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# Feed efficiency in dairy cows

## Individual cow variability in component traits

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# Feed efficiency in dairy cows. Individual cow variability in component traits

## Abstract

Feed efficiency (FE) varies between cows, and this variation is linked to the variation in energy metabolism variables. Respiration chambers are needed for measuring energy metabolism variables while individual cow dry matter intake (DMI) records are necessary for measuring FE, but these are difficult to obtain due to cost and logistic constraints. This thesis evaluated the between-cow coefficient of variation (CV) in the components of FE and their contribution to FE. Also, marker techniques of measuring DMI and the use of an upgraded GreenFeed system (GF) to measure energy balance (EB) in lactating dairy cows were evaluated. Marker-based estimates of DMI underestimated observed DMI. The use of external markers for faecal output estimates gave the best prediction of FE suggesting that faecal output measurements with external markers are enough to determine FE thereby removing the need for analysing feed samples. However, the direct measurement was more precise making it a method of choice unless otherwise not feasible due to facility limitations. The between-cow CV in gross energy (GE) intake was the highest among all component traits while that of digestibility (DE/GE) was small. Although the between-cow CV in methane ($CH_4$) as a proportion of GE was important, it was positively correlated with DE/GE, suggesting that selecting for low $CH_4$ emitters may result in unintended selection for low DE/GE which is an important trait for ruminants. The between-cow CV in residual energy corrected milk (RECM) was double that of residual feed intake (RFI) indicating that RECM is more amenable to genetic selection than RFI. Using respiration chamber data to predict DMI and ECM for RFI and RECM calculations, respectively, the partial regression coefficients were biologically meaningful. About 65% of the difference between low and high-FE (RFI or RECM) cows was due to improved utilisation of metabolisable energy. Residual $CO_2$ could be the FE index of the future as it eliminates the need for measuring individual animal DMI. The replacement of cereal grain with by-product did not have negative effects on production and EB, suggesting that by-product can replace cereal grain in early lactation cow diets. The GF proved to be a promising tool for measuring EB. Milk mid-infrared (MIR) spectral data also gave a good prediction of EB which presents an opportunity to estimate individual cow EB without added investments as MIR is an on-farm routine analysis.

*Keywords:* variation, dairy cow, energy balance, repeatability, residual energy corrected, residual feed intake

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# Feed efficiency in dairy cows. Individual cow variability in component traits

## Abstract

Fodereffektiviteten (FE) varierar mellan kor och variationen är kopplad till skillnader i djurens energimetabolism. För att mäta energimetabolism hos enskilda djur behövs respirationskamrar och för att mäta FE är konsumtionsdata (DMI) från enskilda djur nödvändiga. Individuella data för dessa parametrar är dyra och praktiskt svåra att ta fram. I den här avhandlingen utvärderades variationskoefficienten för FE mellan mjölkkor och olika enskilda komponenters bidrag till FE. Olika markörtekniker utvärderades att mäta konsumtion och ett uppgraderat GreenFeed-system (GF) för att mäta energibalansen (EB). Markörbaserade skattningar av foderkonsumtionen (DMI) underskattade den observerade konsumtionen. Att använda externa markörer för att skatta träckproduktionen gav den bästa skattningen av FE, vilket tyder på att mätningar av mängden träck med externa markörer är tillräckligt för att bestämma FE. Variationen i konsumtion av bruttoenergi (GE) var den viktigaste komponenten för variation i FE hos mjölkkor, medan variationen för smältbarhet hos djuren (DE/GE) var låg. Även om variationen av metan ($CH_4$) som en andel av GE var signifikant, korrelerades den positivt med DE/GE. Väljer vi ut djur med låga metanutsläpp kan det leda till felaktig selektion för djur med låg fodersmältbarhet, en mycket viktig egenskap för mjölkkor. Variationen mellan kor i avvikelse från förväntad mjölkproduktion (RECM) var dubbelt så stor som för avvikelser i avvikelse från förväntad foderkonsumtion (RFI), vilket indikerar att RECM är en bättre egenskap för genetisk selektion än RFI. Data från respirationskamrar visade att RFI och RECM är de biologiskt mest betydelsefulla komponenterna för FE. Mängden $CO_2$ som produceras från varje enskilt djur, skulle kunna bli ett FE-index i framtiden, då det eliminerar behovet av individuella konsumtionsmätningar i stallar om vi vet mjölkmängden. Att byta spannmålsprodukter i fodret mot med biprodukter från industrin hade inte några negativa effekter på mjölkproduktionen eller EB hos korna, vilket tyder på att biprodukter kan ersätta spannmålsprodukter även under tidig laktation. Spektrala data (Mid Infra Red/MIR) från mjölkprover gav också goda förutsägelser för EB, vilket ger en möjlighet att uppskatta EB för enskilda kor utan extra investeringar på gården, eftersom MIR är en rutinanalys av mjölk som görs på gårdar.

*Keywords:* Mjölkko, förväntad mjölkproduktion, förväntad foderkonsumtion, energibalans, variation mellan kor, upprepbarhet

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# Dedication

To my mother, Hajia Zeinabu Alhassan.

*"Verily, with every difficulty, there is relief".*
*Quran 94:6*

# 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 Guinguina A., S. Ahvenjärvi, E. Prestløkken, P. Lund, and P. Huhtanen (2019). Predicting feed intake and feed efficiency in lactating dairy cows using digesta marker techniques. *Animal*, 13 (10), 2277-2288.

II Guinguina A., T. Yan, P. Lund, A. R. Bayat, and P. Huhtanen (2020). Between-cow variation in the components of feed efficiency (Submitted).

III Guinguina A., T. Yan, A. R. Bayat, P. Lund, and P. Huhtanen (2020). The effect of energy metabolism variables on feed efficiency in respiration chamber studies with lactating dairy cows (submitted)

IV Guinguina A., S. J. Krizsan, M. Hetta, and P. Huhtanen (2020). Postpartum responses of dairy cows supplemented with cereal grain or fibrous by-product concentrate (manuscript)

V Guinguina A., T. Yan, E. Trevisi, and P. Huhtanen (2020). The use of an upgraded GreenFeed system to measure energy balance in early lactation cows (manuscript)

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Abdulai Guinguina.

The contribution of Abdulai Guinguina to the papers included in this thesis was as follows:

I Data management and statistical analysis. Wrote the manuscript with regular contribution from the main supervisor and co-authors.

II Contributed in processing and statistical analysis of data. Wrote the manuscript with regular input from supervisors and co-authors.

III Worked jointly with main supervisor in processing data and writing manuscript with regular input from co-authors.

IV Planned the study together with the co-authors. Contributed in the collection, preparation, and analyses of data. Wrote the manuscript with regular inputs from supervisors and co-authors.

V Planned the study together with the co-authors. Contributed in the collection, preparation, and analyses of data. Wrote the manuscript with regular inputs from supervisors and co-authors

# Abbreviations

| | |
|---|---|
| BW | Body weight |
| ΔBW | Body weight change |
| CV | Coefficient of variation |
| DE/GE | Gross energy digestibility |
| DMD | Dry matter digestibility |
| DMI | Dry matter intake |
| EB | Energy balance |
| ECM | Energy corrected milk |
| FCE | Feed conversion efficiency |
| FDMO | Faecal dry matter output |
| FE | Feed efficiency |
| GE | Gross energy |
| GF | GreenFeed system |
| $k_l$ | Efficiency of ME use for lactation |
| ME | Metabolisable energy |
| ME/GE | Metabolisability |
| $ME_m$ | ME requirement for maintenance |
| NEFA | Non-esterified fatty acids |
| RC | Respiration chamber |
| $RCO_2$ | Residual carbon dioxide |
| RECM | Residual energy corrected milk |
| RFI | Residual feed intake |

# 1 Introduction

Improving feed efficiency (FE) is a well-established goal in dairy production as it is expected to increase profitability. It is even more relevant in the present given the ever-decreasing food-producing land base (Berry and Crowley, 2013) and the global concerns regarding greenhouse gas emissions and nutrient losses to the environment (Connor, 2015). Also, because feed accounts for the largest proportion of operating costs in dairy production, variations among animals in converting feed into additional milk are and will continue to be of great importance (Coleman *et al.*, 2010). Therefore improved FE will be realised through the identification of individuals that produce the same quantity of milk using fewer feed resources or individuals that produce increased volumes of milk from similar levels of feed inputs with less waste into the environment without compromising animal health and fertility.

The main factors influencing the FE of dairy cows are diet, genetics and the physiological state. Actually, the contribution of genetics to improvements in FE is the most recognised. Studies in the 1980s showed between-breed and selection-line variation in FE (Korver, 1988). However, the results from old studies may no longer be completely applicable to the modern dairy cow population due to considerable genetic progress (Liinamo *et al.*, 2012). New knowledge of the individual animal variation in FE would be beneficial for future improvements in FE. In this regard, a variety of international research partnerships have been established (Berry *et al.*, 2014; VandeHaar *et al.*, 2016; Pryce *et al.*, 2018) since it will take several years for a single research group to generate the volume of data necessary to perform genetic evaluation. In 2013, an international collaboration among the Nordic countries called, ‘Feed utilisation in Nordic Cattle (FUNC)’ was established. The aim was to pool data and expertise from which the biological basis of FE can be characterized and to assess the possibility of incorporating the trait into breeding programs. Some studies on genetic parameter estimates and the accuracy of genomic evaluation of FE have been published (Li *et al* 2016; Løvendahl *et al.*, 2018). This thesis is part of the research partnership among the Nordic countries and it focuses on the variation among cows and the repeatability estimates of FE and its component traits.

A better understanding of the between-animal variation in a trait is essential for accurate estimation of its breeding value and heritability (Boake, 1989). Repeatability may be an important tool to quantify the variation between animals due to its relationship with heritability. It has often been used to set an upper boundary on heritability, but because its relationship with heritability is not strong enough, they cannot be used interchangeably (Falconer, 1981). However, repeatability is necessary for evaluating the practicality of measuring heritability. For instance, the efforts needed to accurately estimate the heritability of a trait may be laborious and costly if the heritability is low. Therefore, preliminary measures of repeatability are valuable in identifying traits that could be responsive to genetic selection.

## 1.1 Definitions of feed efficiency in dairy cattle

Measuring individual animal or herd FE has many applications other than as a breeding tool, including the assessment of different management strategies (e.g., diet) or monitoring animal or herd health (Berry and Crowley, 2013). It is also useful for benchmarking and elucidating the possible factors contributing to variation among animals in FE. There are numerous definitions of FE, among which the most appropriate definition for dairy production systems is still unclear (Berry, 2009; Connor, 2015). In this thesis, three main categories of FE definitions are studied and discussed; namely: feed conversion efficiency (FCE), residual feed intake (RFI) and residual energy corrected milk (RECM).

### 1.1.1 Feed conversion efficiency

Feed conversion efficiency (FCE; Brody, 1945), or gross feed efficiency (GFE), is the most basic used measure of FE expressed as the ratio of milk yield in kg to DMI in kg. Since the production of milk fat and protein are associated with energy cost, it may be erroneous to compute FCE with only milk yield not taking into account the fat and protein content, which implies the need to standardize the energy content of milk so as to attain a more precise measurement of FCE (Linn, 2006). This standardization facilitates comparison across herds that vary considerably in milk composition. Furthermore, it is often more suitable as many payment systems are based on amounts of protein and fat in milk. An added advantage of improving the accuracy of calculating FCE could be gained by also correcting DMI for energy content. This correction would increase the accuracy of calculating FCE and allow for comparisons among rations of different compositions (Varga *et al*., 2013). Alternatively, the efficiency of specific dietary nutrients such as N use efficiency (NUE) or milk N efficiency (MNE), may be calculated as the ratio of milk N yield to the quantity of N intake. In Ireland and New Zealand, FCE is basically incorporated in cattle breeding programs which favour greater milk solids production and smaller body weight (BW) together (Coleman *et al*., 2010). Earlier studies have described FCE in dairy cows as being a moderately heritable trait, with estimates ranging from 0.14 to 0.47 subject to the stage of lactation (Vallimont *et al*., 2011; Manafiazar *et al*., 2016; Lidauer *et al*., 2018).

### 1.1.2 Residual feed intake

Residual feed intake (RFI) has been applied successfully in growing animals (Koch *et al*., 1963; Berry and Crowley, 2013; Tedeschi *et al*., 2014), and is now being used in lactating cow populations (Pryce *et al*., 2014; Li *et al*., 2017). In dairy cattle, RFI is defined as the difference between the observed DMI (and energy intake) of the cow and her predicted DMI (or energy intake), taking into account her energy costs for body maintenance, BW change (ΔBW), production and possibly pregnancy over a particular production period (Connor, 2015). Predicted feed intake is usually determined from the sample population using a regression model including various energy sinks. Traditionally, the energy sinks used in the calculation of RFI in dairy cattle are BW change (ΔBW), average metabolic BW (MBW), solids- or energy-corrected milk yield and occasionally, body condition score (BCS). Alternatively, RFI may be calculated using standard feed tables (Mäntysaari *et al*., 2012) to allocate the energy demand for each of the energy sinks and subtract the total from the energy intake. Because RFI denotes a difference between actual feed intake and predicted intake, a low or negative RFI value represents high efficiency and is desirable, while a high RFI value represents low efficiency. Documented heritability estimates of RFI generally are low to moderate ranging from 0.01 to 0.40 among lactating cows (Connor *et al*., 2012; Connor *et al*., 2013; Tempelman et al., 2015).

### 1.1.3 Residual energy corrected milk

Using a similar principle to that of RFI, Coleman *et al*. (2010) proposed residual solids production as an alternative measure of identifying between-animal variation in FE among lactating cows. In a recent study, Løvendahl *et al*. (2018), used the term, residual milk yield which is referred to as residual ECM (RECM) in this thesis. It is estimated as the difference between the cow's actual and predicted ECM production represented by the residuals from the regression of ECM yield on cow DMI, MBW, ΔBW and occasionally, BCS. Unlike RFI, where negative or lower values are deemed to indicate more efficient animals, more positive or higher residual values (i.e., animals producing more than expected) are deemed to be more efficient. Due to the favourability of positive values, RECM is easier to comprehend than RFI. In addition, Coleman *et al*. (2010) reported a higher repeatability estimate for residual milk solids production than RFI (0.33 vs. 0.28) over multiple lactations in Holstein–Friesians on pasture.

## 1.2 Production and efficiency

Advances in dairy FE defined by the fraction of feed energy or dry matter captured in milk during the past 50 years are remarkable, as modern dairy cows can produce more milk than what their ancestors did. In Swedish dairy herds, for instance, the annual milk production per cow averaged about 4,700 kg in the 1970s (Figure 1). However, the application of sound scientific principles to nutrition, management, and genetics has initiated a progressive increase in milk production that continues to this day. Presently, annual milk production in Sweden averages over 8,600 kg per cow. In fact, the annual herd average is >11,000 kg of milk per cow on some Swedish dairy farms. Notably, the current world-record Holstein cow named, “Selz-Pralle Aftershock 3918” produced more than 35,000 kg of milk in a year, which is almost 100 kg/d on average (https://www.dairyherd.com/article/how-wisconsin-dairy-raised-top-milk-producing-cow-world; accessed January 20, 2020); enough to feed more than 100 people. In addition, increased production per cow has reduced the number of animals needed to produce the same amount of milk, resulting in feed cost savings, reduced use of natural resources and reduced total carbon footprint of dairy production (Capper *et al*., 2009).

Despite the incredible gains in average milk production, there remains an important variation among cows in FE even within the same herds where genetics, diet, and management style do not differ (Coleman *et al*., 2010; Arndt *et al*., 2015). From an economic standpoint, this is indeed costly because cows on commercial farms are fed based on expected milk production for the herd. As such low producing cows are over-fed while high producing cows are under-fed. As a result, the low producing cows are more likely to gain excess condition and milk production in the high producing cows is probably restricted by nutrient or energy availability. According to VandeHaar *et al*. (2016), the sources of potential variation in FE among cows can be divided into 1) those that alter maintenance and the dilution of maintenance, or the partitioning of net energy

(NE) between milk and body tissues above maintenance, and 2) those that alter the conversion of gross energy (GE) to NE.

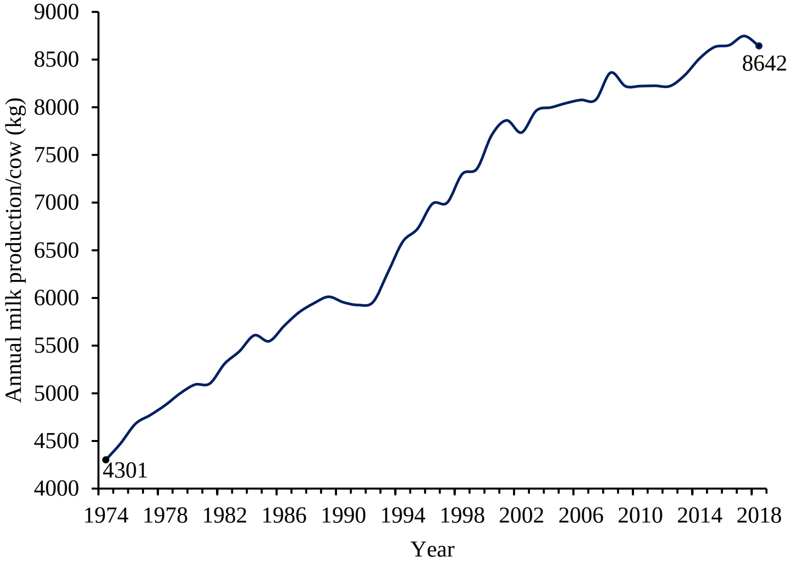


*Figure 1.* Average annual milk production in Sweden per cow. Source: Swedish Board of Agriculture

Increased milk production per cow is associated with increased feed intake per cow, but a greater proportion of the feed is directed towards milk instead of maintenance. This dilution of maintenance has been the main driver of enhanced FE at the animal level in the past, but its advantages have been mostly exploited (VandeHaar and St-Pierre, 2006). At the population level, milk production has been increasing at a decreasing rate since the 1970s (Figure 2). For example, the average annual increase in milk production of Swedish dairy cows in the 1970s was 3.1% but, it has continued to decline since then reaching a nadir of 0.6% between 2000 and 2018. Therefore, further increases in FE must focus on selecting cows directly for their ability to convert feed to milk.

In the conversion of feed energy to milk energy, several steps must occur that are associated with energy losses and utilization (Figure 3). Gross energy is the total chemical energy contained in a feed. Not all of GE intake is useful because some of it is not digested but is lost as faecal energy (FaecalE). Some of the digested energy (DE) is lost as methane energy ($CH_4E$) and as urinary energy (UE). The remaining energy is metabolisable energy (ME). About 33% of ME is lost as heat increment associated with the work of fermenting, digesting and metabolising nutrients. The remaining energy is known as NE, which is the actual energy utilised for maintenance and for production (lactation, body tissue accretion, and conceptus). Altering the proportion of GE intake available for milk production can be achieved by reducing the energy in any of the following components: FaecalE, UE, $CH_4E$, body tissue accretion, or heat. Therefore, quantifying the among-animal variation at each step of energy conversion may provide the basis for future improvements in FE.

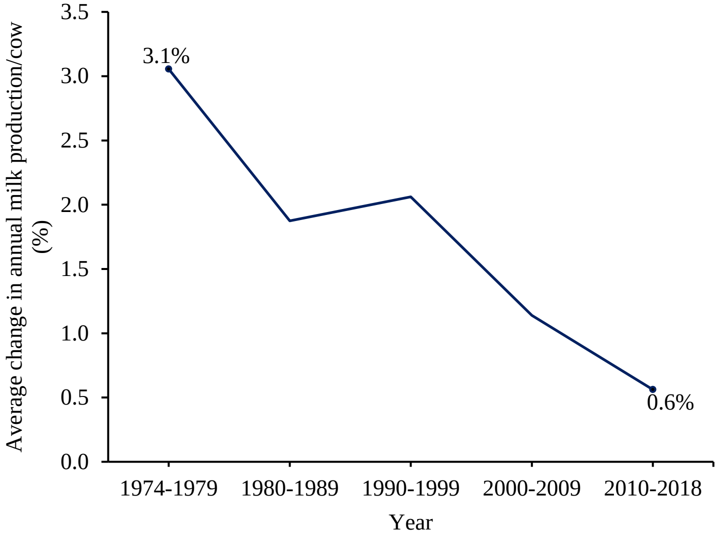


*Figure 2.* The average change in annual milk production in Sweden per cow. Source: Swedish Board of Agriculture.

## 1.3 Sources of variation

The classical energy system used in animal nutrition (Figure 3) is a direct application of the first and second laws of thermodynamics. The first law states that the energy in a system can be transformed, but it can neither be created nor destroyed, and the second law states that the entropy of an isolated system always increases. These two superficially abstract statements are the basis of the NE systems used to formulate diets and evaluate the energy status of animals. In terms pertinent to animal nutrition, the first law can be interpreted as energy intake must equal energy output. The second law can also be construed as no conversion of energy into useful work is completely efficient and the inefficiencies are lost as heat. These two laws are illustrated in Figure 3 and the variation in the components are discussed in this section.

### 1.3.1 Gross energy

Gross energy intake (GEI, expressed in MJ/d) is obtained based on two steps: the measurement of feed intake (on DM basis) and the estimation of GE content of that feed. Gross energy (GE) content is the total amount of chemical energy contained in a feedstuff (expressed as MJ/kg DM of feed). It can be determined in a laboratory by completely burning a sample of feed with a bomb calorimeter. Feed intake is a major determinant of GEI. It is relatively easy to measure in housed animals, as the difference between feed offered and orts or by using automated feed monitoring systems to track and record intakes of individual cows as they visit the feed bunk (Connor *et al.*, 2013). However, quantitative measures of DMI on individual animals are needed for selective breeding and the traditional method of weighing orts will be costly and logistically challenging on a large scale. Moreover, the significant investment in infrastructure and the limited capacity of the automated feed monitoring systems hinder their use in larger groups of lactating cows. Several years of research have been devoted to developing indirect techniques to measure intake with variable success and all methods developed so far have limitations (Lukuyu *et al.*, 2014). Maker techniques are undoubtedly the most widely used indirect methods in the literature but have received many criticisms with regards to preparation works and laboratory analysis of respective markers. However, under experimental conditions, markers provide useful information for advancement in research.

Due to the challenges associated with measuring feed intake, no single standard has been adopted for its estimates. Currently, none of the existing methods is suitable for routine recordings of individual animal DMI in commercial herds. This hinders the application of genetic selection for improved FE, as individual DMI records are prerequisites for accurate estimation of genetic parameters for FE. The phenotypic coefficient of variation (CV) for DMI between cows ranged from 9% to 14% (Berry and Crowley, 2013). In animals given the same diet (particularly a forage-based diet), this between-cow CV could be quite high, ranging from 10 to 30% (Coleman, 2005). The repeatability estimates for DMI across lactation in different dairy cow breeds varied from 0.46 to 0.84 (Søndergaard *et al.*, 2002; Berry *et al.*, 2014). This large variation between cows in DMI points to the effectiveness of including DMI in the breeding goal. However, with most of the variation in DMI being associated with ECM and BW (Spurlock *et al.*, 2012), it should be cautioned that implementation of genetic selection for DMI may improve FE only by reducing the BW or ECM of animals. To prevent this and improve on-farm evaluation of FE, more individual animal DMI data are needed and the methods used in this thesis will serve as a basis for the way forward.

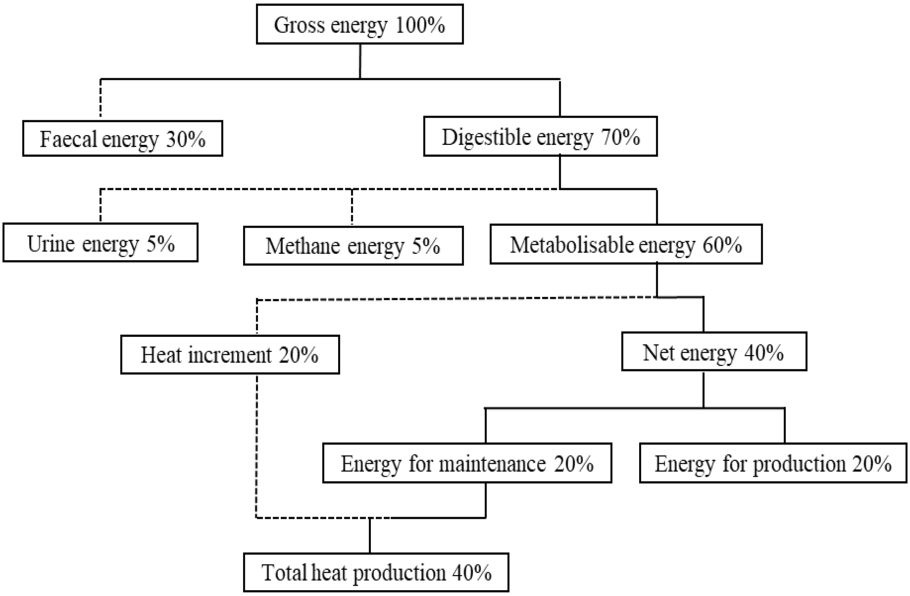


*Figure 3.* The partitioning of food energy in the ruminant. Adapted from McDonald *et al.*, 2002 and Francois & González-Garcia, 2010 (solid lines denote energy usage; dash lines denote energy loss).

### 1.3.2 Digestible energy

Digestible energy (DE) is the energy remaining after the faecal energy is subtracted. Faecal energy is the single greatest loss in the conversion of dietary GE to milk. Just as in the determination of GEI, faecal energy is also determined in two steps: the measurement of faecal output (on DM basis) and the estimation of GE content of faecal samples. The GE content is easily measured in the laboratory with a bomb calorimeter. Faecal output can be measured directly by total collection in pans placed behind animals in metabolic crates or with specialised harness bags attached to animals. However, this is quite cumbersome as it requires the removal and replacement of the bags multiple times during the day, which often obstructs feeding behaviour (Coleman, 2005; Cottle, 2013). In confinement systems, the magnitude of these problems will be exacerbated if unrestricted animals, due to welfare considerations, have to be used. External markers have been used most extensively to estimate faecal output indirectly (Lukuyu *et al.*, 2014). External markers are indigestible substances which are added or bonded to the feed or digesta [e.g. chromium oxide, titanium oxide, rare earth elements (Yb)]. These markers usually are administered orally, through fistulae or by means of controlled-release devices either as a single pulse dose or repeatedly over a period of time in an attempt to reach steady-state conditions where the digesta is labelled uniformly and the ratio of digesta to marker is constant (Marais, 2000). Spot samples of faeces are collected and faecal output is calculated from the concentration of marker in faeces and the daily dose. Digestibility can also be determined indirectly by the use of internal markers. The use of internal markers for estimating digestibility is valuable because the additional step of dosing them is avoided. Several internal markers such as lignin, faecal nitrogen, acid insoluble ash (AIA), indigestible neutral detergent fibre (iNDF) and n-alkanes have been studied. Despite the fact that marker techniques provide animal-specific data on faecal output and digestibility, their feasibility for animal breeding purposes has been limited by the high labour and practical inadequacies.

The digestibility of a diet is an important factor that affects FE in dairy cows. According to Potts *et al.* (2017a), the relationship between digestibility and FE is diet-dependent. They reported a greater effect of digestibility on FE when cows were fed low starch diets than when fed high starch diet (Potts *et al.*, 2017a). As well as diet composition, increased DMI has been shown to reduce digestibility, because of the increased rate of digesta passage through the digestive tract at higher levels of intake (Tyrrell and Moe, 1975). It is well established that increased milk production is associated with increased DMI which may reduce digestibility. Therefore, improving digestive efficiency in dairy cows is desirable. Determining the variation between cows in digestibility could be a means to select cows with both increased production and higher digestive efficiency. Literature values suggest that the phenotypic between-cow CV in digestibility is small (Huhtanen *et al.*, 2016; Mehtiö *et al.*, 2016; Cabezas-Garcia *et al.*, 2017), but there is genetic variation between cows (Berry *et al.*, 2007; Mehtiö *et al.*, 2019) which shows that selection for this trait could be beneficial. In addition, because every percentage decrease in diet digestibility corresponds to an equal amount of losses in energy intake, it may receive more attention in the future (Mehtiö *et al.*, 2019).

### 1.3.3 Methane energy

Methane energy ($CH_4$E) loss from ruminants represents 2 to 12% of dietary GEI (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995). As such strategies that reduce $CH_4$ production are more likely to result in the repartitioning of more energy toward production. A large proportion of the variation in $CH_4$ emission from dairy cows has been attributed to diet composition and DMI (Hristov *et al.*, 2013; Ramin and Huhtanen, 2013). For instance, low $CH_4$ yield (g $CH_4$/kg DMI) have been reported in feedlot growing cattle fed high-concentrate diets (Johnson and Johnson, 1995) and in fat supplemented dairy cows (Eugène *et al*., 2008). There is also evidence of variation between cows in $CH_4$ yield reported in the literature (Blaxter and Clapperton, 1965; Cabezas-Garcia *et al*., 2017). However, there remains a challenge of measuring $CH_4$ production on a population-scale and more data is required for genetic evaluation. In respiration chamber studies, Blaxter and Clapperton (1965) reported a between-cow variation of between 7.2 and 8.1% in $CH_4$ yield. With the GreenFeed system, the average between-cow CV was 10.7% (Cabezas Garcia, 2017). Values up to 30% have been reported with the sniffer method (Garnsworthy *et al*., 2012; de Haas *et al*., 2013). It appears that the large variation is mainly reported when measurements are based on the sniffer method and this could be attributed to the large random errors associated with this method. In general, there is considerable variation in $CH_4$ emissions between cows, giving scope for genetic selection for reduced $CH_4$ to improve FE. Despite the lack of big data, there is evidence of trade-off between digestibility and $CH_4$ yield (Huhtanen *et al*., 2016; Cabezas-Garcia *et al*., 2017; Løvendahl *et al*., 2018), suggesting that increasing digestibility could entail a higher $CH_4$ yield and vice versa.

### 1.3.4 Metabolisable energy

Metabolisable energy (ME) is the energy remaining after urinary energy (UE) and $CH_4E$ are subtracted from DE. Daily urine output can be measured by total collection and the energy content is measured by bomb calorimeter. However, total collection is laborious and expensive and requires that animals are tied in specific stalls, which often restricts the number of animals used in experiments. Therefore, indirect methods of measurement have been used over the years with urine creatinine (de Groot and Aafjes, 1960; Tebbe and Weiss, 2018) or N (Hetta *et al*., 2013; Pang *et al*., 2018) concentration as markers. Although the accuracy of the marker method has been questioned (Shingfield and Offer, 1998), it is advantageous because more animals can be used which allows for reliable evaluation of dietary effects on production as well as nutrient utilization (Broderick and Reynal, 2009). The variation in UE are determined to a large extent by the dietary crude protein concentration (CP), with higher CP components contributing to a larger amount of UE loss (Huhtanen *et al*., 2008). Diets with high CP increase urea synthesis which is excreted via urine thereby increasing the loss of UE (Weiss, 2007).

Current genetic evaluations of dairy cows do not consider information on individual cow ME intake (MEI) or metabolisability (ME/GE), partly because creating such database require accurate measurements of faecal, methane and urinary energy losses which are not very easy to quantify. As such empirical equations are used in practical feed evaluations to convert digestible nutrients to dietary ME concentration (MAFF, 1984). The ME system is widely adopted in many countries in Europe, especially in the UK. The main reason many animal nutritionists have a preference for the ME system is that all energy losses (faeces, urine, and $CH_4$) are measurable in a material sense although there is a paucity of information on ME values.

### 1.3.5 Net energy

In addition to energy losses in faeces, urine, and $CH_4$ production, heat is also lost as a result of the chemical and physical processes associated with digestion and metabolism (Agnew and Yan, 2005). This heat is called heat increment (HI) and is not equivalent to HP. Thus, net energy (NE) is calculated as the difference between ME and HI, which is the actual energy used for maintenance and for production (growth, conceptus, lactation). Therefore, NE of a feedstuff represents that fraction of its energy content that could be realized in animal product or work (Bondi, 1987). Thus, NE is said to be the most accurate method for evaluating the energy value of feedstuffs as it allows different efficiency values to be calculated for different production purposes (growth, conceptus, lactation). At present, only France, Germany, and the Netherlands have developed NE systems to evaluate feed energy values, but several other countries have conducted research into NE. Measurement of NE is much more intricate than that of DE or ME, which may be a reason it has received only limited use.

### 1.3.6 Maintenance energy requirement

Maintenance energy requirement is defined as the energy needed for basal metabolism, voluntary body activity and the generation of heat to maintain body temperature (Korver, 1988). It is the difference between NE and the energy needed for production purposes (growth, conceptus and lactation). Generally, elements of maintenance energy expenditure can be divided into three major classes: 1) 40 to 50% is service functions (heart, kidney, liver, nerve, and respiratory functions), 2) 15 to 25% is cell component synthesis (protein and lipid membrane synthesis), and 25 to 35% is cell maintenance mainly associated with ion transport ($Na^+$, $K^+$) across cell membrane (Baldwin *et al*., 1985).

For several years, the ME requirement for maintenance ($ME_m$) has been estimated by measuring the fasting metabolism of pregnant non-lactating dairy cows and beef steers (AFRC 1990). In the UK ME system, HP was measured at maintenance (≥ 28 days) and fasting (4-5 days). The published data were then used to develop equations to calculate $ME_m$ for lactating dairy cattle. Using a number of respiration chamber studies, ARC (1980) proposed a curvilinear relationship between fasting metabolism (FM) and BW (FM = 0.53 × $(BW/1.08)^{0.67}$). In the implementation of AFRC (1990), an activity allowance (0.0091 × BW) was added to the FM which was defined as NE requirement for maintenance ($NE_m$) in the UK. The $ME_m$ (MJ/day) was calculated as the ratio of $NE_m$ (MJ/day) to the efficiency of utilization of ME for maintenance ($k_m$) using the following equations:

$ME_m = NE_m/k_m = (0.53 \times (BW/1.08)^{0.67} + 0.0091 \times BW)/k_m$ [1]

$k_m = 0.35 \times ME/GE + 0.503$ [2]

The limitations of this approach are the difficulty with keeping the animals at maintenance and the influence of variables such as plane of nutrition, production level, visceral organ mass, breed and sex of animals, and duration of measurement (Graham and McC, 1982). On the other hand, the $ME_m$ of lactating dairy cattle can be estimated by regression of milk energy ($E_l$) adjusted to zero energy balance ($E_{l(0)}$) against ME intake (Yan *et al.*, 1997). There is a wide range of $ME_m$ values published in the literature irrespective of the technique used to estimate $ME_m$. Moe *et al.*, (1970) reported an average $ME_m$ estimate of 0.456 MJ/kg $BW^{0.75}$ from a large dataset of dry and lactating cows fed a range of forage types and proportions. Using a large set of production data assembled from a large number of individual respiration chamber experiments, Yan *et al.* (1997) estimated $ME_m$ values ranging from 0.49 to 0.64 MJ/kg $BW^{0.75}$.

Within the literature, there is evidence that $ME_m$ is directly proportional to feed intake (Dong *et al.*, 2015b) and is affected by diet quality (Yan *et al.*, 1997; Agnew and Yan, 2000; Dong *et al.*, 2015a). Yan *et al.* (1997) and Dong *et al.* (2015a) examined the effect of dietary forage proportion on $ME_m$ using the regression technique. The results from both studies revealed that dairy cows fed high forage diets had significantly higher $ME_m$ (MJ/kg $BW^{0.75}$) than those offered low forage diets. Between-breed variation in $ME_m$ have also been reported, and these variations are related to differences in the productive potential of different breeds (Archer *et al.*, 1999). Münger (1991) recorded variable $ME_m$ values for different breeds of lactating cows fed maize silage and hay or a mixture of fresh grass and clover (0.47, 0.53, and 0.56 MJ/kg $BW^{0.75}$ for Simmental, Holstein/Friesian, and Jersey cows, respectively). There is, however, a dearth of information on between-cow variation in $ME_m$. This is partly due to the difficulty and costs involved in measuring $ME_m$ on many animals to provide evidence of between-animal variation.

### 1.3.7 Efficiency ME utilisation for lactation

The terminology $k_l$ is the partial efficiency of ME use for lactation (i.e. MJ of NE captured in milk per MJ of ME consumed). With the exception of NRC (2001), $k_l$ was designed to be directly proportional to dietary ME/GE in all major energy systems. This positive relationship between $k_l$ and ME/GE was largely cantered on the work of van Es (1975) using large data from energy balance (EB) experiments. The results showed that $k_l$ increased by about 0.40 per unit increase in ME/GE, but this relationship was less accurate due to the limited variation in the values of ME/GE. The calculated $k_l$ values ranged from 0.58 to 0.63 for INRA (1989), and from 0.60 to 0.67 for AFRC (1990) using ME/GE values of between 0.50 and 0.70. The calculation of $k_l$ for AFRC (1990) is expressed as follows:

$k_l = 0.35 \times ME/GE + 0.42$ [3]

In respiration chamber studies, $k_l$ has often been calculated by assuming a fixed $ME_m$ value which is subtracted from MEI to provide the ME available for production ($ME_p$) and then relating this to milk energy output adjusted to zero EB ($E_{l(0)}$):

$k_l = E_{l(0)}/ME_p = (E_l + aE_g)/(MEI - ME_m)$ [4]

Where $E_g$ = tissue energy change. If $E_g$ is positive, a = 1/0.95 (AFRC, 1990), 1 (INRA, 1989), or 0.64/0.75 (NRC, 2001); if $E_g$ is negative a = 0.84 (AFRC, 1990), 0.80 (INRA, 1989) or 0.82 (NRC, 2001).

Alternatively, $k_l$ can be estimated using linear regression ($E_{l(0)}$ against MEI) or multiple regression (relating MEI to MBW, $E_l$ and $E_g$) techniques (Agnew and Yan, 2000). The range in $k_l$ values of lactating dairy cows reported in earlier studies has been variable. Unsworth *et al* (1994) used the equations of AFRC (1990) to calculate $ME_m$ of dairy cows fed grass silage-based diets in 4 respiration chamber studies and reported a $k_l$ of 0.56. Using the regression technique on large sets of production data, each pooled from a large number of different respiration chamber studies, Yan *et al* (1997) reported variable $k_l$ values ranging from 0.60 to 0.67 with a mean of 0.63. The relationship between the latter $k_l$ values and their corresponding $ME_m$ values was strongly positive ($R^2 = 0.77$, $P < 0.05$) suggesting that $k_l$ is largely dependent on the accuracy of the $ME_m$.

There is a substantial body of evidence that $k_l$ values remain relatively constant over a wide range of conditions such as breed, diet composition and level of production (Agnew and Yan, 2000). Earlier studies did not find between-breed (Dong *et al*., 2015b) or within-breed (Gordon *et al*., 1995) variation in $k_l$ values. Yan *et al*. 1997 and Dong *et al*. (2015a) evaluated the effects of diet forage proportion on $k_l$ values and found that $k_l$ values were the same across all diets.

### 1.3.1 Energy balance

From the law of conservation of energy, energy intake is equal to energy output. Thus EB is the energy remaining, after subtracting NE used for maintenance, lactation, growth, and pregnancy from NE intake. When the NE intake is less than NE requirement, the cow is said to be in negative EB and if the reverse is the case, the cow is said to be in positive EB. Effectively, the measurements of all losses depend on the validation of EB trials conducted in respiration chambers. Not many EB studies have been performed because of the cost, labour and technology requirements of respiration chambers. However, there is a renewed interest to measure energy metabolism in dairy cows due to the need to apply knowledge of energetics in the development of recommendations for practical feeding systems. Various research institutions are building facilities for accurate measurements of EB, in many cases with small monetary budgets.

#### *Techniques for measuring energy balance*

For more than 120 years, respiration chambers **(**RC**)** have been used as indirect calorimeters for the measurement of energy metabolism of ruminants (e.g. Armsby, 1903). Respiration chambers have been used as the gold standard method because they are the most accurate (Blaxter and Clapperton, 1965; Grainger *et al.*, 2007). Whole animal open-circuit RC (Figure 4) are now the most widely used with varying degrees of sophistication. They range from poly-tunnels and shower curtains placed over cubicles, to more refined and high-cost calorimeters that are dedicated to long term investments (Hammond *et al.*, 2016).

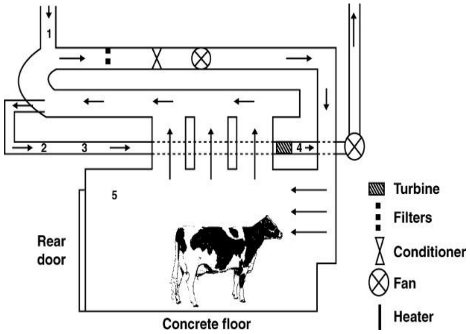


*Figure 4.* Schematic diagram of the open-circuit respiration chamber (adapted from Grainger *et al.* (2007) showing the airflow and conditioning, and release and sampling locations within the circulation system. Locations 1 and 2 are the intake and exhaust ducts sample points for non-calibration periods; location 3 is the injection point enabling the analytical system calibration; location 4 is the sample point for the system calibration, and location 5 denotes the chamber volume.

The principle of these systems is that inflowing air is circulated through the chamber and around the animal to mix incoming air and exhaled air within the volume of the chamber while sampling incoming and exhaust air for gas (i.e. $O_2$, $CO_2$ and $CH_4$) analysis. Gas fluxes are determined by multiplying the airflow through the system by the difference in the concentration of inflowing and outflowing air. Gas concentrations and flow are corrected to standard temperature and pressure (STP) conditions and account for humidity. The gas contained in the chamber at the beginning and end of measurements must also be accounted for. The measured gas values are then used in equations to calculate HP. Respiration chambers have been critiqued for the fact that they do not mimic the natural conditions of the animals and that the restriction could impact feeding behaviour, and could lower HP due to the reduction in physical activity. Moreover, RC are expensive, intricate and not amenable to measurements on a large scale.

Head boxes or ventilated hood chambers have been used to record gas measurements (*e.g.* Odongo *et al*., 2008). Similar to RC, they can be used to obtain continuous measurements over a continuous 24 h periods. However, animals need to be adapted to the hood apparatus, which requires extensive training, thereby limiting their use for screening large numbers of animals.

Alternative spot sampling techniques to RC are enabling scientists to record gas measurements from cattle in their own production settings (e.g., grazing, free stall). Typical examples include quantifications of (1) HP from $O_2$ consumption per heartbeat (Brosh *et al*., 1998), (2) energy expenditure using the $^{13}C$ bicarbonate technique together with $O_2$ consumption and respiratory quotient (**RQ**; Junghans *et al*., 2007).

In 2010, a new method called GreenFeed (GF, C-Lock Inc, Rapid City, South Dakota, USA) was developed to measure real-time $CO_2$ and $CH_4$ mass fluxes ruminants (Figure 5). It was recently upgraded to measure $O_2$ consumption. The number and duration of the visits can be adjusted to serve experimental objectives. One unit can be used for 25-30 animals for a seven day period of measurements, which translates to ~1000 animals per year (Garnsworthy *et al*., 2019). A small amount of concentrate feed dropped from the feed bin is used as a bait to attract animals to the system. During a visit, the exhaled air together with the airflow is pulled into the system via the pipes and gets mixed within a fan. After passing through the fan, a sample of gas is taken and then analysed for $O_2$, $CO_2$ and $CH_4$ concentrations. The system is also equipped with a head position sensor which filters out data when the head of the animal is not in the right position. Earlier studies have shown that the between-animal CV in $CH_4$ from the GF system is comparable with those from RC (Huhtanen *et al*., 2019). In this thesis, the use of the GF system to measure EB in dairy cows is evaluated.

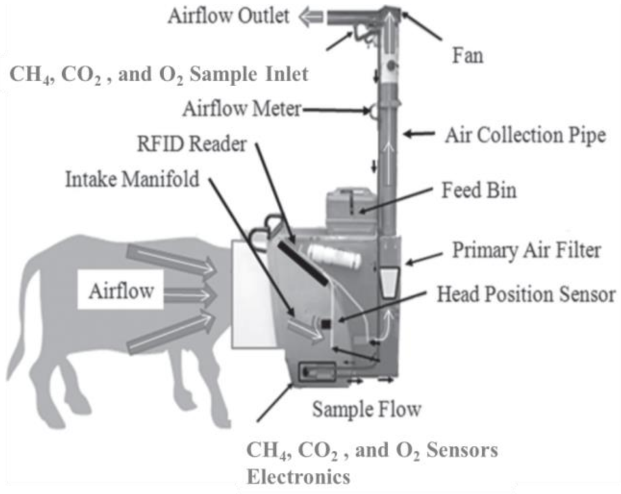


*Figure 5.* Design of the GreenFeed system (C-Lock Inc., Rapid City, SD, USA). Adapted from Huhtanen *et al*. (2015).

# 2 Objectives

The overall aim of the studies presented in this thesis was to investigate the variation in different components traits of feed efficiency and their contribution to the observed variation in feed efficiency of dairy cows. The individual animal variation in feed efficiency was evaluated by studying measurement techniques of component traits. Specific objectives were to:

1. Compare feed marker-based estimates with observed measurements of feed intake, faecal output, and digestibility and to explore the effect of each marker-based estimate in predicting feed efficiency.

2. Evaluate the between-cow variation in different components related to feed efficiency and any potential trade-offs among these components.

3. Quantify the effects of the different components related to feed efficiency on the different feed efficiency measurements

4. Examine the effect of replacing grain concentrate with fibrous by-products on the performance of early lactating dairy cows

5. Evaluate the GreenFeed system for measuring energy balance in lactating dairy cows and examine the relationship between milk fatty acid and determined energy balance with the GF system

# 3 Materials and methods

## 3.1 Paper I

A meta-analysis based on an individual cow dataset was conducted to investigate the performance of digesta marker-based estimates against direct or observed measurements. Equations were also developed for the prediction of FE. Data used included a total of 416 cow-within period observations from 29 change-over studies that were assembled across 3 research stations in Denmark (5), Finland (18) and Norway (6). The experimental diets were based on silages (mainly grass with some legume and whole-crop silage), with the exception of 4 trials where hay was used instead. Concentrates consisted of cereal grains or by-products as energy supplements, and soybean, rapeseed meal or rapeseed expeller as protein supplements. The average forage: concentrate ratio across all diets was 59:41 on DM basis.

Observed DMI was measured as the difference between feed offered and the refusals. Observed faecal DM output (FDMO) and DM digestibility (DMD) were determined by total faecal collection. The marker-based estimate of faecal DM output (eFDMO) was made from the concentration of external marker in faeces and the daily dose. The external markers used in the calculations were Cr-mordanted fibre, Yb, polyethylene glycol (PEG) and Cr- and Co-ethylenediaminetetraacetic acid (EDTA). The marker-based estimate of DMD (eDMD) was made from dietary and faecal concentrations of internal markers. The internal markers used in the calculations were indigestible NDF (iNDF) and acid insoluble ash (AIA). Marker estimated DMI (eDMI) was calculated by dividing eFDMO by the indigestibility of the diet determined from internal markers (1– eDMD). Estimated FE (eFE) of individual animals was also calculated as the quotient of ECM and eDMI.

Variance components analysis was made for both observed and marker-based estimates in the PROC MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary. NC) to calculate the random effects of experiment (Exp), Cow(Exp), Diet(Exp), Period(Exp), and Marker(Exp). In addition, repeatability values were estimated as in Paper I. Single regression models were developed with observed measurements as dependent variables and marker-based estimates as independent variables with random Marker(Exp) effect. The accuracy of the models was determined by calculating the root mean square prediction error (RMSPE) as in Paper I. Mean and slope biases were evaluated from the intercept and slope of the regression of residuals (observed-estimated) on marker-based estimates as described by St-Pierre (2003). Multiple regression models were developed for the prediction of FE using the MIXED procedure in SAS as reported in Paper I.

## 3.2 Paper II

In paper II, a meta-analysis based on RC studies was conducted to evaluate the between-cow variation in the components and measurements of FE as well as to explore the associations among these components. Data used included a total of 841 cow-within period observations from 31 studies across 3 research stations in the UK (20), Denmark (9 studies) and Finland (2 studies). The experimental diets were based on grass or maize silages, fresh grass, a mixture of fresh grass and straw with cereal grains or by-products as energy supplements, and soybean, rapeseed meal or rapeseed expeller as protein supplements. The average forage: concentrate ratio across all diets was 56:44 on DM basis.

Heat production was calculated according to the equation of Brouwer (1965). The ME requirement for maintenance ($ME_m$) and efficiency of ME use for lactation ($k_l$) of individual cows were calculated according to the equations of AFRC (1993). Residual feed intake (RFI) was calculated by regressing DMI on metabolic BW (MBW), milk energy ($E_l$) and energy balance (EB). Residual ECM was also determined by regressing ECM on GEI, MBW and EB.

The relationship between the FE components (DE/GE, $CH_4E$/GE and UE/GE) and the animal variables (DMI and BW) were determined by using MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary. NC) as described in Paper II. Variance components analysis was made for both components and measurements of FE to calculate the random effects of experiment (Exp), Cow(Exp), Diet(Exp), and Period(Exp). In addition, repeatability values were estimated as in Paper II.

The efficiency of ME use for lactation ($k_l$) was also determined using the regression method by regressing $E_l$ adjusted to zero EB ($E_{l(0)}$) on ME intake as in Paper II. The models included one independent variable $X_1$ and one random statement: a random intercept and slope of $X_1$ with SUBJECT = Exp using the TYPE = UN as covariance structure of the random statement. Outlier observations were investigated for leverage and influence and removed from the analysis using the method described by Belsley *et al.* (1980). Partial correlations among the FE components were determined using MANOVA in PROC GLM of SAS while controlling for feeding level (g DMI/kg BW), Exp, Diet(Exp), and Period(Exp).

## 3.3 Paper III

In paper III the influence of energy metabolism variables on FE was evaluated. Details of experimental design, calculations, outlier detection and energy metabolism traits are reported in Paper II. Feed efficiency was calculated as RFI, RECM or feed conversion efficiency (FCE = kg ECM/kg DMI).

Cows were classified into 3 groups of equal sizes (n =279-281) of High-Medium- and Low-FE. For RFI the cows were categorised as high-RFI (RFI > 0.72), Medium-RFI (-0.39 ≤ RFI ≤ 0.72) or Low-RFI (RFI < -0.39). Similarly, they were grouped by RECM value as High-RECM (RECM > 1.2), Medium-RECM (-1.32 ≤ RECM ≤ 1.2) or Low-RECM (RECM < -1.32). Cows with FCE below 1.28 were categorised to group Low-FCE, cows with 1.28 ≤ FCE ≤ 1.51 were categorised to group Medium-FCE, and cows with FCE > 1.51 were categorised to High-FCE. The effects of RFI and RECM groups on intake, production, and energy metabolism variables were determined using the MIXED procedure of SAS. The model included the fixed effect of RFI or RECM group, and random effects of Exp, Diet(Exp) and Period(Exp). In addition, pairwise comparisons of LSM among the efficiency groups were performed using the PDIFF option in the LSMEANS statement.

## 3.4 Paper IV

A study was conducted at Röbäcksdalen research station, Swedish University of Agricultural Sciences, Umeå, Sweden (63°45’N; 20°17’E). The objective was to investigate the effects of replacing cereal grains with fibrous by-products on performance and $CH_4$ emissions of early lactation dairy cows fed a grass silage-based diet. Twenty-two Nordic Red cows (13 multiparous and 9 primiparous cows) were alternately assigned to 1 of 2 dietary treatments post-calving until 18 weeks in lactation. The cereal grain treatment contained 59.3 % of grass silage, 31.7 % of cereal grain mixture (barley, oat, and wheat), 7.9 % of heat-treated canola meal, and 1.1 % of a mineral mix on a DM basis. A mixture of unmolassed beet pulp, wheat middlings, barley fibre, and wheat fibre replaced cereal grains in the by-product treatment. Cows were offered the diets *ad libitum* as TMR, with free access to water, and were milked twice daily.

Daily feed intake and milk yield were recorded during the whole experiment and milk samples were taken for composition analysis at 4 consecutive milkings on lactation week 1 to 8 and every other week after that until lactation week 18. Gas emission data ($CH_4$ and $CO_2$) was recorded daily by the GF system (C-Lock, Rapid City, SD) as described by Huhtanen *et al* (2015).

Feed samples were collected weekly to adjust dietary DM value in the automatic feeding system. Grab faecal samples were collected twice daily for 3 consecutive days every 4 weeks to determine diet digestibility with ash-free iNDF as an internal marker (Huhtanen *et al.*, 1994). The ECM yield and milk energy concentration were calculated according to Sjaunja *et al.* (1990). The human edible fraction of feeds and edible feed conversion efficiency (HeFCE) for energy and for protein were calculated based on recommendations by Wilkinson (2011) and Ertl *et al.* (2015b). Feed conversion efficiency (FCE) was calculated as ECM yield (kg/d)/DMI (kg/d) and milk N efficiency (MNE) as milk N [CP (g/d)/6.38]/N intake (kg/d).

All measurements were averaged within cow and week of lactation and analysed by ANOVA using the MIXED procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC). Treatment, week of lactation, parity and their 2-way interactions were specified as fixed effects. Cow within treatment was specified as a random effect. A REPEATED statement was included in the model as measurements on individual cows were repeated over time (week of lactation). A first-order autoregressive [AR(1)] covariance structure was used as it resulted in the lowest Akaike's information criterion (AIC).

## 3.5 Paper V

The aim of Paper V was to study the effects of the diets used in Paper IV on blood metabolites and milk fatty acids (FA) as well as to examine the relationship between milk FA and determined energy balance by the GF system. Data from this study was derived from the experiment in Paper IV. Animal management, experimental design, diets, feeding and sampling procedures remain strictly the same as for Papers IV.

Milk FA concentration was determined by means of a mid-infrared reflectance (MIR) spectrometer (MilkoScan FT6000, Foss Electric, Hillerød, Denmark). Spot samples of urine were collected at the same time intervals as for faecal samples in Paper IV. Blood samples were collected from the tail vein of all cows once during weeks of lactation 1, 2, 4, 8, and 12 and were analysed for energy metabolism, protein metabolism and inflammation parameters. Gas exchange measurements ($CH_4$, $CO_2$, and O2) were made during the entire experimental period using the GF system. The GE contents of feed, faeces, and urine samples were determined using a bomb calorimeter.

Heat production was calculated following the equation of Brouwer (1965). The ME requirement for maintenance ($ME_m$) and efficiency of ME use for lactation ($k_l$) of individual cows were calculated according to the equations of AFRC (1993).

Data were averaged on a weekly basis before ANOVA using the MIXED procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC). The model included fixed effects of treatment, week of lactation, parity and their interactions. Cow within treatment was included in the model as a random effect. The model included a REPEATED statement with a first-order autoregressive [AR(1)] covariance structure as it resulted in the lowest AIC. For blood metabolites, a spatial power [SP(POW)] covariance function was used as the time intervals between blood samples were unequal. Statistical significant differences between treatment means were determined using the PDIFF from Tukey-Krammer test for pairwise comparison. A multiple linear regression model was developed to predict EB from milk FA using stepwise regression (PROC GLMSELECT in SAS) as described in Paper V. The determined EB from the GF were compared with values calculated from energy requirements in Finnish feed tables.

# 4 Results

## 4.1 Paper I

Energy corrected milk yield and BW were on average 26.1±0.26 kg/d and 609±0.26 kg respectively. The recovery rates of external markers were 0.80, 1.01, 0.99, and 0.94, for Cr-mordanted fibre, Yb, Co-EDTA, and Cr-EDTA respectively. For iNDF and AIA as internal markers, the recovery rates were 0.86 and 0.95, respectively. For observed measurements, the variation due to experiment was the largest source of variation, while the variance component Marker(Exp) was the largest source of variation for marker-based estimates. The repeatability of marker-based estimates was generally smaller than their corresponding observed measurements of repeatability.

The predictions of FDMO with individual external marker-based estimates were associated with errors. Cr-mordanted fibre gave the worst prediction among all external markers. In general external markers overestimated FDMO by 0.22kg/d (RMSPE = 0.55 kg/d). The relationships between DMD and eDMD for individual internal markers were also associated with prediction errors. Acid insoluble ash gave a better prediction than iNDF. Altogether, internal markers underestimated DMD by 36.8 g/kg DM (RMSPE = 47.2 g/kg DM). The combination of internal and external markers overestimated DMI and FE by 1.7 kg/d (RMSPE = 2.9 kg/d) and 147 g ECM/kg DMI (RMSPE = 265 g ECM/kg DMI), respectively.

Energy corrected milk was positively related to FE ($P < 0.01$) while BW was negatively related to FE. Both eFDMO and eDMI were negatively related to FE ($P < 0.01$) while eDMD was positively related to FE ($P = 0.05$). The inclusion of eFDMO and eDMI in the model resulted in lower residual variances. Based on residual variance, the model for predicting FE was the one with ECM, BW and eFDMO as independent variables.

## 4.2 Paper II

The data set covered the expected ranges in dietary chemical composition and feed intake of dairy cows in the Northern European countries. The dietary concentrations of CP and NDF were 175±0.8 and 381±2.5 g/kg DM respectively. The data represented a wide range of GEI and large variations in energy losses (UE, $CH_4E$, and HP). Metabolisable energy intake varied from 84 to 379 MJ/d and $E_l$ from 19 to 163 MJ/d. The range in EB was generally (-49 to 50 MJ/d). The overall coefficient variations (CV) in DE/GE and ME/GE were rather low (CV = 5.3% and 6.0%, respectively). Using the equations of AFRC (1993), the $ME_m$ and $k_l$ averaged 0.68 MJ/kg $BW^{0.75}$ and 0.65 respectively. With the regression technique, the $ME_m$ was 0.73 MJ/kg $BW^{0.75}$ while the slope which represents the $k_l$ was 0.68.

Gross energy digestibility was positively related ($P < 0.01$) to BW and $CH_4E/GE$ but not DMI. Increases in DMI were linearly associated ($P < 0.01$) with decreases in $CH_4E/GE$. Variance component analysis revealed that the effect of experiment was the largest source of variation. The between-cow CV was higher than the variation due to diet for GEI, $CH_4E/GE$, $E_l/GE$, $k_l$ and RECM, while the opposite was observed for UE/GE, DE/GE, ME/GE. The between-cow variation in RECM was 2-fold that of RFI. The greatest between-cow CV and repeatability estimates were observed for GEI and $E_l/GE$. A greater DE/GE was associated with increased $CH_4E/GE$ ( $r = 0.24$) and the correlation between $k_l$ and $CH_4E/GE$ -0.44 suggesting the selection for cows with higher $k_l$ would result in cows with lower $CH_4$ emission.

## 4.3 Paper III

Among RFI groups, high efficient cows (Low-RFI) consumed less feed with lower energy losses in faeces, $CH_4$, and urine ($P < 0.001$). High-RFI cows had a higher ME intake than Low-RFI cows (21 MJ/d; $P < 0.001$). However, this was offset by a greater HP (21 MJ/d; $P < 0.001$) resulting in no differences in EB among groups. Lower ($P < 0.001$) HP in Low- compared with High-RFI cows was associated with improved ($P < 0.001$) $k_l$. Gross energy digestibility, ME/GE, and g $CH_4$/kg ECM improved with decreasing RFI ($P < 0.001$). Milk energy output and $CH_4E/GE$ remained the same among RFI groups. About 65% of the variation in RFI was due to $k_l$ while the contribution if $CH_4E$ was only small (5%)

Among RECM categories, energy losses as $CH_4$ and urine were lower for efficient cows (High-RECM) than Low-RECM cows ($P < 0.001$). High-RECM cows had higher DE/GE, ME/GE, and *kl*, compared to the Low- and Medium-RECM group ($P < 0.001$). Feed intake was not different among all groups of RECM. The contribution of ME/GE to variation in RECM was 33% while DE/GE contributed to most of the variation. The correlation between RFI and RECM was 0.75 suggesting they are not the same trait. The residual of the prediction of RECM with RFI were positively related to DMI and ECM but negatively related to $CH_4E/GE$.

The differences among FCE groups were mainly a result of the differences in nutrient partitioning between milk production and body tissue retention, and partly due to smaller BW of High- than Low-FCE cows.

## 4.4 Paper IV

The equal MP and ME formulation of the diets were as expected as there was no difference in their concentrations between the diets. Silage fermentation quality was good, as indicated by low pH and ammonia-N concentrations. The main differences in diet composition between treatments were related to the higher starch but lower fibre concentrations in the cereal grain concentrate than the fibrous by-product concentrate ($P < 0.01$).

Dry matter and CP intake were not different between treatments, but they were affected by week of lactation ($P < 0.01$) and parity ($P < 0.01$). However, cows receiving the by-product treatment consumed more fibre (NDF and pdNDF), but less starch and NDS compared to cows receiving the grain concentrate ($P < 0.01$). The digestibility of DM, OM, CP, and NDS was reduced with by-product supplementation ($P \leq 0.01$). The lower digestibility observed for by-product diet was consistent with the increased faecal output of nutrients (expressed as g/kg DMI).

Milk and ECM yield were not different between the diets but were affected by week of lactation and parity ($P < 0.01$). Milk composition was not influenced by treatment. Milk fat to protein ratio followed a similar time course as ECM yield. Feed conversion efficiency was not different between the treatments. In terms of HeFCE, the by-product diet was more efficient compared to cereal grain diet ($P < 0.01$). Body weight and body condition score (BCS) were not different between the diets but were affected by week of lactation and parity ($P < 0.01$). Total $CH_4$ emission (g/d), $CH_4$ yield (g $CH_4$/kg DMI) and (g $CH_4$/kg $CO_2$) were reduced with by-product supplementation ($P \leq 0.04$). Treatment had no effect on $CH_4$ intensity (g $CH_4$/ kg ECM), total $CO_2$ production, g $CO_2$/kg DMI and g $CO_2$/kg ECM.

## 4.5 Paper V

The between-cow CV for total $CH_4$, $CO_2$ and $O_2$ (g/d) averaged 10.3%. The lowest residual CV and highest repeatability estimate were observed for $O_2$ consumption (g/d). Replacing cereal grain with by-product did not affect GEI, $E_l$, UE, HP, EB, $k_l$ and RQ. However, faecal energy increased ($P = 0.01$) while DE/GE, ME/GE and $CH_4$E and $CH_4$E yield decreased ($P < 0.01$) with by-product supplementation. Week of lactation and significantly influenced all energy metabolism variables ($P \leq 0.04$). Multiparous cows consumed more energy, digested more energy and produced more faeces and $CH_4$ than primiparous cows ($P < 0.01$). Multiparous cows also produce greater $E_l$ per unit of GE intake ($P < 0.01$). Cows on by-product treatment produced higher proportional faecal N excretion and lower urine N excretion (g/kg N intake) than cows receiving the cereal grain treatment. Milk N efficiency was not different between the dietary treatments.

Blood parameters were not affected by treatment. Week of lactation and parity effects on blood parameters were significant for the majority of blood metabolites ($P \leq 0.04$). Milk short-chain FA (SCFA) and medium-chain FA (MCFA) concentrations were not different between the diets. However, poly-unsaturated FA concentrations were higher for cows on the by-product treatment ($P \leq 0.05$). With the exception of C4:0 and C16:1 *cis*, all SCFA and MCFA were positively correlated with EB. The EB balance estimated from the GF is in close agreement with estimates using the energy requirement from Finnish feed tables. Milk FA from MIR explained 53.2% of the variation in EB measured with the GF system.

# 5 Discussion

The main goal of every dairy production system is to boost productivity or maximise profitability. With feed accounting for about 80% of the total variable costs (Shalloo *et al*., 2004) of milk production, the possible variation between cows in feed intake or in their efficiency of converting feed to milk is of great importance. In the modern dairy cow population, efficiency expressed as feed energy captured in milk has more than doubled through genetic selection for high milk yield and the application of sound feeding and management practices over the past decades (VandeHaar *et al*., 2016). The advantage with increased milk yield is that it is associated with increased feed intake, a greater part of which is directed toward milk production instead of maintenance or body energy repletion (Bauman *et al*., 1985; VandeHaar *et al*., 2016). This phenomenon, known as the dilution of maintenance has been the main basis of improved FE in the past, but this is counterbalanced by possible diminishing marginal returns of FE. With each successive increase in production relative to BW, FE increases at a decreasing rate until it reaches an asymptote. This is because high feed intake is associated with depression in digestibility which may offset the dilution of maintenance effect (Vandehaar, 1998). Despite this, between-cow variation in FE has been observed in earlier studies, which were associated to variation in the processes involved in the pathway of conversion of energy intake to milk energy (Coleman *et al*., 2010; Arndt *et al*., 2015; VandeHaar *et al*., 2016). If any of these existing sources of variation can be identified and quantified, they may serve as a basis for a further selection of FE in the future. Moreover, the emergence of the concept of sustainable agriculture which includes reducing the negative impacts of dairy production on the environment has propelled scientists to find means of lowering GHG production as well as nutrient losses into the environment. Genetic selection against high emitters (without negative effects on production), may help promote a clean environment as well as improve feed efficiency. Therefore, understanding the component traits contributing to the observed between-cow variation in FE is essential for detecting potential correlated responses and knowing what traits to consider in selection. With this knowledge, it may be possible to identify cheaper and easier to measure traits which could be used as markers to aid selection for FE. The five studies presented in this thesis focus on evaluating the measurement of feed intake for FE prediction as well as quantifying the between-cow variation in traits influencing FE and the magnitude of their contribution to FE.

## 5.1 Feed efficiency

The importance of improving dairy FE on farm income and the environment cannot be overemphasized. As such its genetic basis will remain an important subject of discussion on farms. Many definitions of FE have been used in the literature, but no single measure can adequately describe FE or be applicable across systems. Each measure has its peculiar strengths and weaknesses. In this section, the merits and demerits of three of the definitions and their potential effects on genetic selection for FE are discussed.

### 5.1.1 Feed conversion efficiency

Feed conversion efficiency is the most widely used measure of FE and is calculated as the ratio of milk output (kg or MJ) to feed intake (kg DM or MJ). There is ample evidence of genetic variation in FCE whether expressed as between animal variation or heritability estimates (Korver, 1988, Veerkamp and Emmans, 1995, Vallimont *et al*., 2011; Spurlock *et al*., 2012). The between-cow CV in FCE from 661 lactations of Holstein cows was 11% (Hooven *et al*., 1968). In paper II of this thesis, the between-cow CV of the same trait expressed as $E_l$/GE was 8.4% and its repeatability was moderate (0.50) pointing to the genetic basis of this trait. Currently, FCE is incorporated in the cattle breeding program of New Zealand (Coleman *et al*., 2010). Although it is a conceptually easy measure of FE, FCE is faced with many challenges. Because of the existing positive genetic correlation between milk yield and FCE (Spurlock *et al*., 2012), selection for FCE will induce an indirect gain in milk yield which will curtail the added burden of measuring DMI. However, peak milk yields are mainly determined by a genetic propensity to partition DMI and mobilised body energy reserves to milk production. Even with healthy cows fed high energy diets *ad libitum* in early lactation, the DMI is not sufficient to meet the energy demand for lactation. Therefore, the cows assume a negative EB status for a greater part of the first trimester of lactation because the peak yields are partly supported by mobilised body energy reserves. This negative EB status can be detected clinically by the increased concentration of non-esterified fatty acid (NEFA) in blood circulation. It could induce the incidence of ketosis which is antagonistic to fertility and health traits (Coppock, 1985; Berglund and Danell, 1987; De Vries *et al*., 1999). Although this phenomenon is widely acknowledged with unexplained reasons, it may be actually the result of inappropriate feed management peripartum. Better nutritional management may cause an increase in feed intake to support higher production during early lactation and likely alleviate the extent of negative EB.

The benefit of supplementation with starch on the energy status of cows in early lactation has been stressed upon in the literature. Starch fermentation in the rumen is expected to result in an increased production of propionate which is a precursor for glucose production in the liver (Friggens *et al*., 2004). Earlier studies in cows fed higher-starch diets postpartum reported improvements in energy metabolism (Andersen *et al*., 2003, McCarthy *et al*., 2015). However, with grass silage-based diets increasing dietary starch concentration by supplementary grain has failed to increase the proportion of propionate in rumen volatile fatty acid (VFA; Murphy *et al*., 2000; Huhtanen *et al*., 2013). At the animal level, the synthesis of glucose from propionate has been reported to activate an insulin response which favours lipogenesis and inhibit lipolysis (Chilliard *et al*., 2000; Ingvartsen and Andersen, 2000). This has consequences on milk production, as nutrients are used for body tissue deposition instead of milk production. Moreover, given the current decreasing land-base for arable farming, fluctuating cereal grain prices, and the debate on the competition between humans and animals for food, feeding more grains will not be sustainable. Industrial by-products have been evaluated as alternative energy sources for grains. Results on production performance reported in the literature are variable but mostly favourable (Huhtanen *et al*., 1995; Ertl *et al*., 2015; Pang *et al*., 2018). The potential of replacing cereal grain in grass silage-based diets with by-product on postpartum energy metabolism was studied in Paper V of this thesis. Interestingly, the concentration of blood NEFA was not different between the two treatments (Figure 6) despite the lower starch concentration in by-product treatment (Paper IV), suggesting the possibility of replacing cereal grain with by-products in early lactation cows.

Although by-product may replace cereal grain in early postpartum cows, negative EB status was observed in cows in the first seven weeks of lactation. Also, it was during the same period that ECM yield and FCE were high (Paper IV). This further emphasizes the limitation with selecting cows for FCE during early lactation as we risk selecting for cows that are mobilising body energy reserves although they may appear efficient. Spurluck *et al*. (2012) evaluated FCE in mid-lactation cows [75 to 150 days in milk (DIM)] and EB in the first month of lactation and found that they were not genetically correlated. This implies that selection for improved FCE specifically during mid-lactation may be possible without significant adverse effects on EB of lactating dairy cows.

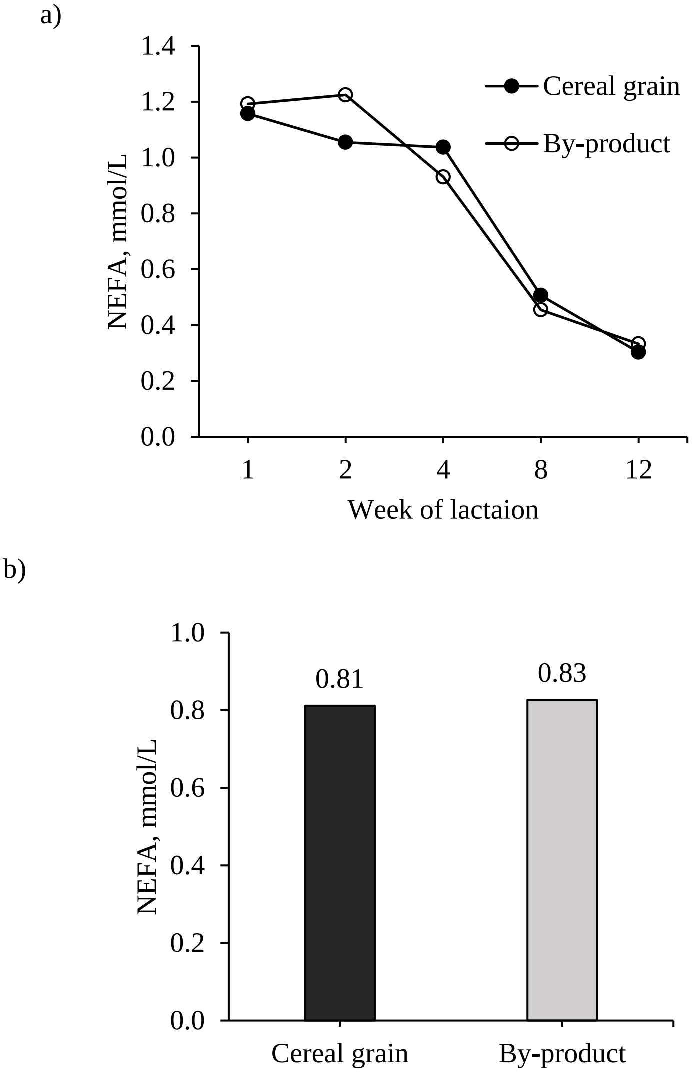


*Figure 6.* The effect of replacing cereal grain with by-product concentrate on blood NEFA concentration (mmol/L) (a) changes with advancing lactation and (b) the average for both diets

An earlier study in the 1970s already demonstrated that FCE in mid-lactation (61-150 DIM) correlated well ($r$ = 0.87 on average) with whole lactation FCE (Hooven *et al.*, 1972). Therefore, efforts should be directed at assessing FCE during this period of established lactation which may reduce the negative impacts of selection for FCE on health and fertility traits. Another limitation of using FCE as an FE index is that it does not differentiate between energy used for separate functions of maintenance, lactation and body tissue depletion or repletion which have been reported to have different partial efficiencies (Veerkamp and Emmans, 1995).

### 5.1.2 Residual feed intake

In an effort to overcome the challenges arising from the use of FCE, RFI was proposed as an alternative measure of FE (Koch *et al*., 1963). Unlike FCE, RFI is designed to measure net FE of the cow. It attempts to allocate a cow's total feed intake to her energy cost for body maintenance, body energy change and production over a course of lactation. Residual feed intake is calculated as the difference between observed and expected feed intake (regression feed intake on a range of energy sinks). This centres RFI around zero, with low or negative values indicating better efficiency and vice versa which can be a source of misperception and limit its acceptance among dairy producers as an FE index (Connor, 2015; Løvendahl *et al*., 2018). Documented heritability estimates of RFI generally are low to moderate ranging from 0.01 to 0.40 among lactating cows (Connor *et al*., 2012; Connor *et al*., 2013; Tempelman *et al*., 2015). This points to the potential of using improving RFI through genetic selection. In Paper II, actual EB measured from RC was used to represent the energy sink of ΔBW. From literature studies, it is clear that this is the first time actual values of EB is used in the prediction of DMI for RFI calculation. The between-cow variation in RFI calculated was 2.0% with a repeatability of 0.22. Much higher estimates of repeatability were observed in earlier production studies with estimates ranging from 0.33 to 0.73 across diets and periods (Kelly *et al*., 2010; Durunna *et al*., 2012). A plausible explanation for the low repeatability observed in Paper II could be that the measurement periods in the chambers are short which can increase the random errors with all errors accumulated in the EB term.

In Paper III, the partial regression coefficient of DMI on ECM was 0.347. Using a dietary ME concentration of 11.8 MJ/kg DM and $k_l$ value of 0.64 from NRC (2001), this partial efficiency would be 0.417. This is within the range of values (0.29-0.47) reported by (Tempelman *et al*., 2015) but higher than the range of values (0.05-0.25) reported by (Li *et al*., 2017) and (Løvendahl *et al*., 2018). Assuming no losses in GE intake to digestion and metabolism, the minimum coefficient would be 0.17. Although it is a less attended-to issue in the literature, the biological significance of the coefficients is very important in having good measures of RFI. Generally, the partial regression coefficients for

DMI prediction in Paper III were much closer to published requirements in different energy systems (e.g. NRC 2001). In addition, earlier studies have reported varying partial coefficients of DMI on ECM at different stages of lactation (Li *et al.*, 2017; Løvendahl *et al.*, 2018; Mehtiö *et al.*, 2018), which contradicts with the concept of constant $k_l$ during lactation (AFRC 1993). Using a section of the data used in Paper III, DIM was negatively associated with $k_l$ although the magnitude was small (0.017 units ~2.7% change per 100 days). The variation in the partial coefficients is a result of fluctuations in the energy requirements of the cow through the course of lactation. Hurley *et al.* (2018) reported weak phenotypic correlation ($r$ = 0.12 to 0.23) among estimates of three different stages of lactation (8-90 DIM, 91-180 DIM and >180 DIM) for grazed dairy cows. Løvendahl *et al.* (2018) evaluated the consistency of RFI over 10 subperiods (4 weeks each) of lactation with full lactation and found that the RFI estimates from the 4th period (week 13-16) were more closely related to RFI for the entire lactation period. In a recent analysis, Connor *et al.* (2019) indicated that a recording period of 64 to 70 days in duration made between 150 to 220 DIM gave the most reliable estimate of RFI for the whole lactation. These results suggest that RFI is best evaluated in the more stable part of lactation when the negative effect of energy balance is eradicated. However, because animals on a farm are not in the same stage of lactation at a point in time, it will be difficult to evaluate RFI for part of lactation as this will limit the number of subjects (Løvendahl *et al.*, 2018). Sufficient numbers of animals are required to obtain reliable estimates of RFI so as to understand correlated responses to selection.

The partial regression coefficients of DMI on ΔBW has also been typically low, variable between studies and among stages of lactation. This reflects the fact that ΔBW is a poor indicator of EB (Tempelman *et al.*, 2015; Li *et al.*, 2017; Løvendahl *et al.*, 2018). A detailed discussion of this is found in Paper III. Using a Monte Carlo simulation, partial regression coefficients of DMI on BW increased while that of ECM decreased when the correlation between DMI and ECM decreased (P. Huhtanen, Swedish University of Agricultural Sciences, Umeå, Sweden, personal communication). This emphasizes the importance of considering the stage of lactation when evaluations are made for RFI. In early lactation when DMI is at its lowest and not adequate to support the increased milk production, mobilised body reserves are used to support the high energy demand for lactation leading to a loss in BW. During this period, the contribution of EB to DMI is the greatest which is further influenced by the errors in estimating ΔBW of which energy content can be variable. In late lactation, DMI, BW and EB are higher while ECM is low which can increase errors in estimating RFI. Theoretically, estimates would be more reliable when determined in established lactation as stated above, a period where the contribution of EB to DMI is rather small compared with BW and ECM.

### 5.1.3 Residual energy corrected milk

Analogous to RFI, Coleman *et al.* (2010) proposed an alternative approach to estimate FE in lactating dairy cows called residual solids production which is calculated as the difference between the actual and expected milk solids production. A similar approach was used in the study of (Løvendahl *et al.*, 2018) called residual milk yield. In this thesis, the term RECM was used as the difference between observed ECM and predicted ECM. The predicted ECM was obtained from the regression of ECM on GE, MBW and EB. An advantage of RECM over RFI is that a positive value is indicative of a greater FE and is desirable, which is more easily appreciated by producers than the negative value in the case of RFI. Also because feed intake is included in the regression model, differences in RECM are independent of feed intake as observed in the similar DMI and GEI among RECM groups in Paper III. The correlation coefficient between RFI and RECM was (-0.75) which clearly indicates that they are not the same trait. Residual feed intake correlates positively with feed intake, but not ECM yield, MBW, ΔBW, suggesting that low RFI (high efficient) cow eat less. On the other hand, high RECM favours high production at a fixed feed intake and MBW. Residual feed intake lays emphasis on production cost while RECM focuses on income (Løvendahl *et al.*, 2018). The use of RECM instead of RFI is economically favourable; assuming a milk price is double that of feed, the difference between the income over feed cost between the most and least efficient cows based on RECM would be about quadruple that based on RFI. In the study of Coleman *et al* (2010), residual solid production in early lactation had a positive influence on conception rate and survival traits. The observed between-cow variation in RECM in paper II was double that observed in RFI. Coleman *et al.* (2010) also reported a higher repeatability estimate of RECM than RFI, suggesting that RECM is more amenable to genetic selection than RFI.

The partial regression coefficients of ECM on various energy sinks were consistent with energy requirements in feed into milk (FiM; Thomas, 2004) and NRC (2001). Using these two systems, the calculated increase in ECM yield was 2.0 kg/kg DMI with an average dietary GE concentration of (18.4 MJ/kg DM), representing ca. 85% of the expected ECM per DMI from both energy systems. With MBW, the partial regression corresponded to 10 kg ECM for cow weighing 600 kg which is approximately 80% of the range of values (12-13 kg ECM) specified in NRC (2001) for maintenance energy requirement for a cow of the same weight. The partial regression coefficients of negative EB and positive EB were approximately 55% of the coefficients presented by NRC (2001). When MEI was used in place of GEI in RECM model, the partial regression coefficients were closer to those presented in NRC (2001). The reason for this is that the model with MEI considers the ME/GE of the diet. Gross energy intake instead of MEI was used in the prediction of ECM because GEI allows for the evaluation of the effects of the losses in faeces, urine and $CH_4$ on FE. Partial regression coefficients for the prediction of intake and ECM in the calculation of RFI and RECM respectively, are not often reported. However, the biological significance of these coefficients is necessary for better evaluation of residual FE traits. For instance, the range of values reported by Løvendahl *et al*. (2018) for the partial regression of DMI on ECM at different stages of lactation was markedly lower than those presented in the energy systems stated above. They (Løvendahl *et al*., 2018) demonstrated that a period of between 3 to 4 months was adequate to evaluate whole lactation RFI.

### 5.1.4 Residual Carbon dioxide

Methane is a product of fermentation in the rumen and to a lesser extent in the hindgut, while $CO_2$ comes from both fermentation and tissue metabolism. Although both $CH_4$ and $CO_2$ are GHG, most of the research on reducing GHG emission from dairy cows have often not accounted for $CO_2$, mainly because it is assimilated by plants and is a less potent gas than $CH_4$ (Steinfeld *et al*., 2006). However, $CO_2$ could be used as a marker of animal efficiency as it is more closely related to whole animal HP (Brouwer, 1965). Using the same approach as for RFI and RECM, Bayat *et al* (2019) introduced the concept of residual $CO_2$ ($RCO_2$). Residual $CO_2$ is defined as the difference between the actual $CO_2$ produced by a cow and her predicted $CO_2$ production. The residual from the regression of $CO_2$ on ECM, MBW and EB is referred to as the $RCO_2$. A low or negative $RCO_2$ represents high efficiency and is desirable while a high or positive $RCO_2$ represents low efficiency. In a meta-analysis of RC data, it was found that $RCO_2$ predicted RFI more accurately (RMSE = 0.42) than it did RECM (Huhtanen *et al*. manuscript under preparation). Given the constraints of measuring individual animal feed intake which is a requirement for determining RFI, $RCO_2$ presents an opportunity to measure individual animal FE without the need for feed intake measurements. However, $CO_2$ production has usually been measured on individual animals in RC, which have been criticised for being expensive, laborious and restraining animals which may affect feeding behaviour. These constraints are being addressed by researchers globally, as new low-cost methods that mimic animals' natural environment have been developed. However, the measurements from some of these methods (discussed later) are variable suggesting their inadequacies in measuring gas production. If $CO_2$ can be determined more accurately, $RCO_2$ can be a good index of measuring FE. Using the GF system in paper V of this thesis, the repeatability estimate of $CO_2$ production was 0.72, suggesting the reliability of this technique in measuring gas production. Besides, the throughput per GF unit can be as large as1000 animals per year on average (Garnsworthy *et al*., 2019). This is an advantage as large data can easily be generated which can be used for ranking cows according to FE with reasonable cost without measuring individual animal feed intake.

## 5.2 Factors affecting feed efficiency

### 5.2.1 Feed intake

Feed intake is an important trait needed for assessing dairy FE regardless of the definition. Moreover, the substantial between-cow variation observed for this trait in Paper I (DMI), Paper II (GEI) and earlier studies (Coleman *et al*., 2010; Li *et al*., 2016) makes it an excellent candidate for consideration when selecting for enhanced FE through genetic selection programs. However, the cost of measuring feed intake for individual animals in commercial dairy farms limits its inclusion in breeding programs. Several indirect methods of measuring DMI have been used over the years and the feed marker technique appears to be the most widely used. In Paper I, the accuracy of using markers to estimate DMI was evaluated. External markers were used for the estimation of FDMO while internal markers were used for the estimation of DMD. The estimated values of both external and internal markers were used to estimate eDMI. The results showed high repeatability estimate for marker-based estimate of DMI (eDMI), which is about 72% of the repeatability estimate for observed DMI. However, the prediction of observed DMI with eDMI showed both slope and mean biases despite a high $R^2$. This indicates that high precision (high $R^2$ and repeatability) does not always imply high accuracy. This lack of accuracy can be related to the problems with incomplete marker recovery and analytical procedures of individual markers used. For instance, the nylon bags used in the *in situ* determination of iNDF as an internal marker varied across studies used in the data analysis while the particle grind size was <2.0 mm. With fragile particles such as faecal samples the likelihood of fine particles escaping from the bags is high. To avoid potential errors, grind size of 2.0 mm was recommended to be used in ruminal *in situ* iNDF determination (Krizsan *et al*., 2015). The markers generally underestimated DMI by 1.69 kg/day and the RMSPE was 15.4% of the observed mean. Based on the classification of relative prediction error values by Fuentes-Pila *et al*. (1996), the predictions provide relatively good estimates. The eDMI was also used in a model to predict FCE with BW and ECM as independent variables in a basal model. The inclusion of eDMI in the basal model improved the prediction by reducing the residual variance by 57%. Which points to the usefulness of using markers for DMI estimates in improving FE measurements. In the same Paper I, however, using external marker-based estimate of FDMO in the model gave the best prediction of FE. Because eDMI requires a combination of both internal and external markers, it would be prudent to use only external markers to estimate faecal output for FE predictions. This will reduce the cost and labour needed for the analysis of double markers. However, it is important to recognise the effort needed for dosing of external markers and caution must be exercised in interpreting results.

### 5.2.2 Digestibility

Digestibility of a diet is an important component of FE and is a function of both animal and diet factors. Individual cow digestibility can be determined directly by total faecal collection, but this method is expensive and laborious, especially when large numbers of animals are needed for selection purposes. An indirect technique is to use different feed markers to determine digestibility. Although this method has been used extensively over the years, its suitability for breeding purposes has been hindered by the cost and procedural demand for laboratory analysis. In Paper I, the accuracy of using marker-based estimate of digestibility was evaluated. The estimate was not entirely accurate as the prediction was associated with both mean and linear biases. Moreover, the repeatability estimate (0.12) for eDMD was 22% of the corresponding estimate for observed DMD, suggesting that direct methods are better than marker methods of estimating digestibility.

Near-infrared reflectance spectroscopy (NIRS) is a relatively simple, and low-cost tool for predicting marker concentrations in faeces, or even directly individual cow digestibility (Nyholm *et al*., 2009; Decruyenaere *et al*., 2012; Mehtiö *et al*., 2019). Mehtiö *et al*. (2016), examined the accuracy of NIRS in predicting three digestibility traits, namely, iNDF concentration is faeces, OMD from faecal samples, and DMD from iNDF concentration in both diet and faeces. The prediction of iNDF was the most accurate with an $R^2$ value of 0.85 and repeatability estimate of 0.46, indicating the possibility to predict diet digestibility using NIRS prediction. In a recent study, Mehtiö *et al*. (2019) demonstrated that NIRS scans of iNDF in faeces adequately predicted genetic variation between cows in digestibility. They (Mehtiö *et al*., 2019) also recorded higher estimates of repeatability and heritability with faecal iNDF, which suggests that the NIRS prediction of iNDF from faeces was more accurate than the prediction of DMD. Therefore, for as long as cows of the same contemporary group consume the same diet, there will be no need to analyse feed samples for digestibility determination with NIRS. However, adjustments need to be made with regards to the sampling protocol and cost so as to establish a suitable genomic prediction cow reference population (Mehtiö *et al.*, 2019).

In Paper I, Paper II, and earlier studies (Huhtanen *et al.*, 2016; Mehtiö *et al.*, 2016; Cabezas-Garcia *et al.*, 2017), the phenotypic between-cow variation in digestibility were small. The between-diet CV was much higher than the between-cow CV (Papers I and II) suggesting that greater improvements in digestibility can be made through diet manipulation. However, the existing between-cow CV cannot be overlooked. It indicates scope for genetic selection of this trait. Besides genetic selection provides a cumulative and long-lasting enhancement in traits and the results are greater and more profitable than those obtained through nutritional manipulation (Richardson *et al.*, 2020). Although small, there is also evidence of genetic variation for this trait (Berry *et al.*, 2007; Mehtiö *et al.*, 2019), suggesting that selection for digestibility could be beneficial.

In Paper III, when FE was expressed as FCE, digestibility remained unchanged across all three FE groups. Similarly, the addition of eDMD to the basal model predicting FCE in Paper I did not improve the accuracy of the model. However, diet digestibility was positively related to improved FE expressed as either RFI or RECM (Paper III). Reduced DMI and improved digestibility accounted for 42 and 58% of lower faecal energy losses in Low- and High-RFI cows respectively. The calculated difference in digestibility between Low- and High-RECM cows accounted for 30% (1.8 kg ECM) of the difference in RECM between the two groups. The results in paper III is consistent with earlier studies that reported negative relationships between diet digestibility and RFI although not always significant (Ben Meir *et al.*, 2018; Fischer *et al.*, 2018). Fischer *et al.* (2018) found a negative correlation (-0.26) between DMD and RFI indicating that the higher the digestive efficiency, the higher the FE. In a recent study by Potts *et al.* (2017b), DMD was found to have declined by 2% from 1970 to 2014. However, when the DMI and diet composition were considered in the model prediction, no differences in DMD were found between the old cows and modern cows (Potts *et al.*, 2017b). The results from Paper I, Paper III, and previous studies suggest that the prospects of improving digestibility by selection have been downplayed and individual cow digestibility has not improved via selection for increased production.

### 5.2.3 Methane

Owing to the global concerns that $CH_4$ emission from dairy cows contribute to climate change, efforts are being directed to selecting animals that emit less. Moreover, $CH_4$ emission is a form of energy loss for the animal. Thus, selecting against it may direct more energy to milk production. Successful breeding of a trait requires the existence of large enough variation between animals. In Paper II the observed between-cow CV in $CH_4$E/GE from RC studies was 6.6%. Using closed-circuit RC, Blaxter and Clapperton, 1965 reported a slightly greater variation (7.2 to 8.1%) in sheep. In the study of Yan *et al*. (2010) an estimate of 17% was recorded, but this included both diet and period effects. Much greater values of at least 30% were reported in studies using the sniffer technique (Garnsworthy *et al*., 2012; De Haas *et al*., 2013; Bell *et al*., 2014; van Engelen *et al*., 2018). In paper V, the between-cow CV (6.2%) observed for $CH_4$ yield from the GF system is consistent with the result in Paper II for RC. Cabezas-Garcia (2017) analysed data from 10 studies conducted with the GF system and reported an average between-cow CV of 10.7% in $CH_4$ yield. It appears that the large between-cow CV was reported with the sniffer method which could be related to the large random errors with the measurements. The close agreement of the GF values with those from RC presents an opportunity to use the GF for $CH_4$ measurements which require lower investment and labour than the RC.

On energetic terms, the effect of $CH_4$ yield is small. For a cow consuming 20 kg/day (GE of 18 MJ/kg DM) of DM, 1 standard deviation in $CH_4$ yield is equivalent to ±1.6 MJ energy i.e. the requirement of about ±0.3 kg of ECM. The effect is likely to be much smaller because of the positive correlation between digestibility and $CH_4$. This positive relationship between digestibility and $CH_4$ is confirmed in Paper IV, where feeding by-product in place of cereal grain in grass silage-based diet reduced digestibility as well as total $CH_4$ production and $CH_4$ yield. It would be expected that $CH_4$ production would be reduced in the cereal grain-fed cows because of the high starch content, which is known to increase the production of propionate in the rumen, thereby reducing $CH_4$ production by acting as an alternative $H_2$ sink (Moss *et al*., 2000). The result in Paper IV provides proof of the long-held view that increasing the starch concentration in grass silage-based diets by supplementary grain does not increase the proportion of propionate in rumen VFA (Murphy *et al*., 2000; Huhtanen *et al*., 2013). Therefore, it can be said that the effect of digestibility on $CH_4$ emission is of greater importance than with starch supplementation on grass silage-based diets.

According to Løvendahl *et al*. (2018), between 25% and 30% of incremental digestible energy can be lost as $CH_4$ in response to lower passage rate and increased digestibility. In Paper II, on average, a percentage increase in DE/GE was associated with 0.04% in $CH_4$/GE. Earlier studies have also reported a positive relationship between $CH_4$ yield and fibre digestibility (Pinares-Patiño and Clark, 2010), rumen pool size (Pinares-Patiño *et al*., 2003), passage rate and digestibility (Huhtanen *et al*., 2016). The direct relationship between $CH_4$ yield and digestibility represents a limitation to enhancing FE by selecting for low $CH_4$ emitters and high digestibility simultaneously. This is because selecting for low emitters may inadvertently result in low digestibility which is a more important characteristic of ruminant nutrition (Løvendahl *et al*., 2018)

Earlier studies in beef cattle showed a positive relationship between RFI and $CH_4$ production (Nkrumah *et al*., 2006; Alemu *et al*., 2017). Similarly, in paper III, cows in the High-RFI groups produced 3.4 MJ more $CH_4$ than their counterparts in the Low-RFI group. However, no difference was observed in $CH_4$ yield (kJ $CH_4$E/MJ GE) among RFI groups. This is unexpected as $CH_4$ yield has been shown to increase with high digestibility and low intake (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013). Lower feeding level generally increases mean retention time of digesta in the rumen (NRC, 2001, Huhtanen *et al*., 2016) and make available more substrate for fermentation (Cabezas-Garcia *et al*., 2017) which leads to increased $CH_4$ production per unit of feed. Goopy *et al*. (2014) reported that sheep with smaller rumens and mean retention time produced less $CH_4$ per unit of feed. This suggests that selecting for low $CH_4$ may as well lead to selecting for smaller animals which may consequently lead to lower digestibility. Body weight has been shown to be positively related to gut volume (Demment and van Soest, 1985). However, more work is needed to elucidate the relationship between retention time and RFI, because data available have failed to show a longer retention time in animals of low RFI (Rius *et al*., 2012; Fitzsimons *et al*., 2014).

With RECM classification, $CH_4$ yield was 3.8 kJ/MJ greater in less efficient than high efficient cows resulting in greater ME/GE in the High-RECM than Low-RECM cows. A positive relationship between $CH_4$ yield and digestibility was observed within RECM groups while a negative relationship was observed between groups. The model from the study of Ramin and Huhtanen (2013), predicted 1.5 kJ/MJ greater $CH_4$ yield for High- than Low-RECM. These findings are not in complete agreement with Freetly *et al*. (2015), who indicated that $CH_4$ yield would not decrease if the improvement in FE is a result of increased metabolic efficiency. However, $CH_4$ yield may increase if the improved efficiency is due to digestibility.

Methane intensity (g $CH_4$/kg ECM) declined with increasing efficiency suggesting that selecting for efficient animals is the most effective way to reduce $CH_4$ emission per unit of product without the need to measure $CH_4$ which is difficult to obtain on commercial farms.

### 5.2.4 ME requirement for Maintenance

The $ME_m$ is an important parameter in the calculation of energy requirements. The $ME_m$ of a cow is difficult to measure. Therefore not many studies have attempted to evaluate the between-cow CV in this trait. The equation $ME_m = NE_m/k_m$ provided by AFRC (1993) has often been used to calculate $ME_m$. Using this relationship with the data from Paper II, the calculated $ME_m$ would be 0.42 MJ/kg $MB^{0.75}$. However, using the regression technique, the estimated $ME_m$ value was 0.74 MJ/kg $BW^{0.75}$. This is proportionately 43% higher than that estimated from the equation of AFRC (1993). Yan *et al*. (1997) also obtained higher values ranging from 0.61 to 0.75 MJ/kg $BW^{0.75}$ (mean was 40% higher than AFRC values) using different regression techniques. The high $ME_m$ values with the regression technique could be attributed to the higher metabolic rates of lactating dairy cows used in this study compared to steers and non-lactating cows used by AFRC (1990). Five decades ago, Moe *et al*. (1970) demonstrated that lactating dairy cows had proportionately 21% greater $ME_m$ compared with dry cows. Agnew and Yan (2000) provided a detailed explanation for the increased $ME_m$ observed in modern lactating dairy cows. Specifically, selection for milk production may have resulted in cows requiring more feed for basal metabolism, which consequently increases their $ME_m$ (Agnew and Yan, 2000; VandeHaar *et al*., 2016). Moraes *et al*. (2015) provided more evidence of increasing $ME_m$ per kg $BW^{0.75}$ with increasing genetic merit of dairy cows over a period of 30 years. An increase in basal metabolism is accompanied by increased activity of internal organs with greater digestive load, cardiac output, and blood flow to digest, absorb and deliver nutrients for increased production resulting in greater oxygen consumption (Reynolds, 1996). The $ME_m$ currently used for formulating dairy cow rations in the UK (AFRC 1993) was developed using calorimeter data obtained from over 4 decades ago. Therefore, it is imperative to update the recommendation specified by AFRC (1993) to reflect the high $ME_m$ of modern dairy cows.

In Paper II, the $ME_m$ was calculated using the relationship from AFRC (1993) but by replacing the $NE_m$ with the intercept obtained from the regression technique. This resulted in an infinitesimal between-cow CV (0.5%, $P < 0.01$) in $ME_m$, which variation was due to the small differences in ME/GE. However, when the equation (assuming fixed $k_l$) in the study of Dong *et al*. (2015a) was used in the calculation of $ME_m$, the between-cow CV in $ME_m$ was 4.9%. Using this $ME_m$ derived from Dong *et al*. (2015a) to calculate $k_l$ resulted in a small between-cow CV (0.9%) in $k_l$. These results indicate that either both $k_l$ and $ME_m$ vary alternately or they vary concurrently. Because $ME_m$ is measured in animals fed only to meet their metabolic functions plus some activity without producing, the simultaneous measurement of $k_l$ and $ME_m$ in lactating cows is technically unattainable. The results in Paper II, however, suggest that there may be an important variation between cows in $ME_m$. Yan *et al* (1997) reported values of between 0.61 to 0.75 MJ/kg $BW^{0.75}$ representing a large range of variation. The few studies that have evaluated between-animal differences in $ME_m$ have reported variable results. In a study with dairy cows and steers, van Es (1961) estimated a between-cow CV in $ME_m$ of 4 to 10%. McNamara (2015), stated that the variation in maintenance requirement is the main cause of variation between animals in FE. Sainz *et al*. (2013) reported a 30% increase in $ME_m$ for High-RFI beef steers relative to Low-RFI steers. In contrast, Low-RFI group had a greater $ME_m$ than the Medium- and High-RFI cows (14 and 18% respectively) with the regression technique (Paper III). Considering the differences in HP among groups (positively related to RFI), it is expected that at low RFI, cows will consume less feed but produce the same amount of milk as the High-RFI cows, which will lead to lower metabolic rates for Low-RFI cows. A likely explanation for the contradictory result could be derived from the study of Hou *et al*. (2012) who stated that there is an existing genetic variation between cows in immunity and response to inflammation, which could affect their ability to show signs during infections. The higher $ME_m$ is likely the result of increased energy expenditure in response to inflammation, but this is beyond the scope of this thesis.

### 5.2.5 Efficiency of ME utilisation for lactation

In paper II, the $k_l$ value estimated from the linear regression technique was 0.68. This is the same as the value obtained by Yan *et al* (1997) using multiple regression of MEI against MBW, $E_l$, and EB. It is, however, higher than the value (0.52) obtained using the $ME_m$ (0.42 MJ/kg $MB^{0.75}$) calculated from AFRC (1993). This could be related to the underestimation of $ME_m$ which inflates the ME requirement for production thereby underestimating $k_l$.

The effects of dietary composition on $k_l$ have been widely studied (Agnew and Yan, 200). There is evidence of increased metabolic activity of internal organs with increasing fibre proportion in the diet, which reduces the energy available for production (Reynolds *et al*., 1991). It is well-established that a high proportion of dietary fibre contributes to increased acetate production while a high proportion of concentrate increases the production of propionate. Propionate is linked to increased milk lactose production, which promotes milk yield while acetate and butyrate basically stimulate milk fat production, which is less energy efficient than lactose production. In view of that Huhtanen *et al*. (1993) demonstrated that increasing butyrate infusion levels at low proportions of propionate reduced $k_l$. However, not much work has been done to show the significant relationship between rumen VFA profile and $k_l$. In Paper II and earlier studies (Ferris *et al.*, 1999; Dong *et al.*, 2015a), there were no differences in $k_l$ for different diet forage proportions. It could be related to the forage type used in these studies being mostly grass silage which has been reported to have little influence on rumen VFA (Murphy *et al.*, 2000; Huhtanen *et al.*, 2013). This is further confirmed in Paper IV where no differences in milk fat and lactose contents were found in grass silage fed cows supplemented with either fibrous by-product cereal grain concentrate. Consequently, no differences were observed in $k_l$ for the two diets.

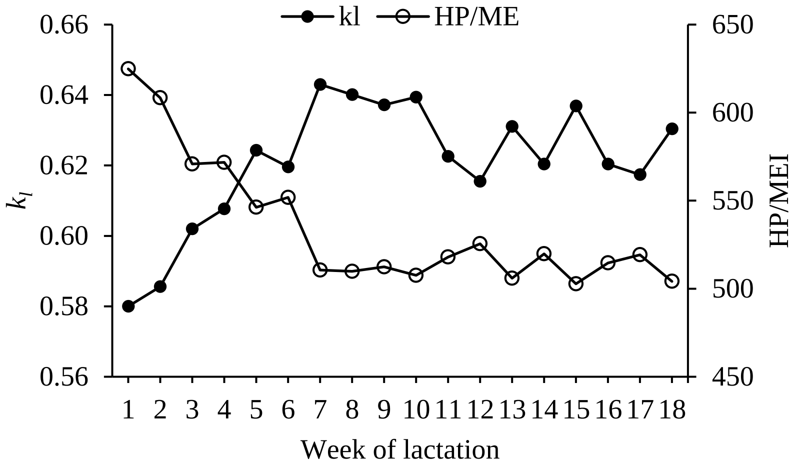


*Figure 7.* The efficiency of metabolisable energy (ME) utilization for lactation ($k_l$) and the heat production as a proportion of ME (HP/ME) from week 1 to 20 of lactation for Nordic Red dairy cows fed mainly cereal grain or fibrous by-product concentrate based diet.

Because $k_l$ of individual cows is difficult to determine, not many experiments have evaluated the between-cow variation in this trait. Earlier studies have reported no differences between cows of the same breed (Gordon *et al.*, 1995a; Ferris *et al.*, 1999) or different breeds (Yan *et al.*, 2006). In Paper II, however, a between-cow variation of 3.8% was observed in $k_l$ which contradicts with the view that $k_l$ remains relatively constant between animal genotypes (Agnew and Yan, 2000). In addition, the $k_l$ of cows observed in Paper V was significantly affected by the week of lactation (Figure 7). The $k_l$ increased from week 1 to week 18 of lactation. This could be attributed to the significantly higher HP as a proportion of MEI in the early weeks of lactation (Figure 7). This result agrees with the findings of Xue *et al.* (2011) in a whole lactation study involving Holstein and Jersey × Holstein dairy cows but contradicts with the studies of

Yan *et al*. (2006) who found that $k_l$ was similar for both Holstein and Norwegian dairy cows in early and late lactation. The inconsistent results obtained for the effects of different factors on $k_l$ suggests more research to better understand this trait.

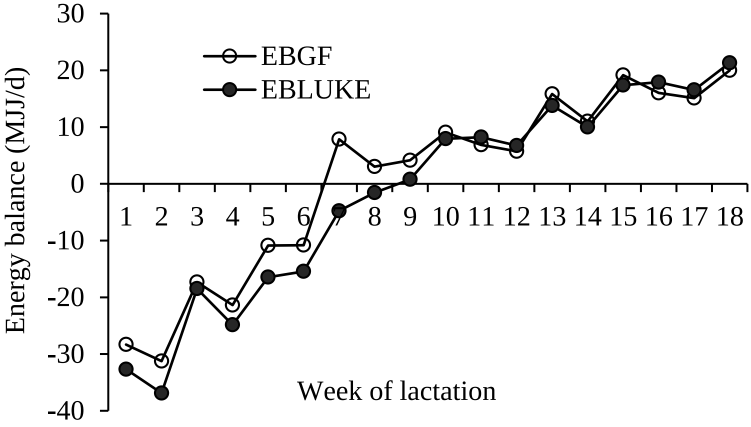
The effect of $k_l$ on FE (RFI and RECM) was greater than the effect of ME/GE on FE. The greater MEI (20.5 MJ/day) of High-RFI compared with Low-RFI cows was offset by a comparable HP. Using an average dietary ME concentration of 11.8 MJ/kg DM, it can be estimated that the loss in HP between the High- and Low-RFI groups was 1.7 kg DMI (20.5 /11.8). Consequently, 65% [1.7 kg/(1.41 kg-(-1.20 kg)] of the difference between Low- (-1.2 kg) and High-RFI (1.4 kg) cows could be ascribed to the differences in their ability to utilise ME for milk production ($k_l$). For RECM classification, the contribution of $k_l$ to the difference between low and high efficient cows was 64%. With these findings, it can be said that $k_l$ is an important trait to consider in genetic evaluation to increase FE. Nevertheless, the determination of $k_l$ involves the use of energy metabolism data which may not always be available.

### 5.2.6 Energy balance

The influence of EB has been a major concern with measuring FCE as discussed earlier. However, the measurements of EB require the use of an RC, which has some limitations (see details in the introduction). The GF system was introduced in 2010 and has since been used to measure $CH_4$ production from ruminants with values being close to those obtained in RC (Huhtanen *et al*., 2019). Five years ago, an upgraded version of this system equipped with $O_2$ analyser in addition to $CH_4$ and $CO_2$ measurements was introduced. In Paper V this new system was used as an indirect calorimeter to determine HP and consequently EB of early lactation dairy cows. Direct comparison of EB measured from the GF with those measured from respiration is practically not possible as each technique requires its own protocol and measurements cannot be made at the same time. Alternatively, it is possible to compare EB data generated from GF system with EB estimated from energy requirements. Energy requirements data are usually based on large datasets, in many cases from RC studies, covering wide ranges in feed intake and diet composition, which is not the case when techniques are compared directly. Therefore, the EB data determined with the GF system ($EB_{GF}$) was compared with EB estimated ($EB_{LUKE}$) from energy requirements for dairy cows specified in the Finnish feed table (LUKE, 2017). Weekly EB ($EB_{LUKE}$) was calculated for each cow using a week average MEI, ECM yield and BW data [$EB_{LUKE}$ = MEI (MJ) – energy required for milk production and maintenance (MJ of ME)], where ME requirement for milk production =

5.15 × ECM (kg), and ME requirement for maintenance = MBW × 0.515. The result showed that EB changes across weeks of lactation were not different for both measurements (Figure 8a). Using a linear regression of $EB_{LUKE}$ against $EB_{GF}$ (Figure 8b), the variation in $EB_{GF}$ explained 76% of the variation in $EB_{LUKE}$. This suggests that estimates of weekly EB from the GF system were in good agreement with values obtained from energy requirement, thus providing the opportunity for using GF in EB measurements.

a)



b)

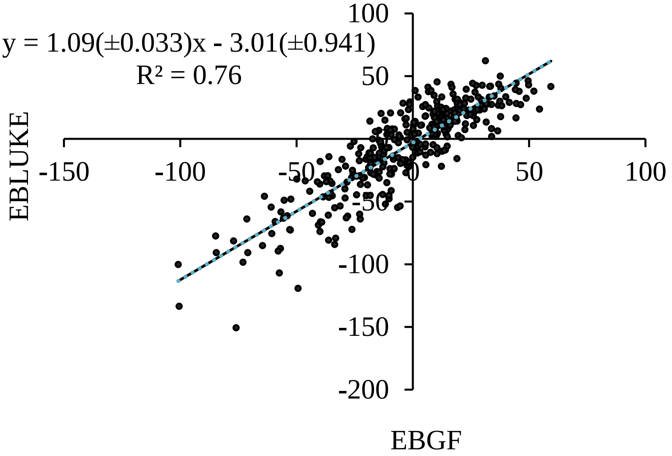


*Figure 8.* Energy balance of Nordic Red dairy cows determined from the GF system ($EB_{GF}$) and energy requirement of Finnish feed tables ($EB_{LUKE}$) during week 1 to 18 of lactation (a), and the relationship between $EB_{GF}$ and $EB_{LUKE}$ (b).

## 5.3 Milk mid-infrared fatty acid profile and energy balance

Energy balance, particularly in early lactation, is well-known to be related to ensuing health and fertility in dairy cows. However, its inclusion in genetic selection programs is hampered by the lack of quick, simple, and cheap measurement techniques. Not long ago, less expensive alternatives using the change in body reserves as feasible methods to quantify EB on a large scale have been suggested (Friggens *et al.*, 2007; Banos and Coffey, 2010). The shortcoming of these methods is that they require regular measurements of BCS and BW, neither of which are routinely obtainable on all commercial farms. Mid-infrared (MIR) spectral data is used regularly by milk recording organisations globally to predict milk composition. In addition, Soyeurt *et al.* (2011) demonstrated that MIR spectroscopy can accurately predict some individual fatty acids. Earlier studies used milk composition measures such as fat-to-protein ratio to measure EB (Heuer *et al.*, 2000; Friggens *et al.*, 2007). Stoop *et al.* (2009) showed that there is a relationship between milk fatty acid composition and EB.

In addition, non-esterified fatty acid (NEFA) concentrations in blood have long been used as indicators of EB in animals clinically. Using the data from Paper V, NEFA was negatively related to EB with an $R^2$ of 0.50. The relationship was much stronger during periods (week 1 to 4) of negative EB ($R^2 = 0.39$) than periods (week 8 to 12) of positive EB ($R^2 = 0.18$). This suggests that EB can best be predicted with NEFA during periods of severe negative EB which helps to identify cows that are apparently efficient due to mobilisation of body reserves. In a recent study, Mäntysaari *et al.* (2019) examined the associations between plasma NEFA concentration and milk MIR with body traits and concluded that NEFA of cows in early lactation can be estimated with reasonably high accuracy by routine milk measurements. In Paper V, the association between $EB_{GF}$ and milk MIR fatty acid data was explored. The model resulted in a cross-validation $R^2$ ($R^2$cv) of 0.53 and RMSE of 19.4 MJ/day. This result indicates that milk MIR fatty acid data can predict EB determined from the GF with an appreciable level of accuracy. As with NEFA, milk MIR fatty acid data predicted EB with higher precision during periods of negative EB ($R^2$cv = 0.52) than periods of positive EB ($R^2$cv = 0.34). With good calibrations and modification to reference data, milk MIR could be a good proxy for EB determination compared to the short-term measurements of BW and BCS changes which are not always reliable. With more data being accumulated on various farms, it will be possible to improve the predictions of EB with MIR which could be a solution to obtaining large data of EB on individual animals for genetic evaluations. This will eliminate the need for investing in additional infrastructure to determine EB.

# 6 Conclusions

- Feed intake determined by the marker method improved predictions of FCE compared to a model based on BW and ECM.
- Faecal DM output predicted FCE better than estimated DMI and digestibility.
- Increased faecal output at a given ECM yield and BW is similarly associated with reduced feed efficiency whether it is derived from increased intake or reduced digestibility.
- Considering difficulties with accurate dosing of external markers and laborious faecal sampling in practical farms, analytical costs and relatively large random errors, it is unlikely that intake estimates based on marker techniques are applicable for ranking the cows according to feed efficiency.
- Greater between-cow variation and repeatability was observed for feed intake which makes it a candidate for genetic selection
- The between-cow variation in digestibility was rather small
- The positive correlation between methane yield and digestibility is a setback for selecting against high emitters
- Selecting for cows with high utilisation of ME for lactation could lead to lower methane emissions
- Regression coefficients of models predicting RFI and RECM agreed much better with published values of energy requirements (energy sinks) compared with estimates derived from production study data.
- The correlation coefficient between RFI and RECM was different from unity indicating that they are not the same trait. High efficiency based on RECM (ECM yield at same DMI, MBW and EB) favoured higher DMI and ECM yield, whereas high efficiency based on RFI favoured reduced DMI at same ECM, BW, and EB.
- About 1/3 of the differences in RFI and RECM between low- and high-efficiency groups was related to improved metabolisability (ME/GE) of the diet and 2/3 to improved efficiency of ME utilization.

- Digestibility accounted for 80% of improved ME/GE, whereas the contribution of $CH_4E$ to ME/GE was about 15% and about 5% to the total difference between low- and high RFI- or RECM groups.
- Methane intensity ($CH_4$/ECM) was 24% lower for high-ECM cows compared with low-ECM cows suggesting that improved feed efficiency is a win-win situation for the dairy industry and environment
- As demonstrated in many studies before, cereal grains can be replaced with fibrous by-products from the food industry without compromising milk production, thereby improving the efficiency of converting non-human edible resources to high-quality human food.
- Energy metabolism measurements by the upgraded GreenFeed system produced realistic values of energy balance and efficiency of ME utilization.
- Weekly mean energy balance measured by GreenFeed or from tabulated ME requirements from tabulated feed values were similar and followed a similar pattern with advancing lactation.
- Feed efficiency traits should be determined in the established stage of lactation when the contribution of the most uncertain energy sink (EB) is relatively smaller than in early or late lactation.
- Blood NEFA concentration or milk fatty acid composition analysed by MIR are useful tools as proxies to eliminate cows that still mobilize body fat and have apparently high efficiency.

# 7 Future perspective

- More reference data is needed to evaluate NIRS prediction of digestibility from faecal samples
- With the limited data size, international collaboration is needed to gather data from a large population which can be used to obtain acceptable levels of repeatability estimates of feed efficiency and related traits for effective genomic evaluation
- The genetic basis for feed efficiency using different definitions should be further investigated to better describe feed efficiency
- The positive relationship between methane and digestibility should be further investigated to find practical ways of selecting for low emitters without compromising on digestive efficiency
- The recommendations in AFRC (1993) should be updated to reflect the high maintenance energy requirement of modern dairy cows.
- Further research is needed on the use of Residual $CO_2$ as a feed efficiency index
- Carbon dioxide production from the GreenFeed system should be evaluated for ranking cows based on $RCO_2$
- Future research should focus on developing on-farm measurement techniques of carbon dioxide and evaluate their reliability
- Develop prediction models for energy balance using the milk MIR spectral data as more data is being accumulated on farms

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# Popular science summary

Modern dairy cows produce more than twice what their ancestors did about 50 years ago. This is the result of milk yield-oriented breeding programs together with the application of sound nutritional and management practices. With increased milk production, feed intake also increases, but a greater portion of it is partitioned to milk instead of maintenance resulting in the dilution of maintenance. This dilution of maintenance has been the major driver of increased feed efficiency in the past. However, it is no more effective at high intake as the digestibility of the diet is hampered with increasing feed intake which has dire consequences on feed efficiency. Therefore efforts should be directed at how to maximise production from each kg of feed.

Individual animal feed intake records are needed for measuring feed efficiency. However, the direct methods of measurement are expensive and laborious, making it difficult to include feed intake in breeding programs. In the first study of this thesis, we evaluated the use of feed markers in estimating individual animal feed intake and feed efficiency. Although the prediction was not accurate, it was very useful in predicting feed efficiency. However, external marker-based estimates of faecal output gave the best prediction of feed efficiency suggesting that faecal output measurements with external markers are adequate to determine feed efficiency thereby removing the need for analysing feed samples. That said, direct measurements were more precise than marker-based estimates. Therefore, if farm conditions are favourable, it is recommended to use direct measures of feed intake in calculating feed efficiency.

The feed energy consumed by a cow goes through several processes of digestion and metabolism before ending up into milk. This trajectory includes components of energy utilization and losses which are believed to have direct effects on feed efficiency. In the second study of this thesis, we quantified the between-cow variation in these components to ascertain their genetic basis. The variation between cows in feed intake was the highest making it the most responsive trait to genetic selection. The variation between cows in digestibility was very small. There was substantial between-cow variation in methane ($CH_4$) energy as a proportion of gross energy intake. With the emphasis being placed on dairy production to reduce $CH_4$ emission into the environment, this result suggests that reduction in $CH_4$ emissions can be achieved by selecting for low emitters. However, caution must be exercised in selecting for low $CH_4$ emitters as we risk selecting for cows with low digestibility because of the positive correlation observed between digestibility and proportional $CH_4$. There was also some variation between cows in their efficiency of utilisation of metabolisable energy for lactation ($k_l$). This variation was negatively correlated with proportional $CH_4$ suggesting that applying selection pressure on $k_l$ will be an effective way to reduce $CH_4$ emissions.

In the third study, we evaluated how the components influence feed efficiency. Despite the low between-cow variation observed in digestibility, we found that the effect of digestibility on feed efficiency was quite substantial. This proposes digestibility is an important trait to consider for future breeding programs. Besides, with low digestibility comes high losses of dietary energy consumed which may have consequences on farm income and the environment.

In the fourth and fifth study, we evaluated the effect of replacing cereal grain with fibrous by-product on the performance of dairy cows in early lactation. The results showed fibrous by-product can replace cereal grain in early lactation cows without impairment on production or energy metabolism. The respiration chamber is currently the gold standard for measuring energy balance of cows, but they are quite expensive and intricate. New methods are being developed by researchers to accurately quantify energy balance with small budgetary allocation. In the fifth study of this thesis, the GreenFeed system was used to estimate the energy balance of cows which was comparable with values obtained from feed requirement tables from LUKE, Finland. The results showed the reliability of using the GreenFeed to measure energy balance. We also found a good correlation between milk fatty acids and energy balance estimated from the GreenFeed system. The use of milk mid-infrared data (MIR) presents a promising means to estimate individual animal energy balance, as MIR is used routinely on farms to determine milk composition which would eliminate the need for additional infrastructure. Combined with measurements of carbon dioxide production the cows could be ranked according to feed efficiency with reasonable cost without measuring feed intake.

# Populärvetenskaplig sammanfattning

Den moderna mjölkkon producerar dubbelt så mycket mjölk som korna gjorde för 50 år sedan. Det är resultatet av avelsprogram som fokuserat på mjölkproduktionen, i kombination med förbättrad utfodring och skötsel. När mjölkproduktionen ökar, ökar också foderintaget, samtidigt som en större andel av fodret går till rpoduktion och en mindre till underhåll. Minskiningen av andel foder som går till underhåll har hittills drivit arbete med fodereffektivitet. Dock blir fodereffektiviteten ofta lägre om foderintaget är mycket högt, eftersom ett högt foderintag kan minska smältbareheten av fodret. Det är därför bättre att fokusera på hur man kan öka effektiviteten av varje kg utfodrat foder.

För att mäta fodereffektiviteten krävs att man kan observera enkilda djurs foderkonsumtion. De direkta mätmetoderna är dock dyra och omständiga, vilket gör det svårt att inkludera foderkonsumtion i avelsprogram. I den första studien i denna avhandling utvärderade vi användningen av fodermarkörer för att uppskatta individuell konsumtion och fodereffektivitet. Även om skattningen inte var helt korrekt, var den mycket användbar för att skatta fodereffektiviteten. Externa markörbaserade uppskattningar av träckproduktion gav den bästa skattningen av fodereffektiviteten, vilket tyder på att mätningar av träckproduktion med externa markörer fungerar för att bestämma fodereffektiviteten och att man minskar behovet av att analysera foderprover. Dock var direkta mätningar mer exakta än markörbaserade uppskattningar. Därför rekommenderas det att använda direkta mått på foderintaget för att beräkna fodereffektiviteten, om förhållandena på gården tillåter det.

Den energi en ko äter går igenom flera processer under matsmältning och metabolism innan den till slut blir till mjölkenergi. Processerna inkluderar komponenter av energianvändning och förluster som tros ha direkta effekter på fodereffektiviteten. I den andra studien i denna avhandling kvantifierade vi variationen mellan kor för dessa komponenter för att fastställa deras genetiska bas. Variationen i foderkonsumtionen mellan kor var störst, vilket gör det till den mest responsiva egenskapen för genetisk selektion. Variationen mellan kor i fodersmältbarhet var mycket liten. Det var stor variation mellan ko i metan ($CH_4$) energi som andel av brutto energiintag. Med hänsyn till tyngdpunkten som läggs på att minska $CH_4$-utsläpp till miljön för mejeriproduktion, tyder detta resultat på att minskning av $CH_4$-utsläpp kan uppnås genom att välja kor med låga utsläpp. Emellertid måste försiktighet iakttas när man väljer ut djur med låga $CH_4$-utsläpp eftersom vi riskerar att välja kor med låg smältbarhet, på grund av den positiva korrelationen som observerats mellan fodersmältbarhet och proportionell $CH_4$. I studien fann vi också en viss variation mellan kor i deras effektivitet vid användning av omsättningsbar energi för amning (kl). Denna variation korrelerades negativt med proportionerliga $CH_4$, vilket tyder på att tillämpning av selektionstrycket på $k_l$ är ett effektivt sätt att minska $CH_4$-utsläpp.

I den tredje studien utvärderade vi hur komponenterna påverkar fodereffektiviteten. Trots den låga variationen i fodersmältbarhet som observerats mellan kor fann vi att effekten av matsmältbarhet på fodereffektiviteten var ganska betydande. Detta visar att smältbarhet är en viktig egenskap att beakta för framtida avelsprogram. Med låg smältbarhet kommer desutom stora förluster av energi som konsumerats, vilket kan ha konsekvenser för jordbrukets inkomster och påverkan på miljön.

I den fjärde och femte studien utvärderade vi effekten på mjölkkornas prestanda vid tidig laktation om man ersätter spannmål med en fiberrik biprodukt. Resultaten visade att en fiberrik biprodukt kan ersätta spannmålsprodukter hos kor i tidig laktations utan försämring av produktionen eller energimetabolismen. Respirationskammaren är för närvarande standarden för att mäta energibalansen hos kor, men de är ganska dyra och komplicerade. Nya metoder utvecklas av forskare för att exakt kvantifiera energibalansen till en låg kostnad. I den femte studien i denna avhandling användes GreenFeed-systemet för att uppskatta energibalansen hos kor och vi erhöll värden som var jämförbara med värden som erhållits från fodertabeller från LUKE, Finland. Resultaten visade att GreenFeed-systemet var pålitlig för att mäta energibalansen hos mjölkkor. Vi fann också en god korrelation mellan mjölkfettsyror och energibalansen uppskattad från GreenFeed-systemet. Användning av mjölk mellaninfraröd (MIR) data är en lovande metod för att mäta individuell eneribalans, då MIR idag används rutinmässigt på mjölkgårdar för att bestämma mjölksammansättning. I kombination med mätning av koldioxidproduktion skulle man kunan ranka korna enligt fodereffektivitet, utan att lägga onödig kostnader på att mäta foderintaget.

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This thesis investigated the between-cow variation in the components of feed efficiency. The variation in feed intake was the largest while the variation in digestibility was rather small. Digestibility was positively related to methane emission which is a drawback to selecting for low methane emitters and high digestibility simultaneously. An effective way to reduce methane emission is to select cows with high feed efficiency.

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