Digestibility and Metabolism in Icelandic Horses Fed Forage-only Diets

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Abstract

The objective of this thesis was to investigate digestibility and metabolism in Icelandic horses fed forage differing in stage of maturity/harvesting date and at two feeding levels. Additional aims were to compare digestibility and metabolic plasma profile in Icelandic and Standardbred horses. Finally, one aim was to estimate the digestible energy (DE) and digestible crude protein (DCP) requirements of Icelandic horses and compare them to widely used recommendations based on estimates from other breeds. Four studies were performed as change-over arrangements and total collection of faeces was performed for 6 days. In addition, urine was collected in two studies to estimate energy and nitrogen losses.

In studies I and II the effects of maturity and harvesting date on the nutritional value of different forages was estimated. Study I was performed on timothy haylage and study II on meadow haylage. In each study 4 horses were used. There was a negative relationship between harvesting date and the coefficient of total tract apparent digestibility (CTTAD) of all measured components. The lignin and acid detergent fibre contents were the main components affecting the CTTAD of organic matter (OM), while the content of neutral detergent fibre (NDF) had less influence.

In study III, 8 horses were used and fed high-energy haylage at two feeding levels. The lower feeding level was set at 1.0 and the higher at 1.5 times maintenance energy intake. For all measured dietary components except crude protein (CP), the CTTAD was higher at the lower feeding level.

In study IV, 6 Icelandic and 6 Standardbred horses were fed 2 different haylages. No overall breed differences were detected for the CTTAD of energy, CP and NDF. However, the Standarbred horses had higher CTTAD of OM. There were also differences between the breeds in total plasma protein, urea and insulin concentrations.

The estimated DE and DCP requirements in sedentary maintenance fed Icelandic horses were comparable to minimum requirements found in general recommendations for horses.

Keywords: digestibility, Icelandic horse, haylage, feeding level, breed, energy, nitrogen, requirements.

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To the Icelandic horse

While the grasse groweth the horse starveth
John Heywood (c. 1497-1580)

Contents

List of Publications

This thesis is based on the work contained in the following papers, refered to by Roman numerals in the text:

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

II Ragnarsson, S. & Lindberg, J.E. (2009). Nutritional value of meadow haylage in Icelandic horses (*Submitted*).

III Ragnarsson, S. & Lindberg, J.E. (2009). Impact of feeding level on digestibility of a haylage-only diet in Icelandic horses. *Journal of Animal Physiology and Animal Nutrition* (*In press*).

IV Ragnarsson, S. & Jansson, A. (2009). A comparison of grass haylage digestibility and metabolic plasma profile in Icelandic and Standardbred horses (*Submitted*).

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Abbreviations

AD	Apparent digestibility
ADF	Acid detergent fibre
BCS	Body condition score
BW	Body weight
CP	Crude protein
CTTAD	Coefficient of total tract apparent digestibility
DCP	Digestible crude protein
DE	Digestible energy
DM	Dry matter
DMD	Dry matter digestibility
GE	Gross Energy
GI	Gastro intestinal
GLM	General linear model
LSM	Least square means
M	Maintenance
MJ	Mega joule
N	Nitrogen
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acids
OM	Organic matter
OMD	Organic matter digestibility
SE	Standard error
TPP	Total plasma protein
VFA	Volatile fatty acids

Thesis at glance

Diet	Horses	Feeding level	Sampling	Measurements	Main results	Paper
4 timothy haylages, first cuts at different stages of maturity	4 Icelandic	M level 10,0 g DM kg^{-1} BW	6 days total collection of faeces and urine	CTTAD, energy and N utilization	Negative relationship between stage of maturity and CTTAD	I
4 meadow haylages, first cuts at different harvest dates	4 Icelandic	M level 9,8 g DM kg^{-1} BW	6 days total collection of faeces and urine	CTTAD, energy and N utilization	Negative relationship between harvest date and CTTAD	II
1 timothy haylage, first cut	8 Icelandic	M level 10,7 g DM kg^{-1} BW M level x 1.5 18,1 g DM kg^{-1} BW	6 days total collection of faeces	CTTAD	Reduced CTTAD at feeding level 1.5 x M	III
2 haylages, first cuts at different maturities	6 Icelandic and 6 Standardbred	Icelandic 15.5 g DM kg^{-1} BW Standardbred 16.3 g DM kg^{-1} BW	2 x 3 days total collection of faeces blood sampling	CTTAD and metabolic plasma parameters	No major breed differences in CTTAD but in some metabolic plasma parameters	IV

Introduction

Horses (*Equus caballus*) are mono-gastric herbivores that have evolved a digestive system which contains symbiotic microorganisms that can digest fibre (Duncan *et al.,* 1990). They have evolved as grazers and their digestive physiology is characterized by a high degree of mastication, a rapid gastric transit, a brief but intense enzymatic digestion in the small intestine and a prolonged microbial action in the large intestine (Hintz, 1975; Janis, 1976; Duncan *et al.,* 1990).

Fibrous forage forms the basis of diets for wild equids and can constitute the entire diet for domesticated horses, either grazed or conserved when pasturing is not possible or horses are confined to stalls (NRC, 2007). In northern Europe pasture grass can generally not be provided all year round due to the cold climate and snow cover. Therefore, the grass has to be harvested and conserved and consequently the nutritional values must be known to ensure that the horse's nutrient requirements are met. The factor with the greatest influence on the nutritional value of horse feeds is its OMD (Cuddeford, 2000).

Digestibility measurements

Digestibility is the proportion of the feed that is not excreted in the faeces and is therefore assumed to be absorbed by the animal, commonly expressed as a coefficient or a percentage (McDonald *et al.,* 2002). The measurement of digestibility *in vivo* is the ideal method (Cuddeford, 2000).

Total tract apparent digestibility is the most common method for estimating the nutritive value of feeds in horses (Goachet *et al.,* 2009) and it is conventionally measured by total collection of faeces for 5-6 consecutive days after 2-3 weeks of adaptation to the feed (Martin-Rosset *et al.,* 1994; Pagan, 1998). In total collection trials the horses are placed in metabolic stalls or equipped with a collection harness. These direct procedures have been found to be expensive, laborious and impractical for studies on athletic horses (Goachet *et al.,* 2009) or in field studies. Therefore alternative indirect methods have been evaluated by performing partial collections using indigestible/in-absorbable markers in the feed (e.g. Miraglia *et al.*, 1999) or by feeding small amount of indigestible markers (Cuddeford & Hughes, 1990). Calculations of digestibility coefficients are then based on the concentration of markers naturally present or added to the feed. Commonly used internal markers for digestibility estimates in horses are acid insoluble ash, lignin and *n*-alkanes. The ideal marker has not yet been found and results from digestibility trials using indigestible markers are contradictory, although sometimes comparable to total collection values. It has been concluded by several authors that using markers for digestibility determinations is acceptable (Miraglia *et al.,* 1999; Ordakowski *et al.,* 2001), however results from marker studies may differ from total collection *in vivo* data (Bergero *et al.*, 2004; Peiretti *et al.,* 2006).

One option to reduce the workload needed to perform direct digestibility trials is to reduce the number of collection days. In the literature the collection days range from 3 (Jansson *et al.*, 2006) to 10 (Smolders *et al.,* 1990) and adaptation periods from 9 (Palmgren Karlsson *et al.,* 2000) to 21 (Van Weyenberg *et al.,* 2007). Hintz & Loy (1966) suggested that 4 days was enough for estimating digestibility of hay-grain rations. In agreement, Vander Noot *et al.* (1967) also concluded that 4 days should be enough for measuring digestibility but to reduce the influence of individual animals a 5 day collection period should be considered. Recently, it has been reported that digestibility coefficients (DM, OM and fibre) were not affected when the total collection period was reduced from 5 to 3 days (Goachet *et al.,* 2009). The purpose of the adaptation periods is mainly to ensure that the residues being excreted in the faeces arise from the digestion of the feed being evaluated and that the microflora is stabilized. Therefore the maximum retention time in the digestive tract and the differences between the diets fed should be considered when establishing the length of the adaptation period.

In this thesis a standard method (6 day total collection and 14 days of adaptation, Martin-Rosset *et al.,* 2004) has been used to estimate *in vivo* digestibility.

Maturity of grasses and digestibility

Generally the variation in the DM digestibility of temperate grasses can to a large extent be explained by the content and digestibility of the cell walls (Fonnesbeck, 1969; Van Soest, 1994). Therefore the stage of growth when the grass is cut is the most important factor determining the nutritional value of the forage in horses (NRC, 2007).

The cell content includes most of the protein, starch, sugars, lipids, organic acids and soluble ash found in the plant. These are highly digestible and are degraded by the enzymes produced by the horse. The cell wall contains the fibrous portion of the plant (primarily cellulose and hemicellulose) which are resistant to the digestive enzymes produced by the horse itself and must be digested by bacterial fermentation (Fonnesbeck, 1969). The proportion of cell wall increases with the maturity of the grass and is modified by the plants environment (Thorvaldsson, 1988; Van Soest, 1994).

The latitude were the forage is grown may influence its nutritional value. Studies performed on cool-season varieties of timothy grass have shown that their rate of production has been superior at higher latitudes (Deinum *et al.,* 1981), at lower temperature and at longer photoperiods (Bertrand *et al.,* 2008).

Temperature during the growing season will affect the digestibility of forages, both directly, by affecting the cell wall digestibility and indirectly, by modifying phenological development (Smith & Jewiss, 1966; Van Soest, 1994). Thorvaldsson *et al.* (2007) reported that *in vitro* cell wall digestibility decreased with increased temperature and Thorvaldsson (1987) concluded that temperature is the climate factor with the strongest influence on the digestibility of grass. Bertrand *et al.* (2008) reported a reduction in NDF digestibility of forage with increased temperature but high temperature had a limited effect on the concentration of NDF. This is in contrast with Thorvaldsson *et al.* (2007) who reported an increased NDF concentration with increased temperature.

Light intensity also influences forage quality directly and also via an effect on morphological development. A longer photoperiod has been shown to stimulate DM production of timothy grass, partly by modifying plant morphology (Heide *et al.,* 1985) and partly by direct effects which may influence digestibility. High light intensity has a positive effect on the content of water soluble carbohydrates (Van Soest, 1994) and thereby improves forage digestibility (Bertrand *et al.,* 2006; Thorvaldsson, 2006). There are indications in the work summarized by Deinum *et al.* (1981) that each hour increase in day length increases herbage digestibility by 0.02 units and is therefore expected to result in herbage with a greater nutritive value. However, Deinum *et al.* (1981) reported that digestible organic matter declined faster at higher latitudes. Nevertheless when compared at the same morphological stage, the forage digestibility was better, because the lignification process of the cell wall was slower than the growth of the plant.

Other factors, environmental and genetic, can affect DM production and the digestibility of grasses. These effects are variable and to some extent interactions are present (Van Soest, 1994; Thorvaldsson, 2006).

In this thesis, the forages used were produced in Iceland at high latitudes, (65°N) and in Sweden at (59°N).

Feeding level

Level of feeding is often expressed in multiples of the amounts needed for maintenance (i.e. prevents animal from loosing weight but does not allow growth) and are defined as unity (McDonald *et al.,* 2002).

Earlier researches in horse nutrition have often used traditional hays and when feeding high energy forages, less feed is needed to fulfill the maintenance requirements. A reduced quantity of feed might influence the digesta retention time and have an impact on the digestibility of the dietary components (Julliand *et al.,* 2008). Pearson *et al.* (2006) reported a longer mean retention time of fibre when restricted amounts of feed were given to ponies compared to *ad libitum* feeding.

Earlier studies on the effect of feeding level on forage digestibility in horses have been contradictory. Higher feeding levels have been reported to have no influence on digestibility (Martin-Rosset & Dulphy, 1987; Martin-Rosset *et al.*, 1990; Todd *et al.,* 1995), or indicated a positive effect (Ott, 1981; Þórhallsdóttir & Eiríksson, 1996). However, it has also been suggested (Cuddeford *et al.*, 1995) that DM intake and nutrient digestibility are related as digestibility of the dietary components tended to be more efficient on lower DM intake in that study.

To my knowledge there are no available data on the impact of feeding level on digestibility of high-energy haylage in horses.

Breed differences

The Icelandic horse is a popular riding horse and is used for breeding, competition and leisure in more than 20 countries. The largest part of the population is found in Iceland, Germany and Scandinavia (Worldfengur, 2009). They stand an average of 134 to 143 cm and generally weigh 330 to

430 kg. This breed has lived isolated on Iceland for over 1000 years and through the centuries, survived natural disasters and harsh condition (Sveinsson, 2003). There is a scarcity of information about the feed utilization of the Icelandic horse although there are indications from research that its digestive tract (Sverrisdóttir *et al.,* 1994) and its digestive capacity (Þórhallsdóttir & Eiríksson, 1996) may be different from that of other horse breeds. Similar indications have been reported for another old breed, the Hokkaido horse (Shingu *et al.,* 2000; Kobayashi *et al.* 2006).

Studies comparing digestive capacity between horse breeds are quite limited. The main purpose has been to study if there are any breed differences or if the size of the horse has an effect on its digestive capacity and feed utilization. In studies comparing various horses and ponies no significant differences were detected (Slade & Hintz, 1969; Vermorel *et al.*, 1997b; Shingu *et al.*, 2001). However, indications have been reported suggesting that ponies might be more efficient in digesting roughage than horses (Barth *et al.,* 1977; Udén & Van Soest, 1982; Shingu *et al.,* 2000). Another possible difference between different breeds is that ponies might have lower requirements for energy than horses (Vermorel *et al.,* 1997b) and it is common belief that Icelandic horses require low amounts of feed to maintain condition (Þórhallsdóttir & Eiríksson, 1996).

There is a scarcity of research comparing metabolic blood parameters between different equine breeds, especially when fed the same diet at the same feeding level. However, lately interest has increased due to possible breed differences related to insulin resistance and obesity (Nadeau *et al.,* 2006; Carter *et al.,* 2009; Pagan *et al.,* 2009).

In this thesis Icelandic horses are used for the *in vivo* measurements and in paper IV they are compared to Standardbred horses. To my knowledge this study is the first attempt to compare haylage digestibility and metabolic plasma parameters between Icelandic horses and another horse breed.

Conserved forage for horses

Through the centuries hay has been the traditional preservation method of grass forage fed to horses. However, it seems that hay has to some extent been replaced by ensiled forage, as this method depends less on weather conditions than hay production and high quality feed can more easily be produced (Moore-Colyer & Longland, 2000; Peiretti & Bergero, 2004). This might primarily be due to an earlier stage of growth at harvest and to more limited losses during harvest and collection (Van Soest, 1994).

The nutritional value of ensiled forages in horses has not been extensively investigated, because historically it has been regarded as unsuitable to horses (NRC, 2007). However, the idea of feeding ensiled forages to horses is not new and feeding recommendations have existed since before the middle of last century (Olsson & Ruudvere, 1955). Anecdotal evidence suggests increased interest in feeding ensiled forages to horses (Moore-Colyer & Longland, 2000; Ellis & Hill, 2005; NRC, 2007) and generally horses in Iceland are fed plastic wrapped forages (Halldórsson, 2001; Harðarson *et al.,* 2002).

Wilted forage is often called haylage instead of silage and has been defined as the product when silage is wilted to 40-65% dry matter content (Frape, 2004; NRC, 2007). However, there is no clear definition of the difference between haylage and silage and in this thesis the same definition is used as the referred authors. In general, fermentation is restricted as dry matter content increases in silages and this is reflected in higher pH and soluble carbohydrates and lower levels of fermentation acids (McDonald *et al.*, 2002). When compared to hay, well preserved silage has less aeroallergens (Vandenput *et al.,* 1997) and in wilted silages clostridial and enterobacterial activities are normally minimal although some growth of lactic acid bacteria occurs, even in herbage wilted to dry matter content as high as 50% (McDonald *et al.*, 2002). By wilting the forage, the risk of adverse fermentation is reduced, as is the risk of botulism (NRC, 2007).

Moore-Colyer & Longland (2000) compared the intake of big-bale silage (DM 50%) and haylage (DM 68%) in horses and found that these products were consumed in larger amounts than hay and clamp silage (DM 34%) had the lowest intake. In contrast, a recent preference study on baled grass forages with different DM content showed that the product with the lowest DM content (31%) was preferred and consumed in larger amounts than hay and haylage (Müller & Udén, 2007). The most successful producers and users of haylage seem to find that haylage with very high DM content is acceptable to most of the horses (Frape, 2004). Recently it has been shown that although simultaneously harvested, silage (45% DM) has higher digestibility in horses than hay (Muhonen *et al.*, 2009a) and might therefore be different in their nutritional value.

In this thesis the nutritive value of plastic wrapped grass forages, mainly haylages, are studied.

Aims of the thesis

The general aim was to study the *in vivo* digestibility of grass haylage differing in botanical composition and maturity. More specific aims were:

- To study the effect of the maturity of timothy grass haylage on *in vivo* digestibility in Icelandic horses (Paper I).
- To study the effect of harvest date on *in vivo* digestibility of meadow grass haylage in Icelandic horses (Paper II).
- To study the effect of feeding level on *in vivo* digestibility of grass haylage in Icelandic horses (Paper III).
- To compare *in vivo* digestibility of grass haylage at different maturities in Icelandic horses and horses of a modern international horse breed. An additional aim was to compare some metabolic plasma parameters between the breeds (Paper IV).
- To estimate the maintenance requirements of mature sedentary Icelandic horses for DE and DCP.
- To evaluate the effect of the number of collection days on the CTTAD of DM (papers I –III) and OM (paper IV).

Hypotheses:

- The nutritional value of timothy haylage will decrease with advancing stage of maturity (Paper I).
- The nutritional value of meadow grass haylage will be decreased with delayed harvest date (Paper II).
- Feeding level will influence the digestibility of haylage (Paper III).
- There will be no differences between Icelandic and Standardbred horses in digestibility of haylage differing in maturity or in metabolic plasma parameters (Paper IV).

- Requirements in Icelandic horses for DE and DCP will be similar to general recommendations for horses.
- The CTTAD will differ depending on the number of collection days.

Materials and Methods

Three of the experiments in this thesis were performed at Hólar University College in Iceland (paper I–III) and one experiment (paper IV) was performed at the National Trotting School at Wången, Nälden, Sweden.

The methods used in this thesis are presented or referred to in the accompanying publications. An overview of the 11 haylage batches used in the thesis is presented in Table 1.

Experimental horses

In total 17 Icelandic horses and 6 Standardbred horses (all geldings) were used in the four papers. Eleven horses participated in the three papers performed in Iceland. One horse took part in all three papers and three same horses were used both in papers II and III. The Icelandic horse's ages ranged from 6 to 14 year (average 10 years), BW from 336 to 456 kg and the height at the withers from 135 to 143 cm. The Icelandic horses weighed 375 kg on average and average size was 139 cm at the withers (all studies). The Standardbred horses were on average 10.4 years old, ranging from 6 to 15 years. Their BW ranged from 466 to 586 kg (average 507 kg) and height at the withers was from 157 to 168 cm with an average of 161 cm.

Experimental forages

In papers I, II and III the forages were produced in Iceland. In paper IV the forages were produced south of Stockholm in Sweden. All forages were plastic wrapped, wilted and all had recorded botanical composition. All feeds were baled in large (250-450 kg) round or square bales and fed in long form. In all papers, water was available *ad libitum*.

Experimental design, feeding and collection of samples

In papers I and II the experiments were arranged as 4×4 Latin-Squares in a change-over design, including 4 horses. Paper III was a change-over experiment using 8 horses, 4 horses allocated to each treatment in each period. In papers I-III the horses were fed two equal meals daily at 08:00 and 18:00. The lengths of the adaptation and collection periods were the same in papers I-III. Each 20-day period consisted of 14 days of adaptation to the diet followed by 6 days total collection of faeces. Urine was collected in papers I and II and acidified to prevent volatile losses.

Paper IV was arranged as a change-over design with two 24 day periods interrupted by a four-day transition period. Twelve horses were used, 6 Icelandic and 6 Standardbred horses. The horses where fed 4 times per day at 06:00 h (10% of allowances), 12:00 h (19%), 17:00 h (25%) and 21:00 h (46%). Faeces were collected for two three-day periods at days 15 to 17 and days 22 to 24 in each of the two experimental periods. Rump fat was estimated according to Westerwelt *et al.* (1976).

In all papers a collection harness (Stablemaid®, Melbourne, Australia) was used to collect faeces and urine. To meet requirements for vitamins and minerals the horses were fed commercial vitamin or mineral mixtures and had access to salt lick stones during non-collection periods until 4 days before faecal collection began.

Chemical analysis

Feed and faecal analyses were performed on freeze-dried samples after milling through a 1-mm screen (except paper IV, which were performed on oven dried samples at 60°C for 24 hours). In papers I-III DM determination during analysis of dietary components was performed on the freeze-dried samples (16-24 h at 103°C) but on oven dried in paper IV. Ash concentration was determined by incineration at 550°C for at least 3 hours. NDF was analysed according to Chai & Udén (1998) using undiluted ND solution, sodium sulphate and amylase. ADF and lignin was analysed according to Goering & Van Soest (1970) using permanganate for lignin. In paper I the Ankom 220 technology was used for lignin determination following the Procedure Operators Manual (1997). The Kjeldahl method was used to determine N content and the value multiplied by 6.25 for estimating CP. GE was determined using an adiabatic calorimeter bomb (Technical manual No 130, 1966). The GE in the urine (papers I and II) was determined by freeze-drying 5 ml of urine in a small paper cup also functioning as primer.

Plasma analysis

In paper IV, blood samples were taken from the vena jugularis by vacutainer in heparinised tubes (10 ml) on the last day of the collection periods at 13:00 – 13:30. They were centrifuged (10 min 950g) and stored at -18°C until analysed. For quantitative determination of NEFA in plasma an enzymatic colorimetric method was used (ACS-ACOD method, Wako Chemicals GmbH, Neuss, Germany) and calculations were made from a standard curve with a linear curve fit. TPP was estimated using a refractometer (Atago, Tokyo, Japan). The plasma urea concentration was analysed using an enzymatic method (Konelab, Thermo Clinical Labsystem, Vantaa, Finland). Plasma cortisol was analysed using radioimmunoassay (Immulite 2000, Siemens, Eschborn, Germany) and plasma insulin by ELISA (Mercodia equine insulin kit, Mercodia, Uppsala, Sweden).

Calculations

Figures and regressions were prepared using Microsoft Office Excel 2003. To estimate the apparent digestibility of dietary components and energy (x), quantitative measurements were made of the amounts of feed consumed and faeces excreted over a 6 day period. AD was calculated on DM basis as follows:

$$AD\ (x) = \frac{\text{Intake } (x) - \text{Faecal excretion } (x)}{\text{Intake } (x)}$$

Statistical analysis

The data obtained were subjected to a GLM analysis, using the statistical software MINITAB® (Meet Minitab, 2003) in papers I–III and SAS (2002) in paper IV. LSM and SE were calculated and Tukey-test was used for comparisons of means, with significance level set to 5 %. Results are presented as LSM with their SE. More detailed descriptions are in the accompanying publications. The models used in this thesis were:

Papers I and II

$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk}$ where Y_{ijk} is the observation, μ the mean value, α_i the effect of feed, β_j the effect of period, γ_k the effect of horse, and e_{ijk} the residuals; e_{ijk} ~IND $(0, \delta^2)$.

Paper III

$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk}$ where Y_{ijk} is the observation, μ the mean value, α_i the effect of feeding level, β_j the effect of period, γ_k the effect of horse and e_{ijk} the residuals; e_{ijk} ~IND $(0, \delta^2)$.

Paper IV

$Y_{ijkl} = \mu + \alpha_i + \varepsilon_j + \beta_k + \gamma_l + (\beta\gamma)_{kl} + e_{ijkl}$ where Y_{ijkl} is the observation, μ the mean value, α_i the effect of period, ε_j the effect of collection period (within period), β_k the effect of breed, γ_l the effect of feed, $(\beta\gamma)_{kl}$ the effect of interaction between breed and feed and e_{ijkl} the residuals; e_{ijkl} ~IND $(0, \delta^2)$.

For comparison of collection days in paper I-IV, the data were analysed using the following model:

$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$ where Y_{ij} is the observation, μ the mean value, α_i the day measurement (either daily values or cumulative), β_j the effect of horses and e_{ij} the residuals; e_{ij} ~IND $(0, \delta^2)$. The Tukey test was not used for comparisons in this model.

Results

General results

During all studies the horses were in good health and in moderately thin to fleshy conditions reflected in their body condition scores. In papers I and II the Icelandic horses lost weight but not in papers III and IV. The Standardbred horses lost weight in paper IV. Feed leftovers were almost none in papers I-III but on average 6% of allowances in paper IV.

Chemical composition and feed intake

The average chemical composition of all forages used is given in Table 1. Increasing the stage of maturity generally increased the content of ADF and lignin. The CP content decreased with stage of maturity in all papers. The GE was high (19.0 to 20.1 MJ/kg DM) for all the Icelandic haylages (papers I-III). The DM content of the haylages ranged from 430 g/kg to 853 g/kg and pH varied from 4.7 to 6.0 (Table 1). There were no differences ($p>0.05$) in DM intake between treatments in papers I and II (Table 5) but the horses consumed more ($p<0.05$) of haylage 2 in paper IV.

Table 1. *Dry matter content (g/kg), chemical composition (g/kg DM), gross energy content (MJ/kg DM) and pH in the experimental haylages of this thesis. More detailed analyses are shown in the accompanying publications*

	Paper I				Paper II				Paper III	Paper IV	
	Cut 1	Cut 2	Cut 3	Cut4	Haylage 1	Haylage 2	Haylage 3	Haylage 4	Haylage	Haylage 1	Haylage 2
DM	469	501	430	447	689	631	853	600	679	788	778
OM	918	940	946	937	921	941	939	922	944	928	952
CP	175	132	102	93	200	165	142	121	125	111	61
NDF	503	593	639	615	507	545	540	526	612	611	627
ADF	299	368	411	405	270	298	287	306	351	362	377
Lignin	20	28	56	57	30	48	33	45	53	51	72
GE	19.3	19.3	19.4	19.2	19.9	20.1	19.3	19.3	19.0	18.6	18.6
pH	5.6	4.7	5.1	5.0	5.7	5.7	5.7	5.8	–	6.0	6.0

Digestibility

Paper I

There was a wide range in the CTTAD of dietary components (Table 2). As stage of maturity increased the CTTAD of all measured dietary components was reduced ($p<0.05$). The CTTAD of cut 1 was higher than that of cut 2 ($p<0.05$). Both cut 1 and 2 had higher CTTAD´s ($p<0.05$) than cuts 3 and 4. Cut 3 had higher ($p<0.05$) CTTAD then cut 4 for DM, NDF and ADF. The lignin and ADF contents were the main components affecting the CTTAD of OM, while the content of NDF had less influence (Table 4).

Paper II

The CTTAD of all measured dietary components were affected ($p<0.05$) by harvest date (Table 2). The CTTAD for haylage 1 was higher ($p<0.05$) than the other haylages for all measured dietary components except for CP. The other haylages were more similar although differences ($p<0.05$) in CTTAD for DM, OM, CP, NDF and ADF were detected and numerically reduced with delayed harvesting. The ADF content was the main component affecting the CTTAD of OM while the contents of NDF and lignin had less influence (Table 4).

Paper III

The CTTAD for DM, OM, NDF, ADF and energy were higher ($p<0.05$) at the Low feeding level. Feeding level did not affect the CTTAD of CP. The largest difference in CTTAD between feeding levels was found for NDF and ADF with an average difference of 0.065 units between treatments for both components (Table 3).

Paper IV

No overall breed differences were detected for the CTTAD of energy, CP and NDF. However, the Standarbred horses had higher ($p<0.05$) overall CTTAD of OM (both haylages included) and the CTTAD´s for OM and energy on haylage 1 was higher compared to the Icelandic horses (Table 3).

Table 2. *Coefficients of total tract apparent digestibility of the experimental haylages in papers I and II*

	Paper I					Paper II				
	Cut 1	Cut 2	Cut 3	Cut4	SE	Haylage 1	Haylage 2	Haylage 3	Haylage 4	SE
DM	0.716[a]	0.626[b]	0.513[c]	0.457[d]	0.011	0.691[a]	0.616[b]	0.619[b]	0.556[c]	0.010
OM	0.747[a]	0.647[b]	0.527[c]	0.485[c]	0.010	0.710[a]	0.630[b]	0.640[b]	0.578[c]	0.010
CP	0.809[a]	0.735[b]	0.642[c]	0.639[c]	0.014	0.765[a]	0.740[ab]	0.708[ab]	0.688[b]	0.015
NDF	0.770[a]	0.646[b]	0.516[c]	0.440[d]	0.009	0.717[a]	0.584[bc]	0.594[b]	0.520[c]	0.013
ADF	0.746[a]	0.629[b]	0.480[c]	0.400[d]	0.008	0.685[a]	0.545[b]	0.555[b]	0.485[b]	0.017
Energy	0.733[a]	0.633[b]	0.515[c]	0.468[c]	0.006	0.670[a]	0.600[b]	0.595[b]	0.560[b]	0.011

a, b, c, Values in the same row without common superscript differ ($p<0.05$).

Table 3. *Coefficients of total tract apparent digestibility of the experimental haylages in papers III and IV*

	Paper III			Paper IV				
	Low	High	SE	Haylage 1 Icelandic	Haylage 1 Standardbred	Haylage 2 Icelandic	Haylage 2 Standardbred	SE
DM	0.610[a]	0.577[b]	0.004	–	–	–	–	–
OM	0.629[a]	0.586[b]	0.004	0.536[a]	0.565[b]	0.431	0.427	0.006
CP	0.691[a]	0.680[a]	0.005	0.636	0.660	0.478	0.479	0.012
NDF	0.608[a]	0.543[b]	0.004	0.517	0.536	0.322	0.320	0.008
ADF	0.576[a]	0.511[b]	0.008	–	–	–	–	–
Energy	0.596[a]	0.556[b]	0.004	0.517[a]	0.540[b]	0.407	0.400	0.007

a, b, Values in the same row without common superscripts differ ($p<0.05$) and indicate breed differences in paper IV.

Table 4. *Regressions between the CTTAD's of OM ($CTTAD_{OM}$) and the concentration of NDF, ADF and lignin (g/kg DM) in papers I and II*

Paper	Component	Regressions	r^2
I	NDF	$CTTAD_{OM}$ = -0.0018x + 1.6614	0.8127
I	ADF	$CTTAD_{OM}$ = -0.0022x + 1.4202	0.915
I	Lignin	$CTTAD_{OM}$ = -0.0061x + 0.8465	0.9526
II	NDF	$CTTAD_{OM}$ = -0.0017x + 1.5614	0.2979
II	ADF	$CTTAD_{OM}$ = -0.0034x + 1.6239	0.9479
II	Lignin	$CTTAD_{OM}$ = -0.0047x + 0.8215	0.5763
I+II	NDF	$CTTAD_{OM}$ = -0.0016x + 1.4955	0.6354
I+II	ADF	$CTTAD_{OM}$ = -0.0016x + 1.1284	0.5658
I+II	Lignin	$CTTAD_{OM}$ = -0.0058x + 0.8543	0.8823

Nitrogen and energy metabolism

Paper I

Nitrogen intake and N-losses in urine were affected ($p<0.05$) by cut (Table 5). The N-balance was affected ($p<0.05$) by cut as well as by horse. No significant effect of cut was detected for N-losses in the faeces. The average DCP intake was 523, 302, 233 and 202 g in cuts 1, 2, 3 and 4, respectively. The stage of maturity had an effect ($p<0.05$) on the energy balance and GE lost (as % of GE) in urine and faeces (Table 6) while there was no effect ($p>0.05$) of energy lost in urine as a percentage of DE intake. The DE content (MJ kg^{-1} DM) was different ($p<0.05$) for cuts 1, 2, 3 and 4 and amounted to 14.1, 12.2, 10.0 and 9.0, respectively.

Paper II

Intake of N was highest ($p<0.05$) for haylage 1, while there were no differences ($p>0.05$) between the other haylages. Faecal N excretion was similar ($p>0.05$) for all haylages (Table 5). The average DCP intake was different ($p<0.05$) and was on average 522, 398, 361 and 295 g DCP for haylages 1, 2, 3 and 4, respectively. There was an effect ($p<0.05$) of haylage on the energy lost in urine (as % of GE), while there was no effect ($p>0.05$) of haylage on energy balance and energy lost in urine as a percentage of DE intake (Table 6). The DE content (MJ kg^{-1} DM) was different ($p<0.05$) for haylages 1, 2, 3 and 4 and amounted to 13.4, 12.1, 11.5 and 10.8, respectively.

Table 5. *DM and N intake, N losses in faeces and urine (g/day), urine excreted (kg/day) and N balance in papers I and II*

	Paper I					Paper II				
	Cut 1	Cut 2	Cut 3	Cut4	SE	Haylage 1	Haylage 2	Haylage 3	Haylage 4	SE
DM intake	3711[a]	3148[a]	3577[a]	3398[a]	184.1	3398[a]	3243[a]	3568[a]	3543[a]	107.8
Urine excreted	14.0[a]	11.3[b]	6.6[c]	7.6[c]	0.393	8.4[a]	6.2[b]	6.8[b]	6.6[b]	0.496
N intake	103.8[a]	65.8[b]	58.1[bc]	50.5[c]	2.779	108.9[a]	85.7[b]	80.8[b]	68.6[b]	3.611
N lost in faeces	19.9[a]	17.5[a]	20.5[a]	18.1[a]	1.374	25.4[a]	22.1[a]	23.1[a]	21.5[a]	0.374
N lost in urine	50.1[a]	32.6[b]	33.9[b]	30.7[b]	2.341	62.6[a]	52.9[b]	53.3[b]	42.4[b]	1.815
N balance	33.8[a]	15.7[b]	3.4[c]	1.7[c]	2.446	21.0[a]	10.7[b]	4.4[b]	4.7[b]	2.090

[a, b, c,] Values in the same row without common superscript differ ($p<0.05$).

Table 6. *Energy intake (MJ per day), and energy losses in faeces and urine in papers I and II*

	Paper I					Paper II				
	Cut 1	Cut 2	Cut 3	Cut4	SE	Haylage 1	Haylage 2	Haylage 3	Haylage 4	SE
GE intake	71.8[a]	60.8[a]	69.5[a]	65.1[a]	3.485	67.7[a]	65.1[a]	68.5[a]	68.2[a]	2.226
DE intake	52.5[a]	36.8[b]	35.6[bc]	30.5[c]	1.608	45.5[a]	39.2[a]	40.8[a]	38.1[a]	1.775
GE lost in faeces as % of GE	26.9[a]	36.8[b]	46.3[c]	53.2[c]	1.210	32.8[a]	40.0[b]	40.5[b]	44.1[b]	1.092
GE lost in urine as % of GE	6.7[a]	5.7[ab]	5.1[b]	4.7[b]	0.236	5.2[a]	4.8[ab]	4.9[ab]	4.2[b]	0.171
GE lost in urine as % of DE	9.2[a]	9.1[a]	9.8[a]	10.1[a]	0.367	11.5[a]	12.5[a]	12.0[a]	11.0[a]	0.821

[a, b, c,] Values in the same row without common superscript differ ($p<0.05$).

Plasma parameters

The Icelandic horses had lower ($p<0.05$) plasma urea, higher ($p<0.05$) plasma insulin and higher ($p<0.05$) TPP concentrations than the Standardbred horses (Table 7). There were no breed differences ($p>0.05$) in the plasma cortisol and NEFA concentrations. However, if the results were not adjusted for a higher TPP (possible smaller plasma volume) the Icelandic horses had higher ($p<0.05$) NEFA concentration. There was a positive correlation between BCS and plasma insulin ($Y= 9.8259x - 26.498$, $r^2=0.65$) and also between rump fat thickness and plasma insulin ($Y=7.063x-13.87$, $r^2=0.41$) across breeds.

Table 7. *Total plasma protein (TPP, g/L), Urea (mmol/L), non-esterfied fatty acids (NEFA µmol/L), Insulin (µU/ml) Cortisol (nmol/L) concentrations and their ratios with TPP and BCS* in Icelandic and Standardbred horses fed two haylage diets in paper IV*

	Haylage 1 Icelandic	Haylage 1 Standardbred	Haylage 2 Icelandic	Haylage 2 Standardbred	Breed effect p - value
TPP	71	65	70	64	0.0004
Urea	5.0	7.0	3.5	5.0	0.0001
NEFA	58.3	49.9	64.9	52.0	0.0145
Insulin	38.4	15.5	54.4	23.3	0.0008
Cortisol	73.7	85.0	70.5	75.7	0.2553
Urea/TPP	0.07	0.11	0.05	0.08	0.0001
NEFA/TPP	0.83	0.77	0.92	0.82	0.1238
Insulin/TPP	0.55	0.78	0.24	0.37	0.0016
Cortisol/TPP	1.04	1.30	1.01	1.20	0.0596
BCS*	7.5	4.6	7.2	4.4	0.0001

* BCS = Body condition score (Henneke *et al.,* 1983).

Collection days

In papers I and II a similar result($p>0.05$) for DMD would have been found by collecting for 2 cumulative days, compared to 3, 4, 5 and 6 days (Table 8). In papers III and IV, a larger variation in CTTAD was detected (Table 9). In paper III, 3 day cumulative collection was required for similar results as for 4, 5 and 6 days. In paper IV the two haylages were different and in haylage 1, one day collection is enough but for haylage 2 at least 4 days would be required to achieve the same result. In paper IV large differences between the first 3 and last 3 days were detected in haylage 2.

Table 8. *Cumulative and daily CTTAD's in papers I and II presented as LSM*

	Paper I				Paper II			
	Cut 1	Cut 2	Cut 3*	Cut 4	Hayl. 1	Hayl. 2	Hayl. 3	Hayl. 4
CTTAD								
1	0.713^{A}	0.625^{A}	0.516^{A}	0.416^{A}	0.700^{A}	0.633^{A}	0.583^{A}	0.540^{A}
1 - 2	0.710^{A}	0.629^{A}	0.510^{A}	0.433^{AB}	0.682^{A}	0.628^{AB}	0.603^{B}	0.559^{AB}
1 - 3	0.713^{A}	0.630^{A}	0.515^{A}	0.438^{AB}	0.683^{A}	0.626^{AB}	0.610^{B}	0.561^{AB}
1 - 4	0.719^{A}	0.637^{A}	0.506^{A}	0.448^{AB}	0.685^{A}	0.625^{AB}	0.614^{B}	0.565^{B}
1 - 5	0.718^{A}	0.629^{A}	0.517^{A}	0.454^{B}	0.687^{A}	0.620^{AB}	0.616^{B}	0.561^{AB}
1 - 6	0.716^{A}	0.626^{A}	0.513^{A}	0.457^{B}	0.690^{A}	0.616^{B}	0.619^{B}	0.556^{AB}
Daily CTTAD								
1	0.713^{AB}	0.625^{AB}	0.519^{A}	0.416^{A}	0.700^{A}	0.633^{A}	0.583^{A}	0.540^{AB}
2	0.707^{AB}	0.633^{AB}	0.509^{A}	0.450^{AC}	0.663^{A}	0.625^{A}	0.624^{B}	0.577^{A}
3	0.719^{AB}	0.633^{AB}	0.529^{A}	0.448^{AC}	0.686^{A}	0.621^{A}	0.623^{B}	0.565^{AB}
4	0.736^{A}	0.658^{A}	0.479^{A}	0.479^{BC}	0.691^{A}	0.621^{A}	0.625^{B}	0.579^{AB}
5	0.704^{B}	0.594^{B}	0.565^{A}	0.476^{BC}	0.694^{A}	0.600^{A}	0.625^{B}	0.547^{AB}
6	0.730^{AB}	0.619^{AB}	0.503^{A}	0.478^{BC}	0.708^{A}	0.598^{A}	0.634^{B}	0.528^{B}

Hayl. = Haylage, $^{A, B, C}$ values in same column without common superscripts differ significantly ($p<0.05$). * missing values in one horse

Table 9. *Cumulative and daily CTTAD's in papers III and IV presented as LSM*

	Paper III		Paper IV*	
	Low	High	Haylage 1	Haylage 2
CTTAD				
1	0.578^{A}	0.571^{A}	0.554^{A}	0.450^{A}
1 - 2	0.588^{AC}	0.555^{B}	0.550^{A}	0.446^{A}
1 - 3	0.601^{BC}	0.567^{A}	0.556^{A}	0.448^{A}
1 - 4	0.605^{B}	0.573^{A}	0.547^{A}	0.435^{B}
1 - 5	0.608^{B}	0.575^{A}	0.536^{A}	0.429^{B}
1 - 6	0.610^{B}	0.577^{A}	0.536^{A}	0.431^{B}
Daily CTTAD				
1	0.578^{A}	0.571^{A}	0.554^{AB}	0.450^{A}
2	0.597^{A}	0.539^{B}	0.545^{AB}	0.442^{AC}
3	0.629^{B}	0.591^{A}	0.569^{A}	0.452^{A}
4	0.617^{B}	0.589^{A}	0.518^{BC}	0.395^{B}
5	0.619^{B}	0.585^{A}	0.494^{C}	0.408^{BCD}
6	0.622^{B}	0.589^{A}	0.537^{ABC}	0.438^{AD}

*Icelandic horses, $^{A, B, C}$ values in same column without common superscripts differ significantly ($p<0.05$)

General Discussion

General

To my knowledge the papers in this thesis are the first attempts to study haylage digestibility in Icelandic horses (papers I-IV). Paper III is the first study measuring the impact of feeding level on the digestibility of haylage-only diet in horses and paper IV is the first study to compare haylage digestibility in Icelandic horses with another horse breed during standardized conditions.

The chemical composition of all the haylages in this thesis was different and a wide range of concentrations of the main chemical components was detected, resulting in a large variation in digestibility and utilization of the experimental haylages. The botanical composition of the studied haylages was different, timothy was the main grass in papers I, III and IV but as intended, more diversity was detected in the botanical composition in study II. Timothy was the main grass used in the experimental haylages of this thesis because it is popular horse feed (e.g. Matsui *et al.,* 2005; Ordakowski-Burk *et al.,* 2006) and the most seeded grass for forage production in Iceland (Helgadóttir & Sveinsson, 2006).

Digestibility

Influence of maturity/harvesting date on the CTTAD

In all papers a negative relationship was found between fibre content and the CTTAD of DM or OM in agreement with earlier reports (e.g. Fonnesbeck, 1969; Smolders *et al.*, 1990). In papers I-III the CTTAD of the fibre fraction was higher than in many earlier studies where hays were fed (Cymbaluk, 1990b; Crozier *et al.,* 1997; Vermorel *et al.,* 1997a). However, similar values are reported in the literature for OMD (Smolders *et al.,* 1990; Moore-Colyer & Longland, 2000) and fibre digestibility (Moore-Coolyer *et al.*, 2003) on silage diets. Earlier studies have to large extent been performed on hay and it has been shown that the preservation method can affect the chemical composition and the utilization of grass forage, although simultaneously harvested (Jakkola & Huhtanen, 1993; Muhonen *et al.*, 2009a). Most likely the low feeding level in papers I, II and III also contributed to the high CTTAD´s as in earlier studies larger amounts have been fed. The grass in papers I-III was grown in high latitude conditions that may also have contributed to the high digestibility values of OM and fibre. Grasses grown at high latitudes are believed to have greater nutritive value than those grown at lower latitudes, mainly because of different environmental conditions (Deinum *et al.,* 1981; Bertrand *et al.,* 2008). High latitudes offer low air temperatures and long photoperiods during the growing season. Under these conditions the cell wall makes up a smaller proportion of the digestible components of the plants and the proportion of soluble carbohydrates and proteins is larger (Van Soest, 1994). At higher latitudes there is a higher rate of dry matter production and even though aging of grasses is relatively fast, the digestibility is higher at all morphological stages of the growth cycle (Deinum *et al.,* 1981). Consequently, referring to the literature comparing forages at the same morphological stages is complicated, as both the chemical composition and digestibility could be affected by the latitude.

The reduction in digestibility of the fibre components observed in papers I and II of this thesis can be explained by an increase in plant maturity, which is accompanied by increased lignification and increased calculated content (g/kg DM) of hemicellulose (NDF-ADF) and cellulose (ADF – lignin). The fact that the ADF content explains the variation of CTTAD of OM better than NDF in papers I and II is not surprising as ADF represents mainly the cellulose and lignin fraction of the cell wall (Van Soest, 1994). Interestingly, the negative relation to the NDF content was much higher for the CTTAD of OM in paper I and when pooling the studies together the ADF no longer explains the fall in the CTTAD of OM better then NDF. This may be due to differences in botanical composition of the haylages between the studies, resulting in different fibre composition, as well as variation in the growth stage of grasses within each haylage. It can be calculated that the content of hemicellulose in the NDF fraction of the meadow haylages in paper II was proportionally larger compared to that of timothy in paper I. As hemicellulose has been shown to be more digestible than cellulose (Fonnesbeck, 1969; Van Soest, 1994), the observed difference between paper I and paper II may partly be explained by the relative proportions of hemicellulose and cellulose. Although the discussion above is based on results from only 8 feeds it seems that the major determinant of the CTTAD of OM is not the content of fibre *per se*, but rather the chemical composition of the fibre fraction as pointed out by Fonnesbeck (1968). The present study confirmed the observations by Udén & Van Soest (1982) that there is an individual difference in the ability of horses to digest fibre, because significant effects of horse ($p<0.05$) were detected on NDF digestibility in all studies except paper II.

Impact of feeding level

In paper III feeding level had a marked impact on the CTTAD of DM, OM and energy, which all were reduced as feeding level increased. The reduced CTTAD of DM, OM and energy could largely be attributed to a reduced fibre digestibility.

These findings are not in accordance with earlier papers on the effect of feeding level on digestibility in horses. In most cases no significant effect of feeding level on digestibility has been reported (Martin-Rosset & Dulpy, 1987; Martin-Rosset *et al.,* 1990; Todd *et al.*, 1995; Pearson *et al.,* 2006). However, in several papers there are large numerical differences in digestibility between feeding levels, indicating that horses on lower feeding levels may digest the diet more efficiently (Martin-Rosset & Dulpy, 1987; Cuddeford *et al.*, 1995). In the paper by Martin-Rosset & Dulpy (1987), the digestibility of crude fibre was 5.3 units higher in horses fed on the maintenance energy level (M) (63.5 g/kg $BW^{0.75}$) compared with horse fed the same diet *ad libitum* (99.1 g/kg $BW^{0.75}$), and the corresponding difference between feeding at M and 1.4 x M (86.3 g/kg $BW^{0.75}$) was 6.4 units.

The main differences between earlier studies on horses and the present study is the combination of a very low DM intake on the low feeding level, a relative large difference between feeding levels, and the type of forage used. An increased feeding level should be expected to increase the digesta rate of passage through the horse GI-tract, which may have an impact on the extent of the digestion within the GI-tract (Van Weyenberg *et al.,* 2006; Julliand *et al.*, 2008). A major impact should be expected on the fibre fraction of the diet due to the reduced time for colonization and digestion by the gut microflora (Julliand, 2005). The CP intake in paper III was high, which has been shown to increase colon VFA concentration in horses indicating increased bacterial activity (Muhonen *et al.,* 2008). Increased bacterial activity and slower passage rate might therefore have contributed to higher digestibility at the low feeding level. Possibly, the type of forage used in paper III has other properties than more mature and dried forages, allowing the gut microbial population to exert more of its potential digestive capacity at low levels of intake.

As shown in papers I and II, grass harvested at an early stage of maturity has a high digestibility of dietary components and energy, and a high DE content. Thus, the DM allowance needed to cover the daily maintenance energy requirements (NRC, 2007) in horses fed this type of haylage is low and comparable to the low feeding level used in the present study.

Breed differences

Paper IV indicates that there are no major differences in the digestive capacity of Icelandic horses compared to Standardbred horses. This is in accordance with earlier studies on different types of horses (Slade & Hintz, 1969; Martin-Rosset *et al*., 1990; Vermorel *et al*., 1997b). However, the OMD was lower in the Icelandic horses, mainly due to the response on haylage 1. The reason for this is not clear.

In both periods the Icelandic horses were in positive energy balance, indicated by increased BW. This is in contrast to the Standardbred horses who, in spite of their higher DE intake on haylage 1, failed to maintain BW. The energy intake of the horses was in accordance to NRC (2007) for requirements of horses with average voluntary activity (33.3 kcal DE/kg BW). The higher energy requirements of the Standardbred horses compared to the Icelandic horses might be due to a higher maintenance requirement because of more energy requiring body tissue composition (10.8 % total body fat in the Standardbred horses compared to 12.8 % in the Icelandic horses), i.e. more muscles and less fat (Blaxter, 1989). However, the negative energy balance and catabolism was not reflected in higher NEFA concentrations in the Standardbreds compared to the Icelandic horses. A higher metabolic rate in the Standardbred horses could be supported by numerically higher cortisol levels (cortisol/TPP) and the higher plasma urea concentration could indicate catabolic metabolism.

The plasma insulin concentrations were higher in the Icelandic compared to the Standardbred horses. This could be a breed difference but it could also be breed independent and influenced by differences in body condition (Pagan *et al.,* 2009). The Icelandic horses had higher BCS and thicker rump fat. The BCS has been shown to be correlated to plasma insulin concentrations in horses and ponies (Carter *et al.,* 2009). In the present study, the r-square explaining the variation in insulin in relation to BCS was 0.65 which supports the suggestion that a BCS measurement could be of value as indicator of altered insulin responses (Carter *et al.,* 2009).

Energy metabolism

Gross energy

The GE of the haylages in this thesis was high and exceeded 19 MJ GE/kg DM for all haylages in papers I and II. In the literature forage fed to horses is usually not that high in GE although comparable values can be found for ensiled forage fed to horses (19.1 MJ GE for silage, Smolders *et al.*, 1990 and 19.5 MJ GE for haylage, Lowman *et al.*, 1999), but often the values are lower (17.5 MJ GE for haylage, Moore-Coolyer & Longland, 2000 and 18.3 MJ GE for haylage, Bergero *et al.*, 2002). The reason for the high GE content of the Icelandic haylages is not clear; however the high CP content and preservation of volatile fermentation products due to the freeze drying might have contributed to the high GE values.

Digestible energy

The CTTAD of energy in paper I was higher in the first cuts than in paper II and then declined to a greater extent with advancing maturity. This reduction in digestibility was reflected in a lowering of the DE values at the later cuts, ranging from 14.1 to 9.0 MJ/kg DM in the timothy haylage compared to values ranging from 13.4 to 10.8 MJ/kg DM in the meadow haylage. This difference could be due to diversity in plant species and therefore heterogeneity of plant maturity in the later cut meadow haylages. Also, the first cut in paper I was harvested earlier than the first cut in paper II which might offer an explanation for the differences as well as possible yearly variation in agronomic conditions.

Energy losses in urine

According to McDonald *et al.* (2002) the gross energy lost in urine on silage diets is variable and ranges from 3 to 7 %, which is in accordance with present findings in papers I and II. The energy loss via urine in horses reported by Vermorel *et al.* (1997a) was less, than that found in papers I and II. This difference can be explained by the difference in feeding level of protein. In the first cuts in papers I and II the horses were highly overfed with protein, which is reflected in significantly higher urine excretion of N compared to the other cuts (Table 6). Interestingly, when the energy lost in urine was expressed in relation to the DE content there were no significant differences between forages. Thus, the urinary energy excretion could be expressed as a ratio to the DE intake. However, it was not the same for paper I and II and might be related to CP intake.

Energy retention

For the purpose of establishing the feeding regime of the current thesis, a pilot study was performed with the aim of estimating the maintenance energy requirements of stabled Icelandic horses. In that study (Ragnarsson, unpublished), three groups of Icelandic horses (3 horses in each group) were fed different amounts of haylage (Table 10) for 3 months (DM 62%, CP 105 g/kg DM, crude fibre 321 g/kg DM and DE 11.8 MJ/kg DM). During this study a total collection of faeces was performed (3 occasions for 4 days) and weight changes were registered using an electronic livestock scale. The individual weight response ranged from gaining 15 kg in the highest energy intake group to loosing 26 kg in the lowest energy intake group. The horses in the middle group had on average zero weight change (one lost 4 kg, one gained 4 kg and one had no BW change).

Table 10. *Energy intake, number of horses (n), BW response and initial average BW in mature sedentary Icelandic horses fed different amounts of DE for 3 months*

Study	MJ DE/kg $BW^{0.75}$	n	BW response	Initial average BW
Paper I	0.49	4	-5	348
Paper II	0.51	4	-9.5	358
Ragnarsson[1]	0.34	3	-18	385
Ragnarsson[1]	0.52	3	0	370
Ragnarsson[1]	0.61	3	+11	391

[1] (Ragnarsson, unpublished)

From this study, the maintenance energy requirement was estimated to be 0.51 MJ DE/kg $BW^{0.75}$/d. However, when applied to the data in papers I and II this estimate is apparently to low as horses in paper I and II were fed on average 0.50 MJ DE/kg $BW^{0.75}$/d for 3 months and lost weight (Table 10). By combining the data from the pilot study and papers I and II, 17 estimates from 3 month periods were obtained and the maintenance requirements of mature sedentary Icelandic horses was estimated to be 0.54 MJ DE/kg $BW^{0.75}$/d (Fig 1).

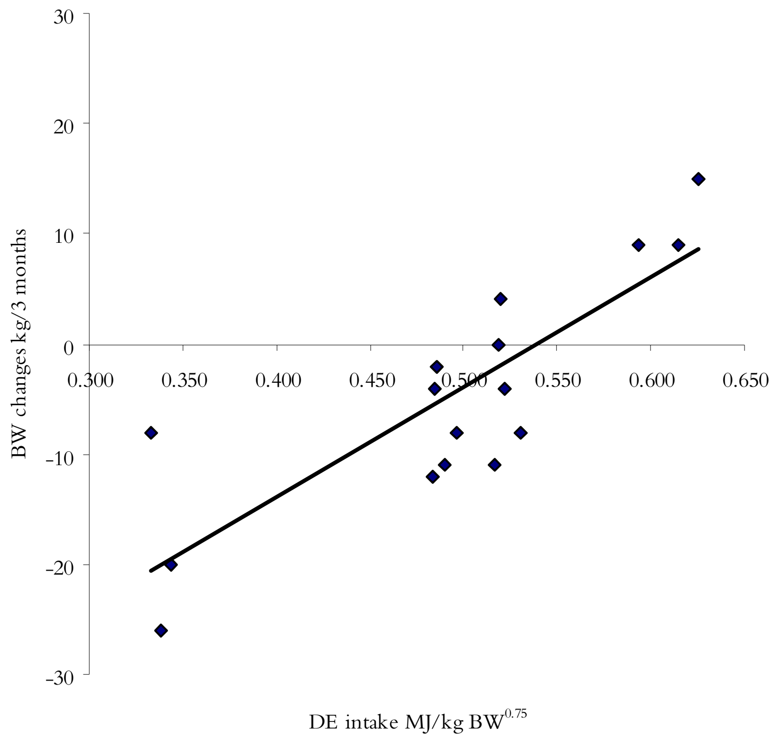

Figure 1. Linear regression between BW changes during 3 months (Ragnarsson unpublished pilot study and papers I and II) and energy intake (Y=100.21x-54.058, r^2=0.69).

When applied to a 370 kg horse (average weight of the horses in Table 10) the calculated maintenance requirements are 46.4 MJ DE/d. The minimum maintenance requirements (0.127 MJ/kg BW/d) recommended by NRC (2007) correspond to 48.3 MJ/day and the German feeding standards (Coenen, 2001) using the standard value (0.6 MJ DE/kg $BW^{0.75}$/d) corresponds to 51.6 MJ DE/kg $BW^{0.75}$/d. However, according to Coenen (2001) the maintenance requirements can range from 0.48 to 0.62 MJ DE/kg $BW^{0.75}$/d and the current estimate (0.54 MJ DE/kg $BW^{0.75}$/d) falls within this range. This large range in maintenance energy requirements probably reflects individual differences within and between horse breeds. In addition, the chemical composition of the feed can affect its utilization (Vermorel *et al.,* 1997a) and the body composition of the horse (Blaxter, 1989) and also the environmental conditions should be accounted for (Cymbaluk, 1990a). No attempt was made to account for the energy cost of carrying the collection harness. Therefore the current estimates should be used with caution and regarded as minimum maintenance requirements for mature sedentary stabled Icelandic horses in moderate body condition.

Nitrogen metabolism

Digestible CP

The CTTAD of CP was to some extent affected by grass maturity (papers I and II), but was not affected by feeding level (paper III) and did not differ between horse breeds (paper IV). Papers I and II are in agreement with earlier reports (e.g. Fonnesbeck, 1969; Gibbs *et al.*, 1988; Olsman *et al.,* 2003) where the CTTAD of CP was reduced with a reduced CP intake. When applying linear regression to the data in paper I and II the N digested and the N intake (g/kg BW/d) were closely related (y=0.9298x + 0.0441; r^2 = 0.99). The N faecal losses were not different ($p>0.05$) although the N could have been of a different origin, resulting in a larger proportion of endogenous N in the faeces on the low N forages.

N losses in urine

The N losses in urine decreased from the first to the second cut and thereafter remained unchanged except in paper II where the urinary N loss was also lower in haylage 4. The amounts of N excreted in urine were closely related to the N intake (Y=0.5035x+5.6546, r^2=0.82). The excess N is metabolized resulting in increased production of heat and urea (Blaxter, 1989). The production of urea may increase the water needs of the horses as reflected in a higher water intake on the first cuts in paper I.

N balance and retention

In papers I and II the calculated N balance was numerically positive for all forages but decreased with advancing stage of maturity. This suggests that N intake and utilization was sufficient to maintain N balance even in the late-cut timothy haylage in paper I (0.91 g CP/kg BW/d). However, the last two cuts in both papers were not different from zero and there are dermal losses and possibly some volatile losses to be accounted for.

From the data presented, it is possible to estimate the N requirements by regressing N retention and apparent digested N from papers I and II. The estimated value is 400 mg digestible N /kg $BW^{0.75}$/d or 2.5 g DCP/ kg $BW^{0.75}$/d for zero N retention (Fig. 2). This corresponds to 0.58 DCP/kg BW/d which is similar to NRC (1989).

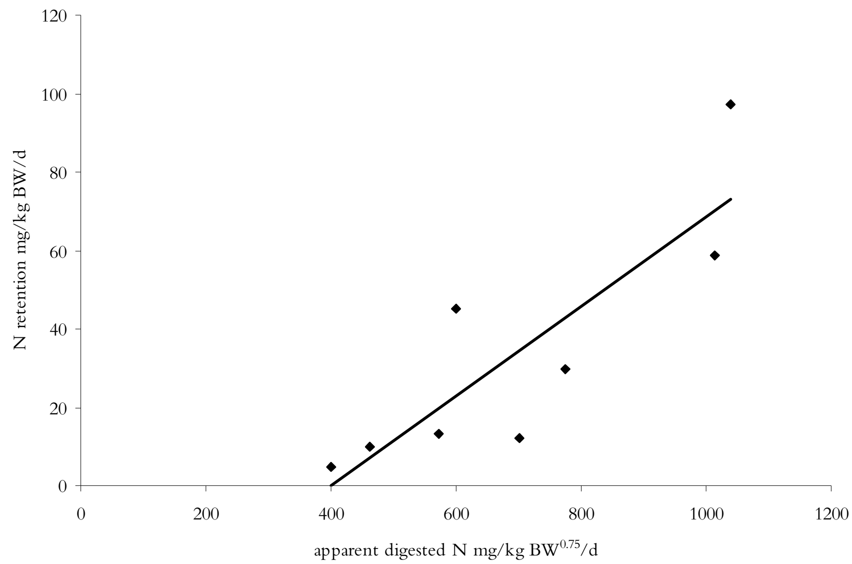

Figure 2 Linear regression between N retention and apparent digested N in papers I and II, (Y=0.1146x-45.854, r^2=0.72)

The results from papers I and II fit well into the German (Coenen, 2002) recommendations that assume that the requirement is 3 g DCP/kg BW$^{0.75}$. The differences between current estimate and the German recommendations are mainly because of "build in" safety margins due to differences in CP digestibility and quality of proteins (Coenen, 2002).

The new NRC (2007) system does not give N requirements in DCP, instead they use CP. For comparison, the data in papers I and II can be used to estimate the N requirements by regressing N retention and N intake as in NRC (2007). The estimated zero N retention was 0.144 g N/kg BW/d (Y=0.4422x-0.0635, r^2=0.66). When expressed as CP (0.144g N x 6.25) the estimated maintenance requirements are 0.90 g CP/kg BW/day.

In NRC (2007) a linear regression is applied for estimating minimum maintenance requirements for N. This implies that the requirements are 0.126 g N/kg BW/d or 0.79 g CP/kg BW/d. This is lower than the current estimate. However, because the N balance can underestimate true nitrogen losses from the body due to dermal and sweat losses, in addition to measurement errors, the NRC suggest that some allowances for nitrogen retention greater then zero should be made when determining maintenance requirements. Therefore, NRC (2007) estimates the requirements by using the non-linear (broken-line) approach. By using that method the estimated requirements becomes much higher and amount to 0.202 g N/kg BW/d or 1.26 g CP/kg BW/d. When applying 95% confidence intervals to this value the minimum maintenance requirements are estimated to be 0.173 g N/kg BW/d or 1.08 g CP/kg BW/d, which is higher than current results. However, in papers I and II the N losses are underestimated because dermal, experimental and possible sweat losses have not been accounted for. Dermal losses have been estimated to be approximately 35 mg/kg $BW^{0.75}$ (Meyer, 1983) and the N content of sweat amounts to 1-1.5 g N/L (Meyer, 1987). Therefore the true N requirements are higher than current estimate and could be similar to the NRC (2007) recommendations.

Collection days

For evaluating the required length of collection periods to determine the digestibility, the daily CTTAD of DM (CTTAD of OM in paper IV) were compared as well as the consecutive cumulative values of CTTAD´s. The results indicate that in papers I and II, two cumulative days would have been sufficient. The reason for this consistency could be that the horses were fed the same amount of DM every day, at a low feeding level with no left-overs and all meals came from the same bale with low DM variation. In paper III there seems to be some irregularity on day 2 with the result that 3 day collection would be required. This might be due to stress from wearing the collection harness, causing the horses to defecate more at the beginning of the collection period. The influence of the collection harness on the CTTAD´s in this thesis is unknown. Data in Paper IV is less comparable to the other papers as the 6 day collection was divided in two 3 day collections which were then pooled into one cumulative 6 day period. Therefore, as day 4 is not really connected to day 3 the flow of the digesta might be different. The period effect was included in the model used for digestibility comparisons. If the collection period had only consisted of one of the 3 day periods the same conclusions as in papers I and II would have been made. The reason for the differences between the papers could be the differences in DM intake and also the different amounts of left-overs in paper IV which might affect the MRT causing irregularity in faecal outflow affecting the estimated CTTAD. These findings indicate that when measuring the CTTAD of DM in maintenance fed horses and left-overs are minimal, only 2-3 collection days are needed. Similar results have been published recently by Goachet *et al.* (2009). However, when using higher feeding levels or if leftovers are present at least 4 consecutive days could be necessary. The total length of the collection period must be viewed in relation to the variation of the diet and possible influences on the digestive tract and its microbes (Julliand, 2005; Muhonen *et al.*, 2009b).

Conclusions

- The nutritional value of timothy and meadow haylages in Icelandic horses will decrease with advancing stage of maturity, mainly due to increased fibre and decreased protein content.

- Early cut Icelandic haylages are highly digestible and can be supplied in low amounts and still meet the maintenance energy requirements of mature sedentary Icelandic horses. Moreover, early cut haylages have the energy value to form the basis of diets for horses with high requirements.

- Feeding level has an impact on the digestibility and energy value of early cut haylage in Icelandic horses.

- There are small or no differences in the digestive capacity between Icelandic and Standardbred horses when fed haylage harvested at different maturity stages. However, differences were observed in blood plasma parameters which could be breed or body condition related.

- The estimated protein and energy requirements of Icelandic horses are comparable to the German and NRC recommendations for horses with low requirements and these recommendations could therefore be used for sedentary maintenance fed Icelandic horses.

- The present data indicates that collection days in total collection digestibility trials measuring DMD could be reduced to 2-3 days in maintenance fed horses fed haylage-only diets with minimum leftovers.

Populärvetenskaplig sammanfattning

Projektets bakgrund och hypoteser

Islandshästen är en populär hästras och finns i mer än 20 länder. På Island har rasen fötts upp i relativt fritt tillstånd under de senaste 1000 åren vilket innebär att djuren utsatts för både strängt klimat och begränsad födotillgång. Idag finns en utbredd uppfattning att islandshästen har en bättre fodersmältningsförmåga än andra raser och att den därför klarar av att leva på lite foder och foder med låg näringsmässig kvalité. Den här uppfattningen har till viss del fått stöd av en undersökning där storleken på islandshästars digestionskanal jämfördes med litteraturuppgifter från andra raser och där man kom fram till islandshästens digestionskanal nog var större och sannolikt gjorde att foderutnyttjande kunde vara bättre. Hittills har det bara funnits ett fåtal studier där islandshästens fodersmältningskapacitet studerats och inte någon publicerad studie där deras fodersmältning jämförts med andra hästar på samma foder. Det finns enstaka studier som antyder att det kan vara skillnad i olika rasers förmåga att smälta foder med det finns också studier där ingen skillnad mellan ras/typ av häst kunnat observeras.

Hästar kan, tack vare de mikroorganismer som lever i deras stora blind- och tjocktarm, utnyttja mycket av den energi- och näring som finns i gräs. I hästens magsäck och tunntarm börjar fodret brytas ner av de digestionsvätskor och enzymer som hästen själv producerar och enkla sockerarter, aminosyror (innehållet i protein) och det fett som finns i gräset tas upp i kroppen. Fibrerna i gräset kan hästen inte själv bryta ner utan de kommer till grovtarmen där mikroorganismer bryter ner dem och avger kortkedjiga fettsyror som hästen använder som energikälla. Generellt gäller att ju mer färdigväxt ett gräs är (från några blad till blad med strå, blomma och färdiga frön) desto mer fibrer och förvedade fibrer innehåller det.

Dessa fibrer kan vara svåra eller omöjliga även för mikroorganismerna att bryta ner. Det är dock känt att gräs som växer i nordiska klimat (kallt och ljust) har en annorlunda åldringsprocess och deras fiberinnehåll kan fortsätta vara ganska tillgängliga för mikroorganismerna fast de är färdigväxta.

I det här forskningsprojektet har man undersökt hur fodersmältningen, energi- och proteinutnyttjandet hos islandshästar påverkas när de erbjuds gräsvallfoder som skördats vid olika tidpunkter och utfodrats i olika mängder. En studie har också genomförts för att jämföra om fodersmältningen skiljer sig mellan islandshästar och varmblodiga travhästar. Projektets hypoteser var att smältbarheten hos islandshästar skulle försämras ju senare fodret skördats, att storleken på fodergivan skulle påverka fodersmältningen och att det inte skulle vara några skillnader i smältbarhetsförmåga mellan islandshästar och varmblodiga travhästar.

Resultat och diskussion

Resultaten visar att mängden smältbar energi och smältbart protein i de isländska vallfodren successivt minskade med ett senare skördedatum och i augusti var energiinnehållet i ett timotejfoder 36 % lägre än i juni och 19 % lägre i ett blandvallfoder. Trots detta var energi- och proteininnehållet i augusti ganska högt i både timotejfodret och blandgräsvallfodret (ungefär 7,7 och 9,3 MJ omsättbar energi och 60 och 77 g smältbart råprotein/ kg ts) om man jämför med vallfoder skördade i södra Sverige i mitten av augusti. Det kan som sagt bero på att gräsens kemiska sammansättning och åldringsprocess är annorlunda i kallare och ljusare klimat.

Utfodring med vallfoder i två olika mängder per dygn visade tydligt att hästarna utnyttjade energin och fibrerna i fodret bättre när de utfodrades den minsta fodergivan (ca 6 % högre fibersmältbarhet). Den ökade smältbarheten innebar att mängden energi som hästarna tillgodogjorde sig från fodret motsvarade ungefär 9,7 MJ med den minsta fodergivan och 9,1 MJ med den höga fodergivan (omsättbar energi/kg ts). Orsaken till detta kan vara att fodrets passage genom digestionskanalen går långsammare om mängden foder är liten och därmed får mikroorganismerna mer tid på sig att bryta ner fodret.

Den studie som gjordes för att jämföra smältbarhetsförmågan hos islandshästar med varmblodiga travhästar kan inte bekräfta att islandshästar har en bättre fodersmältningsförmåga, vare sig på ett sent eller tidigare skördat blandgräsvallfoder. I genomsnitt var det var ingen skillnad i rasernas förmåga att smälta energi, råprotein och fiber i fodren men de varmblodiga travhästarna visade dock en något högre smältbarhet (3 %) på fodrets organiska substans (hela foderinnehållet utom vatten och mineraler), särskilt på det tidigast skördade fodret. Orsaken till detta är oklar. I denna studie togs även blodprov för att undersöka om det fanns några skillnader mellan raserna i några ämnen och hormoner i blodet med betydelse för ämnesomsättningen. Analyserna visade att islandshästarna hade högre insulinnivåer och lägre koncentration av urea (restprodukt från nedbrytning av kroppseget och/eller foderprotein). De förhöjda insulinnivåerna hos islandshästarna kan bero på att de hade mer kroppsfett än travhästarna och det fanns också en positiv korrelation mellan alla hästarnas kroppspoäng (ju fetare häst desto högre poäng) och insulinnivåerna. Den lägre ureakoncentrationen hos islänningarna kan bero på att travhästarna var underutfodrade och därför bröt ner en del kroppseget protein vilket stöds av det faktum att travhästarna gick ner i vikt medan islänningarna ökade i vikt under studien (trots att de fodrades samma giva per kg kroppsvikt och att travhästrana åt mer av givan på det tidigast skördade fodret). Dessa resultat antyder att islandshästar kan vara mer lättfödda än tex travhästar men att det inte beror på en bättre förmåga att smälta fodret.

Med information från det här projektet om islandshästarnas energiintag och viktsförändringar över tid har deras underhållsbehov för energi uppskattats. Den bearbetningen visar att behovet är ungefär i samma storleksordning som de amerikanska och tyska utfodrings-rekommendationerna anger för lättfödda hästar. Även behovet av råprotein har uppskattats och visade sig motsvara de minimirekommendationer som finns för andra hästar både i USA och Europa.

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