems. Livestock Science, 215, 16-20.

Kalck, K.A. 2009. Inflammatory bowel disease in horses. Veterinary Clinics: Equine Practice, 25(2), 303-315.

Karasu, G.K., R. Krabbenborg, A. Einspanier, and I. Vervuert. 2015. Insulinaemic and glycaemic responses to a second meal of a fibre-or starch-enriched compound feed in healthy horses. The Veterinary Journal, 204(2), 220-222.

Kienzle, E., S. Radicke, S. Wilke, E. Landes, and H. Meyer. 1992. Praeileale stärkeverdauung in abhängigkeit von stärkeart und zubereitung. Pferdeheilkunde, 1, 103-106. (In German).

Kienzle, E. 1994. Small intestinal digestion of starch in the horse. Revue de Medecine Veterinaire, 145, 199–204.

Kienzle, E., J. Pohlenz, and S. Radicke. 1997. Morphology of starch digestion in the horse. Journal of Veterinary Medicine Series A, 44.1-10, 207-221.

Lindåse, S., C. Müller, K. Nostell, and J. Bröjer. 2018. Evaluation of glucose and insulin response to haylage diets with different content of nonstructural carbohydrates in 2 breeds of horses. Domestic Animal Endocrinology, 64, 49-58.

Leek, B.F. 1993. Digestion in the Ruminant Stomach. In: Swenson, M.J., and W.O. Reece (Eds.), Dukes Physiology of Domestic Animals. Comstock Publishing Associates, London. pp. 387-416.

Longland, A.C. 2013. Pastures and pasture management. In: Geor, R.J., P.A. Harris, and M. Coenen. Equine applied and clinical nutrition. Elsevier Health Science, United Kingdom. pp. 332-348.

Longland, A.C., C. Barfoot, and P.A. Harris. 2018. Exercised Ponies Fed Hay and Three Concentrate Feeds: Apparent Digestibility, Nutritive Value, Observed Versus Calculated Digestible Energy and Behavior. Journal of Equine Veterinary Science, 62, 32-39.

Loaiza, P.A., O. Balocchi, and A. Bertrand. 2017. Carbohydrate and crude protein fractions in perennial ryegrass as affected by defoliation frequency and nitrogen application rate. Grass and Forage Science, 72(3), 556-567.

López, S., J. France, M.S. Dhanoa, and J. Dijkstra. 1999. Comparison of mathematical models to describe disappearance curves obtained using the polyester bag technique for incubating feeds in the rumen. Journal of Animal Science, 77(7), 1875-1888.

Liu, Y, J. Yu, L. Copeland, S. Wang, and S. Wang. 2019. Gelatinization behavior of starch: Reflecting beyond the endotherm measured by differential scanning calorimetry. Food Chemistry, 284, 53-59.

Luthersson, N., K.H. Nielsen, P.A. Harris, and T.D.H. Parkin. 2009. Risk factors associated with equine gastric ulceration syndrome (EGUS) in 201 horses in Denmark. Equine Veterinary Journal, Supplement, 41, 625-630.

Macheboeuf, D., C. Poncet, M. Jestin, and W. Martin-Rosset. 1996. Use of a mobile nylon bag technique with caecum fistulated horses as an alternative method for estimating pre-caecal and total tract nitrogen digestibilities of feedstuffs. EAAP-47th Annual Meeting, Aug., Lillehammer, Norway. p. 296.

Martin-Rosset, W., and J.P. Dulphy. 1987. Digestibility interactions between forages and concentrates in horses: influence of feeding level-comparison with sheep. Livestock Production Science, 17, 263-276.

Martin-Rosset, W., J. Andrieu, M. Vermorel, and J.P. Dulphy. 1984. Valeur nutritive des aliments pour le cheval. In: Jarrige, R., and W. Martin-Rosset (Eds.), Le cheval-Reproduction, sélection, alimentation, exploitation. INRA publications, Versailles, France. pp. 209-238. (In French).

Martin-Rosset, W., M. Doreau, S. Boulot, and N. Miraglia. 1990. Influence of level of feeding and physiological state on diet digestibility in light and heavy breed horses. Livestock Production Science, 25, 257–264.

Martin-Rosset, W. (Ed.). 2015. Equine nutrition: INRA nutrient requirements, recommended allowances and feed tables. Wageningen Academic Publisher, Netherlands.

McAllister, T.A., R.C. Phillippe, L.M. Rode, and K.J. Cheng. 1993. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. Journal of Animal Science, 71(1), 205-212.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh, C.A. Morgan, L.A. Sinclair and R.G. Wilkinson. 2011. Animal nutrition (7th edition). Prentice Hall/Pearson Education, Harlow. pp. 16-30.

McLean, B.M.L., J.J. Hyslop, A.C. Longland, D. Cuddeford, and T. Hollands. 1999a. In vivo apparent digestibility in ponies given rolled, micronized or extruded barley. In: Proceedings of the British Society of Animal Science. Cambridge University Press. Nov., Cambridge, United Kingdom. p. 133.

McLean, B.M.L., J.J. Hyslop, A.C. Longland, D. Cuddeford, and T. Hollands. 1999b. Development of the mobile bag technique to determine the degradation kinetics of purified starch sources in the pre-caecal segment of the equine digestive tract. In: Proceedings of the British Society of Animal Science. Cambridge University Press. Nov., Cambridge, United Kingdom. p. 138.

McLean, B.M.L., J.J. Hyslop, A.C. Longland, D. Cuddeford, and T. Hollands. 1999c. Effect of physical processing on in situ degradation of maize and peas in the caecum of ponies. In: Proceedings of the British Society of Animal Science. Cambridge University Press. Nov., Cambridge, United Kingdom. p. 134.

McLean, B.M.L., J.J. Hyslop, A.C. Longland, D. Cuddeford, and T. Hollands. 2000. Physical processing of barley and its effects on intra-caecal fermentation parameters in ponies. Animal Feed Science and Technology, 85, 79–87.

Medina, B., I.D. Girard, E. Jacotot, and V. Julliand. 2002. Effect of a preparation of Saccharomyces cerevisiae on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. Journal of Animal Science, 80(10), 2600-2609.

Merritt, A.M., and V. Julliand. 2013. Gastrointestinal physiology. In: Geor, R.J., P.A. Harris, and M. Coenen (Eds.), Equine Applied and Clinical Nutrition: Health, Welfare and Performance. Elsevier Health Sciences, United Kingdom. pp. 3-31.

Métayer, N., M. Lhŏte, A. Bahr, N.D. Cohen, I. Kim, A.J. Roussel, and V. Julliand. 2004. Meal size and starch content affect gastric emptying in horses. Equine Veterinary Journal, 36(5), 436-440.

Meyer, H., S. Radicke, E. Kienzle, S. Wilke, D. Kleffken, and M. Illenseer. 1995. Investigations on preileal digestion of starch from grain, potato and manioc in horses. Journal of Veterinary Medicine Series A, 42(1-10), 371-381.

Meyer, H., S. Radicke, E. Kienzle, S. Wilke, and D. Kleffken. 1993. Investigations on preileal digestion of oats, corn, and barley starch in relation to grain processing. Proceeding 13th Equine Nutrition and physiology symposium, place unknown, pp. 43-48.

Miyaji, M., K. Ueda, H. Hata, and S. Kondo. 2011. Effects of quality and physical form of hay on mean retention time of digesta and total tract digestibility in horses. Animal Feed Science and Technology, 165(1-2), 61-67.

Miyaji, M., K. Ueda, H. Hata, and S. Kondo. 2014. Effect of grass hay intake on fiber digestion and digesta retention time in the hindgut of horses. Journal of Animal Science, 92, 1574–1581.

Moore-Colyer, M.J.S., J.J. Hyslop, A.C. Longland, and D. Cuddeford. 2000. Intra-caecal fermentation parameters in ponies fed botanically diverse fibre-based diets. Animal Feed Science and Technology, 84(3-4), 183-197.

Moore-Colyer, M.J.S., J.J. Hyslop, A.C. Longland, and D. Cuddeford. 2002. The mobile bag technique as a method for determining the degradation of four botanically

diverse fibrous feedstuffs in the small intestine and total digestive tract of ponies. British Journal of Nutrition, 88(6), 729–740.

Moore-Colyer, M.J.S., H.J. Morrow, and A.C. Longland. 2003. Mathematical modelling of digesta passage rate, mean retention time and in vivo apparent digestibility of two different lengths of hay and big-bale grass silage in ponies. British Journal of Nutrition, 90(1), 109–118.

Moore-Colyer, M.J.S., T. Clercq, V. Julliand, and C. Drogoul. 2006. In vivo foregut digestion of starch-based concentrate feeds in horses using the mobile bag technique. BSAP Occasional Publication, 35, 171-174.

Morrison, W.R., T.P. Milligan, and M.N. Azudin. 1984. A relationship between the amylose and lipid contents of starches from diploid cereals. Journal of Cereal Science, 2(4), 257-271.

Muhonen, S., V. Julliand, J.-E. Lindberg, J. Bertilsson, and A. Jansson. 2009. Effects on the equine colon ecosystem of grass silage and haylage diets after an abrupt change from hay. Journal of Animal Science, 87(7), 2291-2298.

Murray, M.J., and G.F. Schusser. 1993. Measurement of 24-h gastric pH using an indwelling pH electrode in horses unfed, fed and treated with ranitidine. Equine Veterinary Journal, 25(5), 417-421.

Murray, M.J., and E.S. Eichorn. 1996. Effects of intermittent feed deprivation, intermittent feed deprivation with ranitidine administration, and stall confinement with ad libitum access to hay on gastric ulceration in horses. American Journal of Veterinary Research, 57(11), 1599-1603.

Murray, S.M., E.A. Flickinger, A.R. Patil, N.R. Merchen, J.L. Brent Jr., and G.C. Fahey Jr. 2001. In vitro fermentation characteristics of native and processed cereal grains and potato starch using ileal chyme from dogs. Journal of Animal Science, 79(2), 435-444.

Murray, J.A.M., A. Longland, P.M. Hastie, M. Moore-Colyer, and C. Dunnett. 2008. The nutritive value of sugar beet pulp-substituted lucerne for equids. Animal Feed Science and Technology, 140(1-2), 110-124.

Müller, C.E. 2012a. Impact of harvest, preservation and storage conditions on forage quality. In: Saastamoinen M., M.J. Fradinho, A.S. Santos, and N. Miraglia (Eds.)., Forages and grazing in horse nutrition, EAAP publication No. 132. Wageningen Academic Publishers. Wageningen. pp. 237-247.

Müller, C.E. 2012b. Equine digestion of diets based on haylage harvested at different plant maturities. Animal Feed Science and Technology, 177(1-2), 65-74.

Nielsen, B.D., C.I. O'Connor-Robison, H.S. Spooner, and J. Shelton. 2010. Glycemic and insulinemic responses are affected by age of horse and method of feed processing. Journal of Equine Veterinary Science, 30(5), 249-258.

Newton, R. 2020. Personal communication. The Micronizing Company UK Ltd., Charnwood Mill, Framlingham, Suffolk, United Kingdom. Tel: +44 1728 723435, E-mail: Newton@micronizing.com.

Næsset, J.A., K. Hove, and R.B. Jensen. 2018. Effects of sensor location during in situ measurements on pH in caecal digesta of the horse, and post-observational treatment of sensor data. Acta Agriculturae Scandinavica, Section A - Animal Science, 68:1, 33-38.

Ørskov, E.R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science, 92(2), 499–503.

Pagan, J.D., P.A. Harris, T. Brewster-Barnes, S.E. Duren, and S.G. Jackson. 1998. Exercise affects digestibility and rate of passage of all-forage and mixed diets in thoroughbred horses. The Journal of Nutrition, 128(12), 2704S–2707S.

Palmgren Karlsson, C., J.-E. Lindberg, and M. Rundgren. 2000. Associative effects on total tract digestibility in horses fed different ratios of grass hay and whole oats. Livestock Production Science, 65, 143-153.

Palmgren Karlsson, C., A. Jansson, B. Essén-Gustavsson, and J.-E. Lindberg. 2002. Effect of molassed sugar beet pulp on nutrient utilisation and metabolic parameters during exercise. Equine Veterinary Journal, 34, 44–49.

Pearson, R.A., R.F. Archibald, and R.H. Muirhead. 2001. The effect of forage quality and level of feeding on digestibility and gastrointestinal transit time of oat straw and alfalfa given to ponies and donkeys. British Journal of Nutrition, 85(5), 599-606.

Pearson, R.A., R.F. Archibald, and R.H. Muirhead. 2006. A comparison of the effect of forage type and level of feeding on the digestibility and gastrointestinal mean retention time of dry forages given to cattle, sheep, ponies and donkeys. British Journal of Nutrition, 95(1), 88-98.

Petry, H., and B.M. Handlos. 1978. Untersuchungen zur Bestimmung der Verdaulichkeit von Nährstoffen und Futterenergie mit Hilfe der Nylonbeutel Technik beim Schwein, Archiv für Tierernaehrung, 28(8), 531-543. (In German).

Philippeau, C., C. Faubladier, A.G. Goachet, and V. Julliand. 2009. Is there an impact of feeding concentrate before or after forage on colonic pH and redox potential in horses. In: Applied equine nutrition and training. Equine Nutr. Training Conf (ENUTRACO). Wageningen Academic Publishers. Madrid, Spain. pp. 203- 208.

Philippeau, C., M. Varloud, and V. Julliand. 2014. Mobile bag starch precaecal disappearance and postprandial glycemic response of four forms of barley in horses. Journal of Animal Science, 92, 2087–2093.

Potter, G.D., F.F. Arnold, D.D. Householder, D.H. Hansen, and K.M. Brown. 1992. Digestion of starch in the small or large intestine of the equine. Pferdeheilkunde, 1(4), 107-111.

Ragnarsson, S., and J.-E. Lindberg. 2008. Nutritional value of timothy haylage in Icelandic horses. Livestock Science, 113(2–3), 202–208.

Ragnarsson, S., and J.-E. Lindberg. 2010. Impact of feeding level on digestibility of a haylage-only diet in Icelandic horses. Journal of Animal Physiology and Animal Nutrition, 94(5), 623-627.

Ragnarsson, S., and A. Jansson. 2011. Comparison of grass haylage digestibility and metabolic plasma profile in Icelandic and Standardbred horses. Journal of Animal Physiology and Animal Nutrition, 95(3), 273-279.

Ringmark, S., and A. Jansson. 2013. Insulin response to feeding forage with varying crude protein and amino acid content in horses at rest and after exercise. Comparative Exercise Physiology, 9(3-4), 209-217.

Roberts, M.C. 1974. Amylase activity in the small intestine of the horse. Research in Veterinary Science, 17, 400-401.

Rodiek, A.V., and C.L. Stull. 2007. Glycemic index of ten common horse feeds. Journal of Equine Veterinary Science, 27(5), 205-211.

Rodrigues, L.M., F.Q.D. Almeida, M.B. Pereira, A.C.T. Miranda, A. Guimarães, and A.M.D. Andrade. 2012. Roughage digestion evaluation in horses with total feces collection and mobile nylon bags. Revista Brasileira de Zootecnia, 41(2), 341–346.

Romano, N., and V. Kumar. 2019. Starch gelatinization on the physical characteristics of aquafeeds and subsequent implications to the productivity in farmed aquatic animals. Reviews in Aquaculture, 11(4), 1271-1284.

Rosenfeld, I., and D. Austbø. 2009. Digestion of cereals in the equine gastrointestinal tract measured by the mobile bag technique on caecally cannulated horses. Animal Feed Science and Technology, 150, 249–258.

Russell, P.L., C.S. Berry, and P. Greenwell. 1989. Characterisation of resistant starch from wheat and maize. Journal of Cereal Science, 9(1), 1-15.

Sales, J. 2012. A review on the use of indigestible dietary markers to determine total tract apparent digestibility of nutrients in horses. Animal Feed Science and Technology, 174(3-4), 119-130.

Santos, A.S., M.A.M. Rodrigues, R.J.B. Bessa, L.M. Ferreira, and W. Martin-Rosset. 2011. Understanding the equine cecum-colon ecosystem: current knowledge and future perspectives. Animal, 5(1), 48-56.

Sauer, W.C., H. Jørgensen, and R. Berzins. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Canadian Journal of Animal Science, 63(1), 233-237.

Schaafstra, F.J.W.C., D.A. van Doorn, J.T. Schonewille, F.C. Wartena, M.V. Zoon, M.C. Blok, and W.H. Hendriks. 2015. Evaluation of methodological aspects of digestibility measurements in ponies fed different grass hays. Journal of Animal Science, 93(10), 4742-4749.

Schaafstra, F.J.W.C., D.A. van Doorn, J.T. Schonewille, M.M.J. van Riet, P. Visser, M.C. Blok, and W.H. Hendriks. 2017. Evaluation of methodological aspects of digestibility measurements in ponies fed different haylage to concentrate ratios. Animal, 11(11), 1922-1929.

Schaafstra, F.J.W.C., D.A. Van Doorn, J.T. Schonewille, E. Roelfsema, C.M. Westermann, O. Dansen, M. Jacobs, J.-Y. Lee, E.A. Spronck, M.C. Blok, and W.H. Hendriks. 2018. Effect of exercise on apparent total tract digestibility of nutrients and faecal recovery of ADL and TiO2 in ponies. Animal, 12(12), 2505-2510.

Sievert, D., and Y. Pomeranz. 1989. Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic method. Cereal Chemistry, 66, 342-347.

Simmons, H.A., and E.J.H. Ford. 1991. Gluconeogenesis from propionate produced in the colon of the horse. British Veterinary Journal, 147(4), 340-345.

Svihus, B., A.K. Uhlen and O.M. Harstad. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Animal Feed Science and Technology, 122(3-4), 303-320.

Stewart, C.S., and M.P. Bryant. 1988. The rumen bacteria. In: Hobson, P.N. (ed). The rumen microbial ecosystem. Elsevier Science Publishers, Essex, United Kingdom. pp. 21.

Sykes, B.W., M. Hewetson, R.J. Hepburn, N. Luthersson, and Y. Tamzali. 2015. European College of Equine Internal Medicine Consensus Statement—equine gastric ulcer syndrome in adult horses. Journal of Veterinary Internal Medicine, 29(5), 1288.

Theander, O., P. Åman, E. Westerlund, and H. Graham. 1994. Enzymatic/chemical analysis of dietary fiber. Journal of AOAC International, 77, 703–709.

Udén, P., and P.J. Van Soest. 1984. Investigations of the in-situ bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. Journal of Animal Science, 58(1), 213–221.

Van der Poel, A.F.B. 1990. Effects of processing on bean (phaseolus vulgaris) protein quality. PhD thesis. Animal Nutritional Group, Wageningen Institute of Animal Science, Wageningen Agricultural University, The Netherlands. pp. 75-77.

Van Soest, P.J., J.B. Robertson, and B. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74(10), 3583-3597.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant, 2nd. edn, Cornell University Press, Ithaca, New York, p. 476.

Varloud, M., A. de Fombelle, A.G. Goachet, C. Drogoul, and V. Julliand. 2004. Partial and total apparent digestibility of dietary carbohydrates in horses as affected by the diet. Animal Science, 79(1), 61-72.

Varloud, M., A. Roussel, P. Boisot, and V. Julliand. 2007. A technique for the collection and the study of biochemical and microbial characteristics of postprandial

gastric contents from conscious horses. Animal Feed Science and Technology, 133(3-4), 259-274.

Vander Noot, G.W., L.D. Symons, R.K. Lydman, and P.V. Fonnesbeck. 1967. Rate of passage of various feedstuffs through the digestive tract of horses. Journal of Animal Science, 26(6), 1309-1311.

Van Straalen, W.M., F.M.H. Dooper, A.M. Antoniewicz, I. Kosmala, and A.M. Van Vuuren. 1993. Intestinal digestibility in dairy cows of protein from grass and clover measured with mobile nylon bag and other methods. Journal of Dairy Science, 76, 2970–298.

Van Weyenberg, S., J. Sales, and G.P.J. Janssens. 2006. Passage rate of digesta through the equine gastrointestinal tract: A review. Livestock Science, 99(1), 3–12.

Van Weyenberg, S., J. Buyse, and G.P. Janssens. 2007. Digestibility of a complete ration in horses fed once or three times a day and correlation with key blood parameters. The Veterinary Journal, 173(2), 311-316.

Vanzant, E.S., R.C. Cochran, and E.C. Titgemeyer. 1998. Standardization of in-situ techniques for ruminant feedstuff evaluation. Journal of Animal Science, 76, 2717–2729.

Varvikko, T., and J.-E. Lindberg. 1985. Estimation of microbial nitrogen in nylon-bag residues by feed 15 N dilution. British Journal of Nutrition, 54, 473–481.

Varvikko, T., and A. Vanhatalo. 1989. Critical aspects in intestinal digestion estimates obtained by the mobile bag method. Asian-Australasian Journal of Animal Sciences, 2(3), 404-406.

Vervuert, I., M. Coenen, and C. Bothe. 2003. Effects of oat processing on the glycaemic and insulin responses in horses. Journal of Animal Physiology and Animal Nutrition, 87, 96–104.

Vervuert, I., M. Coenen, and C. Bothe. 2004. Effects of corn processing on the glycaemic and insulinaemic responses in horses. Journal of Animal Physiology and Animal Nutrition, 88, 348–355.

Vervuert, I., C. Bothe, and M. Coenen. 2007. Glycaemic and insulinaemic responses to mechanical or thermal processed barley in horses. Journal of Animal Physiology and Animal Nutrition, 91(5-6), 263-268.

Vervuert, I., K. Voigt, T. Hollands, D. Cuddeford, and M. Coenen. 2008. Effects of processing barley on its digestion by horses. Veterinary Record, 162(21), 684- 688.

Vervuert, I., K. Voigt, T. Hollands, D. Cuddeford, and M. Coenen. 2009a. Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. The Veterinary Journal, 182(1), 67-72.

Vervuert, I., S. Klein, and M. Coenen. 2009b. Effect of mixing dietary fibre (purified lignocellulose or purified pectin) and a corn meal on glucose and insulin responses in healthy horses. Journal of Animal Physiology and Animal Nutrition, 93(3), 331-338.

Vervuert, I., S. Klein, and M. Coenen. 2009c. Effects of feeding state on glycaemic and insulinaemic responses to a starchy meal in horses: a methodological approach. Animal, 3(9), 1246-1253.

Vieira, R.A., J. Pereira, P.A. Malafaia, and A.C. de Queiroz. 1997. Application of non-linear models in the description of in situ degradation profiles of the elephant grass (Pennisetum purpureum Schum., Mineiro variety). Animal Feed Science and Technology, 66(1-4), 197-210.

Warzecha, C.M., J.A. Coverdale, J.E. Janecka, J.L. Leatherwood, W.E. Pinchak, T.A. Wickersham, and J.C. McCann. 2017. Influence of short-term dietary starch inclusion on the equine cecal microbiome. Journal of Animal Science, 95(11), 5077-5090.

Weisbjerg, M.R., P.K. Bhargava, T. Hvelplund, and J. Madsen. 1990. Anvendelse Af Nedbryddningsprofiler I Fodermiddelvurderingen, 679. Beretning fra Statens Husdyrbrugsforsøg, Frederiksberg, Danmark. pp. 8-31. (In Danish).

Wilcox, G. 2005. Insulin and insulin resistance. Clinical Biochemist Reviews, 26(2), 19-39.

Willard, J.G., J.C. Willard, S.A. Wolfram, and J.P. Baker. 1977. Effect of diet on cecal pH and feeding behavior of horses. Journal of Animal Science, 45(1), 87-93.

Wolever, T.M.S., and C. Mehling. 2002. High-carbohydrate–low-glycemic index dietary advice improves glucose disposition index in subjects with impaired glucose tolerance. British Journal of Nutrition, 87, 477–487.

Wolter, R., J.P. Valette, A. Durix, J.C. Letourneau, M. Carcelen, A. Villard, and A. Bruny. 1982. Digestibilité comparée de quatre céréales (avoine, orge, maïs, blé), selon le mode de présentation, chez le poney. In Annales de zootechnie, 31(4), 445-458. (In French).

Zhu, F. 2017. Barley starch: composition, structure, properties, and modifications. Comprehensive Reviews in Food Science and Food Safety, 16(4), 558-579.

Paper I

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

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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 ($\mathbf{D_t}$) 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.

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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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.

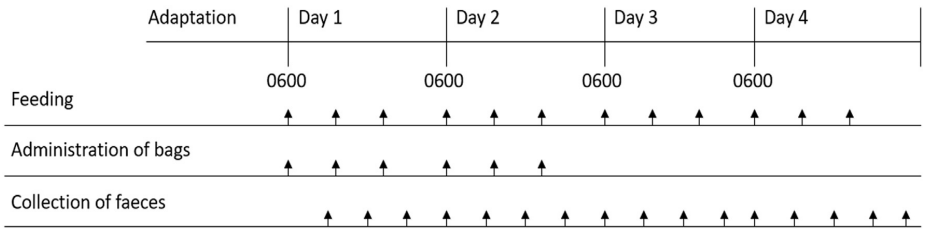

Fig. 1. The experimental set-up illustrating feeding times (0600, 1400 and 2000 h), faecal collection times (every 6 h) and times mobile bags were administered (at every meal on days 1 and 2).

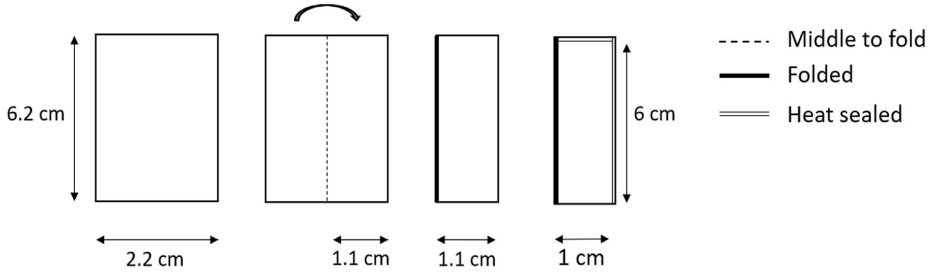

Fig. 2. Illustration of mobile bag construction with example of bag size 1 × 6 × 2 cm (height × length × side).

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:

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

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):

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

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:

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

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

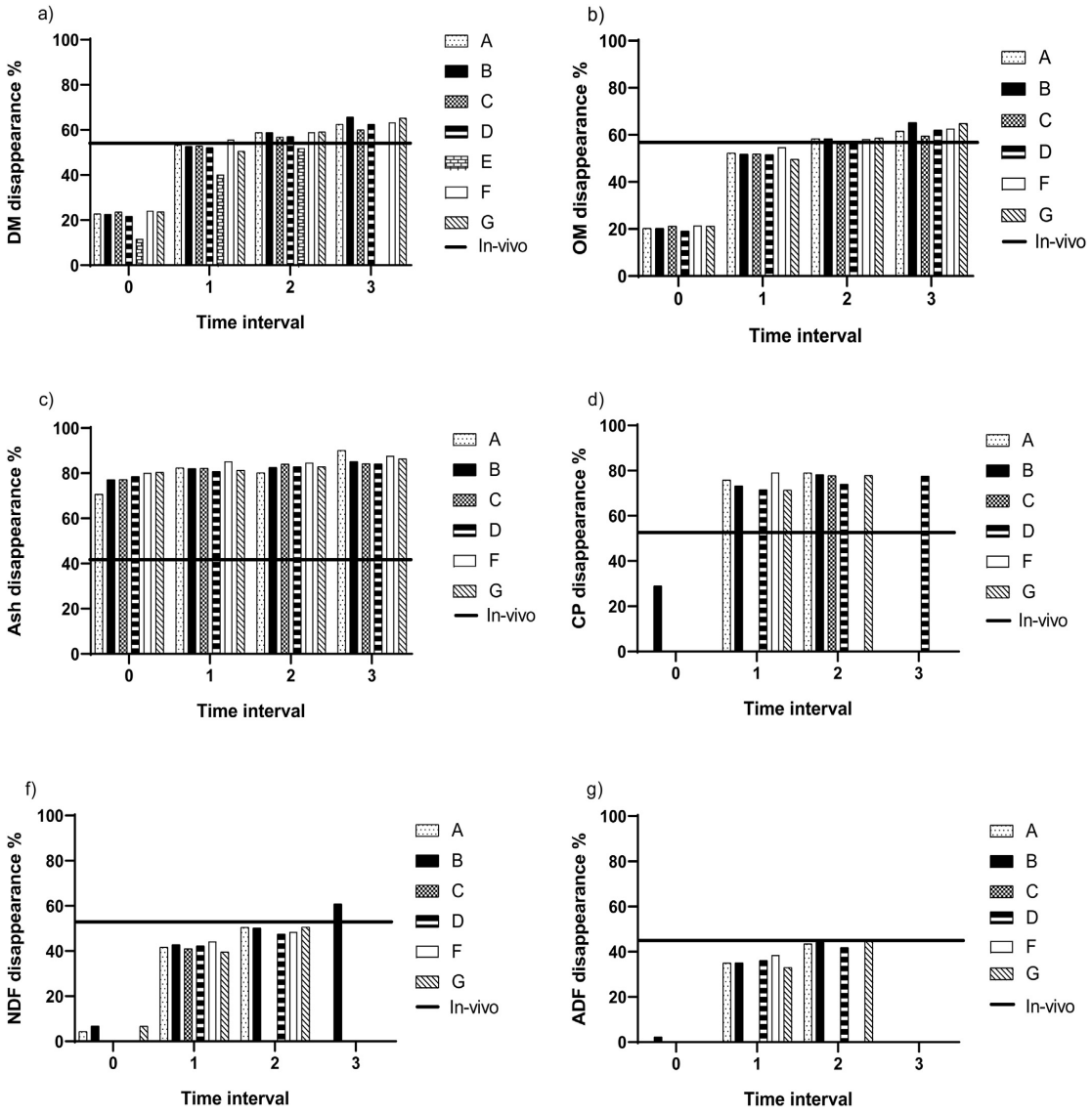

Fig. 3. *In-vivo* apparent total tract digestibility of nutrients and *in-situ* nutrient disappearance from different mobile bag types and time intervals (0 = control bags; 1 = 15–30 h; 2 = 31–50 h; 3 = 51–115 h).

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

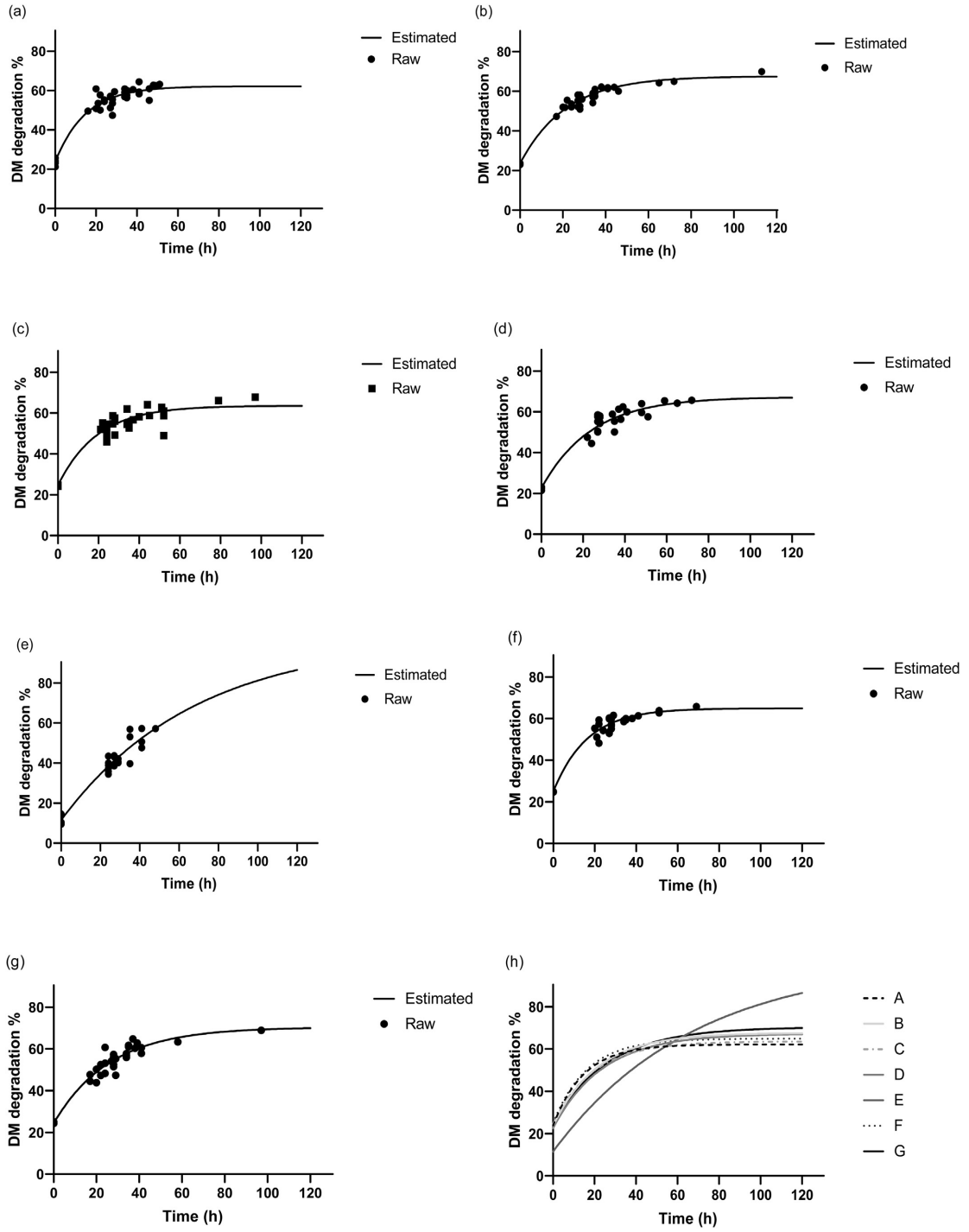

Fig. 4. Ørskov and McDonald (1979) degradation curves of DM from hay, based on mobile bags with different feed to surface area (FSA) and sizes. (a) bag size 1.2 × 10 × 2 with FSA 10.4 mg/cm^2; (b) bag size 1.2 × 10 × 2 with FSA 20.8 mg/cm^2; (c) bag size 1 × 6 × 2 with FSA 10.4 mg/cm^2; (d) bag size 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/cm^2). 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/cm^2 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/cm^2 than of 10.4 mg/cm^2. 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/cm^2 when evaluating digestion in the small intestine and of 10 mg/cm^2 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/cm^2 as the bags with an FSA close to 40 mg/cm^2 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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Author contributions

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.

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References

Åkerlind, M., Weisbjerg, M., Eriksson, T., Tøgersen, R., Udén, P., Olafsson, B.L., Harstad, O. M., Volden, H., 2011. Feed analyses and digestion methods. In: Volden, H. (Ed.), NorFor: the Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 41–54.

ANKOM, 2017a. Acid detergent fiber in feeds – filter bag technique (for A300 and A200I). Retrieved on 01.05.2018 from. https://www.ankom.com/sites/default/files/document-files/Method_5_ADF_A200.pdf.

ANKOM, 2017b. Neutral detergent fiber in feeds – filter bag technique (for A200 and A200I). Retrieved on 01.05.2018 from. https://www.ankom.com/sites/default/files/document-files/Method_6_NDF_A200.pdf.

Cherian, G., Sauer, W.C., Thacker, P.A., 1989. Factors affecting the apparent digestibility of protein for swine when determined by the mobile nylon bag technique. Animal Feed Science and Technology 27, 137–146.

Clauss, M., Schiele, K., Ortmann, S., Fritz, J., Codron, D., Hummel, J., Kienzle, E., 2014. The effect of very low food intake on digestive physiology and forage digestibility in horses. Journal of Animal Physiology and Animal Nutrition 98, 107–118.

Dhanoa, M.S., France, J., Lopez, S., Dijkstra, J., Lister, S.J., Davies, D.R., Bannink, A., 1999. Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. Journal of Animal Science 77, 3385–3391.

Drogoul, C., Poncet, C., Tisserand, J.L., 2000. Feeding ground and pelleted hay rather than chopped hay to ponies: 1. Consequences for *in-vivo* digestibility and rate of passage of digesta. Animal Feed Science and Technology 87, 117–130.

Faichney, G.J., 1975. The use of markers to partition digestion within the gastro-intestinal tract of ruminants. Digestion and metabolism in the ruminant 1974. Proceedings of the 4th International Symposium on Ruminant Physiology. Univiversity of New England Publishing Unit, Armidale, NSW, Australia, pp. 277–291.

Hummel, J., Scheurich, F., Ortmann, S., Crompton, L.A., Gerken, M., Clauss, M., 2017. Comparative selective retention of particle size classes in the gastrointestinal tract of ponies and goats. Journal of Animal Physiology and Animal Nutrition 102, 429–439.

Hvelplund, T., Weisbjerg, M.R., Andersen, L.S., 1992. Estimation of the true digestibility of rumen undegraded dietary protein in the small intestine of ruminants by the mobile bag technique. Acta Agriculturae Scandinavica Section A Animal Science 42, 34–39.

Hvelplund, T., Madsen, J., Misiciattelli, L., Weisberg, M.R., 2003. Proteinomsætning I mavetarmkanalen og dens kvantificering. In: Hvelplund, T., Nørgaard, N. (Eds.), Kvægets ernæring og fysiologi. Danmarks Jordbrugs Forskining, Denmark, pp. 281–308.

Hymøller, L., Dickow, M.S., Brøkner, C., Austbø, D., Jensen, S.K., 2012. Cereal starch, protein, and fatty acid pre-caecal disappearance is affected by both feed technological treatment and efficiency of the chewing action in horses. Livestock Science 150, 159–169.

Hyslop, J.J., 2006. In situ and mobile bag methodology to measure the degradation profile of processed feeds in different segments of the equine digestive tract. Livestock Science 100, 18–32.

Hyslop, J.J., Cuddeford, D., 1996. Investigations on the use of the mobile bag technique in ponies. Proceedings of the British Society of Animal Science. 62. Cambridge University Press, p. 99.

Jensen, R.B., Austbø, D., Knudsen, K.B., Tauson, A.H., 2014. The effect of dietary carbohydrate composition on apparent total tract digestibility, feed mean retention time, nitrogen and water balance in horses. Animal 8, 1788–1796.

Macheboeuf, D., Poncet, C., Jestin, M., Martin-Rosset, W., 1996. Use of a mobile nylon bag technique with caecum fistulated horses as an alternative method for estimating pre-caecal and total tract nitrogen digestibilities of feedstuffs. EAAP-47th Annual Meeting, 25–29 August, Lillehammer, Norway, p. 296.

Miyaji, M., Ueda, K., Hata, H., Kondo, S., 2014. Effect of grass hay intake on fiber digestion and digesta retention time in the hindgut of horses. Journal of Animal Science 92, 1574–1581.

Moore-Colyer, M.J.S., Hyslop, J.J., Longland, A.C., Cuddeford, D., 2002. The mobile bag technique as a method for determining the degradation of four botanically diverse fibrous feedstuffs in the small intestine and total digestive tract of ponies. British Journal of Nutrition 88, 729–740.

Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science 92, 499–503.

Philippeau, C., Varloud, M., Julliand, V., 2014. Mobile bag starch precaecal disappearance and postprandial glycemic response of four forms of barley in horses. Journal of Animal Science 92, 2087–2093.

Randby, Å.T., Nørgaard, P., Weisbjerg, M.R., 2010. Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing Norwegian Red bulls. Blackwell Publishing Ltd. Grassland Science 65, 273–286.

Rosenfeld, I., Austbø, D., 2009. Digestion of cereals in the equine gastrointestinal tract measured by the mobile bag technique on caecally cannulated horses. Animal Feed Science and Technology 150, 249–258.

Udén, P., Van Soest, P.J., 1984. Investigations of the *in-situ* bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. Journal of Animal Science 58, 213–221.

Van Straalen, W.M., Dooper, F.M.H., Antoniewicz, A.M., Kosmala, I., Van Vuuren, A.M., 1993. Intestinal digestibility in dairy cows of protein from grass and clover measured with mobile nylon bag and other methods. Journal of Dairy Science 76, 2970–2981.

Vanzant, E.S., Cochran, R.C., Titgemeyer, E.C., 1998. Standardization of *in-situ* techniques for ruminant feedstuff evaluation. Journal of Animal Science 76, 2717–2729.

Varvikko, T., Lindberg, J.E., 1985. Estimation of microbial nitrogen in nylon-bag residues by feed 15 N dilution. British Journal of Nutrition 54, 473–481.

Volden, H., Harstad, O.M., 1995. Effect of rumen incubation on the true indigestibility of feed protein in the digestive tract determined by nylon bag techniques. Acta Agriculturae Scandinavica Section A Animal Science 45, 106–115.

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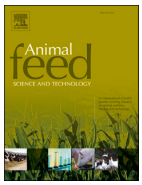

Mobile bag technique for estimation of nutrient digestibility when hay is supplemented with alternative fibrous feedstuffs in horses

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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 subsamples 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 ± standard deviation of mean (SD)) of 558 ± 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 × 3 m) with rubber mats and wood shavings as bedding material. During the adaptation periods, the horses were allowed access to a gravel

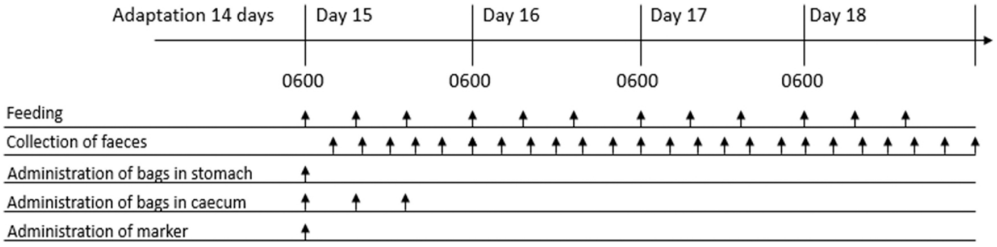

Fig. 1. The experimental setup illustrating feeding times (06.00, 14.00 and 22.00 h), faecal collection times (every 4th h), administration of mobile bags in the stomach and the caecum and administration of marker in caecum during the 4 days of data collection in each of the two experimental periods.

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

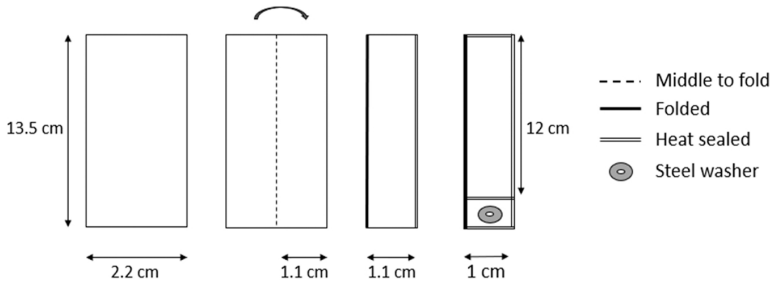

Fig. 2. Illustration of mobile bag construction with example of bags administered in the stomach.

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.

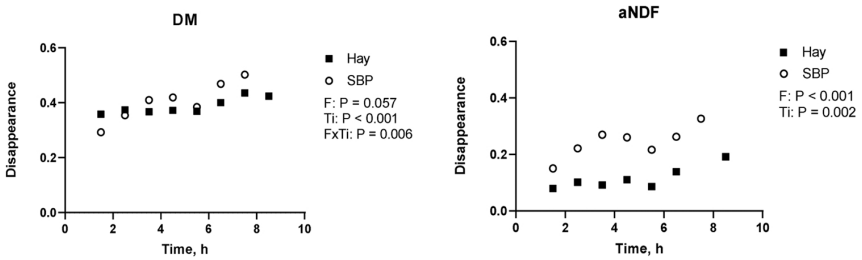

Fig. 3. Pre-caecal disappearance of dry matter (DM) and neutral detergent fibre assayed with heat-stable amylase and expressed inclusive of residual ash (aNDF) determined from the mobile bag technique for the two feedstuffs (F): sugar beet pulp pellets (SBP) and hay (mainly Timothy, first cut) over time (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						P-value	
		In-vivo	In-situ						
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

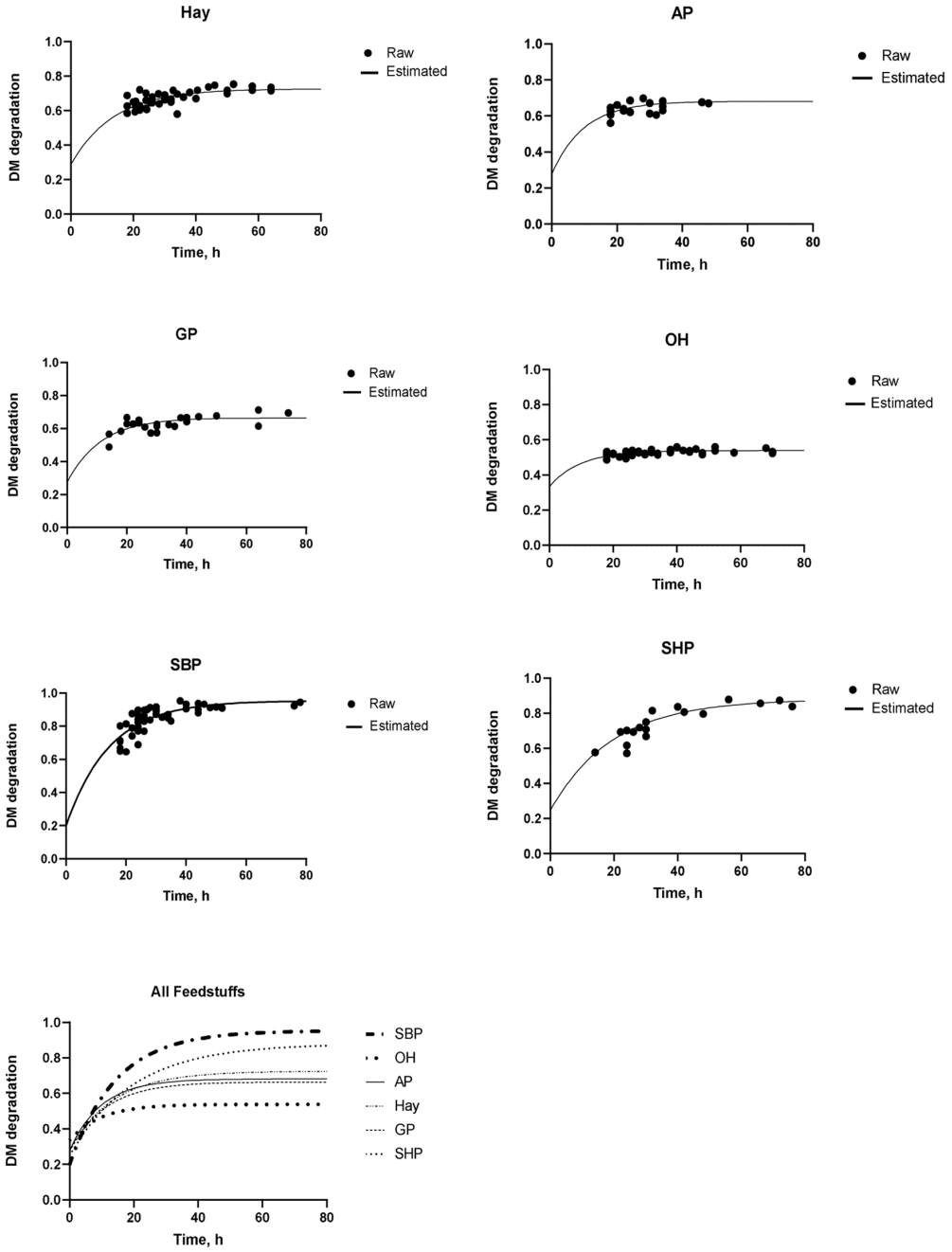

Fig. 4. Ørskov and McDonald (1979) degradation curves and raw data of dry matter (DM) for hay (mainly Timothy, first cut), alfalfa pellets (AP), grass pellets (GP), oat hulls (OH), sugar beet pulp (SBP) and soya hull pellets (SHP) based on mobile bags administrated into the caecum and collected in faeces.

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

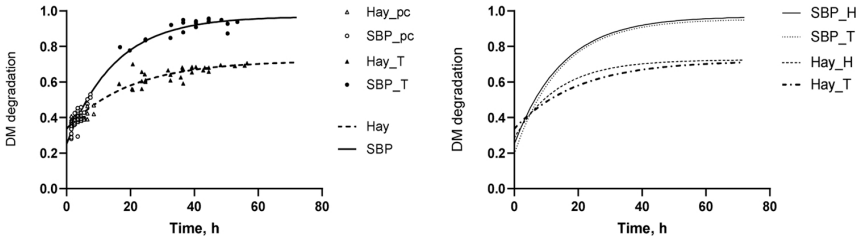

Fig. 5. Ørskov and McDonald (1979) degradation curves of dry matter (DM) for hay (mainly Timothy, first cut) and sugar beet pulp pellets (SBP) fitted to raw data from mobile bags administered in the stomach and found in caecum (pre-caecal, pc) or found in faeces (total tract, T) and administered in caecum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

ANKOM , Neutral detergent fiber in feeds - filter bag technique (for A2000 andA2000I) NDF Method – Method 13 2017a 2.⟨https://www.ankom.com/sites/default/files/documentfiles/Method_13_NDF_⟩A2000.pdf.

ANKOM Acid detergent fiber in feeds - filter bag technique (for A2000 andA2000I) ADF Method – Method 12 2017b 2.⟨https://www.ankom.com/sites/default/files/documentfiles/Method_12_ADF_⟩A2000.pdf.

AOAC, 1990. Official methods of analysis of the AOAC. Methods 996.11, 15th ed. AOAC, Arlington, VA, USA.https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf. (Accessed 28 May 2019).

Argenzio, R.A., Southworth, M., Stevens, C.E., 1974. Sites of organic acid production and absorption in the equine gastrointestinal tract. Am. J. Physiol. 226, 1043–1050. https://doi.org/10.1152/ajplegacy.1974.226.5.1043.

Bach Knudsen, K.E., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67, 319–338. https://doi.org/10.1016/S0377-8401(97)00009-6.

Bach Knudsen, K.E., 2001. The nutritional significance of "dietary fibre" analysis. Anim. Feed Sci. Technol. 90, 3–20. https://doi.org/10.1016/S0377-8401(01)00193-6.

Brøkner, C., Knudsen, K.B., Karaman, I., Eybye, K.L., Tauson, A.H., 2012. Chemical and physicochemical characterisation of various horse feed ingredients. Anim. Feed Sci. Technol. 177, 86–97. https://doi.org/10.1016/j.anifeedsci.2012.06.005.

Clauss, M., Schiele, K., Ortmann, S., Fritz, J., Codron, D., Hummel, J., Kienzle, E., 2014. The effect of very low food intake on digestive physiology and forage digestibility in horses. J. Anim. Physiol. Anim. Nutr. 98, 107–118. https://doi.org/10.1111/jpn.12053.

Coverdale, J.A., Moore, J.A., Tyler, H.D., Miller-Auwerda, P.A., 2004. Soybean hulls as an alternative feed for horses. J. Anim. Sci. 82, 1663–1668. https://doi.org/10.2527/2004.8261663x.

Dhanoa, M.S., France, J., Lopez, S., Dijkstra, J., Lister, S.J., Davies, D.R., Bannink, A., 1999. Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. J. Anim. Sci. 77, 3385–3391. https://doi.org/10.2527/1999.77123385x.

Doehlert, D.C., McMullen, M.S., Riveland, N.R., 2010. Groat proportion in oats as measured by different methods: analysis of oats resistant to dehulling and sources of error in mechanical dehulling. Can. J. Plant Sci. 90 (4), 391–397. https://doi.org/10.4141/CJPS09184.

Drogoul, C., Poncet, C., Tisserand, J.L., 2000. Feeding ground and pelleted hay rather than chopped hay to ponies: 1. Consequences for in vivo digestibility and rate of passage of digesta. Anim. Feed Sci. Technol. 87, 117–130. https://doi.org/10.1016/S0377-8401(00)00187-5.

Faichney, G.J., 1975. The use of markers to partition digestion within the gastro-intestinal tract of ruminants. Digestion and metabolism in the ruminant 1974. Proceedings of the 4th International Symposium on Ruminant Physiology, 277–291. Univ. of New England Publishing Unit., Armidale, NSW, Australia.

Farley, E.B., Potter, G.D., Gibbs, P.G., Schumacher, J., Murray-Gerzik, M., 1995. Digestion of soybean meal protein in the equine small and large intestine at various levels of intake. J. Equine Vet. Sci. 15 (9), 391–397. https://doi.org/10.1016/S0737-0806(07)80483-7.

de Fombelle, A., Varloud, M., Goachet, A.G., Jacotot, E., Philippeau, C., Drogoul, C., Julliand, V., 2003. Characterization of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets. Anim. Sci. 77 (2), 293–304. https://doi.org/10.1017/S1357729800059038.

de Fombelle, A., Veiga, L., Drogoul, C., Julliand, V., 2004. Effect of diet composition and feeding pattern on the prececal digestibility of starches from diverse botanical origins measured with the mobile nylon bag technique in horses. J. Anim. Sci. 82 (12), 3625–3634. https://doi.org/10.2527/2004.82123625x.

Harris, P.A., Ellis, A.D., Fradinho, M.J., Jansson, A., Julliand, V., Luthersson, N., Santos, A.S., Vervuert, I., 2017. Review: feeding conserved forage to horses: recent advances and recommendations. Animal 11 (6), 958–967. https://doi.org/10.1017/S1751731116002469.

Hudson, J.M., Cohen, N.D., Gibbs, P.G., Thompson, J.A., 2001. Feeding practices associated with colic in horses. J. Am. Vet. Med. Assoc. 219, 1419–1425. https://doi.org/10.2460/javma.2001.219.1419.
Hummel, J., Scheurich, F., Ortmann, S., Crompton, L.A., Gerken, M., Clauss, M., 2017. Comparative selective retention of particle size classes in the gastrointestinal tract of ponies and goats. J. Anim. Physiol. Anim. Nutr. 102, 429–439. https://doi.org/10.1111/jpn.12763.
Hyslop, J.J., 2006. In situ and mobile bag methodology to measure the degradation profile of processed feeds in different segments of the equine digestive tract. Livest. Sci. 100 (1), 18–32. https://doi.org/10.1016/j.livprodsci.2005.11.007.
Hyslop, J.J., Cuddeford, D., 1996. Investigations on the use of the mobile bag technique in ponies. In: Proceedings of the British Society of Animal Science, 62. Cambridge University Press, UK, p. 647.
Janis, C., 1976. The evolutionary strategy of the Equidae and the origins of rumen and cecal digestion. Evolution 30 (4), 757–774. https://doi.org/10.1111/j.1558-5646.1976.tb00957.x.
Jarosz, L., Hvelplund, T., Weisbjerg, M.R., Jensen, B.B., 1994. True digestibility of protein in the small intestine and the hind gut of cows measured with the mobile bag technique using 15N-labelled roughage. Acta Agric. Scand. A Anim. Sci. 44 (3), 146–151. https://doi.org/10.1080/09064709409410891.
Jensen, R.B., Austbø, D., Knudsen, K.B., Tauson, A.H., 2014. The effect of dietary carbohydrate composition on apparent total tract digestibility, feed mean retention time, nitrogen and water balance in horses. Animal 8 (11), 1788–1796. https://doi.org/10.1017/S175173111400175X.
Julliand, V., de Fombelle, A., Varloud, M., 2006. Starch digestion in horses: the impact of feed processing. Livest. Sci. 100 (1), 44–52.
Karlsson, C.P., Jansson, A., Essén-Gustavsson, B., Lindberg, J.E., 2002. Effect of molassed sugar beet pulp on nutrient utilisation and metabolic parameters during exercise. Equine Vet. J. Suppl 34, 44–49. https://doi.org/10.1111/j.2042-3306.2002.tb05390.x.
Lorenzo-Figueras, M., Preston, T., Ott, E.A., Merritt, A.M., 2005. Meal-induced gastric relaxation and emptying in horses after ingestion of high-fat versus high-carbohydrate diets. Am. J. Vet. Res. 66 (5), 897–906. https://doi.org/10.2460/ajvr.2005.66.897.
Luthersson, N., Nielsen, K.H., Harris, P., Parkin, T.D.H., 2009. Risk factors associated with equine gastric ulceration syndrome (EGUS) in 201 horses in Denmark. Equine Vet. J. Suppl. 41, 625–630. https://doi.org/10.2746/042516409×441929.
Macheboeuf, D., Poncet, C., Jestin, M., Martin-Rosset, W., 1996. Use of a mobile nylon bag technique with caecum fistulated horses as an alternative method for estimating pre-caecal and total tract nitrogen digestibilities of feedstuffs. EAAP-47th Annu. Meet. 25–29, 296 (August, Lillehammer, Norway).
Martin-Rosset, W., Andrieu, J., Vermorel, M., Dulphy, J.P., 1984. Valeur nutritive des aliments pour le cheval (In French). Le Cheval – Reproduction, Sélection, Alimentation, Exploitation. INRA Publications, Versailles, France, pp. 208–238.
Moore-Colyer, M.J.S., Hyslop, J.J., Longland, A.C., Cuddeford, D., 2002. The mobile bag technique as a method for determining the degradation of four botanically diverse fibrous feedstuffs in the small intestine and total digestive tract of ponies. Br. J. Nutr. 88 (6), 729–740. https://doi.org/10.1079/BJN2002734.
Moore-Colyer, M.J.S., Morrow, H.J., Longland, A.C., 2003. Mathematical modelling of digesta passage rate, mean retention time and in vivo apparent digestibility of two different lengths of hay and big-bale grass silage in ponies. Br. J. Nutr. 90 (1), 109–118. https://doi.org/10.1079/bjn2003869.
Müller, C.E., 2012. Equine digestion of diets based on haylage harvested at different plant maturities. Anim. Feed Sci. Technol. 177, 65–74. https://doi.org/10.1016/j.anifeedsci.2012.06.002.
National Research Council, 2007. Energy. In: Nutrient Requirements of Horses, sixth ed. National Academies Press, Washington, DC, USA, pp. 3–33.
Norwegian Government, 2015. Regulations on the Use of Animals in Experiments (In Norwegian: Forskrift om bruk av dyr i forsøk). Oslo, Norway.
Oliveira, C.A.A., Azevedo, J.F., Martins, J.A., Barreto, M., Silva, V.P., Julliand, V., Almeida, F.Q., 2015. The impact of dietary protein levels on nutrient digestibility and water and nitrogen balances in eventing horses. J. Anim. Sci. 93 (1), 229–237. https://doi.org/10.2527/jas.2014-6971.
Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92 (2), 499–503. https://doi.org/10.1017/S0021859600063048.
Pagan, J.D., Harris, P., Brewster-Barnes, T., Duren, S.E., Jackson, S.G., 1998. Exercise affects digestibility and rate of passage of all-forage and mixed diets in thoroughbred horses. J. Nutr. 128 (12), 2704S–2707S. https://doi.org/10.1093/jn/128.12.2704S.
Philippeau, C., Varloud, M., Julliand, V., 2014. Mobile bag starch prececal disappearance and postprandial glycemic response of four forms of barley in horses. J. Anim. Sci. 92 (5), 2087–2093 http://doi.org//10.2527/jas.2013-6850.
Ragnarsson, S., Lindberg, J.E., 2008. Nutritional value of timothy haylage in Icelandic horses. Livest. Sci. 113 (2–3), 202–208. https://doi.org/10.1016/j.livsci.2007.03.010.
Randby, Å.T., Nørgaard, P., Weisbjerg, M.R., 2010. Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing Norwegian Red bulls. Grass Forage Sci. 65, 273–286. https://doi.org/10.1111/j.1365-2494.2010.00745.x.
Rodrigues, L.M., Almeida, F.Q.D., Pereira, M.B., Miranda, A.C.T., Guimarães, A., Andrade, A.M.D., 2012. Roughage digestion evaluation in horses with total feces collection and mobile nylon bags. Rev. Bras. De. Zootec. 41 (2), 341–346. https://doi.org/10.1590/S1516-35982012000200016.
Rosenfeld, I., Austbø, D., 2009. Digestion of cereals in the equine gastrointestinal tract measured by the mobile bag technique on caecally cannulated horses. Anim. Feed Sci. Technol. 150, 249–258. https://doi.org/10.1016/j.anifeedsci.2008.09.002.
Saastamoinen, M.T., Hellämäki, M., 2012. Forage analyses as a base of feeding of horses. In: Saastamoinen, M., et al. (Eds.), Forages and Grazing in Horse Nutrition, 132. EAAP publication Wageningen Academic Publishers, The Netherlands, pp. 305–313.
RStudio Team, 2020. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA, USA.
Theander, O., Åman, P., Westerlund, E., Graham, H., 1994. Enzymatic/chemical analysis of dietary fiber. J. AOAC Int. 77, 703–709. https://doi.org/10.1093/jaoac/77.3.703.
Thorringer, N.W., Jensen, R.B., 2021. Methodical considerations when estimating nutrient digestibility in horses using the mobile bag technique. Animal 15 (1). https://doi.org/10.1016/j.animal.2020.100050.
Udén, P., Van Soest, P.J., 1984. Investigations of the in-situ bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. J. Anim. Sci. 58 (1), 213–221. https://doi.org/10.2527/jas1984.581213x.
Van Weyenberg, S., Sales, J., Janssens, G.P.J., 2006. Passage rate of digesta through the equine gastrointestinal tract: a review. Livest. Sci. 99 (1), 3–12. https://doi.org/10.1016/j.livprodsci.2005.04.008.

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Non Ruminant Nutrition

NON RUMINANT NUTRITION

The effects of processing barley and maize on metabolic and digestive responses in horses

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Abstract

The competition for customers increases the search for new grain processing methods for equine feed, but the effect on starch digestibility and metabolic responses varies. Therefore, to evaluate the effect of the processing methods, toasting and micronizing, on starch digestion and the effect on metabolic responses, the mobile bag technique (**MBT**) and plasma glucose and insulin concentrations in the blood were used to estimate nutrient disappearance and metabolic responses pre-cecally. Further, cecal pH, ammonium nitrogen (N), and short-chain fatty acid (**SCFA**) concentrations were used to estimate the metabolic response in the cecum. Four cecally cannulated horses (body weight [**BW**] 565 ± 35 kg) were used in a 4 × 4 Latin square design with four periods of 8 d of diet adaptation and 2 d of data collection. Diets were formulated using hay and processed grains: micronized barley (**MB**), toasted barley (**TB**), micronized maize (**MM**), and toasted maize (**TM**) and were balanced to provide 1 g starch/kg BW in the morning meal. On day 9 in each period, blood and cecal fluid samples were taken before the morning meal and hourly thereafter for 8 h. On day 10 in each period, 15 bags of either MB, TB, MM, or TM (1 × 1 × 12 cm; 15 µm pore size; 1 g feed) were placed in the stomach, respectively. The dry matter disappearance was highest for the MM at all time points compared with the other feedstuffs ($P < 0.001$). Maize and micronizing had the highest starch disappearance ($P = 0.048$) compared with barley and toasting. No treatment effect was measured for any of the glucose and insulin parameters. No feed effect was measured for the insulin parameters. Plasma glucose peaked later ($P = 0.045$) for maize than for barley, and TB had a larger area under the curve for glucose than MB, MM, and TM ($P = 0.015$). The concentration of total SCFA increased after feeding ($P < 0.001$), with a higher concentration for barley than for maize ($P = 0.044$). No treatment or feed effects were measured for ammonium N or pH, but both were affected by time ($P < 0.001$). In conclusion, toasting was not as efficient as micronizing to improve pre-cecal starch digestibility; therefore, the preferred processing method for both barley and maize is micronizing. Further, the amount of starch escaping enzymatical digestion in the small intestine was higher than expected.

Key words: glucose, insulin, mobile bag technique, pH, short chain fatty acid

Introduction

The apparent total tract digestibility of starch in grains is found to be nearly 100% in horses (Jensen et al., 2014), whereas larger variations (21.5% to 90.1%) are found for pre-cecal starch digestion (Meyer et al., 1995). In horses, the pre-cecal starch digestion depends on several factors, such as the type of grain and its characteristics, meal size, and passage rate of digesta (Kienzle, 1994). Further, grain processing

Abbreviations

ADF	acid detergent fiber
AUC	area under the curve
BW	body weight
Cfat	crude fat
CP	crude protein
DG	degree of gelatinization
DM	dry matter
DSC	differential scanning calorimetry
MBT	mobile bag technique
NIR	near-infrared radiation
NDF	neutral detergent fiber
SCFA	short-chain fatty acid
WSC	water-soluble carbohydrates

involving heat and moisture is associated with improving the availability of starch for enzymatic degradation, thereby increasing starch digestion in the small intestine (Svihus et al., 2005). Using the mobile bag technique (**MBT**), Philippeau et al. (2014) found that pre-cecal starch digestion depended on processing, with the lowest digestion for untreated barley and the highest for ground barley, 55.1% and 97.4%, respectively. Enzymatic starch digestion in the small intestine is preferred, as starch fermentation in the hindgut is associated with a higher concentration of short-chain fatty acids (**SCFA**) and lactate, decreased pH, and microbial disturbance in equines (Willard et al., 1977; de Fombelle et al., 2003). Therefore, compound feeds and grains used for horses are often processed, and one of the most common processing methods is micronizing (Julliand et al., 2006). It includes thermal heat processing with high temperatures (85 to 125 °C) for a short time using near-infrared radiation (Farrell et al., 2015). Processing methods that include endosperm disruption and heat above 80 °C in combination with moisture will restructure the starch granules, causing gelatinization (Svihus et al., 2005). Gelatinization increases amylolytic degradation because part of the crystalline structure is lost (Svihus et al., 2005). Holm et al. (1988) found that the degree of starch gelatinization and digestion rate in rats to be positively correlated, assuming more starch to be digested and thereby change the metabolic responses, as more glucose will be absorbed in the small intestine. Vervuert et al. (2008) found that thermal processing increased serum glucose and insulin responses when horses were fed extruded barley compared with rolled barley or micronized barley (**MB**), reflecting a higher digestibility of starch in the small intestine with extrusion than with the other methods. However, from the literature, it is unclear whether the degree of gelatinization (**DG**) from processing is followed by higher glucose and insulin responses (Vervuert et al., 2003, 2007, 2008). The competition for customers increases the search for other processing methods so that feed producers can achieve a differential product. Toasting is one of the "new" processing methods employed by some equine feed companies. This method is often used in products for human consumption, such as breakfast cereals, flour, and wine (Fares and Menga, 2012; Chira and Teissedre, 2013), primarily to enhance the taste as a result of the Maillard reaction (Martins et al., 2001), and it includes temperatures ranging from 90 to 240 °C (Grala et al., 1994; Mosenthin et al., 2016). Hence, toasting could potentially be as effective as micronizing for improving the small intestine's digestibility of starch. Nonetheless, to our knowledge, no study has been conducted on toasting's effect on nutrient digestibility in horses. Therefore, the objective of this experiment was to compare the effects of micronizing and toasting on starch digestion of barley and maize. It is hypothesized that: 1) toasting is as efficient as micronizing for improving the small intestine's digestibility of starch; 2) starch digestibility in the small intestine is highly reflected in the blood glucose and insulin responses after feeding, independent of processing method; 3) the amount of starch escaping digestion in the small intestine is low; and 4) fluctuations in cecal pH and SCFA concentrations and proportions after feeding are small, independent of processing method.

Materials 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, July 2015). The experiment was designed as a 4 × 4 Latin square experiment with four experimental periods. Each period consisted of 8 d of diet adaptation followed by 2 d of data collection. Blood and cecal samples for pH and SCFA analyses were collected on day 9, and digestibility in the small intestine was measured on day 10 in each period.

Animals

Four healthy cecum-cannulated Norwegian cold-blooded trotter geldings (age 14 to 26 yr) with an initial body weight (**BW** ± SEM) of 565 ± 35 kg were used in the experiment. Horses were followed routinely with veterinarian checkups, including vaccinations, dental examinations, and teeth floating. All horses were housed in individual stalls (3 × 3 m) with rubber mats and wood shavings as bedding material. In the adaptation period, horses were allowed access to a gravel paddock for 3 to 4 h/d. In the collection periods, one outdoor visit for 1 h was allowed daily after sampling had ended.

Diets

Treatments were arranged as 2 × 2 factorial, with two processing methods: micronizing and toasting. Two feeds were used: barley and maize. The chemical composition of the feedstuffs is presented in Table 2. Four experimental diets were formulated using hay and processed grains (same batches): MB, toasted barley (**TB**), micronized maize (**MM**), and toasted maize (**TM**) (Table 3). The micronizing and toasting processes are described below. All concentrate was fed once a day at 0600 hours. Seven days prior to the first adaptation period, a mix of the four diets was fed to gradually increase starch intake from 0 to 1 g/kg BW per day. Thereafter, all diets were balanced to provide 1 g starch/kg BW, and the amount of hay was adjusted to a total DM intake of 3 g/kg BW in the meal at 0600 hours. The horses were fed a total of 15.7 ± 0.03 g DM/kg BW per day, which was divided into three meals fed at 0600, 1400, and 2000 hours (Table 3). A commercial supplement of vitamins and minerals (Champion Multitiskud, Felleskjøpet Forutvikling, Trondheim, Norway) and sodium chloride (80 and 25 g/d, respectively) was included with the morning meal. Water was available in the individual stalls' automatic water troughs and from buckets in the gravel paddock.

Processing

Micronizing and toasting of barley and maize occurred at Felleskjøpet Agri (Skansen, Norway). Approximately 14.5 h prior to the micronizing treatment, the raw maize was preconditioned with water to raise the moisture content to 15.5%. The barley did not receive any preconditioning with water, as it had a moisture content of 11.2%. The barley and maize were then micronized for approximately 45 s at 90 to 105 °C using an infrared micronizer with a heat output of 525 kW (M600/72/HRS, Micronizing Company UK Ltd, Suffolk, UK; Table 1). After micronizing, the heated barley and maize were run through a roller (0.15 mm, TECOM AB, X, Sweden) to produce a flaked product and then cooled down (custom-made cooler; Felleskjøpet Agri, Skansen, Norway). Prior to the toasting treatment (approximately 15 and 12.5 h for maize and barley, respectively), the raw grains were preconditioned with water to raise the moisture content to 20.6% and 22.6% (maize and barley, respectively). Thereafter, the grains were toasted for 30 min at 150 °C (ECOTOAST 600, agrel GmbH agrar Entwicklungs labor, Germany). After toasting, the heated barley and maize were run through a roller (0.35 and 1 mm for barley and maize, respectively; Strukturvalse T80, Vestjysk Smede, Denmark) to produce a flaked product and then cooled down.

Data collection

Feedstuffs

Samples of all feedstuffs were collected regularly during the four data collection periods and stored in sealed plastic bags for later analysis.

Mobile bag technique

The MBT was used to estimate the small intestinal starch digestibility. Bags (1 × 1 × 12 cm) were made from precision-woven open mesh fabric with a porosity of 15 µ (Sefar Nitex, 03-15/10, Sefar AG, Heiden, Switzerland). The bags were prepared by cutting a piece of mesh (large enough for the heat sealing) and folding it in the middle. The mesh was then heat sealed at one end and one side, and then turned inside out to avoid sharp edges. A steel washer (1 cm external diameter, weight 0.3 g) was sealed into the end of each bag, allowing for capture with a magnet in the cecum. Lastly, the bags were marked with a permanent marker for identification. The weights of the bags when empty and when filled with individual feed (1 g/bag) were recorded. All feeds were milled to pass a 1.5-mm screen. The bags (15 bags per horse per period) were soaked in cold tap water before they were placed in the stomach with a nasogastric tube flushed with approximately 1.5 liters of tap water. Bags were administered after feeding half of the morning meal and before feeding hay. The rest of the morning meal and the hay were fed afterward. A string (40 cm long) with a double-sided magnet (approximately 2 cm in diameter) was introduced into the cecum through the cannula to retrieve the bags upon arrival. The bags were removed from the magnet at hourly intervals for 8 h after feeding. Bags not harvested in the cecum were collected in the feces throughout the following days. The capture time of each bag was noted as soon as the bags were collected and, thereafter, hand-rinsed in cold tap water and stored at –20 °C. At the end of the experiment, all bags were thawed at room temperature, washed in cold water for 35 min (Woolprogram, Avantixx 7 Varioperfect, Bosch, Gerlingen-SchillerhÖhe, Germany), and then dried at 45 °C for 48 h. The bags were left at room temperature (approximately 25 °C) for equilibration for 24 h prior to weighing. Control bags (4 bags per feedstuff) were soaked for 1 h before washing and drying as described above to determine their nutrient loss. To obtain enough residue for chemical analyses, the collected bags of each feedstuff were pooled to a specific collection time (0 to 3, 4 to 6, and 7 to 9 h), regardless of which horse they came from. All bags found in the feces were pooled for each feedstuff.

Blood samples

Blood samples were collected by jugular vein puncture into 10-mL heparinized tubes (BD Vacutainer, Becton, Dickinson and Company, USA) before the morning meal (time: 0) and hourly thereafter (time: 1 to 8 h). The blood samples were centrifuged (Heraeus labofuge 300, Thermo Fisher Scientific, Waltham, USA) immediately after sampling at 3000 × *g* for 10 min, and plasma was harvested and stored at –20 °C for later analysis of insulin and glucose concentrations.

SCFA, ammonium nitrogen, and pH

Cecal fluid was collected before the morning meal (time: 0) and thereafter hourly (time: 1 to 8 h). A collection tube and a pH electrode (Sentix 41, WTW, Weilheim, Germany) attached to a data logger (ProfiLine 340i, WTW, Weilheim, Germany) were placed in the cecum according to Jensen et al. (2016) approximately 30 min before the first collection (time: 0). Cecal fluid was sampled (~100 mL) with a 400-mL syringe connected to the tube placed in the cecum. The pH was measured immediately as cecal fluid samples were taken and in situ in the cecum every minute throughout the 8 h time frame with the pH electrode. From this, two subsamples of each 9.5 mL cecal fluid were mixed with 0.5 mL of formic acid and stored at 3 °C for later analysis of SCFA and ammonium nitrogen (N) concentrations.

Chemical analyses

Feed samples from each period were analyzed in duplicate for DM, starch, and crude protein (**CP**) (Table 2). Samples were milled to pass a 1-mm screen (Cutting mill SM 200, Retsch GmbH, Haan, Germany). For starch, feed samples were milled to pass a 0.5-mm screen before analysis. Dry matter (**DM**) content was measured by drying to a constant weight (24 h at 105 ± 2 °C), and samples were incinerated at 550 °C for 16 h for ash determination. Starch was measured according to the Association of Official Analytical Chemists (AOAC, method 996.11.) by using heat-stable α-amylase, and water-soluble carbohydrates (**WSC**) were determined by the

Table 1. Processing conditions for barley and maize

	Toasting				Micronizing			
	Temp.[1]	Duration, min	Heat source	Roller, mm	Temp.	Duration, s	Heat source	Roller, mm
Barley	150	30	Steam	0.35	90 to 105	45	NIR	0.15
Maize	150	30	Steam	1	90 to 105	45	NIR	0.15

[1] Temp, temperature in °C.

Table 2. DM (g/kg), chemical composition (g/kg DM), and DG (%) of hay, micronized maize (MM) or toasted maize (TM), and micronized barley (MB) or toasted barley (TB) (mean ± SEM)

						P-value[1]	
Nutrient	Hay	MM	TM	MB	TB	F	T
DM	898 ± 1.46	874 ± 2.47[a]	840 ± 4.27[b]	881 ± 1.27[A]	830 ± 3.03[B]	0.338	<0.001
Ash	78.2 ± 0.85	14.2 ± 0.31	13.8 ± 0.65	19.8 ± 0.12	20.4 ± 0.30	<0.001	0.862
CP	147 ± 5.59	86.3 ± 2.42[a]	84.2 ± 1.77[b]	120 ± 2.10[B]	126 ± 0.71[A]	<0.001	0.302
CFat	18.6 ± 1.59	43.4 ± 3.25[a]	36.0 ± 1.10[b]	14.3 ± 0.70	15.6 ± 0.57	<0.001	0.058
Starch	28.9 ± 0.80	721 ± 7.89	719 ± 9.69	601 ± 5.00	577 ± 7.88	<0.001	0.145
WSC	84.9 ± 2.18	27.7 ± 0.88[b]	35.4 ± 1.55[a]	32.6 ± 0.50	38.5 ± 0.60	0.557	0.003
NDF	616 ± 6.62	95.8 ± 4.61[b]	119 ± 1.30[a]	224 ± 2.46	227 ± 7.32	<0.001	0.051
ADF	341 ± 4.92	46.7 ± 0.89	47.8 ± 1.10	78.6 ± 0.56	77.1 ± 1.94	<0.001	0.859
DG		56.8 ± 1.49	39.1 ± 3.10	−12.7 ± 12.0	−34.3 ± 1.53	<0.001	0.021

[1]The effect of feedstuff (F) and treatment (T).

[a,b or A,B]Values within a row for each feedstuff are different if superscript differs ($P < 0.05$).

Table 3. Feed intake (kg) and daily nutrient intake (g/kg BW) for the four diets[1] (mean ± SEM)

	MM, $n = 4$	MB, $n = 4$	TM, $n = 4$	TB, $n = 4$
Morning, 0600 hours				
Hay	1.10 ± 0.03	0.91 ± 0.03	1.13 ± 0.04	0.95 ± 0.03
Supplement	0.88 ± 0.03	1.05 ± 0.03	0.90 ± 0.03	1.10 ± 0.03
Lunch, 1400 hours				
Hay	3.95 ± 0.12	3.95 ± 0.12	3.95 ± 0.12	3.95 ± 0.12
Evening, 2000 hours				
Hay	3.95 ± 0.12	3.95 ± 0.12	3.95 ± 0.12	3.95 ± 0.12
Daily nutrient intake[1]				
DM	15.6 ± 0.02	15.6 ± 0.02	15.7 ± 0.03	15.7 ± 0.03
Ash	1.13 ± 0.01	1.12 ± 0.01	1.14 ± 0.01	1.13 ± 0.01
CP	2.21 ± 0.08	2.25 ± 0.08	2.22 ± 0.08	2.27 ± 0.08
Cfat	0.32 ± 0.02	0.29 ± 0.02	0.31 ± 0.02	0.29 ± 0.02
Starch	1.39 ± 0.02	1.39 ± 0.02	1.37 ± 0.02	1.34 ± 0.02
WSC	1.25 ± 0.03	1.25 ± 0.03	1.27 ± 0.03	1.26 ± 0.03
NDF	8.91 ± 0.09	8.97 ± 0.09	8.97 ± 0.09	9.01 ± 0.09
ADF	4.92 ± 0.08	4.89 ± 0.08	4.94 ± 0.08	4.91 ± 0.08

[1]MM, micronized maize; TM, toasted maize; MB, micronized barley; TB, toasted barley.

method described in the study of Randby et al. (2010). Nitrogen was determined according to the Dumas method (Elementar Analysensysteme GmbH, Hanau, Germany), and CP was calculated as N × 6.25. Crude fat was analyzed according to the accelerated solvent extractor method (Dionex ASE 350, Thermo Fisher Scientific, Waltham, USA). Neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) were analyzed using the filter bag technique described by ANKOM (2017a, 2017b). Residues from the mobile bags were analyzed for starch and N as described above. Plasma glucose was analyzed by the hexokinase method according to Tietz et al. (1995), and insulin was analyzed using the ELISA test (Mercodia AB, Uppsala, Sweden). Cecal fluid was analyzed for the concentration of SCFA (times: 0, 1, 3, 5, and 7 h) and ammonium N (times: 0 and 3 h). The concentrations of SCFA were determined by gas chromatography (Trace 1300 GC, Thermo Fisher Scientific, Waltham, USA), and ammonium N was measured according to AOAC International (2002) method 2001.11, besides the first digestion step. The DG was evaluated using the differential scanning calorimetry (**DSC**) method. The DSC method relies on the enthalpy measurement of non-processed and processed samples, and the difference between the two represents the extent of gelatinization with a greater difference indicating greater gelatinization. A DM feed sample weighing approximately 30 mg (ground through a 0.5-mm screen) was weighed in a stainless-steel pan, and deionized water (1:2, feed/water, wt/wt, total weight 90 mg) was added. Thermal scans were conducted using a differential scanning calorimeter (DSC 823, Mettler Toledo, Stockholm, Sweden). The measurement was performed by heating the pan in the DSC from 10 to 120 °C at a heating rate of 10 °C/min. The onset, peak, and conclusion gelatinization temperatures and the enthalpy of gelatinization (ΔH) were then determined. The DG is calculated as $DG\ (\%) = [(\Delta H_0\ \Delta H_1) / \Delta H_0] \times 100$, in which ΔH_0 is the gelatinization enthalpy of starch (J/g starch) in a non-processed sample and ΔH_1 is the gelatinization enthalpy of starch in a processed sample (J/g starch). A 100% DG equates to completely processed starch, whereas 0% equates to unprocessed starch, and negative values indicate lower DG in the processed sample than the non-processed sample. All measurements were performed in duplicate.

Statistical analyses

All statistical analyses were performed in Rstudio (version 1.1.456, Rstudio Inc., Boston, USA). Analysis of variance was done on the

chemical composition of the feedstuffs with a model comprising nutrient as response and feed and treatment as predictors. The DM, starch, and CP disappearance were subjected to ANOVA, with the nutrient disappearance as response and feed, and treatment and time (DM) or time interval (starch and CP) and their interactions as predictors. Mean concentrations, peak concentration, time to peak, and number of peaks were calculated for plasma glucose and insulin. Calculations of area under the curve (**AUC**) above baseline (without considering area beneath) were performed for glucose and insulin in GraphPad Prism (version 8.0.1, GraphPad Software, San Diego, USA), and ANOVAs were performed in a model compromising either mean concentration, peak concentration, time to peak, or number of peaks and AUC as response, with feed, treatment, and their interactions (if present) as predictors. Analyses of SCFA, ammonium N concentrations, and pH were performed using mixed models for repeated measurements. The model comprised the fixed effect of feed (barley or maize), treatment (micronizing or toasting), time (after feeding), interaction (feed × treatment), and the random effect of horse. Significant differences of least-square means were analyzed by Tukey's Honest Significant Difference test (Rstudio, version 1.1.456, Rstudio Inc., Boston, USA). All results are presented as least-square means with SEM as a measure of variance. Effects are considered significantly different if $P < 0.05$ and a tendency if $P < 0.10$.

Results

All horses remained healthy and in good condition throughout the experiment. Residues from the previous evening meal were collected for two horses on the day of sampling (one horse in period 3:1.6 kg DM and two horses in period 3:0.7 and 1 kg DM, respectively). The residue was offered to the horses and eaten after sampling had ended.

Chemical composition of the feedstuffs

The chemical composition of the feedstuffs is presented in Table 2. Hay has the highest numerical DM content compared with maize and barley. An effect of treatment ($P < 0.001$) was measured for DM, with micronizing having the highest content for both maize and barley. Barley had the highest content of CP ($P < 0.001$) compared with maize. Toasting had the highest ($P = 0.003$) WSC content for both barley and maize. The starch content was highest in maize compared with barley ($P < 0.001$), whereas hay had the lowest numerical content. Crude fat was highest in maize compared with barley ($P < 0.001$). NDF and ADF were highest in barley compared with maize ($P < 0.001$). The DG was highest for MM compared with the other diets (Table 2). However, DG for processed barley was negative, indicating that processed barley had a lower DG than whole barley. The negative DG for barley was interpreted as zero DG for barley. The DM intake for each meal and daily nutrient intake is shown in Table 3. The size of the grain meal within each diet varied to ensure similar starch intake.

Nutrient disappearance

The DM loss from the control bags was 7.3 ± 1.4%, 9.9 ± 1.9%, 6.5 ± 1.6%, and 9.6 ± 1.0% for MM, TM, MB, and TB, respectively. The effects of feed, treatment, time, and their interactions on DM, starch, and CP pre-cecal disappearance are shown in Figure 1. There was an effect of the interaction, feed × treatment × time ($P < 0.001$), and the DM disappearance from the mobile bags increased over time; it was at all times highest for the MM compared with the other feedstuffs. Starch disappearance increased with later time intervals, and an interaction between feed × treatment ($P = 0.048$) was measured with maize and micronizing having the highest disappearances compared with barley and toasting. Disappearance of CP increased over time ($P = 0.041$), regardless of feed or treatment.

Metabolic response in plasma

The effects of feed, treatment, and their interaction on plasma glucose and insulin measurements are presented in Table 4. Treatment did not affect any of the measured variables for plasma glucose and insulin. Feed had no effect on the measured variables for plasma insulin. There was no effect of feed on peak and the number of peaks for plasma glucose. However, plasma glucose peaked later ($P = 0.045$) for maize than for barley. Regarding AUC, an interaction between feed and treatment was found for glucose ($P = 0.015$), with a larger AUC for TB than for MB and MM or TM.

Digestive response in the cecum

The effects of feed, treatment, time, and their interactions on SCFA concentrations and molar proportions are shown in Figure 2. The concentration of total SCFA increased after feeding ($P < 0.001$), with a higher concentration for barley than for maize ($P = 0.044$; Figure 2a). Generally, the molar proportion of acetate was the greatest, followed by propionate and then butyrate for all diets at all time points. However, the molar proportion of acetate ($P = 0.004$) first increased and then decreased with time (Figure 2b), whereas the opposite was found for propionate ($P = 0.006$; Figure 2c). Firstly, the proportion of butyrate ($P = 0.086$) tended to increase and thereafter decrease with time (Figure 2d), whereas iso-butyrate ($P < 0.001$; Figure 2e) and iso-valerate ($P < 0.001$; Figure 2g) decreased after feeding. Further, butyrate tended to be higher ($P = 0.058$) for micronizing than for toasting (Figure 2d). An interaction between feed and time ($P < 0.001$) was present for valerate, as the proportion after feeding increased for barley; however, maize remained the same (Figure 2f). The (C2 + C4)/C3 ratio ($P = 0.055$) tended to first increase and then decrease after feeding, reflecting the changes in molar proportions of acetate, propionate, and butyrate over time (Figure 2h). No effects of feed, treatment, or their interaction were found on ammonium N. But the mean concentrations of ammonium N decreased over time ($P < 0.001$), with MM from 57.5 to 23.2 mg/L, MB from 65.7 to 22.3 mg/L, TM from 65.9 to 17.2 mg/L, and TB from 65.8 to 19.5 mg/L. The pH decreased after feeding, reaching a minimum pH after 195, 173, 180, and 150 min for MM, MB, TM, and TB, respectively (Figure 3). The pH then fluctuated before increasing again. Feed, treatment, and their interaction had no effect on cecal pH.

Discussion

Starch digestion has been previously investigated in horses using different direct and indirect methodologies. Small intestinal cannulated horses (Meyer et al., 1995), slaughter experiments (de Fombelle et al., 2003), and the MBT (Philippeau et al., 2014) have been used as more direct methods for quantifying starch digestion in different segments of the gastrointestinal tract of horses. Blood glucose and insulin responses (Healy et al., 1995; Vervuert et al., 2004, 2007; Jensen et al., 2016) and changes in fermentation parameters in the cecum (McLean et al., 2000) of horses have been used as a proxy to evaluate the degree of starch digestion in the small intestine and cecum, respectively. However, the results have been inconclusive. To the authors' knowledge, this is the first

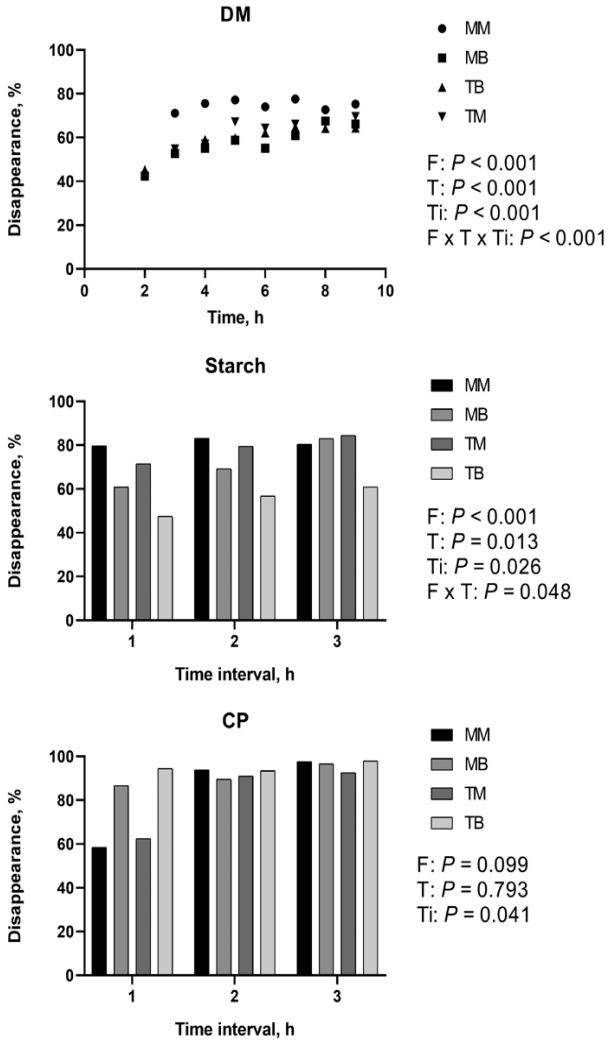

Figure 1. DM, starch, and CP pre-cecal disappearance for each of the four diets (MM, MB, TM, and TB) for each hour or time interval (1 = 0 to 3 h, 2 = 4 to 6 h, and 3 = 7 to 9 h), respectively. Differences are given for feed (F), treatment (T), and time/time interval (Ti) and interactions.

study to include both metabolic responses in blood and the digestive responses in cecum in combination with results from the MBT. The results presented here show the complexity of evaluating starch digestion in horses by only including one of the above-mentioned methodologies.

Pre-cecal disappearances of starch and protein

It is assumed that nutrients lost from mobile bags harvested in the cecum are digested in the small intestine. In the present study, the pre-cecal disappearance of starch and protein varied from 55% to 81% and 82% to 95%, respectively. This is in accordance with previous studies using the MBT (Hymøller et al., 2012; Philippeau et al., 2014). Protein digestion was relatively high and not affected by processing, while high starch digestibility was expected due to the maize and barley being processed. However, some variation was measured in the starch disappearance. In the present study, the average starch intake was 565 g/d, and according to MBT, starch measurements of approximately 107, 164, 122, and 254 g/d escaped digestion in the small intestine for MM, MB, TM, and TB diets, respectively. Since the apparent total tract digestibility of starch in grains is found to be nearly 100% (Jensen et al., 2014), it is expected that the undigested starch was fermented mainly in the hindgut. Some starch might be fermented by gastric microbiota present in the saccus cecus in the nonglandular region of the stomach (Coenen et al., 2006; Varloud et al., 2007). However, to what extend starch is fermented in the stomach still needs to be quantified. The site of starch digestion in the gastrointestinal tract of the horse (pre-cecal or hindgut) is expected to influence the metabolic responses, as discussed below.

Metabolic response in plasma

In the present study, it was hypothesized that starch digestion in the small intestine was reflected in the blood glucose and

Table 4. Mean ± SEM peak (ng/L), time to peak (h), and AUC (ng × h/L) for glucose (G) and insulin (I) with different diets

						P-value[2]		
Feed treatment[1]		MB	TB	MM	TM	F	T	F × T
Peak	G	5.88 ± 0.13	5.85 ± 0.18	5.85 ± 0.19	5.78 ± 0.23	0.794	0.794	0.794
	I	386 ± 56.8	354 ± 26.5	460 ± 64.7	394 ± 65.0	0.325	0.397	0.765
No. of peaks	G	1.75 ± 0.48	1.25 ± 0.25	1.25 ± 0.25	1.50 ± 0.29	0.712	0.712	0.279
	I	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00			
Peak time	G	1.00 ± 0.00[b]	1.25 ± 0.25[b]	1.50 ± 0.29[a]	2.00 ± 0.41[a]	0.045	0.205	0.663
	I	1.25 ± 0.25	1.25 ± 0.25	1.00 ± 0.00	1.25 ± 0.25	0.574	0.574	0.574
AUC	G	2.32 ± 0.28[ab]	3.48 ± 0.44[a]	2.89 ± 0.57[ab]	1.75 ± 0.25[b]	0.177	0.983	0.015
	I	1,373 ± 156	1,433 ± 74.9	1,444 ± 119	1,220 ± 112	0.562	0.502	0.256

[1]MM, micronized maize; TM, toasted maize; MB, micronized barley; TB, toasted barley.
[2]The effect of feedstuff (F), treatment (T), and their interaction (F × T).
[a, b]Values within a row are different if superscript differs ($P < 0.05$).

insulin responses after feeding, independent of the processing method. This was the case, as both plasma glucose and insulin increased after feeding. This was also measured in earlier studies (Vervuert et al., 2003, 2004, 2009). In the present study, MM had a higher pre-cecal DM and starch disappearance from mobile bags compared with the other diets, but no differences were found between feeds or treatments for either plasma glucose or insulin. Similar findings for whole vs. thermally processed barley on starch disappearance and glucose and insulin responses were measured by Philippeau et al. (2014). This contradicts the theory that increased starch digestibility should increase the glucose concentration in the blood and further increase the insulin response (Palumbo et al., 2013). Yet, it is unclear to what degree the disappeared starch from MM was enzymatically digested or possibly degraded by microbes, as they are present along the entire gastrointestinal tract including the stomach (de Fombelle et al., 2003).

The AUC is often used as a parameter to describe both the overall plasma glucose and insulin responses after feeding. However, contradicting results are found for grain processing on AUC. Vervuert et al. (2003) and Vervuert et al. (2004) did not measure any effect of processing oats or maize (untreated vs. thermal processing) on glucose or insulin AUC, respectively. Yet, Vervuert et al. (2008) measured a larger glucose AUC for extruded compared with rolled barley and MB, along with a larger insulin AUC for extruded and MB compared with rolled barley. In the present study, an interaction between feed × treatment was found for AUC, with TB having a higher AUC for glucose compared with MB, MM, and TM. TB peaked twice during the sampling time, whereas MB, MM, and TM only peaked once. The time for peaks to occur and the number of peaks could indicate the differences in gastric contractions and thereby gastric emptying. Lorenzo-Figueras et al. (2005) describe gastric emptying as a combination of relaxation of the proximal portion of the stomach, suppression of antral motility, and stimulation of the pyloric contractions, all working together at once. The composition of the meal combined with volume, physical structure, energy density, and osmolarity can affect the rate of gastric emptying (Meyer et al., 1986). Slower gastric emptying is measured with a starch-rich meal (1.25 g starch/kg BW) compared with a meal low in starch (0.66 g starch/kg BW; Métayer et al., 2004). However, in the present study, all meals were similar in starch content. Yet, plasma glucose peaked later for maize than for barley. In general, meals containing maize were smaller in volume compared with those containing barley, as the starch content was higher in maize than barley; thereby, less was required to obtain 1 g starch/kg BW per meal. This contradicts smaller meals resulting in faster gastric emptying compared with larger meals (Métayer et al., 2004). On the other hand, the difference in meal size is small in the present study, and the effect on gastric emptying may have been limited. Another approach could be physical structure, osmolarity, or even the ratio between amylose and amylopectin in the grains. In general, maize has a higher swelling- and water-binding capacity than barley (Brøkner et al., 2012). This suggests a higher ratio of amylopectin to amylose, as it is easier to solubilize (Cowieson et al., 2019). Furthermore, Hymøller et al. (2012) measured a longer average pre-cecal passage time of mobile bags containing soaked maize compared with soaked barley (7.99 and 6.82 h, respectively), supporting the theory of why plasma glucose peaked later for maize than for barley. Maize and barley contain approximately similar ratios between amylose and amylopectin (approximately 25% and 75%, respectively; Svihus et al., 2005; Cowieson et al., 2019), but it cannot be excluded that maize had a higher amylopectin ratio, as it was not measured in the present study.

Digestive response in the cecum

In general, plasma glucose and insulin concentrations are parameters of pre-cecal digestion, whereas the cecal SCFA concentration together with pH gives an indication of fermentation in the hindgut of the horse. Further, the time to reach maximum SCFA concentration and minimum pH in cecum can indicate the passage rate of the feed from the stomach to the cecum and the fermentability of the escaped starch. In the present study, SCFA concentrations increased relatively fast after feeding (approximately 1 to 2 h), and maximum SCFA concentrations were measured approximately 3 h after feeding. Jensen et al. (2016) measured both an increase in SCFA concentration and a corresponding pH drop approximately 3 h after feeding horses a pelleted barley meal (2 g starch/kg BW). In the present study, barley had a higher total SCFA concentration compared with maize, with TB having the highest SCFA concentration, and, furthermore, a lower pre-cecal starch disappearance up to 6 h after administration, reflecting starch being fermented in the cecum. The proportions of acetate and propionate also indicate the fermentation of starch. McLean et al. (2000) measured higher lactate and total SCFA with both higher acetate and propionate concentrations and lower cecal pH 4 to 8 h after feeding rolled barley compared with micronized and extruded barley, indicating that less starch reached the

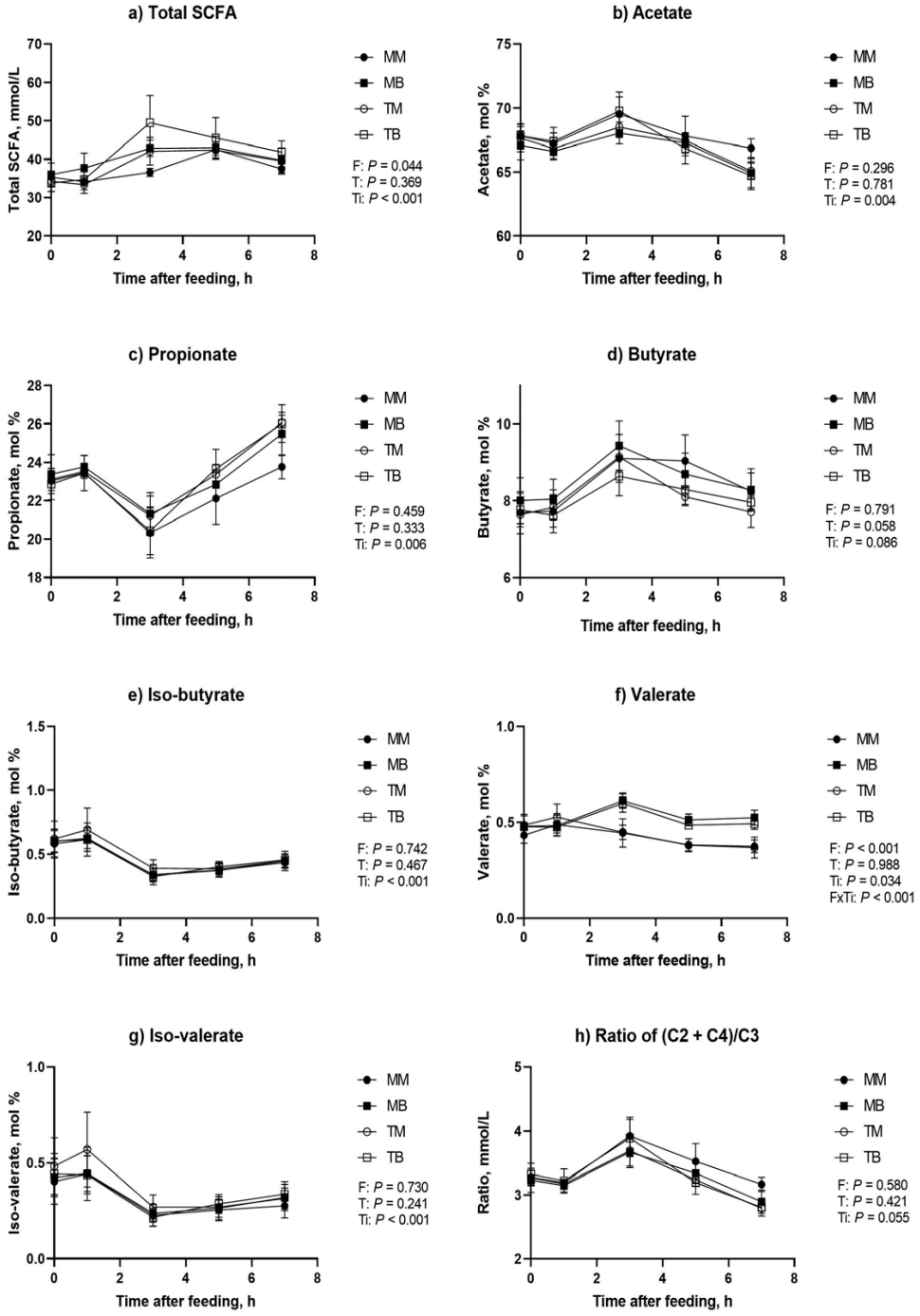

Figure 2. Concentration of SCFA (mmol/L) and molar proportions (%) measured hourly (mean ± SEM) in the cecal fluid after feeding MM, TM, MB, and TB. Differences are given for feed (F), treatment (T), and time (Ti) and interactions.

cecum when using these processing techniques compared with rolling. Similar results are measured for propionate, lactate, and pH by increasing rolled barley in the ration, thereby increasing daily starch intake (Julliand et al., 2001). Starch intake was approximately 2 g/kg BW per meal in the studies by Julliand et al. (2001), McLean et al. (2000), and Jensen et al. (2016), and the

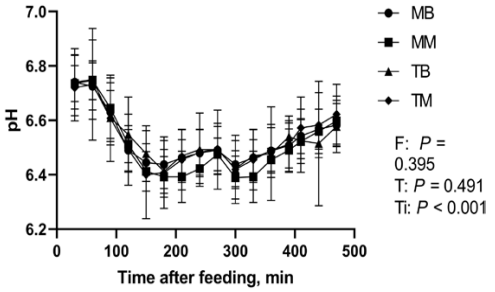

Figure 3. pH fluctuations in cecum measured in 30-min intervals for the average of the four diets after feeding MM, TM, MB, and TB. Differences are given for feed (F), treatment (T), and time (Ti).

minimum pH varied from 6.26 to 6.40, which is lower than the minimum pH in the present study. When feeding either starch at approximately 2 g/kg BW per meal or hay-only diets, cecal pH varied from 6.26 to 6.40 and 6.50 to 6.74, respectively (McLean et al., 2000; Julliand et al., 2001; Jensen et al., 2016). In this study, the decrease in cecal pH was in between the above studies. Altogether, this indicates that processed starch meals fed at a level of 1 g/kg BW can to some extent escape the enzymatic digestion in the small intestine, thereby interfering with the microbiota and concentrations and ratios of SCFA and pH.

In this study, it is possible that the processing methods that included thermal heat increased the pre-cecal starch digestibility as a result of an increased DG. When comparing the DG in the present study, no gelatinization occurred for either of the two barley diets. Whereas, for maize, micronizing had a larger impact on DG compared with toasting. Vervuert et al. (2004) also measured an increased DG when maize was micronized compared with untreated maize. In general, maize has a higher gelatinization enthalpy, meaning that lower temperatures and moisture content are required to gelatinize maize starch compared with barley starch (Tan et al., 2008). However, both Vervuert et al. (2007) and Philippeau et al. (2014) measured the effect of processing barley on DG. From these two studies, ground barley had a DG varying from 15% to 18%, indicating a possibility of a lower DG for TB and MB in the present study. Yet, Rosenfeld and Austbø (2009) did not measure an effect of micronizing grains on pre-cecal starch disappearance as in the present study. An in vitro study demonstrated lower starch digestibility of peas when toasted compared with being extruded and expanded (Masoero et al., 2005). This is also confirmed in pigs, where a lower ileal starch digestibility of toasted peas compared with dried was measured (Canibe and Knudsen, 1997). However, it can be difficult to compare results across studies, as the processing conditions (moisture content, duration, temperature, and pressure) vary.

Methodical and practical recommendations

In summary, the results presented here show the complexity of evaluating starch digestion in horses. Future studies should include detailed information regarding processing (duration, temperature, moisture content, pressure, and machinery), diet characteristics (composition and DG), and feeding management (g/kg BW per meal, number of meals, and feeding order of hay and concentrate), as well as information regarding the techniques used to study starch digestion. This would provide a better basis for comparing and interpreting results.

From a practical point, the results presented in this study indicate that processing affected the DG in maize more than in barley. Furthermore, compared with toasting, the preferred processing technique for improving the starch digestion based on the disappearance of starch from the mobile bags is micronizing. The metabolic responses in plasma and digestive responses in the cecum revealed more of a change over time than an effect of processing and type of grain on the measured variables. However, the SCFA concentration was highest in the TB compared with the MB, TM, and MM, supporting the lower digestibility of starch in the small intestine from the TB. The effect of the changes measured in the cecum in this study on the hindgut health can be questioned. Whereas, the energy value of starch is lower when fermented to SCFA than with enzymatical digestion in the small intestine with the absorption of glucose. The results from this study revealed that when feeding only 1 g processed starch/kg BW per meal, starch escapes the enzymatic digestion in the small intestine, and there is still a lack in our knowledge regarding the diet effects on gastric emptying and passage rate through the small intestine for improving enzymatical starch digestion.

Conclusions

In the present study, it was hypothesized that toasting was as efficient as micronizing to improve starch digestibility. However, this was not the case when evaluating the small intestinal digestibility of starch. Therefore, to increase the pre-cecal starch digestibility, the preferred processing method is micronizing for both barley and maize when measured by the MBT. Further, starch digestibility for both barley and maize was highly reflected in the metabolic responses of plasma glucose and insulin after feeding, but no effect of processing method was measured. Fluctuations in both cecal pH and SCFA concentrations after feeding were significant, and the starch escaping the enzymatical digestion in the small intestine was higher than expected.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Literature Cited

ANKOM. 2017a. Neutral detergent fiber in feeds – filter bag technique (for A200 and A200I). Available from https://www.ankom.com [accessed May 5, 2018].

ANKOM. 2017b. Acid detergent fiber in feeds – filter bag technique (for A300 and A200I). Available from https://www.ankom.com [accessed May 5, 2018]

Association of Official Analytical Chemists (AOAC) International. 2002. *Official methods of analysis*. Gaithersburg (MD): AOAC International. (Method 2001.11).

Brøkner, C., K. B. Knudsen, I. Karaman, K. L. Eybye, and A. H. Tauson. 2012. Chemical and physicochemical

characterisation of various horse feed ingredients. *Anim. Feed Sci. Technol.* **177**:86–97. doi:10.1016/j.anifeedsci.2012.06.005

Canibe, N., and K. B. Knudsen. 1997. Digestibility of dried and toasted peas in pigs. 1. Ileal and total tract digestibilities of carbohydrates. *Anim. Feed Sci. Technol.* **64**:293–310. doi:10.1016/S0377-8401(96)01032-2

Chira, K., and P. L. Teissedre. 2013. Extraction of oak volatiles and ellagitannins compounds and sensory profile of wine aged with French winewoods subjected to different toasting methods: behaviour during storage. *Food Chem.* **140**:168–177. doi:10.1016/j.foodchem.2013.02.049

Coenen, M., A. Mösseler, and I. Vervuert. 2006. Fermentative gases in breath indicate that inulin and starch start to be degraded by microbial fermentation in the stomach and small intestine of the horse in contrast to pectin and cellulose. *J. Nutr.* **136**:2108S–2110S. doi:10.1093/jn/136.7.2108S

Cowieson, A. J., S. L. Vieira, and C. Stefanello. 2019. Exogenous microbial amylase in the diets of poultry: what do we know?. *J. Appl. Poult. Res.* **28**:556–565. doi:10.3382/japr/pfy044

Fares, C., and V. Menga. 2012. Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea (*Cicer arietinum* L.) flour added to durum wheat pasta. *Food Chem.* **131**:1140–1148. doi:10.1016/j.foodchem.2011.09.080

Farrell, R. R., M. Wellinger, A. N. Gloess, D. S. Nichols, M. C. Breadmore, R. A. Shellie, and C. Yeretzian. 2015. Real-time mass spectrometry monitoring of oak wood toasting: elucidating aroma development relevant to oak-aged wine quality. *Sci. Rep.* **5**:17334. doi:10.1038/srep17334

de Fombelle, A., M. Varloud, A. G. Goachet, E. Jacotot, C. Philippeau, C. Drogoul, and V. Julliand. 2003. Characterization of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets. *Anim. Sci. J.* **77**:293–304. doi:10.1017/S1357729800059038

Grala, W., L. Buraczewska, J. Gdala, and B. Pastuszewska. 1994. Effect of the thermal processing on the protein value of double-low rapeseed products. 1. Effect of toasting temperature on protein value of rapeseed oil meal for pigs. *J. Anim. Feed Sci.* **3**:33–42. doi:10.22358/jafs/69817/1994

Healy, H. P., P. D. Siciliano, and L. M. Lawrencez. 1995. Effect of concentrate form on blood and gastric fluid variables in ponies. *J. Equine Vet. Sci.* **15**:423–428. doi:10.1016/S0737-0806(06)81833-2

Holm, J., I. Lundquist, I. Bjorck, A. C. Eliasson, and N. G. Asp. 1988. Relationship between degree of gelatinization, digestion rate in vitro and metabolic response in rats. *Am. J. Clin. Nutr.* **47**:1010–1016. doi:10.1093/ajcn/47.6.1010

Hymølle, L., M. S. Dickow, C. Brøkner, D. Austbø, and S. K. Jensen. 2012. Cereal starch, protein, and fatty acid pre-caecal disappearance is affected by both feed technological treatment and efficiency of the chewing action in horses. *Livest. Sci.* **150**:159–169. doi:10.1016/j.livsci.2012.08.016

Jensen, R. B., D. Austbø, K. E. Bach Knudsen, and A. H. Tauson. 2014. The effect of dietary carbohydrate composition on apparent total tract digestibility, feed mean retention time, nitrogen and water balance in horses. *Animal* **8**:1788–1796. doi:10.1017/S175173111400175X

Jensen, R. B., D. Austbø, D. Blache, K. E. Bach Knudsen, and A. H. Tauson. 2016. The effect of feeding barley or hay alone or in combination with molassed sugar beet pulp on the metabolic responses in plasma and caecum of horses. *Anim. Feed Sci. Technol.* **214**:53–65. doi:10.1016/j.anifeedsci.2016.02.003

Julliand, V., A. de Fombelle, C. Drogoul, and E. Jacotot. 2001. Feeding and microbial disorders in horses: part 3—effects of three hay: grain ratios on microbial profile and activities. *J. Equine Vet. Sci.* **21**:543–546. doi:10.1016/S0737-0806(01)70159-1

Julliand, V., A. de Fombelle, and M. Varloud. 2006. Starch digestion in horses: the impact of feed processing. *Livest. Sci.* **100**:44–52. doi:10.1016/j.livprodsci.2005.11.001

Kienzle, E. 1994. Small intestinal digestion of starch in the horse. *Rev. Med. Vet. (France)*. **145**:199–204.

Lorenzo-Figueras, M., T. Preston, E. A. Ott, and A. M. Merritt. 2005. Meal-induced gastric relaxation and emptying in horses after ingestion of high-fat versus high-carbohydrate diets. *Am. J. Vet. Res.* **66**:897–906. doi:10.2460/ajvr.2005.66.897

Martins, S. I. F. S., W. M. F. Jongen, and M. A. J. S. Van Boekel. 2001. A review of Maillard reaction in food and implications to kinetic modeling. *J. Food Sci. Technol.* **11**:364–373. doi:10.1016/S0924-2244(01)00022-X

Masoero, F., A. M. Pulimeno, and F. Rossi. 2005. Effect of extrusion, espansion and toasting on the nutritional value of peas, faba beans and lupins. *Ital. J. Anim. Sci.* **4**:177–189. doi:10.4081/ijas.2005.177

McLean, B. M. L., J. J. Hyslop, A. C. Longland, D. Cuddeford, and T. Hollands. 2000. Physical processing of barley and its effects on intra-caecal fermentation parameters in ponies. *Anim. Feed Sci. Technol.* **85**:79–87. doi:10.1016/S0377-8401(00)00132-2

Métayer, N., M. Lhôte, A. Bahr, N. D. Cohen, I. Kim, A. J. Roussel, and V. Julliand. 2004. Meal size and starch content affect gastric emptying in horses. *Equine Vet. J.* **36**:436–440. doi:10.2746/0425164044868468

Meyer, J. H., E. A. Mayer, D. Jehn, Y. Gu, A. S. Fink, and M. Fried. 1986. Gastric processing and emptying of fat. *Gastroenterology* **90**:1176–1187. doi:10.1016/0016-5085(86)90383-5

Meyer, H., S. Radicke, E. Kienzle, S. Wilke, D. Kleffken, and M. Illenseer. 1995. Investigations on preileal digestion of starch from grain, potato and manioc in horses. *Zentralbl. Veterinarmed. A* **42**:371–381. doi:10.1111/j.1439-0442.1995.tb00389.x

Mosenthin, R., U. Messerschmidt, N. Sauer, P. Carré, A. Quinsac, and F. Schöne. 2016. Effect of the desolventizing/toasting process on chemical composition and protein quality of rapeseed meal. *J. Anim. Sci. Biotechnol.* **7**:36. doi:10.1186/s40104-016-0095-7

Palumbo, P., S. Ditlevsen, A. Bertuzzi, and A. De Gaetano. 2013. Mathematical modeling of the glucose–insulin system: a review. *Math. Biosci.* **244**:69–81. doi:10.1016/j.mbs.2013.05.006

Philippeau, C., M. Varloud, and V. Julliand. 2014. Mobile bag starch prececal disappearance and postprandial glycemic response of four forms of barley in horses. *J. Anim. Sci.* **92**:2087–2093. doi:10.2527/jas.2013-6850

Randby, Å. T., P. Nørgaard, and M. R. Weisbjerg. 2010. Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing Norwegian Red bulls. *Grass Forage Sci.* **65**:273–286. doi:10.1111/j.1365-2494.2010.00745.x

Rosenfeld, I., and D. Austbø. 2009. Digestion of cereals in the equine gastrointestinal tract measured by the mobile bag technique on caecally cannulated horses. *Anim. Feed Sci. Technol.* **150**:249–258. doi:10.1016/j.anifeedsci.2008.09.002

Svihus, B., A. K. Uhlen, and O. M. Harstad. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: a review. *Anim. Feed Sci. Technol.* **122**:303–320. doi:10.1016/j.anifeedsci.2005.02.025

Tan, I., P. J. Torley, and P. J. Halley. 2008. Combined rheological and optical investigation of maize, barley and wheat starch gelatinisation. *Carbohydr. Polym.* **72**:272–286. doi:10.1016/j.carbpol.2007.08.018

Tietz, N. W. 1995. *Clinical guide to laboratory tests*. Philadelphia (PA): WB Saunders Company; p. 268–269.

Varloud, M., G. Fonty, A. Roussel, A. Guyonvarch, and V. Julliand. 2007. Postprandial kinetics of some biotic and abiotic characteristics of the gastric ecosystem of horses fed a pelleted concentrate meal. *J. Anim. Sci.* **85**:2508–2516. doi:10.2527/jas.2006-182

Vervuert, I., C. Bothe, and M. Coenen. 2007. Glycaemic and insulinaemic responses to mechanical or thermal processed

barley in horses. *J. Anim. Physiol. Anim. Nutr. (Berl)*. **91**:263–268. doi:10.1111/j.1439-0396.2007.00703.x

Vervuert, I., M. Coenen, and C. Bothe. 2003. Effects of oat processing on the glycaemic and insulin responses in horses. *J. Anim. Physiol. Anim. Nutr. (Berl)*. **87**:96–104. doi:10.1046/j.1439-0396.2003.00420.x

Vervuert, I., M. Coenen, and C. Bothe. 2004. Effects of corn processing on the glycaemic and insulinaemic responses in horses. *J. Anim. Physiol. Anim. Nutr. (Berl)*. **88**:348–355. doi:10.1111/j.1439-0396.2004.00491.x

Vervuert, I., K. Voigt, T. Hollands, D. Cuddeford, and M. Coenen. 2008. Effects of processing barley on its digestion by horses. *Vet. Rec.* **162**:684–688. doi:10.1136/vr.162.21.684

Vervuert, I., K. Voigt, T. Hollands, D. Cuddeford, and M. Coenen. 2009. Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. *Vet. J.* **182**:67–72. doi:10.1016/j.tvjl.2008.04.011

Willard, J. G., J. C. Willard, S. A. Wolfram, and J. P. Baker. 1977. Effect of diet on cecal pH and feeding behavior of horses. *J. Anim. Sci.* **45**:87–93. doi:10.2527/jas1977.45187x

10 Appendix

Calculation example for energy in hay for **paper I**, bag type A[1]:

Degradation to time *t* (D*t*, *t* = 20 h) = 52.3% for bag type A.

[1]Surface area = 1.2×10×2, feed to surface area = 10.4 mg/cm^2.

Energy calculations are given in Table 16 (4.8 Practical considerations) as a mean of the six different bag types (A, B, C, D, F, and G).

Digestible organic matter for horses (dOMhorse):

Dt + mean (dOM – dDM)[1] → 52.3% + 1.6 = 53.9%

[1]Calculation specified in Table A1.

Digestible energy (DE, MJ/kg DM):

(0.034 - 1.1 + 0.9477 × dOMhorse) / 100 × gross energy (GE, MJ/kg DM) →

(0.034 - 1.1 + 0.9477 × 53.9%) / 100 × 19.1 MJ/kg DM = 9.55 MJ/kg DM

Metabolizable energy (MJ/kg DM):

DE (MJ/kg DM) × (93.96 – 0.02356 × crude fibre (CF, g/kg DM) – 0.0217 × crude protein (CP, g/kg DM)) / 100 →

9.55 × (93.96 – 0.02356 × 298 – 0.0217 × 136) / 100 = 8.02 MJ/kg DM

Net energy at maintenance (NEm, MJ/kg DM):

(K_m[3] × (ME (MJ/kg DM) × 1000 – 31.33 × crude fat (Cfat, g/kg DM)) + 0.8 × 31.3 × Cfat (g/kg DM)) / 1000 →

(0.74 × (8.02 × 1000 – 31.33 × 22.7) + 0.8 × 31.3 × 22.7) / 1000 = 5.96 MJ/kg DM

[3]65.21 – 0.0178 × Cfat (g/kg DM) + 0.0181 × CP (g/kg DM) + 0.0452 × (starch (g/kg DM) + water soluble carbohydrates (WSC, g/kg DM)) / 100 →

65.21 – 0.0178 × 22.7 + 0.0181 × 136 + 0.0452 × (28.9 + 114) / 100 = 0.74

Feed Units (FU):

NE_m / 9.414 = 0.63 FU

Calculation example for hay in **paper II**:

Degradation to time *t* (D*t*, 20 h) = 62.4%

Digestible organic matter for horses (dOM_{horse}):

Dt + mean (dOM – dDM)[1] → 62.4% + 1.6 = 64%

[1]Calculation specified in Table A1.

Digestible energy (DE, MJ/kg DM):

(0.034 - 1.1 + 0.9477 × dOMhorse) / 100 × GE (MJ/kg DM) →

(0.034 - 1.1 + 0.9477 × 64%) / 100 × 19.1 MJ/kg DM = 11.38 MJ/kg DM

Metabolizable energy (MJ/kg DM):

DE (MJ/kg DM) × (93.96 – 0.02356 × crude fibre (CF, g/kg DM) – 0.0217 × crude protein (CP, g/kg DM)) / 100 →

11.38 × (93.96 – 0.02356 × 298 – 0.0217 × 145) / 100 = 9.5 MJ/kg DM

Net energy at maintenance (NE_m, MJ/kg DM):

(K_m[3] × (ME (MJ/kg DM) × 1000 – 31.33 × crude fat (Cfat, g/kg DM)) + 0.8 × 31.3 × Cfat (g/kg DM)) / 1000 →

(0.72 × (9.5 × 1000 – 31.33 × 22.7) + 0.8 × 31.3 × 22.7) / 1000 = 6.9 MJ/kg DM

[3]65.21 – 0.0178 × Cfat (g/kg DM) + 0.0181 × CP (g/kg DM) + 0.0452 × (starch (g/kg DM) + water soluble carbohydrates (WSC, g/kg DM)) / 100 →

65.21 – 0.0178 × 22.7 + 0.0181 × 145 + 0.0452 × (28.9 + 74.2) / 100 = 0.72

Feed Units (FU):

NE_m (MJ/kg DM) / 9.414 (NE for barley)

6.9 / 9.414 = 0.74 FU

Table A1. Apparent total tract digestibility of dry matter (DM) and organic matter (OM) and the difference between them in percentage (%).

Reference	DM	OM	Difference
Drogoul et al. (2000)	51.5	52.1	0.6
	53.8	55.2	1.4
Palmgren Karlsson et al. (2000)	48	49	1
	55	56	1
	58	59	1
	58	60	2
Bergero et al. (2002)	54.7	55	0.3
	48.9	49.7	0.8
Ragnarsson and Lindberg (2008)	71.6	74.7	3.1
	62.6	64.7	2.1
	51.3	52.7	1.4
	45.7	48.5	2.8
Ragnarsson and Lindberg (2010)	61	62.9	1.9
	57.7	58.6	0.9
Goachet et al. (2009)	53.2	55	1.8
Jensen et al. (2010)	69.3	70.4	1.1
	58.2	59.2	1
De Marco et al. (2012)	42.1	46.1	4
	50.1	55.9	5.8
	40.4	45.8	5.4
Schaafstra et al. (2018)	66.3	66.5	0.2
	64.2	64.4	0.2
Vasco et al. (2021)	69.2	70	0.8
	60.7	62	1.3
	46.2	46.2	0
Paper I	55.9	56.7	0.8
	MEAN±SD		1.6 ± 1.5

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