Improved utilisation of grass silage in milk production

Harvesting strategy and feeding value

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Abstract

The aim of the studies reported in this thesis was to optimise grass silage utilisation in milk production. In the first paper of this thesis data from digestibility trials with sheep fed at maintenance level were used to investigate the effect of forage type and harvesting time of silage on energy and N metabolism. The assumption of constant loss of energy of digestible organic matter from energy losses in urine and CH_4 in evaluation of silage metabolisable energy (ME) did not seem valid for highly-digestible primary growth grass silages. Urinary excretion of high-energy phenolic acids related to solubilisation of lignin could not be related to increased urinary energy excretion from sheep fed highly digestible grass silage. In the second paper a concentrate made from agro-industrial by-products was used in comparison with a conventional grain-based concentrate to dairy cows. The concentrates were fed with early or late harvested grass silage. A concentrate made of by-products could replace a conventional grain-based concentrate without impairing milk production, diet digestibility and CH_4 emissions. Feeding agro-industrial by-product with highly digestible grass silage increased edible feed conversion ratios. Silage digestibility had a stronger effect on production performance by dairy cows than the source of concentrate. In the third paper the effect of strategy for harvesting regrowth (RG) grass silage on performance in dairy cows was studied. Increasing maturity stage in both primary growth (PG) and RG silage decreased intake potential, dairy performance and energy utilisation of lactating dairy cows, but improved N utilisation efficiency and herbage dry matter (DM) yield. Feeding more digestible silages from three-cut system promoted better dairy performance and energy utilisation compared with silages from two-cut systems. In the fourth paper data of intake and milk yield responses with different grass ley harvesting strategies of dairy cows were combined in a meta-analysis. The average increases in milk yields was 0.303 kg d^{-1} per 10 g kg^{-1} DM increase in silage digestible organic matter concentration (*D*-value). But each 10-unit increase in *D*-value reduced milk yield 0.092 kg when dietary ME intake was the same, suggesting the ME concentration of high *D*-value silages was overestimated. Cows fed RG compared to PG silage produced 0.55 kg d^{-1} more energy-corrected milk when dietary ME intake was the same, suggesting more efficient digestion of RG silage. Overall, results from this thesis can be used to improve ration formulation and grassland management, or in economic models optimising milk production.

Keywords: dairy cow, grass silage, harvesting strategy, feed intake, milk production, energy utilisation, nitrogen utilisation.

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Syftet med studierna som omfattas i den här avhandlingen var att optimera utnyttjandet av gräsensilage inom mjölkproduktion. I den första uppsatsen användes data från smältbarhetsförsök med får, utfodrade vid underhållsbehov, för att undersöka effekten av typ av grovfoder samt tidpunkt för skörd av ensilage på energi och N-omsättning. Antagandet av konstant förlust av energi från smältbar organisk substans genom urin och CH_4 i utvärdering av omsättbar energi (ME) i ensilage verkade inte giltigt för förstaskördens gräsensilage med hög smältbarhet. Utsöndring av fenolsyror relaterade till löslighet av lignin kunde inte relateras till en ökad utsöndring av energi i urinen hos får utfodrade med gräsensilage av hög smältbarhet. I den andra uppsatsen jämfördes ett kraftfoder baserat på agro-industriella biprodukter med ett konventionellt kraftfoder, baserat på spannmål, till mjölkkor. Kraftfodren utfodrades med tidigt eller sent skördat gräsensilage. Ett kraftfoder från biprodukter skulle kunna ersätta ett spannmålsbaserat kraftfoder utan att försämra mjölkproduktion, fodrets smältbarhet eller CH_4-utsläpp. Utfodring med agro-industriella biprodukter och gräsensilage av hög smältbarhet gav en ökning av omvandlingsförhållandet för näringsämnen som är ätliga för människor. Ensilagets smältbarhet hade en tydligt starkare effekt på mjölkkornas produktion än kraftfoderkällan. I den tredje artikeln studerades effekten av skördestrategi av gräsensilagets återväxt (RG) på mjölkkors prestation. Ökat mognadsstadie hos gräsensilage vid både förstaskörd (PG) och RG minskade foderintag, avkastning och energianvändning hos mjölkkor, men ökade N-utnyttjandet och avkastad torrsubstans. Utfodring av ensilage med högre smältbarhet från tre-skörde-system främjade mjölkavkastning och energianvändning jämfört med ensilage från två-skörde-system. I den fjärde uppsatsen utfördes en meta-analys genom att kombinera data om foderintag och mjölkavkastning med olika strategier för att skörda gräsvallar. Ökningen i mjölkavkastning var i medeltal 0,303 kg d^{-1} per 10 g kg^{-1} torrsubstans ökning av koncentrationen av ensilagets smältbara organiska substans (*D*-värde). Men varje 10 enheters ökning i *D*-värde minskade mjölkavkastningen 0,092 kg när smältbart ME var den samma, vilket tyder på att koncentrationen av ME hos ensilage med högt *D*-värde var överskattat. Kor som utfodrades med RG ensilage jämfört med PG ensilage producerade 0,55 kg d^{-1} mer energikorrigerad mjölk, när intaget av smältbart ME var den samma, vilket antyder en mer effektiv digestion av RG. Resultaten från den här avhandlingen kan användas för att förbättra foderstatsberäkning och skötsel av gräsvall, eller i ekonomiska modeller för att optimera mjölkproduktion.

Keywords: dairy cow, grass silage, harvesting strategy, feed intake, milk production, energy utilisation, nitrogen utilisation

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There are no secrets to success. It is the result of preparation, hard work, and learning from failure.

Colin L. Powell

Contents

List of publications

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

I Krizsan, S.J., Pang, D., Fatehi, F., Rinne, M. & Huhtanen, P. Metabolisable energy in grass and red clover silages fed to sheep. (Manuscript)

II Pang, D., Yan, T., Trevisi, E. & Krizsan, S.J. (2018). Effect of grain- or by-product-based concentrate fed with early or late harvested first-cut grass silage on dairy cow performance. *Journal of Dairy Science* 101, 7133-7145.

III Pang, D., Krizsan, S.J., Yan, T. & Huhtanen, P. (2018). Effect of strategy for harvesting regrowth grass silage on performance in dairy cows. *Grass and Forage Science* (Revised manuscript submitted).

IV Pang, D., Krizsan, S.J., Nousiainen, J., Sairanen, A. & Huhtanen, P. (2018). Modelling feed intake and milk yield responses with different grass ley harvesting strategies. *Grass and Forage Science* (Submitted).

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The contribution of Degong Pang to the papers included in this thesis was as follows:

I. Worked jointly with the main supervisor in preparing samples for analysis and processing the data. Participated in writing the manuscript jointly with the co-authors.

II. Planned the study together with the co-authors, collected, prepared and analysed samples, processed the data and was responsible for writing the manuscript.

III. Planned the study together with the co-authors, collected, prepared and analysed samples, processed the data and was responsible for writing the manuscript.

IV. Planned the research jointly with the co-authors, collected the dataset in collaboration with the co-authors, analysed the data and wrote the manuscript.

Abbreviations

CP	Crude protein
DE	Digestible energy
DEI	Digestible energy intake
DM	Dry matter
DMI	Dry matter intake
D-value	Digestible organic matter
ECM	Energy-corrected milk
GE	Gross energy
GEI	Gross energy intake
HP	Heat production
iNDF	Indigestible neutral detergent fibre
k_l	Efficiency of metabolisable energy use for lactation
ME	Metabolisable energy
MEI	Metabolisable energy intake
MP	Metabolisable protein
NDF	Neutral detergent fibre
OM	Organic matter
OMD	Organic matter digestibility
PBV	Protein balance in the rumen

1 Introduction

1.1 Grass utilisation in the Nordic countries

In northern Europe, grassland-based milk production systems are biologically more sustainable than intensive systems based on high concentrate diets. Dry matter (DM) yields are generally much higher for grass than for grain at northern latitudes. For example, average DM yield was 5.0 and 10.8 tonnes ha^{-1} for barley and timothy, respectively, in Finnish variety testing trials in 2009-2016 (Laine *et al*., 2017). Grass silage is the main ingredient and forage source in dairy cow diets in the Nordic countries, due to its high feeding value (Huhtanen *et al*., 2013). The northern climate benefits from production of forages with a high digestibility. The long day length allows plants to build up energy-rich carbohydrates by photosynthesis virtually round the clock, and the low temperature in early summer reduces energy consumption from cellular respiration and inhibits lignification of plant cells. The silage dry matter intake (SDMI) by high-producing dairy cows can be up to 17 kg d^{-1} and intake of early-harvested grass silages can be even higher (Kuoppala *et al*., 2008; Randby *et al*., 2012). In addition, only moderate (approximately 8 kg d^{-1}) levels of concentrate supplementation to a forage-based diet are needed to maximise energy-corrected milk (ECM) yield (Kuoppala *et al*., 2008; Randby *et al*., 2012).

At northern latitudes, the digestibility of grass decreases very rapidly in early summer, by about 0.4-0.6% per day (Lockhart & Wiseman, 1988; Huhtanen *et al*., 2006). At the same time, DM yield increases rapidly. Gustavsson and Martinsson (2001) and Virkajärvi *et al*. (2003) reported increases of up to 200 kg DM ha^{-1} d^{-1} during spring growth. Therefore, optimising the harvesting of grass silage is crucial for the economics of dairy farming at northern latitudes. Increased length of growing season due to global warming is an additional factor. The growing season for grass (average temperature above +5°C) in Sweden is now more than 10 days longer than it was 50 years ago (Gustavsson, 2017). The extended growing season is generating more interest in the utilisation of leys for forage production, including new varieties, and is creating a demand for more knowledge of regrowth forages and alternative harvesting regimes. Grass is most commonly harvested twice a year at northerly latitudes, but the advice is now to harvest leys three times a year for high-quality silage production (Sairanen *et al*., 2016; Krizsan *et al*., 2017), in order to efficiently utilise the longer growing season.

The early harvesting of grass generally produces highly digestible silage that promotes high dry matter intake (DMI) and milk yield with less concentrate supplementation (Ferris *et al*., 2001; Kuoppala *et al*., 2008; Randby *et al*., 2012). Analysis of data from four factorial studies indicated that 10 g kg^{-1} DM differences in digestible organic matter concentration (*D*-value) when the level of concentrate supplementation was increased from moderate to high levels corresponded to 0.84 kg of concentrate DMI (Huhtanen *et al*., 2013). However, in single studies milk production responses to increased *D*-value have been variable, probably due to differences in silage dry matter concentration, fermentation quality, level of concentrate supplementation and production potential of cows.

1.1.1 Primary growth compared with regrowth grass

The feeding value or quality of the harvested grass is ultimately determined by the time of harvest and the actual climate characteristics setting the highly year-specific conditions for growth. Organic matter digestibility (OMD) is the most important trait of forages in feeding value determinations (Huhtanen *et al*., 2006; Krizsan *et al*., 2012). In general, digestibility of regrowth silages is reported to be lower than for primary growth silages (presented as *D*-value in Table 1). However, the indigestible neutral detergent fibre (iNDF) concentration is usually higher in regrowth than primary growth silages, reflecting changes in fibre composition throughout the growing period (Van Soest, 1994). In the Nordic countries, in spring both temperature and day length are increasing. After midsummer, the temperature is still increasing but light is starting to decline and in late summer both temperature and light are declining. In addition, intake potential of grass silages made from regrowth compared with primary growth is lower, even when differences in digestibility, DM and neutral detergent fibre (NDF) concentrations and fermentation quality are taken into account (Huhtanen *et al*., 2007). These factors result in lower milk production in cows fed regrowth silages compared with those fed primary growth silages (Peoples & Gordon, 1989; Khalili *et al*., 2005; Kuoppala *et al*., 2008). However, most of these earlier studies have only compared one silage from each cut (primary growth and regrowth), which precludes separating the effects of cut and grass maturity on milk production response.

Table 1. *Differences between regrowth silages and primary growth silages on chemical compositions (g kg^{-1} DM) and digestibility in selected studies*

Reference	CP	NDF	iNDF	*D*-value
Castle & Watson, 1970	+31			-12
Peoples & Gordon, 1989	+28			-9
Heikkilä *et al*., 1998	-78	+43	+87	-123
Bertilsson & Murphy, 2003	-19	+58	+95	-81
Khalili *et al*., 2005	-78	+43	+71	-81
Kuoppala *et al*., 2008	-6	-15	+2	-34
Sairanen & Juutinen, 2013	+13	-24	-9	+2
Alstrup *et al*., 2016	+31	+84	+26	-69
Larsen *et al*., 2016	+33	-45	+17	-26
Naadland *et al*., 2017	+22	-28	+34	-48

CP, crude protein; NDF, neutral detergent fibre; iNDF, indigestible NDF; *D*-value, digestible organic matter.

Fagerberg (1988) and Gustavsson and Martinsson (2004) have found that the proportion of leaves is higher in regrowth grass than in primary growth grass. Fagerberg (1988) observed that the phasic development in the regrowth periods was different, so that the changes were slower and the leaf stages had more leaves before the elongation phase began. Higher proportions of weeds and dead tissues were also found in regrowth grass by Fagerberg (1988). The *in vitro* digestibility of dead grass material has been reported to be very low and thus a high proportion of dead grass can reduce the digestibility of the whole herbage (Heikkilä *et al*., 2000; Pakarinen *et al*., 2008). Furthermore, Heikkilä *et al.* (2000) analysed the composition of brown dead leaves and found that concentrations of crude protein (CP), water-soluble carbohydrate, potassium and phosphorus were lower and those of ash, calcium and magnesium were higher, than in green leaves. Cell soluble matter in regrowth herbages also contains more waxes and cutins (Van Soest, 1994), which have a low availability in animals and may cause the lower true digestibility of neutral detergent solubles for regrowth compared with primary growth silages (Huhtanen *et al*., 2006).

In recent decades the three-cut system is becoming popular in the Nordic countries, since it provides better utilisation of the whole growing season than traditional two-cut system (Hyrkäs *et al*., 2015). The feeding value of the third-cut grass is usually higher than the second-cut grass, and the high digestibility of the third-cut grass is a consequence of the low amounts of fibre due to the low average temperature and radiation during late-summer (Thorvaldsson *et al*., 2007; Hyrkäs *et al*., 2015). In some cases, the *D*-value of the third-cut silage can be even greater than primary growth silage (Huuskonen and Pesonen, 2017). However, the good intake potential of the third-cut silage is not always in line with the results in feeding trials on both dairy cows (Sairanen *et al*., 2016) and beef cattle (Huuskonen and Pesonen, 2017). This suggests that the current intake predicting models do not represent all the factors affecting SDMI of the third-cut silage (Huhtanen *et al*., 2007; Huuskonen *et al*., 2013). Botanical factors and animal behaviour can also affect feed intake and should be taken into consideration when evaluating the intake potential of third-cut silage.

1.1.2 Development of grass in primary growth

Considerable changes occur in forage chemical composition and feeding values with progressing growth. The effect of maturity has been well-investigated by delaying the harvest of forages, with the majority of research in this area focuses on primary growth grass (Van Soest *et al*., 1978; Thomas & Thomas, 1988; Beever *et al*., 2000). In general, the typical effects of delayed harvest on primary growth are an increase in the concentrations of NDF and iNDF, and a decrease of concentration of CP and *D*-value (Rinne, 2000).

During the development of primary growth grass, the ratio of stems to leaves increases and the composition of stems changes, causing an increase in cell wall concentration at the expense of cell contents. The proportion of stem increases and the digestibility of stems decreases considerably, causing the major part of the decrease in whole plant digestibility (Kuoppala *et al*., 2010). Nordheim-Viken *et al*. (2009) reported that the iNDF concentration of leaves did not change, whereas that of the stems increased, when grass was harvested at three stages of primary growth. The iNDF concentration of the whole plant followed that of the stems. The primary growth of grass is characterised by stem elongation, as tillers produce seed heads. The changes observed in the feeding quality of the plants are partly caused by changes occurring within the stems and the leaves, and partly by the rapidly increasing proportion of stems, which at the time of harvest are nutritionally less valuable than leaves (Salo *et al*., 1975; Rinne & Nykänen, 2000).

Table 2. *Daily changes of digestibility expressed as D-value (g kg^{-1} DM) of grass silage due to delay in harvest in selected studies*

Reference	Nation	Grass species*	Primary growth	Regrowth
Salo *et al*., 1975	Finland	ti, mf	-6.7	
Gordon, 1980	UK	pr		-3.9
Thomas *et al*., 1981b	UK	pr	-3.5	
Åman & Lindgren, 1983	Sweden	ti	-2.9	-1.9
Åman & Lindgren, 1983	Sweden	mf	-4.9	-0.4
Lindberg & Lindgren, 1988	Sweden	ti	-4.3	-1.2
Gordon *et al*., 1995	UK	pr	-3.1	
Rinne *et al*., 1999	Finland	ti, mf	-4.8	
Rinne & Nykänen, 2000	Finland	ti	-5.7	
Dawson *et al*., 2002	UK	pr	-3.7	
Dawson *et al*., 2002	UK	pr, rb		-4.3
Rinne *et al*., 2002	Finland	ti, mf	-4.8	
Rinne *et al*., 2007	Finland	ti, mf	-4.8	+0.9
Kuoppala *et al*., 2008	Finland	ti, mf	-5.0	-3.1
Kuoppala *et al*., 2010	Finland	ti, mf	-4.6	
Tahir *et al*., 2013	Sweden	ti	-7.3	
Sairanen *et al*., 2016	Finland	ti, mf		-2.7
Warner *et al*., 2016	The Netherlands	pr, ti		-0.7
Cabezas-Garcia *et al*., 2017	Sweden	ti	-5.6	
Paper II	Sweden	ti	-5.9	
Paper III	Sweden	ti		-3.5
Average			-4.9	-2.1

*Grass species: ti = timothy (*Phleum pratense*); mf = meadow fescue (*Festuca pratensis*); pr = perennial ryegrass (*Lolium perenne*); rb = rough bluegrass (*Poa trivialis*).

1.1.3 Development of grass in regrowth

The reported effects of maturity on the digestibility of regrowth silages are quite variable (see Table 2). The highest daily decrease in digestibility has been observed with perennial ryegrass (Gordon, 1980; Dawson *et al*., 2002) and the lowest with timothy and meadow fescue (Åman & Lindgren, 1983; Rinne *et al*., 2007). However, the effect of grass species on digestibility in those studies may be confounded with geographical location as evidenced by Warner *et al*. (2016), because the data on perennial ryegrass are mainly from the UK and the Netherland, whereas the data on timothy and meadow fescue are from the Nordic countries. As shown in Table 2, the rate of decline in the digestibility of regrowth grass is slower than in primary growth. This results in a smaller range in the nutritive value of regrowth silage than in primary growth silage, even when the time difference between the harvests within cuts is similar (Beever *et al*., 2000).

The different development of regrowth digestibility compared with that of primary growth may be caused by the differences in growth pattern, since the proportion of leaves in the total herbage mass does not decline with advances in regrowth grass growth during autumn (Åman & Lindgren, 1983). The range of variation in chemical composition caused by maturity is also smaller in regrowth than primary growth grass (Lindberg & Lindgren, 1988; Huhtanen *et al*., 2006). Kuoppala *et al*. (2008) and Nordheim-Viken and Volden (2009) reported a marginal increase in NDF concentration in regrowth grass with delayed harvest, but a substantial increase in iNDF concentration. In the Finnish dataset from digestibility studies in sheep (Huhtanen *et al*., 2006), *D*-value, OMD and CP concentration decreased by 1.6, 1.8 and 1.2 g d^{-1} and concentrations of NDF and iNDF increased 0.87 and 1.23 g d^{-1} of delayed harvest of 27 regrowth silages harvested at seven different years.

The change of feeding quality in the third-cut grass is unique due to the absence of true stem and the slower accumulation of temperature sum and radiation sum towards the end of the growing season (Thorvaldsson *et al*., 2007). The negative effect of delay in harvest on digestibility is very small for the third-cut grass. According to a regression analysis of long-term field trial (2009-2015) by Hyrkäs *et al*. (2016), the average daily decline in grass *D*-value of the third-cut grass is 0.7 g kg^{-1} DM, equivalent to half of the average value in second-cut grass (1.4 g kg^{-1} DM) reported by Kuoppala (2010).

1.2 Silage feeding value evaluation

The benefit of improving silage feeding quality is economically significant and measurable in practice. In cows fed *ad libitum* with a silage-based diet, a 10-unit change in silage OMD corresponds to 0.15 kg SDMI, equivalent to 0.5-1.0 kg of supplementary concentrate or 0.3-0.5 kg milk per day depending on silage fermentation quality (Huhtanen, 1998; Rinne, 2000). Silage digestibility coefficients are usually measured by total faecal collection from sheep digestibility trials at maintenance feeding level. In some studies, the silage digestibility in cattle can then be accurately predicted using the data from sheep (Rinne, 2000). There have been several other attempts to predict forage OMD by using chemical composition (CP, NDF, acid detergent fibre and lignin). However, none of the regressions based on single or multiple chemical entities offers a satisfactory prediction of digestibility, since the models only account for about half the variation in digestibility (De Boever *et al*., 1999; Nousiainen, 2004). Therefore, several laboratory methods have been developed to accurately and precisely predict silage digestibility.

1.2.1 *In vitro* methods

The two-stage *in vitro* method was first reported by Tilley & Terry (1963) and involved anaerobic incubation of a sample with strained and buffered rumen fluid in the first step, followed by an acid-pepsin treatment in the second step. The digestibility estimates obtained from this method are often highly correlated with *in vivo* values for a range of forage species under a wide range of environmental conditions (Van Soest, 1994), but between- and within-animal variation in the digestion activity of the rumen fluid is the most serious drawback. In addition, the need for fistulated animals and technical problems in relation to maintaining anaerobic conditions during *in vitro* incubations make the method less attractive to any forage research laboratory. To minimise the variation caused by rumen fluid, it is necessary to replace rumen fluid with commercial fibrolytic enzymes. A commercial mixed fungal (*Trichoderma* sp.) product including both hemicellulases and cellulases has changed this situation (Jones & Theodorou, 2000).

Enzymatic digestion procedures for fibrous forages basically use two different modifications; pre-treatment of a dried and milled sample with either neutral detergent or acid-pepsin solution for 24 h, followed by 24 or 48 h of incubation with buffered enzymes at 35-40 ºC (Dowman & Collins, 1982). The rationale behind both methods is that the cell solubles are completely extracted by the pre-treatment and that the cell wall residues (hemicellulose and cellulose) are partially solubilised in a manner that corresponds or correlates to NDF digestion *in vivo*. However, McQueen and Van Soest (1975) point out that in contrast to rumen microbes, fungal enzymes may respond differently to environmental factors and species differences that underlie the nutritional non-uniformity of forage NDF, since the relative proportion of solubilised NDF is dependent on the forage type. Jones and Hayward (1975) found that 24 h of pepsin treatment plus 48 h of cellulase treatment solubilised 87% of grass DM compared with *in vivo* digestibility. Therefore, enzymatic digestion should be corrected using a statistical equation to obtain digestibility coefficients that correspond to those *in vivo*. Thus it may not be possible to develop a universal correction covering a wide range of forage species and environmental conditions within the same species. The relationship between pepsin-cellulase solubility and OMD varies between forage types and therefore different corrections equations are needed (Nousiainen, 2004; Huhtanen *et al*., 2006).

1.2.2 Indigestible NDF method

Fractionating the forage cell walls into potentially indigestible and digestible fractions is essential in mechanistic feed evaluation models (Huhtanen *et al*., 2006). The iNDF approach provides precise empirical predictions of *in vivo* organic matter digestibility (Nousiainen *et al*., 2003) that are less forage-specific than those obtained using other laboratory methods (Huhtanen et al., 2006; Krizsan *et al*., 2012). There are several *in vitro* and *in situ* methods aiming to measure iNDF directly in relation to digestibility of NDF and/or OM, or in estimates of digestibility through empirical predictions of iNDF. One of the most consistent methods to determine iNDF is by extended *in situ* incubations using nylon bags of small pore size to minimise particle inflow and outflow, while still allowing adequate microbial activity inside the bag (Huhtanen *et al*., 1994; Krizsan *et al*., 2015).

The bag residues after *in situ* incubation need to be treated with neutral detergent to remove microbial and endogenous matter. A definite advantage of this method is that measuring only the end-point of digestion is less labour-intensive than the conventional approach (at least six time points). In addition, estimates may be considered biologically sound, since faecal recovery of iNDF is close to 100% in digestion marker studies (Huhtanen *et* *al*., 1994). There are large variations between cloth type and pore size of the *in situ* bags that are used around the world. Krizsan *et al*. (2015) compared the incubation results of samples displaying a range in fiber concentration and composition for two different grind sizes (0.8 and 2 mm) and incubated in polyester bags of four different qualities. They found that iNDF concentration determined using the 12-µm Saatifil PES bag (Saatitech S.p.A., Veniano, Como, Italy) provided the more accurate iNDF concentration of feed samples than the other bags. The regressions of *in vivo* OMD on iNDF in grass and in silage samples from the study by Krizsan *et al*. (2015) are presented in Figure 1. Neither intercepts nor regression coefficient estimates differed between the two equations ($P>0.05$). The major drawback of the *in situ* iNDF method is the need for surgically modified cows. In addition, outflow of a proportion of soluble lignified material may result in underestimates of the iNDF content of highly digestible grass silages, but despite losses of lignin in iNDF the improvements in predictions of OMD are marginal (Krizsan *et al*., 2014).

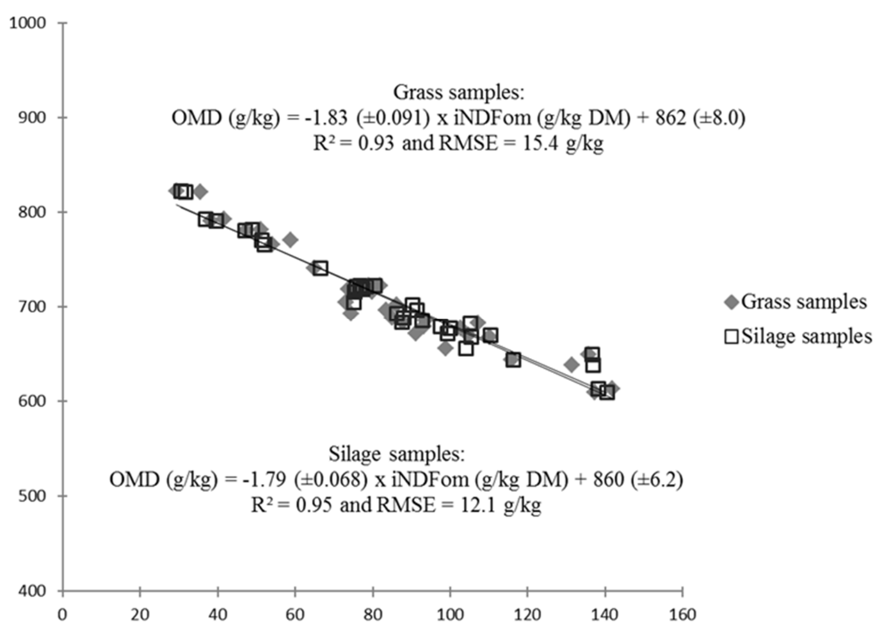

Figure 1. Linear relationship between *in vivo* organic matter digestibility (OMD) in g/kg and ash-free indigestible neutral detergent fibre (iNDFom) in grass and corresponding silage samples in g/kg dry matter (DM). The coefficient of determination (R^2) and root mean square error (RMSE) of the regression equations are given (adapted from Krizsan *et al*., 2015).

1.2.3 The NIRS method

Near infrared reflectance spectroscopy (NIRS) is a simple physical method in which near infrared light (400-2500 nm) is applied to feed samples (Williams & Norris, 1990). Part of the light energy is absorbed by the bending and stretching vibrations of O–H, C–H and N–H bonds in feed chemical components. The methods for quantitative analysis of forage quality (*i.e*. OM, NDF, iNDF and lignin) by NIRS had been developed during the past three decades by carefully calibrating the reflectance spectrum against known chemical composition or biological characteristics of samples by means of regression analysis and mathematical transformations of spectral information (Givens *et al*., 1997; Deaville & Flinn, 2000; Krizsan & Nyholm, 2012).

Published studies show the potential of NIRS in predicting forage OMD *in vivo* (Coleman & Murray, 1993) or *in vitro* (De Boever *et al*., 1996) and grass silage OMD (Nousiainen, 2004). Currently the NIRS method has been well accepted in predicting forage OMD by farm service laboratories in the Nordic countries. In addition, Nyholm *et al*. (2009a, b) also reported the NIRS method could be applied in forage digestibility determinations in sheep and of total tract digestibility of dairy cow diets, i.e. NIRS calibrations of OMD, iNDF and NDF from sheep and dairy cow faecal samples.

The major limitation of the NIRS method in prediction precision is the reference method used for calibration and validation. (Deaville and Flinn (2000) suggested that the calibration sample population must cover all sources of variation in chemical, physical and botanical characteristics of the forage samples to be analysed. However, Krizsan *et al*. (2015) showed that the iNDF concentration was not altered during ensiling of grass, and that the NIRS spectra generated from the herbage material and subsequent silage samples were very similar. They concluded that one common calibration data set was enough for NIRS predictions of iNDF in grass silages, even if feeding value determination is made from herbage material sampled before ensiling. Further, the lowest error from NIRS predictions of iNDF would be achieved with calibration reference data from one laboratory with a standardised and biologically validated *in situ* procedure, thereby, avoiding large inter-laboratory variation as has been pointed out in the latest Nordic ring-tests of the iNDF *in situ* procedure (Lund *et al*., 2004; Eriksson *et al*., 2012).

1.3 Silage energy utilisation

In the current feed evaluation system adopted in the Nordic countries, energy allowances for ruminants are expressed in terms of metabolisable energy (ME) or net energy (NE), and the relationship between ME and NE is shown in Figure 2. The metabolisable energy intake (MEI) represents that portion of the feed energy that can be metabolised by the animal, which is defined as the gross energy (GE) intake from feed minus energy outputs as faeces, urine and combustible gases (mostly methane) as shown in Figure 2. In practical feed evaluations, urine and methane energy are not usually determined, and therefore empirical equations are used to convert digestible nutrients to ME. The forage ME concentration can be estimated by *D*-value (MAFF, 1984), and the coefficient for grass silage is given as 16.0. Alternatively, the ME concentration can be also predicted from total digestible nutrients using a regression equation which takes account of variations in methane yield from different levels of total digestible nutrients (NRC, 2001).

1.3.1 Energy losses as faeces, urine and methane

The energy partitioning in lactating dairy cows are shown in Figures 2 & 3. The single greatest loss in converting dietary gross energy to metabolisable energy by ruminants is that voided in faeces. When cows are fed grass silage-based diets, greater faecal energy output is always observed with more mature silage (Gordon, 1980; Gordon *et al*., 1995; Warner *et al*., 2016), which may be due to the higher amount of undigested cell wall carbohydrate within faeces. Part of the digested energy (DE) is lost in urine and methane. High urinary energy (UE) output is always associated with less matured grass silage-based diets (Beever *et al*., 1988; Givens *et al*., 1989; Hindrichsen *et al*., 2006; Van Dorland *et al*., 2006). Givens *et al*. (1989) reported large variation in UE as a proportion of GE intake (mean 0.06, range 0.01-0.11) in sheep fed different silages, with UE output positively related to *D*-value and CP concentration. In the study by Beever *et al*. (1988), UE output was almost doubled in growing cattle fed early-cut compared with late-cut grass silage. The major increase in UE output can be attributed to elevated urinary nitrogen excretion when cows fed protein-rich early cut silages (Castillo *et al*., 2000; Huhtanen *et al*., 2008) approximately 84.4% of the incremental dietary nitrogen intake is excreted in urine. It may also be explained by the greater amount of phenolic acids and their metabolites excreted in urine by ruminants fed early-cut, highly digestible grass silage (Martin, 1969).

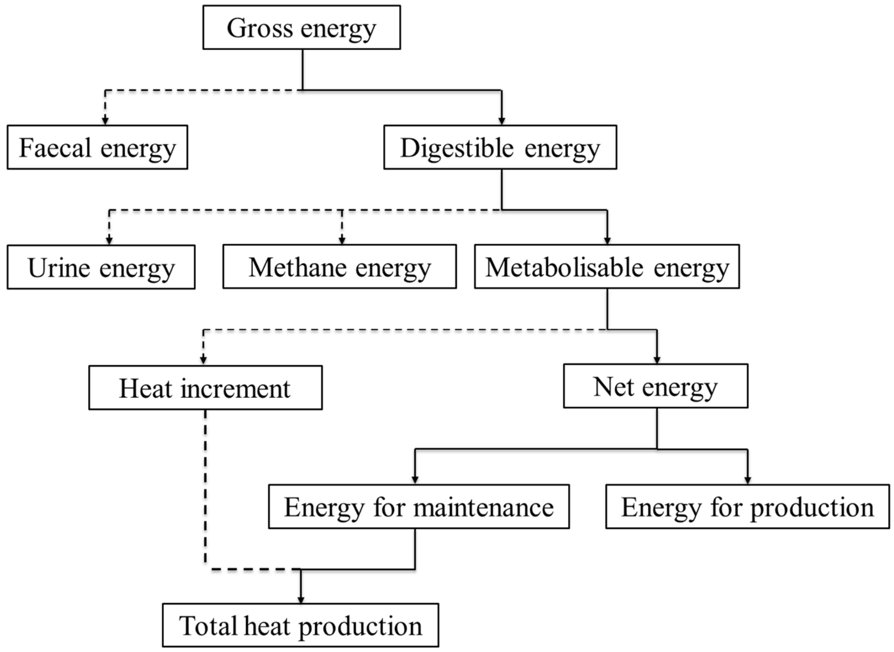

Figure 2. The partition of the feed energy in animal (adapted from McDonald *et al*., 1978)

Energy loss in methane emission represents 2 to 12% of the dietary GE intake of ruminants depending on intake level and dietary composition (Johnson & Johnson, 1995). When cows are fed grass silage-based diets, total methane energy loss is usually higher in cows fed early-cut primary growth silage than late-cut (Beever *et al*., 1988). But for cows fed regrowth silages the effect of growth interval on total methane emissions is not always significant (Warner *et al*., 2016). Cows fed early-cut grass silage usually have a lower methane yield (g kg^{-1} DMI) than cows fed late-cut silage (Brask *et al*., 2013; Warner *et al*., 2016), it can be partly explained by the increased levels of nitrate in early-cut grass silages (Keady *et al*., 2000). However, some studies report greater methane yield with more digestible early-cut silage than late-cut silage (Beever *et al*., 1988). In a meta-analysis by Ramin & Huhtanen (2013) diet digestibility was positively associated with methane yield. Warner *et al*. (2016) concluded that shorter regrowth interval decreases the methane intensity (g kg^{-1} ECM), since feeding the more digestible regrowth silages from shorter regrowth intervals in that study always improved ECM yield. The CH_4/CO_2 ratio in the breath is a good variable for expressing the microbial fermentation efficiency of the feed, since it directly describes the proportion of the carbon excreted that is not metabolised to CO_2 (Madsen *et al.*, 2010). The CH_4/CO_2 ratio is usually lower in cows fed early- than late-cut grass silage, which suggests more of the metabolisable energy is used for productive purposes, despite greater urinary and methane energy losses.

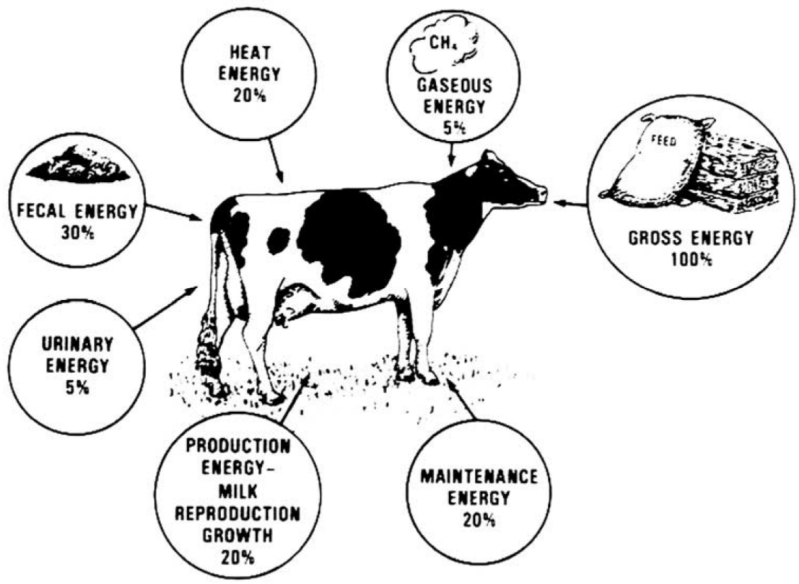

Figure 3. Energy utilisation by a lactating cow showing average partitions of feed energy (Francois & González-García, 2010).

1.3.2 Efficiency of metabolisable energy use for lactation

The efficiency of metabolisable energy use for lactation (k_l) can be determined using regression analysis on large sets of calorimetric data. In the literature, two regression equations are often used: i) linear regression relating milk energy output (adjusted to zero energy balance) to MEI, and ii) multiple regression relating MEI to metabolic live weight, milk energy output and energy balance. In earlier studies based on grass silage diets, the value of k_l ranges from 0.62 (Van Es *et al.*, 1970) to 0.67 (Unsworth *et al.*, 1994). According to a meta-analysis by Agnew and Yan (2000) based on calorimetric data on dairy cows drawn from 42 studies across the world, the average k_l for lactating dairy cows was 0.66. In a more recent meta-analysis by Moraes *et al*. (2015), the average k_l reported was 0.63.

The k_l value of dairy cow is relatively constant over a wide range of conditions such as dietary composition, animal genotype, and production level (Agnew and Yan, 2000), whether it is calculated from linear regression or from multiple regression. When cows are fed with grass silage-based diets, diets with greater forage proportion or more mature grass silage are usually more fibrous, and a greater proportion of energy intake is used to support the chewing, rumination, fermentation, and digestion of these more fibrous diets (AFRC, 1993). Evidence indicates that maintenance metabolic rates can be influenced by dietary composition (Reynolds, 1996). However, Agnew and Yan (2000) suggested that lower dairy performance with dairy cows offered diets containing a higher forage proportion might be due to a higher metabolisable requirement for maintenance, leaving less energy available for production, rather than the high forage diet resulting in a lower k_l value. Yan *et al*. (1997) and Dong *et al*. (2015) also found that increasing the dietary proportion of grass silage increased the metabolisable requirement for maintenance, but had no significant effect on k_l in lactating dairy cows.

1.4 Silage protein utilisation

The protein metabolism in dairy cow is shown in Figure 4. In extreme condition, dairy cows can survive and even produce milk using nitrogen only from urea and ammonium slats (Virtanen, 1966) due to the unique ability to produce microbial protein from non-protein nitrogen (Van Soest, 1994). In grassland-based milk production systems in the Nordic countries, good quality forage usually contains a sufficient CP concentration to meet the requirement for nitrogen metabolism in dairy cows, which can bring economic benefits by reducing the use of expensive protein supplements. However, dairy cows have low overall nitrogen utilisation efficiency (NUE, milk nitrogen output/dietary nitrogen intake) compared with monogastric animals, with an average of around 26-28% (range 16.4-40.2%, Huhtanen & Hristov, 2009). In addition, increasing dietary CP concentration has a strong negative effect on NUE of ruminants. It only marginal increases milk protein yield, most of the incremental nitrogen intake is excreted in urine (Castillo *et al*., 2000; Huhtanen *et al*., 2008). The basic principle for improving feed protein value is to provide more metabolisable protein (MP), the sum of microbial protein (MPS) synthesised in the rumen and ruminal undegraded protein (RUP) from feed, to the animal. The MP requirement of dairy cows should be met at low CP concentration without compromising rumen functions, feed intake and milk production (Huhtanen & Broderick, 2016).

Therefore, improving the forage protein value, which represents a high proportion in total protein intake, is an available strategy to reduce the requirement of protein supplements in lactating dairy cows. In the Nordic countries, silages made from grass or legumes are the main forage source for lactating dairy cows during the indoor feeding period, and the silage protein value can be affected by forage factors and ensiling treatments.

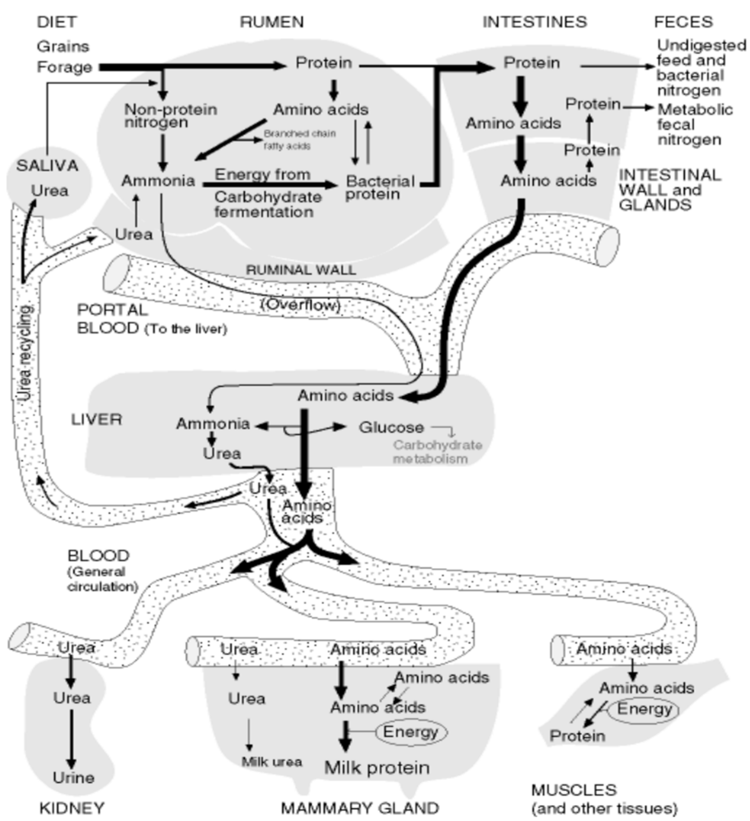

Figure 4. Protein metabolism in dairy cow. (Wattiaux, 1998).

1.4.1 Forage factors

The forage protein value can be influenced by forage species. Among the common forage species in the Nordic countries, red clover (*Trifolium pratense*) is high in CP and is considered as a good forage source for dairy production. As a leguminous plant, red clover can fix atmospheric nitrogen (N_2) and require less nitrogen fertiliser (Wilkins & Jones, 2000). In addition, red clover contains polyphenol oxidase (Lee, 2014), which can deactivate plant proteases and prevent protein breakdown during both ensiling and rumen degradation (Broderick *et al*., 2001; Vanhatalo *et al*., 2009). The recovery of incremental protein in the ruminal outflow is high in cows fed red clover silages and the non-ammonia nitrogen (NAN) flow from the rumen is greater with red clover than grass silages (Dewhurst *et al*., 2003; Vanhatalo *et al*., 2009). However, despite that the milk protein yield is not improved and the milk protein concentration actually decreases in cows fed red clover silages (Vanhatalo *et al*., 2009). This suggests a poor utilisation of incremental NAN flow to the small intestine when cows fed red clover. It can be partly explained by the lower intestinal digestibility of red clover protein than grass protein, and by lower NUE in cows fed red clover increasing the amount of nitrogen excreted in manure (Bertilsson & Murphy, 2003; Vanhatalo *et al*., 2009). The lower intestinal utilisation of red clover protein is problematic, but Moorby *et al*. (2009) reports that feeding dairy cows a grass-clover mixture with 50:50 or higher red clover/grass ratio can decrease dietary CP concentration but provide a greater milk yield and NUE than using red clover as a sole forage source.

In addition, feeding cows with more matured forage will decrease dietary digestibility and consequently decrease MPS, but reduced ruminal CP degradation can increase the proportion of ruminal undegraded protein from feed in total MP supply. Practical studies have confirmed that less matured silage is usually highly digestible and high in CP, which promotes greater intake potential and yields of milk and milk protein, but lower NUE (Rinne *et al*., 1999; Kuoppala *et al*., 2008; Randby *et al*. 2012; Warner *et al*., 2016). Huhtanen *et al*. (2011) suggests that the increase in milk protein yield in cows fed early cut silages is mainly due to the increased MEI. Following a meta-analysis based on production trial results, Huhtanen (2013) concludes that the milk protein yield is not related to CP concentration in cows fed basal diets without supplemental protein. Feeding cows with early cut silages increases the yield of milk and milk protein, mainly due to the improved MEI without any extra benefits from higher CP concentration.

1.4.2 Ensiling treatments

Preserving forage as silage or hay for the long indoor feeding period are the main strategy in the Nordic countries. However, in recent decades hay production has become less popular because of the requirement for weather and facilities during processing. Studies have confirmed that the protein value of well-fermented grass silages can be as good as that of grass hay (Jaakkola & Huhtanen, 1993; Shingfield *et al*., 2002; Vaga, 2017), and that differences in milk production responses are mainly due to the limited energy supply in silage compared with hay diets (Broderick, 1995; Vagnoni & Broderick, 1997). The basic idea of ensiling is to preserve grass in acidic conditions caused by microbial fermentation of sugars under anaerobic conditions. However, the ensiling process is usually accompanied by energy (ATP) losses through volatile fatty acids (VFAs) and lactic acid production (Chamberlain, 1987), and degradation of available feed nitrogen for rumen microbes to ammonia (Van Soest, 1994). The VFAs and lactic acid provide almost no energy for rumen microbes, which results in a lower efficiency of MPS when cows are fed extensively fermented silages.

Adding formic and/or propionic acid-based additives during ensiling is a common way to improve the silage quality in practice, because formic acid can rapidly decrease silage pH and restrict microbial activity, and propionic acid can inhibit growth of yeast and moulds and thus improve the aerobic stability of silage (Castle & Watson, 1970; Gildberg & Raa, 1977). The beneficial effects of these ensiling additives on silage protein value have been examined in earlier studies. Jaakkola *et al*. (2006) reported that preservation of grass with formic acid effectively decreases protein degradation in the silo (soluble nitrogen decreased from 746 in control to 610 g kg^{-1} with the highest dose of additive) and that treating silage with formic acid increased the flow of microbial nitrogen to the duodenum (from 49.0 to 65.4 g d^{-1}). In another study, Nagel and Broderick (1992) reported 65% greater dietary non-ammonia nitrogen flow from the rumen of lactating dairy cows when fed formic acid-treated alfalfa silage compared with untreated silage, while milk production increased by 3.4 kg d^{-1} and milk protein content increased by 1.6 g kg^{-1}. Other silage additives, such as formaldehyde, can also reduce ruminal protein degradation and increase duodenal NAN flow

(Thomas *et al*., 1981a; Siddons *et al*., 1984), but the beneficial effects of formaldehyde-treated silage in terms of production responses are more related to improved energy intake rather than improved protein utilisation (Thomas *et al*., 1981a).

Wilting of forage material before ensiling is another strategy to improve the silage protein value, since a higher DM concentration will restrict the microbial fermentation at ensiling (McDonald, 1981) and decrease ruminal protein degradation when fed to animals (Tamminga *et al*., 1992; Verbič *et al*., 1999). Edmunds *et al*. (2014) evaluated the effect of silage DM concentration on nitrogen flow and found significantly increased estimated utilisable CP flow to the duodenum with increased silage DM. However, in a meta-analysis (n = 90) by Huhtanen and Nousiainen (2012) based on production trials, silage DM concentration has a non-significant effect on milk protein yield when included in a bivariate model with MEI, and had a negative effect on NUE when included in a model with dietary CP concentration. This suggests that production responses to wilting are mediated mainly by increased MEI, and the lower ruminal protein degradation of wilted silages is not realised as improved milk protein yield.

2 Objectives

The aim of the studies reported in Papers I to IV in this thesis was to optimise grass silage utilisation in milk production by contributing to development of practical farm-scale feeding models. Specific objectives of the studies were to:

- Determine the metabolisable energy value of grass and red clover silages for sheep and analyse urine samples for nitrogen and phenolic compounds that could derive from solubilisation of part of the lignin polymer attached to plant cell walls.
- Examine the effects of replacing concentrate based on grain and soybean meal with a range of agro-industrial by-products on the performance of early lactation dairy cows fed silage harvested at different maturity stages.
- Investigate the production responses to second- and third-cut grass silages harvested in different two- and three-cut harvesting systems in terms of milk production and efficiency of nitrogen and energy utilisation when fed to lactating dairy cows.
- Analyse the effects of *D*-value and harvest time (primary growth compared with regrowth) of grass silage on feed intake and milk production in dairy cows using meta-data, and investigate the effects of different factors on these responses.

3 Material and methods

The general overview of the feeding trials and data analysis in this thesis is presented in Figure 5.

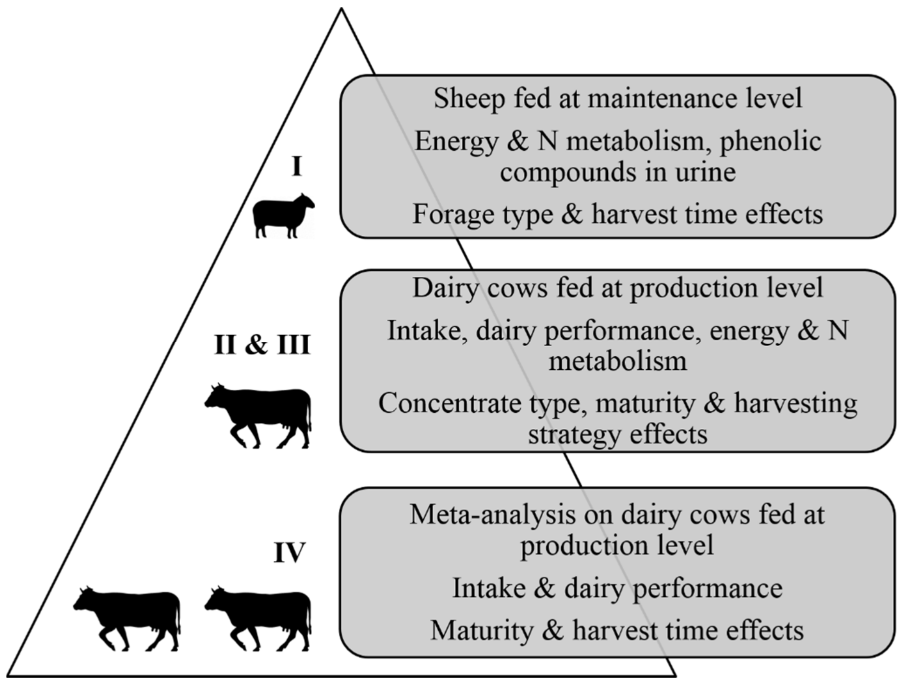

Figure 5. General layout of the work in this thesis. Results from the Paper II & III are also included in the meta-analysis in Paper IV.

3.1 Paper I

Paper I investigated the effect of forage type (grass or red clover) and harvest time (primary growth or regrowth) of silage fed to sheep at maintenance level on intake, energy and nitrogen utilisation, and excretions of urinary nitrogen and phenolic compounds. Samples of 25 primary growth and regrowth silages of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) grass mixtures and red clover (*Trifolium pratense*) from six digestibility trials conducted at the Natural Resources Institute Finland, Jokioinen (60°48´N, 23°29´E), Finland, were used in this study.

Silages, faeces and urine were sampled by period, and analysed for energy and nitrogen concentrations. Urine samples for phenolic compound analysis were determined according to Olthof *et al*. (2003) by using a liquid chromatography-tandem mass spectrometry (LC-MS) application (1290 Infinity system and Agilent 6490 Triple quadrupole mass spectrometers; Agilent Technologies, Santa Clara, CA, USA). Methane emissions produced by silage samples during *in vitro* fermentation were measured according to Ramin and Huhtanen (2012), and rumen fluid for *in vitro* incubation was collected from two Nordic Red cows fed *ad libitum* on a diet consisting of 60% grass silage and 40% concentrate on DM basis.

The effects of grass silage compared with red clover silage and of primary growth compared with regrowth on silage intake and energy and nitrogen utilisation were analysed by applying orthogonal contrasts using the GLM procedure of SAS (SAS Inc. 2007, Release 9.3; SAS Inst., Inc., Cary, NC) to the data, with a model only correcting for the effect of diet. Regressions of UE and phenolic compounds on silage parameters comparing cut and forage types were evaluated based on the 95% confidence intervals for the regression parameters.

3.2 Paper II

The experiment described in Paper II was conducted at Röbäcksdalen research station, Swedish University of Agricultural Sciences in Umeå, Sweden (63°45’N; 20°17’E). Twenty lactating Nordic Red cows with mean body weight (BW) 595 ± 17.4 kg, at 81 ± 6.7 days in milk (DIM) and producing 31.9 ± 1.01 kg d^{-1} milk at the beginning of the experiment were used in a replicated 4 × 4 Latin square design. Each experimental period lasted 21 days, and data recordings and samplings were conducted during the last seven days. The cows were offered the diets *ad libitum* with free access to water, and milked twice daily.

The dietary treatments were in a 2 × 2 factorial arrangement and formulated to have the same forage to concentrate ratio (67:33) without considering extra concentrate supplementation from the GreenFeed system. The diets consisted of a grain-based concentrate (GC) or a concentrate made from agro-industrial by-products (BC), fed with either early-harvested (ES) or late-harvested (LS) primary growth silage, harvested two weeks apart from a third-year timothy (*Phleum pratense*) ley. The concentrates were composed to be isonitrogenous and the ingredients were shown in Table 3. The diet combining GC and ES was formulated to provide ME and MP for 35 kg ECM yield.

Table 3. *Ingredients of grain-based concentrate and agro-industrial by-products concentrate used in Paper II (g kg^{-1} feed).*

Ingredient	Grain-based concentrate (GC)	By-products concentrate (BC)
Energy source		
Oats meal	273	-
Barley meal	273	-
Wheat meal	273	-
Sugar beet pulp	-	579
Wheat bran	-	42
Protein source		
Soybean meal	141	-
Dried distillers grains	-	160
Heat-treated rapeseed meal	-	141
Palm kernel cake	-	30
Premix	40	48

Both concentrates were produced by Lantmännen Lantbruk AB, Malmö, Sweden.

Individual intake and milk yield were recorded daily during the whole experiment, and milk samples were taken for milk composition analysis at four consecutive milkings. Mass fluxes of CH_4, CO_2 and O_2 were recorded daily by the GreenFeed system (GreenFeed, C-Lock, Rapid City, SD), as described by Huhtanen *et al*. (2015b). A commercial concentrate was given to the cows (on average 1.2 kg d^{-1}) to ensure regular visits to the GreenFeed system. Feed samples were collected weekly to correct the DM value in the automatic feeding system and for further laboratory analysis. Spot samples of faeces and urine from 12 multiparous cows were collected during the registration week to determine faecal and urinary energy and nitrogen excretion.

Apparent diet digestibility was calculated using iNDF as an internal marker. The ECM yield and milk energy concentration were calculated according to Sjaunja *et al*. (1990). The human-edible proportion of feeds and edible feed conversion ratio for energy and for protein were calculated according to Wilkinson (2011) and Ertl *et al*. (2015b). Daily faecal nitrogen excretion was calculated from faecal nitrogen concentration and daily faecal output. Daily urinary excretion was estimated from urinary nitrogen concentration and estimated urinary nitrogen excretion (g d^{-1}), which was calculated as described by Huhtanen *et al*. (2015a) including subtraction of scurf nitrogen. Heat production (HP) was calculated according to Brouwer (1965), using data from the GreenFeed devices and urinary nitrogen excretion. The k_l value was calculated according to AFRC (1990). Experimental data were subjected to ANOVA using the General Linear Model of SAS (SAS Inc. 2007, Release 9.3; SAS Inst., Inc., Cary, NC) by applying a model correcting for the effect of block, period and cow within block to evaluate the effects of silage maturity, concentrate type and their interaction.

3.3 Paper III

Grass harvesting and the feeding experiment were conducted at Röbäcksdalen Research Farm, Umeå, Sweden. Regrowth grass silages from one three-cut system and three two-cut systems are used in this study as shown in Figure 6. Thirty lactating Nordic Red cows with mean BW 612 ± 14.4 kg, at 72 ± 4.4 DIM and yielding 31.5 ± 0.93 kg of milk per day at the beginning of the experiment were used in this study. The cows were fed diets *ad libitum* with free access to water, and milked twice daily.

The experiment was conducted as a replicated incomplete 5 × 4 Latin square design with five treatments and four periods. The four second-cut silage treatments were fed in a 2 × 2 factorial arrangement, to investigate the effects of harvesting time of the first cut (EE and EL vs. LE and LL), length of the regrowth interval (EE and LE vs. EL and LL) and their interaction on dairy cow performance. The fifth dietary treatment was the third-cut silage (TC). Each experimental period lasted for 21 days and recordings and samplings were conducted during the last seven days. The experimental diets were formulated to have the same forage to concentrate ratio as (g kg^{-1} DM): silage 565, crimped barley 340, heat-treated rapeseed meal 80, and mineral and vitamin feed 15 excluding extra concentrate supplementation from the GreenFeed system. The diet based on EE silage was formulated to provide ME and MP for 35 kg of ECM yield.

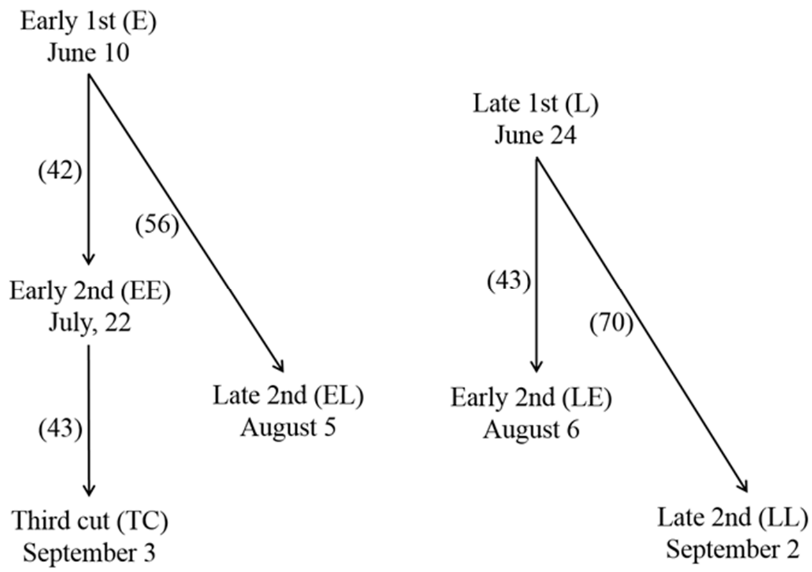

Figure 6. Schematic presentation of the strategies for harvesting regrowth materials for ensiling used in this study. Growing days from previous cut of each regrowth material are shown in brackets.

Data recording and sample collection were conducted as described in Paper II. In addition to the calculations mentioned in Paper II, SDMI index was calculated according to Huhtanen *et al*. (2007). Oxygen consumption for HP calculations was estimated using the relationship between carbon dioxide and oxygen derived from data in Aubry and Yan (2015). Experimental data were subjected to analysis of variance using the General Linear Model of SAS (SAS Inc. 2007, Release 9.3; SAS Inst., Inc., Cary, NC). Least square means are reported and mean separation was done by orthogonal contrasts. The four second-cut treatments were analysed as a 2 × 2 factorial arrangement, to evaluate the effects of harvesting time of the first cut, length of the regrowth interval and their interaction on dairy cow performance. The third-cut treatment was compared with the four second-cut diets to evaluate the effect of second and third cuts on dairy cow performance.

3.4 Paper IV

In Paper IV, a meta-analysis was conducted to investigate the effects of forage maturity and harvest on feed intake and milk production in dairy cows. For the meta-analysis, mean treatment values (n = 226) were taken from 39 dairy cow studies conducted in Europe using *ad libitum* feeding of grass silage. The diets were supplemented with concentrate feeds differing both in amount and composition between experiments, but not within experiments. The data were divided into two subsets based on the grass silage treatments used in the experiment: digestibility influenced by the maturity of grass ensiled (*D*-value; n = 157), and comparison of silages made from primary growth or regrowth grass (harvest; n = 69).

The minimum criteria for an experiment to be included in the dataset were reported values of feed intake, milk yield, milk composition and adequate silage characterisation (*D*-value, DM and chemical composition concentrations). For the *D*-value subset, the different maturity stages of the grass ensiled had to be compared within the same experiment, represented as differences in silage *D*-value. For the harvest subset, it was essential that the comparisons of primary growth and regrowth silages were made within the same experiment. The SDMI index was calculated according to Huhtanen *et al*. (2007). The MEI was estimated at maintenance level, silage ME concentration was calculated assuming 16 MJ kg^{-1} of digestible organic matter (DOM) according to MAFF (1975). The ME concentration of concentrate feeds was calculated using tabular digestibility coefficients (LUKE, 2018) and reported chemical composition. The ECM yield was calculated according to Sjaunja *et al*. (1990).

The data were subjected to simple or multiple linear regression analysis using the mixed effects model procedure of SAS (PROC MIXED; SAS Institute, 2008). In models for harvest comparison, the fixed effect of cut (primary growth compared with regrowth) had to be taken into account. The underlying rationale and further details on using mixed model analysis to integrate quantitative findings from multiple studies can be found in St-Pierre (2001).

4 Results

4.1 Paper I

The energy intake was not affected by forage type, but sheep fed grass silage was low ($P \leq 0.03$) in CH_4 yield (MJ kg^{-1} DMI), proportions of methane energy losses from both GE and DE and urinary energy loss from DE compared with sheep fed red clover silage. The ratio of ME/DE was much greater ($P \leq 0.01$) in primary growth grass silage than in the other forage treatments, which also introduced significant differences between forage type and harvest time ($P \leq 0.03$). Sheep fed regrowth silage excreted more nitrogen in faeces ($P<0.01$) than primary growth silage, and daily faecal nitrogen output was greater ($P<0.01$) on the red clover silage than the grass silage. There was also a strong uniform relationship between urinary energy and silage nitrogen output for both the grass and red clover silages, and a rather high proportion of incremental DE from improved OMD was lost as urinary energy.

The phenolic compounds excreted in urine was dominated by N-benzoylglycine. Greater ($P<0.01$) amount of benzoic acid, but less ($P<0.01$) phenylacetic acid were excreted in urine of sheep fed grass silage compared with red clover silage. More ($P=0.02$) benzoic acid was excreted by sheep fed primary growth compared with regrowth silage.

4.2 Paper II

A two-week delay in harvesting decreased the CP concentration and digestibility, but increased the concentrations of NDF and iNDF in grass silage. Feed intake and total tract digestibility were lower ($P<0.01$) when cows fed LS than ES diets. Replacing GC with BC in the diet decreased (P<0.01) CP digestibility, but increased (P<0.01) NDF digestibility. Yields of milk and milk content and milk protein concentration were higher (P<0.01) for cows fed ES than LS diets. Replacing GC with BC decreased (P=0.02) milk protein concentration. Feeding ES diets improved (P<0.01) feed efficiency, but reduced (P<0.01) NUE compared with feeding LS diets, and the edible feed conversion ratio for protein and energy increased more with ES than LS when GC was replaced with BC (P≤0.05).

Feeding ES diets increased (P<0.01) total CH_4 emissions, but decreased (P<0.01) CH_4 intensity and CH_4/CO_2 ratio compared with feeding LS diets. Lower (P<0.01) CH_4/CO_2 ratio was also observed when cows were fed BC rather than GC. Cows fed ES had lower (P<0.01) faecal energy output, but greater (P<0.01) UE and HP losses than fed LS diets. Feeding ES resulted in a more positive energy balance in cows than feeding LS and the difference between silage sources was smaller when cows were fed BC than GC (P=0.02). Feeding ES combined with GC gave the highest (P=0.03) k_l in experimental cows.

4.3 Paper III

Postponing the first cut (EE & EL compared with LE & LL) and increasing the regrowth interval (EE & LE compared with EL & LL) decreased (P≤0.03) DMI and CP intake, but intake of NDF and iNDF was higher (P<0.01) for the regrowth silage diets after the late first cut. Feeding the TC silage diet decreased (P<0.01) intake of NDF and iNDF, but increased (P<0.01) intake of CP and MP compared with second-cut silage diets. The total tract digestibility decreased (P<0.01) with postponed first cut and longer regrowth interval. The TC silage diet was more (P≤0.01) digestible than second-cut silage diets.

Postponing the first cut and increasing the regrowth interval decreased (P<0.01) the milk yield, and the decrease in ECM yield with increased regrowth interval was greater after early than late first cut. Cows fed the TC silage gave higher (P<0.01) milk yields than those fed second-cut silages. Postponing the first cut decreased (P<0.01) the milk protein concentration, while increasing the regrowth interval decreased (P≤0.03) the milk fat concentration. Feeding the TC silage diet increased (P<0.01) the milk protein concentration compared with cows fed the second-cut silage diets. The NUE increased (P<0.01) with postponed first cut and longer regrowth interval, but increasing the regrowth interval also led to lower (P=0.01) feed efficiency. Feeding the TC silage diet improved (P<0.01) feed efficiency, but decreased (P<0.01) NUE compared with feeding the second-cut silage diets.

Total CH_4 emissions decreased by 16 and increased by 16 g d^{-1} with increased regrowth interval after early and late first cut, respectively. Postponing the first cut increased (P≤0.02) CH_4 yield and CH_4/CO_2 ratio. With longer regrowth interval, CH_4 intensity and CH_4/CO_2 ratio increased (P≤0.02). Feeding the TC silage diet decreased (P≤0.01) methane intensity and CH_4/CO_2 ratio compared with the second-cut silage diets. Postponing the first cut and increasing the regrowth interval decreased (P<0.01) urinary nitrogen excretion. The decrease in urinary nitrogen excretion with increased regrowth interval was greater (P≤0.01) after early than late first cut. Faecal nitrogen output decreased by 15 and increased by 19 g d^{-1} with increased regrowth interval after early and late first cut, respectively. Feeding the TC silage diet increased (P<0.01) outputs of urinary and faecal nitrogen compared with the second-cut silage diets. Postponing the first cut and increasing the regrowth interval decreased (P≤0.04) UE excretion and energy balance. Postponing the first cut also decreased (P<0.01) k_l. The energy balance decreased by 24 and increased by 1 MJ d^{-1} with increased regrowth interval after early and late first cut, respectively. The decrease in UE excretion with increased regrowth interval was greater (P=0.04) after early than late first cut. Feeding the TC silage diet increased (P≤0.03) energy outputs as UE and HP, but decreased (P=0.03) faecal energy output compared with second-cut silage diets.

4.4 Paper IV

The mean response in DMI and SDMI to improved D-value was 0.175 and 0.161 kg per 10 units increase in D-value. The average increase in milk and ECM yield was 0.303 and 0.369 kg per 10 units increase in D-value, respectively. Silage total acid (VFAs + lactic acid) concentration had a negative effect on milk and ECM yield when included in the models with D-value. D-value had a significantly negative coefficient with milk and ECM yield when included in the models with MEI. The milk content concentrations increased (P≤0.02) with increasing D-value and intake. Silage CP concentration had a significantly negative coefficient (-0.0168) in the model with *D*-value, but a positive coefficient (0.0055) in the model with DMI. Silage NDF concentration had a significantly positive effect on milk fat concentration when included in the models with *D*-value.

Differences in most silage parameters between primary growth and regrowth silages were small. Silage OMD was greater ($P<0.01$) in primary growth than regrowth silage, but there was no significant difference ($P=0.74$) in *in vivo* dietary OMD. Daily SDMI, total DMI and MEI was 1.07 kg, 1.03 kg and 16.6 MJ lower, respectively, when cows were fed regrowth rather than primary growth silage. Including silage CP and NDF concentrations in the MEI model reduced the residual standard error compared with MEI models with a single variable. Cows fed primary growth silage produced 0.85 kg more milk and 1.23 kg more ECM per day than fed regrowth silage. The residual standard error of the milk and ECM yield models was reduced when MEI was included as an independent variable, and the milk and ECM yield response was 0.54 and 0.55 kg lower, respectively, for cows fed primary growth silage than regrowth silage when MEI was the same. Including *D*-value in the milk yield model with MEI tended to decrease ($P=0.06$) the difference between regrowth and primary growth silage from 0.54 to 0.47 kg d^{-1}. The milk protein concentration was 0.59 g kg^{-1} higher when cows were fed primary growth silage rather than regrowth silage.

5 Discussion

5.1 Energy and nitrogen utilisation in sheep fed silage

In Paper I, the average CH_4 conversion factor (CH_4-E/GE ratio) was 0.064, which compared well with the 0.065 recommended by IPCC (2006) and 0.067 reported by Zhao *et al*. (2016a). The CH_4 yield (MJ kg^{-1} DMI) was positive related to *in vivo* OMD ($R^2 = 0.36$). This observation is in agreement with Beever *et al*. (1988), who reported greater CH_4 yield with more digestible early cut than late cut grass silage. Ramin and Huhtanen (2013) also showed a positive relationship between digestibility and CH_4 yield based on a meta-analysis with 207 observations.

The lower average CH_4 yield (1.14 vs. 1.22 MJ kg^{-1} DMI) from sheep fed grass silage compared to red clover silage can be explained by the lower molar proportion of acetate and greater proportion of propionate in ruminal VFAs when sheep fed grass than red clover (Vanhatalo *et al*., 2009; Navarro-Villa *et al*., 2011). On the other hand, in general grasses are more likely to accumulate nitrates than legumes (Crowley, 1985). The grass silages in Paper I may have contained greater nitrate concentrations than the red clover silages. Nitrate can inhibit enteric methane production by replacing the reduction of carbon dioxide to methane as a major sink for disposal of hydrogen in the rumen (Allison *et al*., 1981; Akunna *et al*., 1994).

In assessments of silage ME values, the UK system assume a constant loss of energy of 3.0 MJ kg^{-1} of DOM in the DM (DOMD) from energy losses in urine and CH_4 (MAFF, 1975; ARC, 1980). However, it was not supported from the results in Paper I. The sum of energy losses in urine and CH_4 in DE was lower in sheep fed grass silage (on average 0.183) than red clover silage (on average 0.218), which resulted in a greater regression coefficient for grass silages ME calculation (17.1) than the default value of 16.0 set by MAFF (1975) and ARC (1980) and red clover (16.7).

Total nitrogen excretion has been shown to be related to the energy content of the diet (Firkins & Reynolds, 2005). The key energy parameters that may influence nitrogen excretion in faeces, urine and milk are dietary ME concentration and ME/GE ratio (Kebreab *et al*., 2010), which is defined as an indicating factor for feed quality (AFRC, 1993). In Paper I, sheep fed grass silages had higher ME concentration (11.1 vs. 10.6 MJ kg^{-1} DM) and ME/GE ratio (0.568 vs. 0.546), but lower faecal nitrogen (7.12 vs. 8.77 g d^{-1}) and faecal nitrogen: nitrogen intake ratio (0.266 vs. 0.291) than red clover silages. Similarly, sheep fed primary growth silages had greater ME concentration (11.0 vs. 10.7 MJ kg^{-1} DM) and ME/GE ratio (0.570 vs. 0.544), but lower faecal nitrogen output (7.41 vs. 8.48 g d^{-1}), than sheep fed red clover silages.

Low-quality diets (*e.g*. red clover silages, regrowth silages in Paper I) may contain less digestible protein, which increasing the amount of nitrogen excreted in faeces. The increase in dietary quality (*e.g*. high ME concentration) may give a better match in supplying fermentable energy and nitrogen to microbial organisms in the rumen. Fermentable energy supply has been associated with advanced microbial protein synthesis and less urinary nitrogen excretion because it improves ammonia utilisation from rumen microbes (Dijkstra *et al*., 2013). On the other hand, a shift in nitrogen excretion from faeces to urine was also observed with increasing nitrogen digestibility (R^2 = 0.93). Zhao *et al*. (2016b) demonstrated a positive relationship between total tract nitrogen digestibility and the proportion of urinary nitrogen excretion in total nitrogen excretion in sheep. High nitrogen digestibility may be associated with a large proportion of nitrogen being absorbed as ammonia from the rumen. Excess ammonia nitrogen above the requirements for microbial activity would be excreted in urine as urea nitrogen rather than in faeces, thereby increasing the proportion of nitrogen excreted in urine.

5.2 Urinary excretion of phenolic compounds in sheep fed silage

The quinic acid, shikimic acid and hydroxycinnamic acids (*p*-coumaric acid, ferulic acid and caffeic acid) in plant feeds are the main precursors of benzoic acid excreted in ruminant urine (Martin, 1973, 1982). Martin (1973) reported that the decarboxylation by ruminal microbes was the major metabolic pathway for hydroxycinnamic acids converted to benzoic acid in the rumen. Benzoic acid and its conjugates represent 45-85% of total urinary phenolic compounds, and the major form of urinary benzoic acid is N-benzoylglycine (hippuric acid), a condensate of benzoic acid conjugation with glycine formulated in the kidney (Martin, 1982; Silanikove & Brosh, 1989). Hippuric acid is high in energy (23.6 MJ kg^{-1} DM; Oleinik *et al*., 1979), is considered one of the contributors to urinary energy excretion. Black (1971) suggested that the greater value of urinary energy/nitrogen ratio in ruminants than in monogastric animals could be explained by the greater amount of hippuric acid excreted in ruminant urine. The nitrogen excreted as hippuric acid represents approximately 6% (range 2-32%) of total urinary nitrogen excretion in cattle, sheep and goats (Topps & Elliott, 1965; Martin, 1969; Bristow *et al*., 1992). The proportion of hippuric acid nitrogen in total urinary nitrogen is negatively correlated with the dietary CP concentration (Szanyiová *et al*., 1995).

In Paper I, hippuric acid represented 88-96% of the benzoic acid-based compounds in the urine, which is in line with the earlier study by Silanikove and Brosh (1989). The total urinary excretion of benzoic acid-based phenolic compounds was higher in sheep fed grass silage than red clover silage (5.10 vs. 4.44 g kg^{-1} DMI), which is in line with the trends in ratio of urinary energy to urinary nitrogen (0.057 vs. 0.040 MJ g^{-1}). It can be explained by the higher concentration of hydroxycinnamic acids (*e.g.* ferulic acid and *p*-coumaric acid) in grass than in legumes (Cherney *et al*., 1989). Total urinary excretion of benzoic acid-based phenolic compounds was slightly higher in sheep fed primary growth silages than regrowth silages (5.23 vs. 4.73 g kg^{-1} DMI), which was negatively correlated with the silage iNDF concentration (R^2 = 0.63). Martin (1970) reported that higher lignin concentrations in grass resulted in lower quinic acid concentration. Cherney *et al*. (1989) also reported a decrease in soluble hydroxycinnamic acid concentration with the development of lignification in forage. Lower availability of precursors for benzoic acid synthesis results in lower urinary concentration of total benzoic acid-based phenolic compounds in cows fed regrowth silages.

Hippuric acid nitrogen in Paper I represented 1.4-2.3% of total urinary nitrogen excretion. This is close to the value of 2.6% for sheep under pure ryegrass grazing conditions, but lower than the 4.8% for sheep grazing a ryegrass/white clover mixture, reported by Bristow *et al*. (1992). Based on the energy concentration of hippuric acid reported by Oleinik *et al*. (1979), the hippuric acid excreted daily in urine contains approximately 0.09-0.12 MJ energy, which represents 6.3-10.8% of total urinary energy excretion. In addition, Van Groenigen *et al*. (2006) reported that increasing the hippuric acid concentration in urine can decrease urinary nitrous oxide (N_2O) emissions, which suggests that feeding ruminants with primary growth grass diet can reduce greenhouse gas emissions from manure.

5.3 Energy and nitrogen utilisation in dairy cow fed grass silage

In this thesis the total methane emissions decreased with postponed first cut but were not affected by the regrowth interval, which agrees with earlier findings by Warner *et al*. (2016). Differences between the diets in total methane emissions are mainly related to DMI, OMD and dietary fat concentration (Ramin & Huhtanen, 2013). The CH_4/CO_2 ratio was much more closely related to carbon dioxide production than methane production in both Paper II (R^2 = 0.76 vs. 0.37) and III (R^2 = 0.68 vs. 0.04). Therefore, the higher CH_4/CO_2 ratio when postponing the first cut or extending the regrowth interval was mainly from the increased carbon dioxide production from metabolism of the increased MEI. This is also supported by the greater energy digestibility and metabolisability in cows fed early rather than late cut silages from both primary growth and regrowth. Kuoppala *et al*. (2008) and Yan *et al*. (2010) suggest that increasing MEI by feeding cows with more digestible silages gives better milk production, because ME requirement for maintenance will not be changes by increasing MEI, all incremental ME is used for milk production and changes in energy balance. The average CH_4-E/GE ratio and CH_4-E/DE ratio in Paper II and III was 0.061 and 0.090, respectively, which compared well with the values (0.068 and 0.089) reported in a meta-analysis (n = 247) by Yan *et al*. (2000) based on dairy cows fed grass silages. The CH_4-E/DE ratio decreased with increased dietary OMD, in agreement with Ramin and Huhtanen (2013). This suggests a shift in digestion from rumen to intestine (Moss *et al*., 2000) and proportionally more NDF is digested post-ruminally when cows are fed both primary growth and regrowth silages from an early first cut or regrowth silages with a shorter regrowth interval. It can be explained by a change in rumen fermentation pattern toward increased propionate and reduced acetate (Johnson & Johnson, 1995), and the MPS efficiency may be improved with increased DMI when cows fed those more digestible silages (Ramin & Huhtanen, 2013).

On the other hand, the CH_4-E/DE ratio was negatively related to k_l (R^2 = 0.48 in Paper II, R^2 = 0.82 in Paper III) and positively related to inefficiency of ME used for production (HP/MEI ratio; R^2 = 0.77 in Paper II, R^2 = 0.92 in Paper III based on treatment means), which indicates greater energy expenditure for maintenance rather than production in cows fed less digestible silages. The greater amount of structural carbohydrate intake with less digestible silage diets is associated with greater work in rumination and digestion, which increases the energy cost of digestion by enhanced secretion of salt in digestive fluids, such as saliva and enzymes, accompanied by greater desquamation through physical action (Lobley, 1986). Mean k_l value in this thesis was 0.65, which is close to values derived in respiration chamber studies (Agnew & Yan, 2000; Moraes *et al*., 2015). This suggests that HP estimated from gas values measured by the GreenFeed system resulted in a reasonable mean estimate of the k_l value. Increased maintenance requirement with high-fibre diets has been reported (*e.g*. Agnew & Yan, 2000). The differences in k_l values is associated more with dietary iNDF concentration than total NDF concentration, which can be related to greater gut fill and greater work in rumination and digestion, factors which all contribute to greater maintenance requirement (Reynolds *et al.*, 1991).

The NUE increased with postponed first cut and increasing regrowth interval in Paper II & III. This trend agrees with findings in studies investigating the effects of maturity of grass silage at harvest (*e.g*. Rinne *et al.*, 1999; Kuoppala *et al*., 2008; Randby *et al*., 2012) and regrowth interval (Warner *et al*., 2016). The greater NUE when cows fed less digestible silages is mainly due to its lower concentrations of CP and protein balance in the rumen (PBV). In this thesis the NUE decreased by 1.23 and 1.58 g kg^{-1} with every unit increase in dietary concentrations of CP and PBV, respectively, which compares well with the value of 1.20 and 1.58 g kg^{-1} reported in a meta-analysis (n = 998) by Huhtanen *et al*. (2008). Although decreases in dietary CP concentration can increase the risk of restricted microbial activity in the rumen, the PBV values for all diets in studies of this thesis were still above zero (≥5.9 g kg^{-1} DM), the recommended minimum allowance (LUKE, 2017).

Higher incremental nitrogen intake with improved digestibility is excreted as urinary nitrogen, which is supported by the differences in milk urea nitrogen concentration, a good predictor of urinary nitrogen output, between treatments (Jonker *et al*., 1998; Nousiainen *et al*., 2004). According to a meta-analysis (n = 277) by Huhtanen *et al*. (2008), on average 84.4% of the incremental nitrogen from the diet is excreted in urine when using nitrogen intake and DMI as independent variables in a bivariate model. In Paper II & III, there were large differences in UE excretion between the diets. High UE excretion is associated with highly digestible silages from three-cut system (ES, EE & TC) with greater CP concentration, which is in line with the greater UE excretion reported by in cows (Gordon *et al*., 1995) and growing cattle (Beever *et al*., 1988) fed less than more mature silage. In addition to increased urinary nitrogen excretion, it is also possible that the greater UE excretion when cows fed highly digestible silages is due to the greater amount of phenolic acids and their metabolites that are absorbed, but not metabolised and excreted in urine (Martin, 1969). Bristow *et al*. (1992) found that approximately 7% of total urinary nitrogen was excreted as hippuric acid nitrogen in dairy cows fed grass silage-based diets. In this thesis, this equates to 9.1-19.7 g urinary hippuric acid nitrogen excretion. Based on the energy concentration of hippuric acid reported by Oleinik *et al*. (23.6 MJ kg^{-1} DM, 1979), the UE contributed by hippuric acid was 2.7-5.9 MJ d^{-1} in cows fed silage-based diets.

5.4 Effects of grass harvesting strategy on feed intake

5.4.1 Early cut compared with late cut

It is well established in the literature that early harvested, highly digestible grass silage is beneficial for dairy cow production performance in terms of improved SDMI (see Table 4). In this thesis, delaying the harvest time of both primary growth and regrowth silages decreased digestibility and subsequently SDMI. Each 10 units increase in silage *D*-value resulted in a 0.183 and 0.180 kg increase in SDMI in Paper II and III, respectively, which is close to the 0.175 kg increase reported in a meta-analysis (n = 81) by Huhtanen *et al*. (2007). The results of the meta-analysis (n = 157) in Paper IV confirmed that silage *D*-value is a better predictor of SDMI than other forage variables. Mature grass silage with lower *D*-value increase rumen fill due higher iNDF/NDF ratio (iNDF disappears only by passage) and slower digestion rate of pdNDF (requires longer rumen residence retention times). Microbes need enough time to digest the slowly degradable material, which restricts the intake potential of low digestibility forages (Van Soest, 1994). A logarithmic *D*-value model was better than a linear model in predicting intake, based on lower AIC value. This indicates that the negative effect of reduced *D*-value on intake is greater for low than high digestibility silages. It may also indicate that with less digestible silages the intake is limited by rumen fill, whereas the intake of more digestible silages is regulated by the interplay between physical (rumen fill) and metabolic factors. However, in single studies the intake responses to increased silage *D*-value can still vary due to differences in other factors such as concentrate supplementation level and production potential of cows (Rinne *et al*., 1999; Kuoppala *et al*., 2008; Randby *et al*., 2012; Papers II & III).

5.4.2 Primary growth compared with regrowth

Regrowth silages usually have lower intake potential than primary growth silage (Peoples & Gordon, 1989; Khalili *et al*., 2005; Kuoppala *et al*., 2008). In Paper IV, the SDMI of regrowth silage was on average 1.07 kg d^{-1} lower than that of comparable primary growth silage. This difference in SDMI well matched the difference between primary growth and regrowth silage in SDMI index between these (11 units) according to the default value of 0.10 kg per unit given by Huhtanen *et al*. (2007).

The lower intake potential of regrowth silage cannot be fully explained by the models based on *D*-value developed by both Huhtanen *et al*. (2007) and Paper IV. Besides physical and metabolic regulation of intake, it can be attributable to some factors such as silage microbiological quality, which possibly affects the intake by changing the psychogenic modulators such as taste, smell, texture and visual appeal of silage. The microbiological quality of regrowth silage may differ from that of primary growth silage, since the weather is typically warmer later in the summer and the forage material for regrowth silage contains more weeds and dead plants (Kuoppala *et al*., 2008). These variables involve an animal's behavioural response to inhibitory or stimulatory factors in the feed or feeding environment that are not related to the energy value or filling effect of the feed (Mertens, 1994). In this thesis it's possible that the taste or smell of regrowth silage deteriorated due to an increased amount of decomposing and infected leaf material and caused decreased intake, but the effects of those subjective variables cannot be evaluated by the current model.

Table 4. *Changes of silage dry matter intake (kg d^{-1}) of dairy cows per 10 units decrease in silage D-value (g kg^{-1} DM) due to delay in harvest in selected studies*

Reference	Nation	Grass species*	Primary growth	Regrowth
Gordon, 1980	UK	pr		-0.23
Thomas *et al*., 1981b	UK	pr	-0.04	
Gordon *et al*., 1995	UK	pr	-0.20	
Rinne *et al*., 1999	Finland	ti, mf	-0.22	
Rinne *et al*., 2002	Finland	ti, mf	-0.16	
Kuoppala *et al*., 2008	Finland	ti, mf	-0.48	-0.18
Kuoppala *et al*., 2009	Finland	ti, mf	-0.29	
Kuoppala *et al*., 2010	Finland	ti, mf	-0.28	-0.11
Tahir *et al*., 2013	Sweden	ti	-0.15	
Sairanen *et al*., 2016	Finland	ti, mf		+0.08
Warner *et al*., 2016	The Netherland	pr, ti		-0.30
Paper II	Sweden	ti	-0.18	
Paper III	Sweden	ti		-0.18

*Grass species: ti = timothy (*Phleum pratense*); mf = meadow fescue (*Festuca pratensis*); pr = perennial ryegrass (*Lolium perenne*).

Despite the lower OMD determined at maintenance (723 vs. 750 g kg^{-1}) for regrowth silage, OMD determined at production level in dairy cows is not influenced by harvest. When the silage OMD was used a covariate, cows fed the regrowth diet had on average 14.5 g kg^{-1} higher dietary OMD than those fed the primary growth diet. In agreement with this, Kuoppala *et al*. (2008) reported higher pdNDF digestibility for regrowth silages compared with primary growth silages. This may be related to the slower passage rate of iNDF in cows fed regrowth silages (Kuoppala *et al*., 2010). It suggests that regrowth silage is digested more efficiently in cows than primary growth silage, and indicates higher milk yield and feed efficiency when cows are fed more digestible regrowth silages.

A comparison is also conducted between the second- and the third-cut grass silage in this thesis. Interestingly, the feed intake of the third-cut silage diet was lower than expected, as also observed in other studies on the third-cut silage conducted in the Nordic countries (Sairanen & Juutinen, 2013; Sairanen *et al*., 2016). Although the *D*-value of the third-cut silage was close to that of the best second-cut silage (EE; 662 vs. 666 g kg^{-1} DM), the SDMI index of the third-cut silage diet was 8.6 units lower than for EE silage due to the lower DM concentration and higher total acid concentration. However, the lower SDMI index only explains about 50% of the observed difference in SDMI. Moreover, the lower NDF intake in cows fed the third-cut silage despite high digestibility suggests that factors other than rumen fill limited intake of the third-cut silage diet. One possible explanation is that the humid weather conditions in late summer and early autumn can favour the epiphytic flora activity in herbage (Ercolani, 1991). Possible metabolites of these microbes may affect feed intake in dairy cows.

5.5 Effects of grass harvesting strategy on milk production

5.5.1 Early cut compared with late cut

In Papers II and III, every 10 units increase in silage *D*-value resulted in a 0.39 and 0.24 kg increase in milk yield in cows fed primary growth and regrowth silages, respectively. These values are close to the 0.26 kg in Huhtanen (1994), 0.27 kg in Rinne (2000) and 0.30 kg in Paper IV. Every 10 units increase in silage *D*-value resulted in a 0.46 and 0.31 kg increase in ECM yield in cows fed primary growth and regrowth silages, respectively, which compares rather well with those estimated by Rinne (0.32 kg increase; 2000), Huhtanen *et al*. (0.45 kg increase; 2013) and Paper IV (0.37 kg increase) in factorial studies evaluating the effects of silage digestibility.

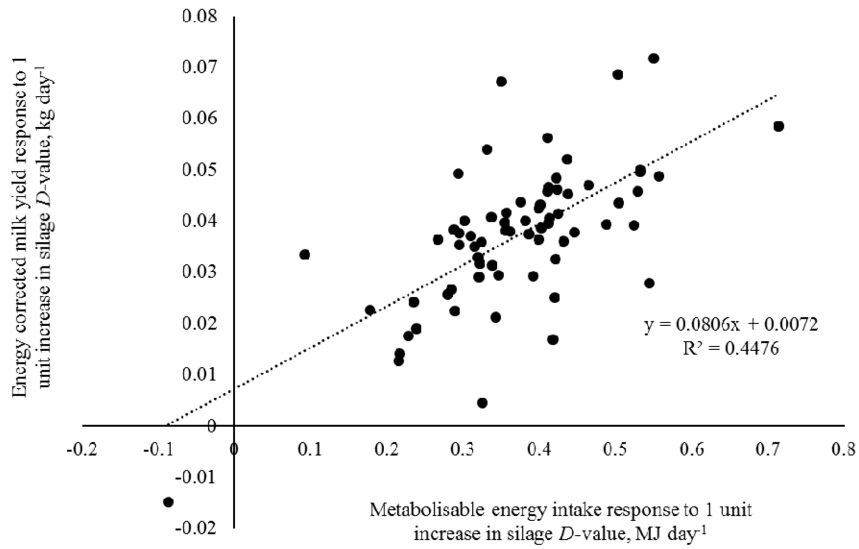

Figure 7. Relationship between responses of ECM yield and MEI to one unit increase in silage *D*-value based on the forage maturity subset in Paper IV. The coefficient of determination (R^2) of the regression equation is given.

The ECM yield is positively related to dietary MEI, and their responses to changes in silage *D*-value is positively correlated ($R^2 = 0.45$, see Figure 7). The response of 0.109 kg ECM per MJ additional MEI observed in Paper IV compares well with the 0.114 per MJ in Huhtanen *et al*. (2003). This suggests that the additional ME from increased feed intake resulting from improved silage quality is used at least with the same relative efficiency for milk production as additional ME on average. These values indicate that about 50% of the incremental MEI was partitioned towards milk production. In respiration chamber studies by Agnew *et al*. (1998) estimated milk energy output at zero energy balance was 0.27 MJ per MJ MEI, *i.e.* approximately 0.086 kg ECM per MJ MEI. The effect of *D*-value on ECM yield was negative when included in bivariate model with metabolisable energy intake. This in contrast to feed energy evaluation models (*e.g.* NRC, 2001) that assume improved ME utilisation efficiency with increased ME concentration. One reason can be overestimation of silage ME concentration from *D*-value using a constant coefficient due to greater UE excretion with highly digestible grass silages (Beever *et al*., 1988; Gordon *et al*., 1995; Paper II & III).

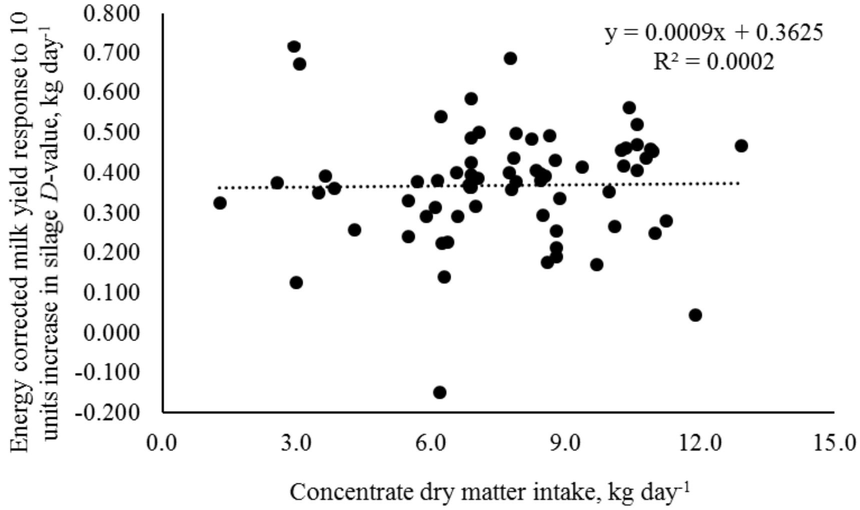

Figure 8. ECM yield responses to 10 units increase in silage *D*-value at different concentrate supplementation level based on the dataset of Paper IV.

In Paper IV the ECM yield response to silage *D*-value is not affected by concentrate supplementation level (see Figure 8), since the comparison on *D*-value is conducted at the same concentrate supplementation level within each study in the *D*-value dataset. But concentrate sparing effect of increased *D*-value is estimated from the selected factorial studies within the *D*-value subset in Paper IV investigating the effects of *D*-value and level of concentrate supplementation. It is noticeable that the importance of silage *D*-value on ECM yield is reduced as the concentrate supplementation level in diet increases (see Figure 9), which is in agreement with the results of earlier studies by Ferris *et al*. (2001) and Kuoppala *et al*. (2008). This interaction between silage digestibility and concentrate supplementation level can be attributed to the smaller marginal increase in total DMI per unit increase in concentrate supplementation with the high digestibility silage-based diets (Ferris *et al*., 2001). On the other hand, the ME concentration is higher in high than low digestibility silage when using a constant factor for converting silage *D*-value to ME (MAFF, 1975). Increasing the concentrate supplementation level in low digestibility grass silage-based diet can significantly increase the dietary ME concentration, while such increase is not always observed in those based on the high digestibility silage, because the difference in ME concentration is much smaller between more digestible grass silage and concentrate supplementation (Ferris *et al*., 2001; Kuoppala *et al*., 2008; Cabezas-Garcia *et al*., 2017).

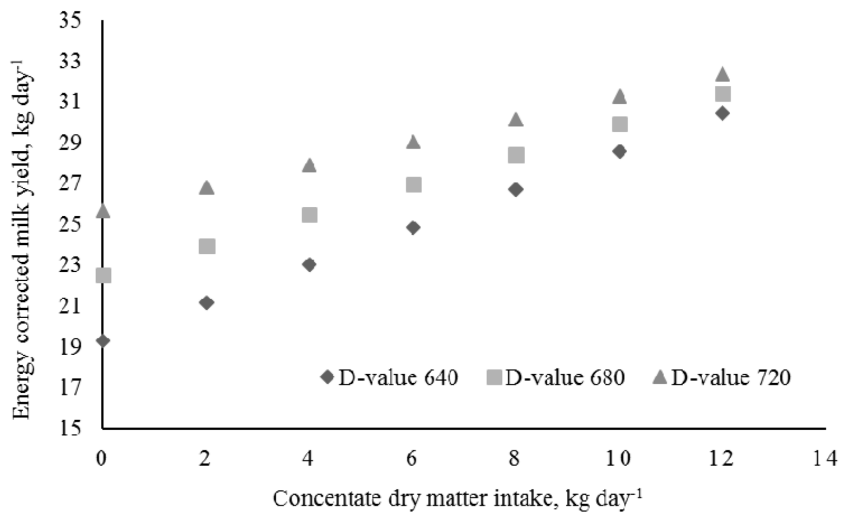

Figure 9. ECM yield responses to different concentrate supplementation levels when cows fed grass silages with different digestibility (*D*-value: 640, 680 and 720 g kg^{-1} DM) based on the dataset of Paper IV.

The milk composition responses to changes in silage *D*-value is not affected by concentrate supplementation level in Paper IV (see Figure 10). Increased silage *D*-value increases the milk fat concentration, and dietary NDF concentration is positively related to milk fat concentration when included in a bivariate model with *D*-value, which is in line with the earlier studies by Beauchemin (1991) and Rinne (2000). However, the effects of decreasing silage *D*-value on milk fat concentration are more inconsistent than those observed with primary growth silages. In paper III the milk fat concentration decreased with extended regrowth interval, while Kuoppala *et al*. (2008) and Warner *et al*. (2016) only observed marginal differences on it. It may be attributed to the silage-based factors which can influence milk fat synthesis in dairy cows other than *D*-value and NDF concentration. For example, Huhtanen *et al*. (2003) reported that increased concentrations of lactic acid in silage decrease milk fat concentration by reflecting fermentation of lactic acid to propionate in the rumen. Higher milk protein concentration has regularly been associated with high digestibility silages due to the improved energy status and increased intestinal supply of amino acids to the cows (Huhtanen, 1994). In Paper II & III, milk protein concentration was significantly higher in cows fed early than late cut silages, which was mainly attributable to their higher silage *D*-value and MEI. In Paper IV, the milk protein concentration increased by 0.09 g kg^{-1} per 10 units increase in silage *D*-value, which is close to the value of 0.10 in Rinne (2000). Silage CP concentration has no effect on milk protein concentration when included in bivariate model with MEI, suggesting energy intake is the main factor influencing milk protein concentration, whereas the effect of dietary CP concentration is negligible (Broderick, 2003).

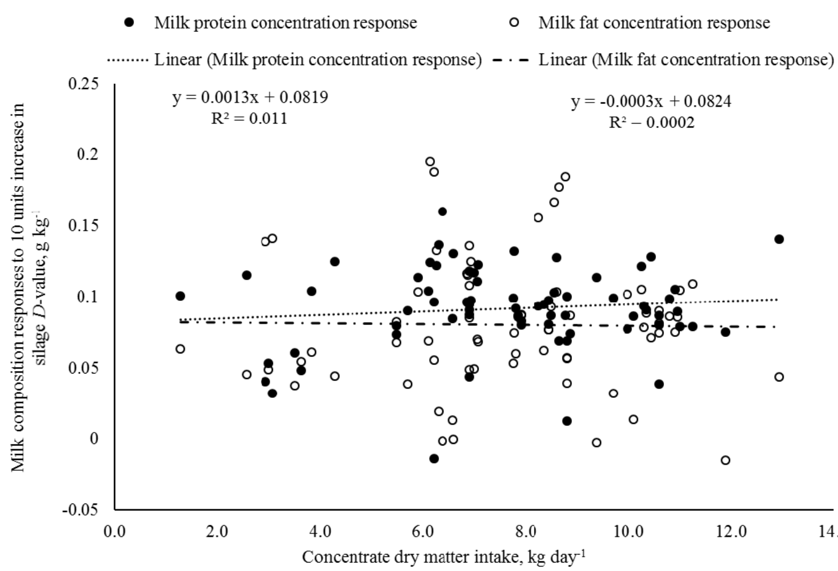

Figure 10. Milk composition responses to 10 units increase in silage *D*-value at different concentrate supplementation level based on the dataset of Paper IV.

5.5.2 Primary growth compared with regrowth

As summarised in Table 5, milk yield and concentrations of milk fat and protein are usually lower in cows fed regrowth silage compared with primary growth silage. The higher milk and ECM yields in cows fed primary growth silage are mainly attributable to the higher dietary MEI derived from both increased DMI and higher digestibility. However, when MEI is used as a covariate in Paper IV, milk and ECM yields are higher in cows fed regrowth than primary growth silages. This can be attributed to the higher digestibility of DOM with regrowth silages, *i.e.* the decline in digestibility from maintenance to production level is smaller. Khalili *et al*. (2005) and Naadland *et al*. (2017) also reported that the ME utilisation efficiency is poorer in cows fed primary growth than regrowth silage in production trials. The difference in ME utilisation efficiency between harvests indicates that there may be a change in the partitioning of energy from milk to tissues. The higher ME utilisation efficiency of regrowth silage suggests energy mobilisation from adipose tissues, which is supported by a lower milk protein concentration (33.2 vs. 33.8 g kg^{-1}) than in cows fed primary growth silage.

Table 5. *Difference between cows fed regrowth silages and primary growth silages on dairy performance in selected studies*

Reference	Milk yield, kg d^{-1}	Milk fat, g kg^{-1}	Milk protein, g kg^{-1}
Castle & Watson, 1970	-0.4	-1.8	+0.4
Peoples & Gordon, 1989	-0.5	-2.1	-0.5
Heikkilä *et al*., 1998	-0.8	-3.1	-0.7
Bertilsson & Murphy, 2003	-2.0	+3.4	-2.2
Khalili *et al*., 2005	-2.4	+2.1	-0.6
Kuoppala *et al*., 2008	-1.9	-2.7	-1.2
Sairanen & Juutinen, 2013	0.0	+0.1	-0.6
Alstrup *et al*., 2016	-0.7	+1.5	-1.0
Larsen *et al*., 2016	+1.1	-1.4	+0.2
Naadland *et al*., 2017	-0.6	-1.9	+0.1
Paper IV	-0.9	-0.7	-0.6
Average	-0.8	-0.6	-0.6

Milk fat concentration in cows fed primary growth silage diets tended to be higher than fed regrowth silage, which is in agreement with Kuoppala *et al*. (2008) and Naadland *et al*. (2017). This can be mainly related to the higher *D*-value of primary growth than regrowth silage (692 vs. 660 g kg^{-1} DM), since the rumen fermentation pattern is high in butyrate when cows are fed more digestible silage (Rinne *et al*., 2002). Intraruminal butyrate infusions have been shown to increase milk fat concentration (Miettinen & Huhtanen, 1996). Silage NDF and lactic acid concentrations are also important factors influencing milk fat concentration (Beauchemin, 1991; Huhtanen *et al*., 2003), but the differences between primary growth and regrowth silage are rather small ($P \geq 0.28$). The higher milk protein concentration in cows fed primary growth than regrowth silage is mainly attributable to higher ME concentration (11.1 vs. 10.6 MJ kg^{-1} DM) in primary growth silage and greater dietary MEI (241 vs. 224 MJ d^{-1}) in cows fed primary growth silage. On the other hand, including dietary MEI in the model improved the prediction accuracy, but the effect of grass harvest is no longer significant, which indicates that energy intake has a greater influence on milk protein concentration than any other single variable.

Within the regrowth silages, the ECM yield of cows fed the third-cut silage is as good as that of cows fed the best second-cut silage (31.2 vs 31.3 kg d^{-1}). However, this good production response cannot be fully explained by the feed intake, since the ECM yield of cows fed the third-cut silage diet is about 1.5 kg higher than estimated from the relationship between dietary MEI and ECM yield. Similar results have been reported by Sairanen *et al.* (2016), but the reasons for the better performance of cows fed diets based on third-cut silage are still unclear. One possible reason is different partitioning of nutrients between milk and body tissues. The energy metabolism data in Paper III suggest that cows fed the second-cut silages partitioned more energy to tissues than cows fed the third-cut silage. In addition, the lower CH_4/CO_2 ratio in cows fed the third-cut silage diet could also indicate mobilisation of body tissues, which produces CO_2 but not CH_4.

5.6 Effects of grass harvesting strategy on herbage yield

5.6.1 Early cut compared with late cut

The opposing changes in forage quality and quantity during herbage growth make harvest time important in forage production. From a nutritional point of view, herbage DM yield is clearly an inadequate measure of herbage output. Greater herbage DM yield can be achieved by delaying the harvest, but meanwhile the forage digestibility decreases very rapidly in the Nordic environmental conditions. Therefore, it is important to balance the grass feeding value and DM yield in a farming system. It is possible to obtain high DOM yield in situations where the digestibility is very low, but DM yield is great. However, in such cases a high level of expensive concentrate supplementation will be needed to sustain milk production, which will result in reduced forage intake and extra cost for feed.

The daily changes in herbage DM yield observed in this thesis are comparable to those reported in studies conducted in the Nordic countries. A two-week delay in harvesting primary growth resulted in an increase in herbage DM yield of 2.3 tonnes ha^{-1}, which equates to an increase of 166 kg DM ha^{-1} for each day of delay in harvest. This value is close to the 146 kg DM ha^{-1} d^{-1} reported by Rinne *et al*. (2000) and the 150 kg DM ha^{-1} d^{-1} reported by Kuoppala *et al*. (2008). During the regrowth season, extending the regrowth interval resulted in an average increase in herbage DM yield of 2.1 tonnes ha^{-1}, corresponding to an increase of 115 kg DM ha^{-1} for each day of delay in the second cut. This is close to the 107 kg DM ha^{-1} d^{-1} reported in a Finnish study by Sairanen *et al*. (2016), but higher than the 79 kg DM ha^{-1} d^{-1} obtained in a previous study in Northern Ireland (Dawson *et al*., 2002). This difference can be attributed to environmental factors, *e.g*. greater day length during the regrowth period under the Nordic conditions. However, the environmental conditions during the regrowth period are usually more variable than in the primary growth season. Moreover, additional factors such as disease, weeds *etc*. can have confounding effects on regrowth herbage yield. For example, in this thesis, the increase in yield for each day of delay in the second cut was greater after an early (148 kg DM ha^{-1}) than late first cut (81 kg DM ha^{-1}), due to the higher average temperature during midsummer. However, in a Finnish study, Kuoppala *et al*. (2008) reported the harvest time of the first cut had no influence on the daily DM yield increase within the extended regrowth interval.

5.6.2 Two-cut compared with three-cut system

Increased length of growing season due to global warming is an additional factor and the change is predicted to be even greater in the Nordic countries (Olesen *et al*., 2011). The management of grasslands needs to be gradually adapted in order to utilise the longer growing season than before. In this thesis, a comparison was made between one three-cut system and three two-cut systems in Northern Sweden. The two-cut system is more commonly used at northern latitudes, but the three-cut system can better utilise the whole growing season. The grass silages (ES, EE & TC) produced from the three-cut system were more digestible and contained higher metabolisable and crude protein concentrations than those from the two-cut systems. However, the total herbage DM yield of the three-cut system was 6.2 tonnes ha^{-1}, which was lower than that of the three two-cut systems (7.0, 9.0 and 11.2 tonnes DM ha^{-1}). Similar result was also reported in a comparable Finnish study on pure timothy-meadow fescue leys (Sairanen *et* *al*., 2016) as shown in Figure 11. It is noticeable that the growth rate of the third-cut grass is much lower in the current study (30 kg DM ha^{-1} d^{-1}) than those of first-cut (166 kg DM ha^{-1} d^{-1}) and second-cut grass (115 kg DM ha^{-1} d^{-1}). Slower growth rate of the third-cut grass has also been reported in a Finnish long-term field trial (2009-2015), where within the whole regrowth season the mean growth rate of third-cut grass was only 35 kg DM ha^{-1} d^{-1} (Hyrkäs *et al*., 2016).

Therefore, like the effect of grass maturity on herbage quality and quantity, the harvesting strategy should be based on the adjusted economic conditions of the individual dairy farm, after taking all the important factors into consideration. For dairy farmers, the main advantages in choosing a three-cut system are the higher milk production and the need for less concentrate enabled by high forage quality. On the other hand, there are risks when applying three-cut system, such as reduced herbage DM yield, greater labour and time requirements for harvesting and greater fertiliser requirements, which can reduce areal gross income from forage production, but increase the areal payment.

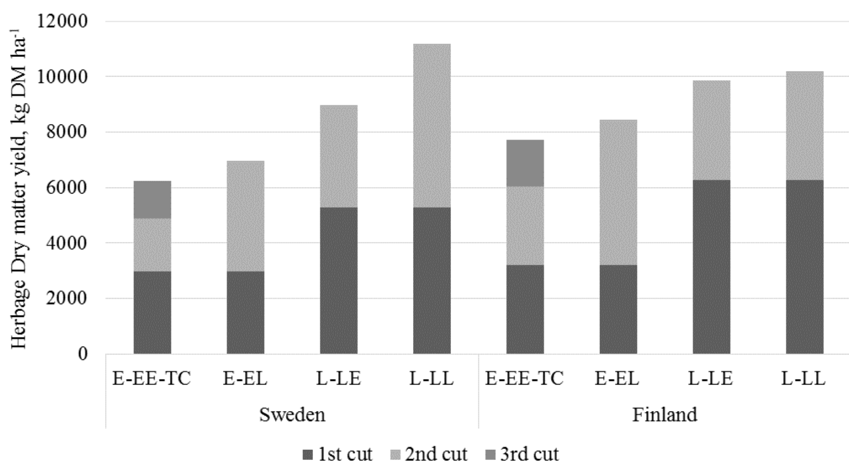

Figure 11. The herbage dry matter yields from different two- and three-cut systems in this study conducted in Sweden (Left) and in Sairanen *et al*. (2016) conducted in Finland (Right). Four second-cut herbages were harvested at early (EE, LE) or late (EL, LL) stage of growth after an early (E) or late (L) first cut, the third-cut (TC) herbage was harvested after the early first and second cut.

Unfortunately, an economic comparison between the three and two-cut system was not conducted in this thesis. However, according to a Norwegian farm economics study by Flaten (2002), the profit (revenues - variable costs) of farms applying a three-cut system is greater than that of farms applying a two-cut system (331,472 vs. 318,626 NOK, 1999 values), despite the greater costs of crop management and labour input for the three-cut system. In that study, the dairy herd consisted of moderate to low-yielding cows and milk production was greater in the three than two-cut system (6603 vs. 6500 kg milk cow^{-1}). However, the PBV was lower for grass silages produced from the two-cut (-20 vs. 21 g kg^{-1} DM) than the three-cut system (Flaten, 2002). Therefore, in order to ensure a particular milk performance level, more expensive concentrate feeds which are high in PBV have to be supplied in the two-cut system to meet the requirements of dairy cows. In addition, the extra profitability of the three-cut system than the two-cut system proved quite insensitive to reduced milk price (-0.25 NOK L^{-1}) and increased area payment for forage production (+1000 NOK ha^{-1}) according to the farm economic model (325,405 vs. 312,229 NOK, 1999 values) (Flaten, 2002).

5.7 Replacing grain with by-products in concentrate

In conventional farming systems in Sweden, large proportions of feed resources are fed to dairy cows and these could instead be used directly as human foods, or utilised more efficiently in poultry and pig production. In addition, there are restrictions on the use of animal sources of protein and genetically modified crops in the European Union. Replacement of human-edible grain-based concentrate with agro-industrial by-products has been suggested as a viable option to improve the sustainability of dairy systems (Ertl *et al*., 2015b). On the other hand, agro-industrial by-products are reported to be an economical source of supplementary feed. In a study by Whelan *et al*. (2017) based on grazing system, replacing barley and soybean meal in concentrate with by-products reduced the concentrate cost by approximately €0.04 kg^{-1}, which offers the opportunity for cost saving at the farm level.

In this thesis, the energy source of a grain-based concentrate was fully replaced by agro-industrial by-products without impairing intake and milk yield of dairy cows, which is in line with recent studies by Dann *et al*. (2014), Ertl *et al*. (2015b, 2016) and Whelan *et al*. (2017). However, in some earlier studies, Mayne and Gordon (1984) reported that replacing barley meal with sugar beet pulp in concentrate decreased DMI in cows, with no negative effect on milk production. Huhtanen (1993) replaced 60% of crushed barley with a mixture of fibrous grain by-products and observed elevated DMI and milk yield when cows fed by-products-based concentrate.

The inconsistent effects of by-products as concentrate energy source on dairy performance depend strongly on the type and amount of by-products included in the ration, since the by-products can differ widely in chemical composition. In addition, a recent meta-analysis concluded that the effect of sugar beet pulp, one of the most commonly used by-products energy sources, depends on the cow's feed intake potential (Münnich *et al*., 2017). In that study sugar beet pulp decreased DMI and dairy performance with high feed intake level (>3.5% of BW) and had the opposite effect with low feed intake level (<3.5% of BW). Huhtanen *et al*. (1993, 1995) suggest that the variable production responses in dairy cows fed by-products supplements can be explained by the rumen fermentation profile. In cattle fed grass silage-based diets, unmolassed sugar beet pulp and barley fibre supplements resulted in greater duodenal non-ammonia nitrogen flow and a higher proportion of propionate than barley, while barley-supplemented diets promoted a higher proportion of butyrate (Huhtanen, 1992). Ertl *et al*. (2015a) observed a lower acetate to propionate ratio *in vitro* for a diet supplemented with fibrous by-products compared with a starch-rich control concentrate. They attributed this to more easily fermentable fibre such as pectin and hemicellulose in the by-products, which is assumed to stimulate propionate formation. The by-products which stimulate propionate formation and gluconeogenesis is beneficial for dairy cows, particularly during early lactation, through improving energy utilisation.

The effect of by-products as a protein source is rather consistent. The most commonly used by-product protein source, rapeseed meal, is reported to be more effective as a protein supplement than soybean meal for dairy cows (Shingfield *et al*., 2003; Broderick *et al*., 2015; Gidlund *et al*., 2015). According to a meta-analysis by Huhtanen *et al*. (2011), the increase in milk yield (3.4 vs. 2.1 kg d^{-1}) and milk protein yield (136 vs. 98 g d^{-1}) with increasing CP concentration is greater with rapeseed meal than soybean meal. Martineau *et al*. (2013) also reported that yields of milk and milk protein increased when rapeseed meal replaced soybean.

However, in this thesis there were no remarkable changes in the yield of milk or milk constituents, or in nitrogen utilisation efficiency when grain-based concentrate was replaced by by-products, which is in line with earlier findings by Ertl *et al*. (2015b, 2016) and Karlsson *et al*. (2018). The unchanged production responses with by-products-based concentrate can be expected to originate from slightly lower feed intake (*P*=0.06) than in cows fed grain-based concentrate. The intake was reported to be higher in cows fed rapeseed meal in those studies reported better dairy performance than fed soybean meal (Shingfield *et al*., 2003; Gidlund *et al*., 2015). The milk protein concentration is slightly lower when cows fed by-products, which is in line with findings by Gordon *et al*. (1995) and Karlsson *et al*. (2018). Lower milk protein concentration in cows fed by-products is sometimes ascribed to their higher crude fat content (Thomas, 1984). However, there is only a small difference in crude fat concentration between grain- and by-products-based concentrate in this thesis (42 vs. 54 g kg^{-1} DM). Another explanation for the reduced milk protein content in cows fed by-product-based diets is lower CP digestibility than for grain-based concentrate. Some agro-industrial by-products have the same CP concentration as soybean meal, but they generally have lower rumen protein degradability (Maxin *et al*., 2013). The heat damage to the protein in dry distillers' grains during the drying process usually leads to a reduction in CP digestibility. Gidlund *et al*. (2015) found that a diet supplemented with rapeseed by-products was more fibrous and had higher iNDF concentration, which could be expected to decrease digestibility. Rinne *et al*. (2015) also found increased total apparent nitrogen digestibility with soybean expeller compared with rapeseed expeller. Therefore, there's no doubt that replacing the protein source in conventional concentrate with by-product-based protein can save human-edible protein for human food or feed for monogastric livestock, but the feed nitrogen utilisation may not improve as much as expected due to the lower CP digestibility than with conventional protein sources in concentrate feeds.

6 Conclusions

- The metabolisable energy (ME)/digestible energy (DE) ratio was not constant across primary growth grass silages of different maturities. The production response of ME might be smaller from highly digestible primary growth grass silages, but couldn't be clearly related to urinary monophenols excretion derived from lignin solubilisation.
- Urinary energy lost excretion was not clearly defined for red clover silage and was much more predictable for grass silage from silage nitrogen concentration. Sheep excreted more benzoic acid and its conjugates but less phenylacetic acid when fed grass silage than red clover silage.
- The human-edible ingredients in conventional concentrate could be fully replaced by agro-industrial by-products in dairy cow diets without impairing feed intake, milk production, diet digestibility or methane emissions.
- Cows fed by-products-based concentrate had better energy status than cows fed grain-based concentrate. However, silage maturity had a stronger effect on production performance by dairy cows than source of concentrate.
- Feeding regrowth silage in two- or three-cut systems harvested after an early first cut and after a short regrowth interval promoted better dairy performance and feed intake and higher efficiency of feed and energy utilisation, but poorer nitrogen efficiency. The third-cut silage gave higher milk yield and feed efficiency than the second-cut silages.

- Apparent digestibility and metabolisability of energy were higher in cows fed more digestible silages. However, higher digestibility of silage also elevated the proportion of DE excreted as urinary energy.
- Feed intake and milk production increased with improved digestibility of grass silage. Silage intake was more related to digestibility than neutral detergent fibre (NDF) concentration, with indigestible NDF (iNDF) being a better predictor of silage dry matter intake than total NDF, due to its greater filling effect.
- Incremental energy intake from improved silage digestibility increased energy-corrected milk yield by 0.11 kg MJ^{-1} ME, indicating that almost 50% of additional energy was partitioned toward body tissues.
- Feed intake was lower for cows fed regrowth silage than for cows fed primary growth silages, even when differences in digestibility and chemical composition were taken into account.
- Lower milk yield with regrowth silages was related to decrease in ME intake. When ME intake estimated at maintenance levels was used as a covariate, the cows fed regrowth silages produced about 0.5 kg more milk and energy-corrected milk than the cows fed primary growth silages.

7 Future perspectives

Based on the results achieved in this thesis, further studies in this area should:

1. Investigate the effects of forage type, maturity and harvest on phenolic compounds excreted in urine and milk from lactating dairy cows fed at different feeding levels.
2. Evaluate the effect of agro-industrial by-products / grain mixture on dairy performance and feed intake when supplied to lactating dairy cows.
3. Develop an economic model to quantify the profits of different two- and three-cut systems under northern Swedish conditions, to provide net income estimates for different harvesting strategies and support decision making by local dairy farmers.
4. Examine why the intake potential of third-cut silage is restricted when fed to lactating dairy cows.
5. Compare the profitability of a three-cut system and two-cut + late grazing system on farm scale in northern Swedish conditions.
6. Compare respiration chambers and the GreenFeed system in energy partitioning evaluations of lactating dairy cows. Evaluate the effect of sampling methods (total collection vs. spot sampling) on calculated faecal and urinary excretion of energy and nitrogen.
7. Develop prediction models for milk yield and feed intake that include animal behaviour and environmental variables.
8. Conducting more detailed studies to understand increased urinary energy with improved grass digestibility.

Popular science summary

Grass silage is the main ingredient and forage source in dairy cow diets during the long indoor feeding period in the Nordic countries, due to its high feeding value. However, the grass digestibility changes rapidly during the short growing season, and global warming brings new challenges to the current grassland based milk production systems.

The three-cut system is becoming popular in the Nordic countries, since it provides better utilisation of the whole growing season than traditional two-cut system. In this thesis the highly digestible silages from the three-cut system promote better dairy performance, greater energy digestibility and metabolisability, and less methane emission per kg energy-corrected milk yield when fed to dairy cows than silages from two-cut systems. However, the herbage dry matter yield is lower in three- than two-cut systems, and the incremental protein from the three-cut system silages cannot be fully utilised by dairy cows for milk protein synthesis, which results in a lower nitrogen utilisation efficiency and greater urinary nitrogen excretion. When adapting two-cut system for silage production, it's better to have an earlier first cut and reduce the length of regrowth interval in silage production from a nutritional point of view. A late harvest increases the herbage dry matter yield, but also reduces the silage feeding value and raises the requirement of expensive concentrate supplementation for milk production. In addition, the conventional concentrate feeds usually contains a great proportion of human-edible ingredients. In this thesis a comparison on concentrate type suggests that the grain ingredients in a conventional concentrate can be fully replaced by agro-industrial by-products in diets without impairing intake or dairy performance of lactating dairy cows.

Although red clover is known to be a protein rich forage, the results from a sheep study in this thesis show that the indigestible proportions of

both carbohydrate and protein in red clover silages are greater than in grass silages. It also shows that sheep fed regrowth grass silage has a lower energy metabolisability than primary growth silage. In a meta-analysis study in this thesis on cows show a lower intake potential and dairy performance when fed with regrowth than primary growth silage. However, an interesting observation from that study also suggests that if the metabolisable energy intake is the same, cows fed regrowth silages produced more milk than fed primary growth silages. The efficiency of metabolisable energy used for lactation is high in cows fed regrowth than primary growth silages, but further studies are still needed on improving the intake potential of the regrowth silages.

Populärvetenskaplig sammanfattning

Ensilage baserat på gräs är den enskilt viktigaste ingrediensen i foderstaten för mjölkkor under den långa inomhusperioden i Norden, till stor del på grund av det höga fodervärdet. Gräsets smältbarhet förändras dock snabbt under den korta växtsäsongen och den globala uppvärmningen skapar nya utmaningar för de nuvarande vallbaserade mjölkproduktionssystemen.

Treskördesystemet ökar i popularitet i de nordiska länderna, eftersom det ger bättre utnyttjande av hela växtsäsongen än traditionellt tvåskördesystem. Resultaten i denna avhandling visar att de lättsmälta ensilagen från treskördesystemet främjar produktionsprestandan hos mjölkkorna, har större smältbarhet och omsättbarhet av energin och mindre metanutsläpp per kg energikorrigerad mjölk när de ges till mjölkkor, än ensilage från tvåskördesystem. Emellertid är skörden i kg torrsubstans lägre i tre- än tvåskördesystemet, och proteinet från treskördesystemets ensilage kan inte utnyttjas fullt ut av mjölkkorna för mjölkproteinsyntes, vilket resulterar i en lägre effektivitet för kväveutnyttjandet och större försluster via urinen. Vid anpassning av tvåskördesystem för ensilageproduktion är det bättre att ha en tidigare första skörd och minska tidsintervallet för återväxten ur näringssynpunkt. En senare skörd ökar skörden, men reducerar även fodervärdet på ensilaget och ökar därför kravet på dyrt koncentrattillskott för mjölkproduktionen. Dessutom innehåller de konventionella foderkoncentraten vanligtvis en stor andel ingredienser som är ätbara för människor. I denna avhandling presenteras även en jämförelse som antyder att spannmålsingredienser i ett konventionellt koncentrat fullständigt kan ersättas av agro-industriella biprodukter i foderstaten utan att försämra konsumtion eller mjölkproduktion hos lakterande mjölkkor.

Även om rödklöver är känd för att vara en proteinrik föda visar resultaten från en fårstudie i denna avhandling att de osmältbara delarna av både kolhydrater och protein i rödklöverensilage är större än i gräsensilage. Studien visar också att gräsensilage från återväxt och har lägre omsättbar energi än ensilage från en förstaskörd vid utfodring till får. En meta-analys som presenteras i denna avhandling visar att mjölkkor har en lägre konsumtionspotential och mjölkprestanda när de utfodras med ensilage från återväxten än när de utfodras med ensilage från första skörd. En intressant observation från studien antyder dock att om konsumtionen av omsättbar energi är lika hög, producerar kor som utfodras med återväxten mer mjölk än de som utfodras med första skörd. Effektiviteten för den omsättbara energin som används för laktation är alltså högre hos kor som har fått ensilage från andraskörd än från förstaskörd. Ytterligare studier behövs dock fortfarande för att förbättra konsumtionspotentialen hos återväxten av gräs.

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